Nitrification bioaugmentation in mainstream flocculent activated sludge systems using sidestream aerobic granular sludge

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Abstract

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Increased nitrification capacity in flocculent activated sludge wastewater treatment systems may be required to meet lower effluent ammonia-nitrogen (NH_3-N) concentration limits, to handle higher influent flows, and/or for conversion to nitrogen removal. A novel approach to increase nitrification capacity in flocculent activated sludge systems is through bioaugmentation with nitrifying granular sludge grown in a sidestream treatment reactor fed anaerobic digester dewatering centrate. Granules from the sidestream reactor are added to the mainstream flocculent activated sludge system to increase the nitrifying biomass concentration without increasing aeration tank volume. The granular sludge solids retention time (SRT) can be decoupled from the low SRT of the flocculent sludge by selective granule retention due to the larger size and faster settling velocity of granules. Retention and accumulation of the granule biomass results in a high volumetric nitrification capacity. The main research goal was to demonstrate viability of the granular sludge nitrification bioaugmentation process and address fundamental scientific and

process application considerations pertaining to sidestream and mainstream treatment performance, nitrification kinetics, and granule biopopulations.

Different types of nitrifying granules were grown on simulated centrate and evaluated for mainstream bioaugmentation potential based on granule nitrification capacity yield and physical characteristics favorable for mainstream retention. Granules performing a) nitrification only and b) nitrification, denitrification and enhanced biological phosphorus removal showed the greatest potential and were further studied in centrate treatment systems. Successful growth of both granule types was obtained with centrate feeding and consistent production of waste granular sludge for bioaugmentation. In separate bioaugmentation tests using the different granule types, addition and selective retention of granules sustained nitrification and allowed nitrogen removal by denitrification in non-nitrifying flocculent activated sludge. Mainstream effluent NH_3-N concentrations near 1 mg/L were achieved at 12°C. Granule removal at the end bioaugmentation immediately resulted in near-complete loss of nitrification. Molecular microbial analyses showed changes to the granule microbial community composition in mainstream treatment and low abundance of ammonia oxidizing bacteria in waste flocculent sludge. The overall results of this research demonstrate that nitrification bioaugmentation with granular activated sludge from sidestream centrate treatment can greatly intensify treatment capability of flocculent activated sludge systems by enabling nitrification and nitrogen removal in otherwise non-nitrifying flocculent activated sludge.

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Chapter 1. OVERVIEW

Nitrogen removal has been and may be required at an increasing number of municipal water resource recovery facilities (WRRFs) to prevent surface water impairment due to eutrophication. Well-established flocculent activated sludge processes have been used in the last four decades for nitrogen removal via aerobic biological nitrification and anoxic biological denitrification. The reactor volume required for these biological processes is greatly governed by the solids retention time (SRT) necessary to maintain the growth of slower-growing nitrifying organisms in the nitrification step. Increasing the nitrification capacity of activated sludge processes in WRRFs to meet lower effluent ammonia-nitrogen (NH_3-N) concentrations, to handle higher influent flows, and/or for conversion to nitrogen removal requires either increased aeration tank volume or intensification of the biological process. Conversion of existing municipal WRRFs to nitrification and nitrogen removal may involve increasing the flocculent activated sludge tank volume by a factor of 3 to 4. Intensification of the biological process is of great interest to limit the capital cost of facility upgrades and because suitable site space to increase the aeration tank volume is often not available.

This research examines a novel approach to increase the nitrification and nitrogen removal capacity in flocculent activated sludge systems by bioaugmentation with nitrifying granular sludge grown in a compact, highly-loaded sidestream treatment reactor fed anaerobic digester dewatering centrate. Nitrifying granules from the sidestream reactor are added to the mainstream flocculent activated sludge system to provide a substantial increase in nitrification capacity without increasing tank volume. The granular sludge solids retention time (SRT) can be

decoupled from the low SRT of the flocculent sludge by selective granule retention due to the larger size and faster settling velocity of granules. Retention and accumulation of the nitrifying granule biomass results in an intensified treatment process with high volumetric nitrification capacity. The main research goal was to demonstrate viability of the granular sludge nitrification bioaugmentation process and address fundamental scientific and process application considerations pertaining to the bioaugmentation potential of different types of nitrifying granules, sidestream and mainstream treatment performance, granule nitrification kinetics and biopopulations, and fate of bioaugmented granules in mainstream treatment.

Chapter 2 reviews relevant literature for this research, including a discussion of the challenges of biological nitrogen removal in flocculent activated sludge systems lacking nitrogen removal capability, nitrification bioaugmentation using flocculent activated sludge from sidestream treatment, aerobic granular sludge growth, and centrate treatment using aerobic granular sludge. The proposed granular activated sludge bioaugmentation process is presented and discussion of the limited prior work on nitrification bioaugmentation using aerobic granular sludge leads to introduction of pertinent research needs addressed in this study.

A product of this effort was a comprehensive report on the use of aerobic granular sludge for biological nutrient removal to advance the state of knowledge within the wastewater treatment industry and provide guidance for future research. The following topics were addressed in detail: 1) physicochemical characteristics of aerobic granular sludge, 2) selective pressures for aerobic granular sludge growth, 3) the effect of different operating conditions on granular sludge growth, 4) microbial diversity in aerobic granular sludge, 5) nutrient removal in bench-scale systems, 6)

removal of micropollutants and industrial chemicals in bench-scale systems, 7) full-scale aerobic granular sludge process applications and nutrient removal performance, 8) modeling of aerobic granular sludge processes, 9) resource recovery opportunities and 10) research needs. **Chapter 3** consists of an edited version of the report's Executive Summary, and the entire report is included in the Supplemental Information as Appendix A.

Although different types of nitrifying granules have been grown in laboratory reactors, metrics of relevance to bioaugmentation have not been reported in prior studies. To inform future research phases, three types of nitrifying granules were grown on simulated centrate and evaluated for mainstream bioaugmentation potential based on granule nitrification capacity yield to support bioaugmentation and physical characteristics favorable for mainstream retention. This is the topic of the research paper in **Chapter 4**: "Comparison of different aerobic granular sludge types for activated sludge nitrification bioaugmentation potential." Granules were grown under the following conditions: 1) anaerobic feeding followed by aeration for nitrification and denitrification and enhanced biological phosphorus removal by phosphorus-accumulating organisms (NDN-PAO granules), 2) anoxic step feeding with sequential aerobic periods for nitrification and anoxic periods with denitrification by ordinary heterotrophic organisms (NDN-OHO granules), and 3) aerated feeding without external organic carbon addition for nitrification only (NIT granules). NDN-OHO granules were not suitable for bioaugmentation due to excessive detrimental growth of *Zoogloea* and *Thauera* and production of extracellular polymeric substances, which resulted in poor settling characteristics and low density, hindering their ability to be separated from flocs. NDN-PAO and NIT granules showed the greatest potential for mainstream bioaugmentation and were further studied in centrate treatment systems.

Sidestream granule growth on centrate and mainstream nitrification bioaugmentation was studied in parallel tracks with NIT and NDN-PAO granules, respectively. Research associated with sidestream NIT granules is the topic of **Chapter 5**, "Bioaugmentation with nitrifying granules in low-SRT flocculent activated sludge at low temperature", and a similar study using NDN-PAO granules is covered in **Chapter 6**, "Bioaugmentation of sidestream nitrifying-denitrifying phosphorus-accumulating granules in a low-SRT activated sludge system at low temperature." Successful growth of both granule types was obtained with centrate feeding and supplemental alkalinity or external organic carbon, respectively. NH_3-N and TN removal efficiencies were greater than 80% in the sidestream NIT and NDN-PAO systems, respectively, at influent total nitrogen (TN) loading rates near 0.6 kg/m^3-d. Waste granular sludge production to support bioaugmentation was consistent for both granule types, but nitrifying granule capacity yield was higher for NDN-PAO granules.

In separate bioaugmentation tests using sidestream NIT or NDN-PAO granules, addition of granules initiated and sustained nitrification and nitrogen removal in non-nitrifying flocculent activated sludge at 12°C with aerobic SRT of 2.5 d and total SRT of 4 to 5 days. Bioaugmentation occurred with an initial slug addition of granules followed by continuous daily addition of granules at a rate proportionate to the observed sidestream granule yield and typical sidestream nitrogen load ratios at full-scale municipal WRRFs. Mainstream effluent NH_3-N concentrations were less than 1 mg/L throughout NIT granule bioaugmentation and near 1 mg/L after 20 days of bioaugmentation with NDN-PAO granules. In both mainstream reactors, the rate of NH_3-N oxidation slowed as NH_3-N concentrations approached the apparent half saturation

concentration of 2.3 mg/L for both granule types. Removal of granules from the mainstream system after 30 to 40 days of bioaugmentation immediately resulted in near-complete loss of nitrification. Together with molecular microbial analyses indicating low abundance of ammonia oxidizing bacteria in the waste flocculent sludge, data indicated that nitrifiers remained with granules with little nitrifier loss to flocculent sludge. In both bioaugmentation tests, granule mass at the end of bioaugmentation was greater than that added as sidestream granules and indicated that seeded granules persisted and new granule growth occurred in mainstream treatment. Granule microbial community composition changed in mainstream treatment and was more similar to that of the flocculent sludge than the original sidestream granules. These overall results indicated that the granular sludge bioaugmentation can provide elevated levels of nitrification and enable nitrogen removal in non-nitrifying flocculent sludge and that both NIT and NDN-PAO sidestream granules are potentially viable for mainstream nitrification bioaugmentation.

The methods, results, and conclusions of this research are presented in Chapters 4-6 as manuscripts accepted or submitted for publication. Additional perspectives, research needs, and engineering implications are discussed in Chapter 7. Supplemental information associated with Chapters 4 and 6 are presented in Appendices B and C, respectively.

The publication associated with the research in Chapter 3 is:

Figdore, B. A., Stensel, H. D., Winkler, M. K. H., Neethling, J. B. (2017) *Aerobic Granular Sludge for Biological Nutrient Removal*. Project No. NUTR5R14h. Water Environment and Reuse Foundation: Alexandria, VA.

The planned publication associated with the research in Chapter 4 is:

Figdore, B. A., Stensel, H. D., Winkler, M. K. H. (2017). Comparison of different aerobic granular sludge types for activated sludge nitrification bioaugmentation potential. *Accepted and pending publication in Bioresource Technology* as of October 2017.

The planned publication associated with the research in Chapter 5 is:

Figdore, B. A., Stensel, H. D., Winkler, M. K. H. (2017). Bioaugmentation with sidestream nitrifying granules in low-SRT flocculent activated sludge at low temperature. *Accepted and pending publication in Water Environment Research* as of October 2017.

The planned publication associated with the research in Chapter 6 is:

Figdore, B. A., Stensel, H. D., Winkler, M. K. H. (2017). Bioaugmentation of sidestream nitrifying-denitrifying phosphorus-accumulating granules in a low-SRT activated sludge system at low temperature. *Submitted to Water Research* in September 2017.

Chapter 2. BACKGROUND

2.1 CHALLENGES OF BIOLOGICAL NITROGEN REMOVAL

The deleterious impacts of eutrophication in aquatic ecosystems including hypoxic zones, fish kills, and production of toxins and offensive taste and odor compounds arising from algal blooms caused by excessive nutrient inputs has long been recognized (Oglesby and Edmondson, 1966). Nitrogen and phosphorus are the limiting macronutrients in aquatic ecosystems, with phosphorus typically being the limiting nutrient in freshwater ecosystems and nitrogen typically being the limiting nutrient in brackish and marine ecosystems (Conley et al., 2009). Point source discharges from domestic and industrial WRRFs represent a significant source of potential nutrient loads to receiving water bodies. Consequently, nutrient removal requirements have been and continue to be promulgated as part of regulatory discharge permits.

While phosphorus can be removed chemically or biologically with minimal modifications or expansion of a conventional flocculent activated sludge WRRF that has been designed for biochemical oxygen demand (BOD) removal only, upgrading it to nitrogen removal requirements presents greater challenges and potential high increase in space needed and cost impacts. Biological nitrogen removal from domestic wastewater is by far considered the economical and viable approach over physical-chemical treatment alternatives. Numerous biological nitrogen removal processes have been developed and widely implemented (USEPA, 2009). Nitrification, the biologically mediated oxidation of ammonia to nitrate, is the rate-limiting step in these processes and ultimately governs the tank volume required for the biological treatment process (Tchobanoglous et al., 2014). For a winter process temperature of 10°C in the northern U.S., the solids retention time (SRT) and tank volume required for a

biological nitrogen removal may be 3 to 4 times greater than that that required for BOD removal only assuming the bioreactor mixed liquor suspended solids (MLSS) concentration is not increased. Therefore, upgrades to achieve nitrogen removal can be difficult to achieve in available site space and can also result in a significant capital cost.

Strategies and technologies to minimize the bioreactor volume expansion necessary to achieve biological nitrogen removal in flocculent activated sludge systems have been developed. Examples include step-feed activated sludge, integrated fixed-film activated sludge (IFAS), and membrane bioreactors (MBRs). A common aspect of these approaches is that the total biomass concentration in the biological reactor is increased. Another approach is to bioaugment nitrifier-enriched biomass from sidestream treatment to the mainstream treatment to increase the nitrification capacity and ratio of nitrifying to non-nitrifying biomass in mainstream treatment. Experiences with nitrification bioaugmentation using flocculent activated sludge from sidestream treatment are discussed in the subsequent section.

2.2 NITRIFICATION BIOAUGMENTATION USING FLOCCULENT ACTIVATED SLUDGE FROM SIDESTREAM TREATMENT

WRRFs with mesophilic anaerobic digestion and sludge dewatering generate a reject stream from the dewatering process characterized by high NH_3-N concentration that can be in the range of 700 to 1500 mg/L. When centrifugation is used for dewatering, the reject liquid stream is referred to as centrate. When untreated, the centrate return stream added to the influent to the mainstream secondary treatment can be 15 to 30% of the total nitrogen load.

Centrate treatment processes can reduce the recycle NH_3-N load and add nitrifying bacteria to the main secondary treatment process. Different approaches have been developed to use the nitrifying biomass generated in centrate treatment to increase the nitrification capacity in mainstream treatment through nitrifier bioaugmentation. Successful nitrifier bioaugmentation using flocculent activated sludge has been demonstrated for both separate sidestream centrate treatment and integrated centrate treatment processes. Aspects and examples of these processes for nitrification bioaugmentation are discussed below.

In separate sidestream centrate treatment processes, centrate is fed to a reactor that is external to the mainstream treatment process and operated as a separate activated sludge system at a different SRT than that of the mainstream treatment process. The schematic of a basic separate sidestream centrate process for nitrification bioaugmentation is shown in Figure 2.1. The sidestream centrate process may be for nitrification only, in which case supplemental alkalinity is required to achieve high ammonia removal efficiency, or for nitrification-denitrification, in which case supplemental carbon is required for denitrification and associated alkalinity recovery. Waste activated sludge (WAS) containing nitrifying biomass from centrate treatment is bioaugmented to mainstream treatment.

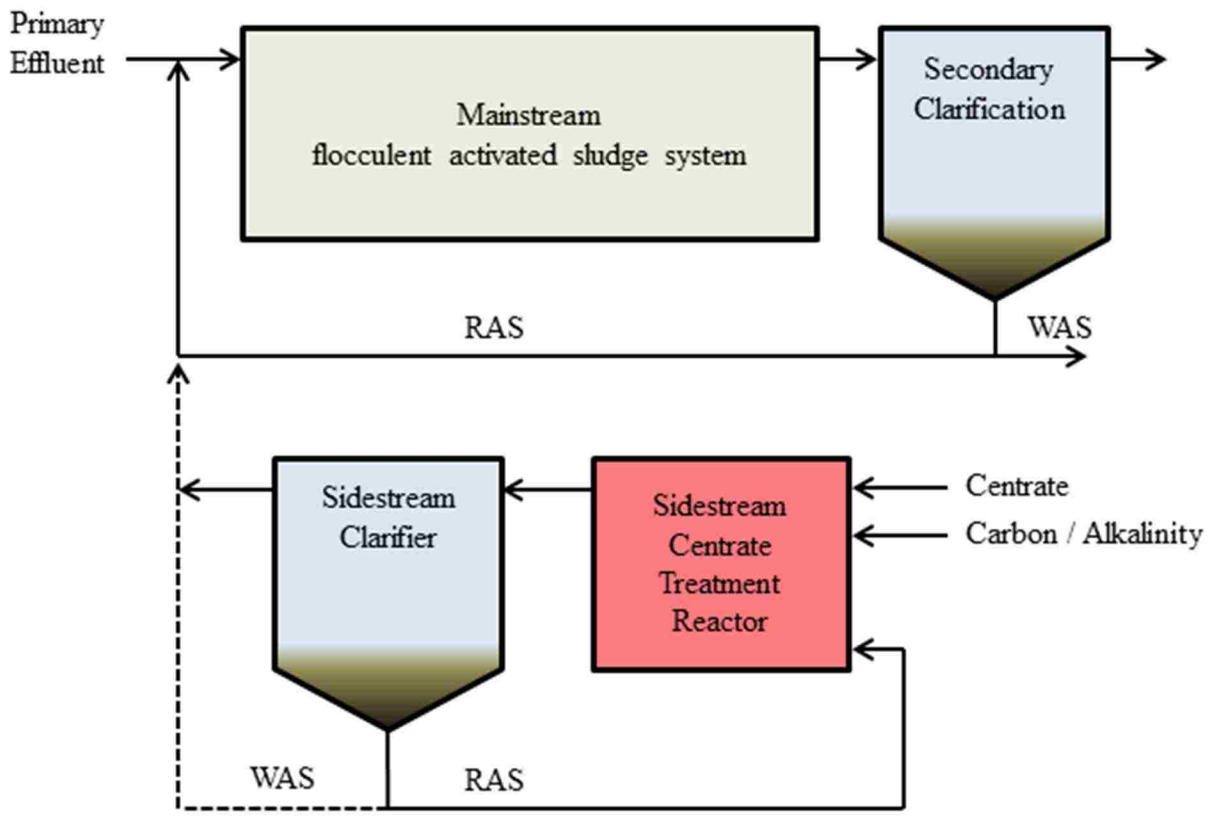

Figure 2.1. Schematic of separate sidestream centrate treatment process for nitrification bioaugmentation.

Nitrifier bioaugmentation using flocculent activated sludge from separate sidestream centrate treatment was initially described by Kos (1998) and later marketed commercially as the InNitri® process. Bioaugmentation by the InNitri® process has been shown to increase mainstream nitrification in a laboratory study (Head and Oleszkiewicz, 2004), but has yet to be shown in larger-scale systems. Aspects of the original InNitri® reactor concept that distinguish it from other separate sidestream centrate treatment processes include operation at high temperature near 35°C, similar to that of mesophilic anaerobic digester centrate, and absence of a return activated sludge (RAS) slipstream from mainstream to sidestream treatment.

Bioaugmentation from separate sidestream treatment processes operated at slightly lower temperature achieved by diverting a small slipstream of mainstream RAS to the sidestream treatment reactor has been shown in larger-scale systems. In a full-scale demonstration study, a mainstream activated sludge train bioaugmented with nitrifying sludge from a sequencing batch sidestream treatment process performing nitritation-denitritation, termed BABE for **b**io**a**ugmentation **b**atch-**e**nhanced, had approximately 60% higher specific nitrification activity and 50% lower effluent NH_3-N concentration than the control train without bioaugmentation or centrate return (Salem et al., 2004). During a 6-week operating period in May and June 2002, influent and effluent NH_3-N concentrations in the BABE-bioaugmented train averaged 57 and 5.2 mg/L, respectively. Average mainstream process temperature and aerobic SRT were 17.9°C and 6.9 d, respectively. Effluent NH_3-N concentration averaged 9.9 mg/L in the control train during this period under similar operating conditions. A different study investigated the bioaugmentation benefit from a flow-through sidestream treatment process performing nitritation-denitritation, termed AT3 for **a**eration **t**ank number **3**, in pilot-scale sidestream and mainstream reactors (Stinson et al., 2007). Parallel mainstream reactors were operated in step-feed nitrogen removal mode with and without bioaugmentation from AT3 centrate treatment. The AT3-bioaugmented mainstream pilot had 30% higher specific nitrification activity than the control mainstream pilot without bioaugmentation. During a one-month operating period beginning on Feb. 15, 2006, effluent NH_3-N concentration averaged about 5.1 mg/L in the AT3-bioaugmented mainstream pilot with process temperature and total SRT near 15°C and 6 d, respectively. Effluent NH_3-N concentration in the parallel mainstream pilot without centrate treatment and bioaugmentation averaged about 15 mg/L during the same period under similar operating conditions.

In the case of integrated centrate treatment processes for nitrification bioaugmentation, the centrate treatment reactor is part of the same activated sludge system as mainstream treatment. Therefore, the same microbial consortium is treating the centrate and primary effluent streams. The fundamental approach in these processes is to increase aerobic SRT through the inclusion of a "regeneration" or "reaeration" basin that receives centrate and the majority or entirety of the RAS flow as shown in Figure 2.2, resulting in a high MLSS concentration in the regeneration basin (up to 10 g/L) and significant increase in aerobic SRT to allow for the growth of nitrifying organisms. This approach has been used at full scale in the United States and Czech Republic (Krhutková et al., 2006; Parker and Wanner, 2007). Operating data presented in Figure 2.3 for the Appleton, Wisconsin WRRF using this nitrification bioaugmentation process in a completely aerobic mainstream activated sludge process show that an average monthly effluent NH_3-N concentration of approximately 5 mg/L was achieved at an SRT of 5 days and winter process temperature of 12°C.

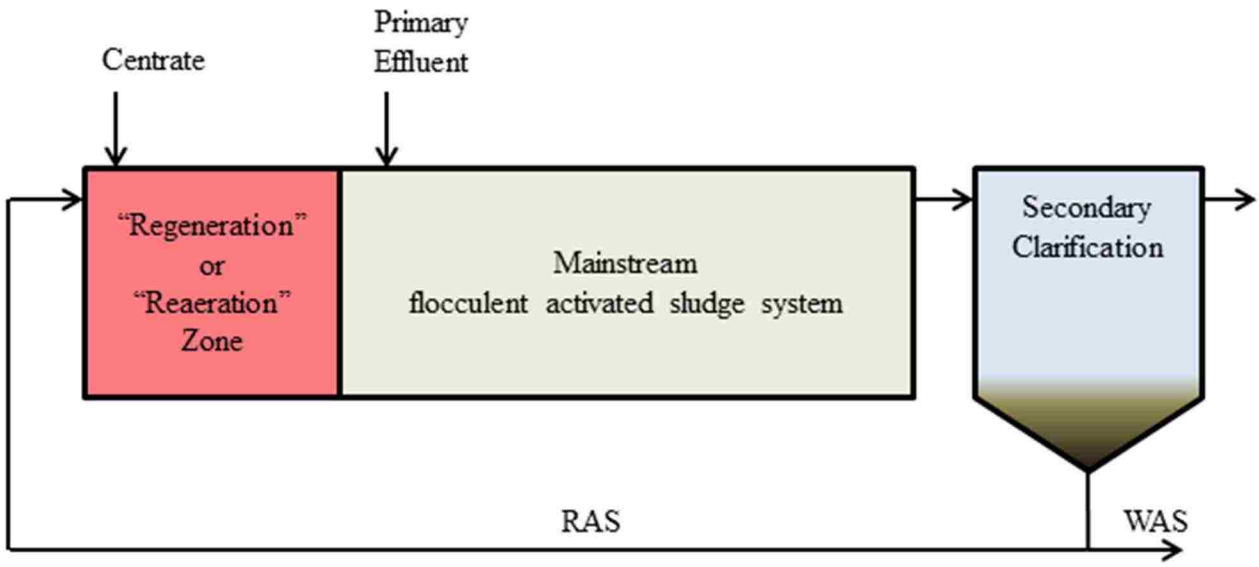

Figure 2.2. Schematic of integrated centrate treatment process for nitrification bioaugmentation.

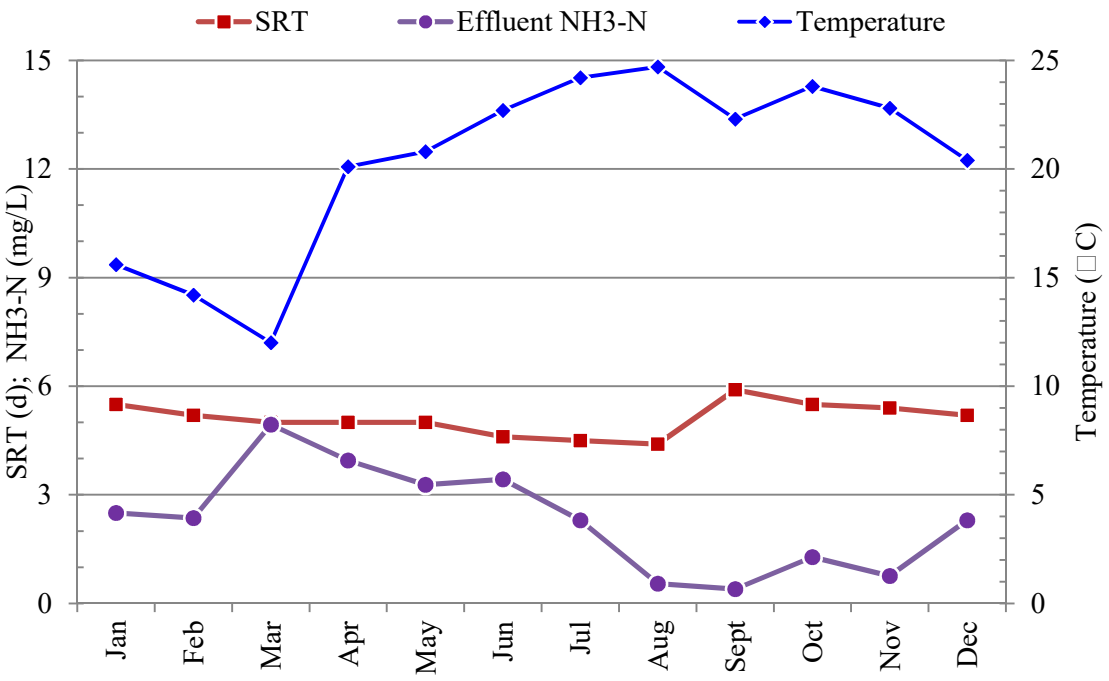

Figure 2.3. Average monthly process temperature, solids retention time, and effluent ammonia concentration in 2003 for Appleton, Wisconsin WRRF.
(Adapted from Parker and Wanner, 2007)

The pilot and full-scale performance data cited above show that nitrification bioaugmentation processes using flocculent activated sludge cannot achieve low effluent NH_3-N concentrations (less than 1.0 mg/L) at winter process temperatures less than 15°C despite moderate SRTs between 5 and 7 days. Effluent NH_3-N concentrations would be even higher for bioaugmentation into systems with low SRTs in the range of 3 to 4 days. The challenge of achieving low effluent NH_3-N concentrations at low SRTs is illustrated in Figure 2.4, which compares effluent NH_3-N concentrations for complete-mix activated sludge (CMAS) systems at 12°C with and without bioaugmentation from a sidestream centrate treatment system. Assumptions made in developing Figure 2.4 include the use of conventional nitrifier kinetic and stoichiometric parameters (Tchobanoglous et al., 2014), a typical sidestream nitrogen load at 20% of the total plant nitrogen

load, and no reduction in bioaugmented nitrifier activity in mainstream treatment. At an aerobic SRT of 4 days, the effluent NH_3-N concentration in the bioaugmented system is 3.0 mg/L, while the non-bioaugmented system would not nitrify. To achieve an effluent NH_3-N concentration of 1.0 mg/L, the bioaugmented system would still require an aerobic SRT of 7 days. The relative reduction in aerobic SRT because of bioaugmentation is also illustrated in Figure 2.4. To achieve an effluent NH_3-N concentration of 1.0 mg/L, the SRT is reduced from 9 to 7 days with sidestream bioaugmentation, corresponding a reduction of only 20%, which is consistent for effluent NH_3-N concentrations greater than 1.0 mg/L and proportionate to the sidestream nitrogen load treated, which was 20% of the total plant nitrogen load.

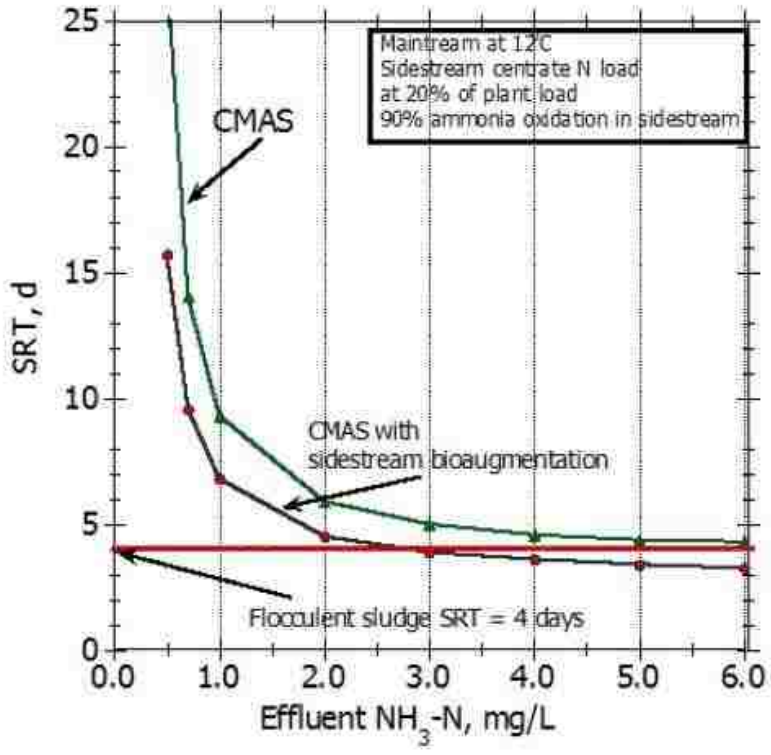

Figure 2.4. Comparison of effluent ammonia concentrations for complete mix activated sludge reactors with and without bioaugmentation from sidestream treatment.

In the successful pilot and full-scale bioaugmentation processes cited above, a common process element was mainstream RAS integration with the sidestream treatment process. Several studies have investigated the effect of bioaugmentation and RAS integration on the nitrifying organisms present in mainstream and sidestream treatment. Podmirseg et al. (2010) showed differences in mainstream and sidestream communities of ammonia oxidizing bacteria (AOB) without RAS integration, while AOB communities tended to converge with an increasing amount of RAS integration. Other studies have shown that bioaugmentation caused a shift in the dominant mainstream nitrifier genera (Pei et al., 2015; Yu et al., 2012). Because RAS integration also lowers the sidestream treatment reactor temperature, it is not known if the effect on the changes in the nitrifier community were due to temperature or the RAS integration or both.

Based on observations of different sidestream and mainstream nitrifying communities, researchers have investigated the possibility of distinct AOB populations that are "mainstream specialists" and "sidestream specialists". A model-based study incorporated two AOB populations with different kinetic parameters (Wett et al., 2011). In three different model formulations, two AOB populations were defined by: (1) different growth and decay temperature sensitivities (i.e., "high-temperature" versus "low-temperature" AOB; (2) different values for μ_{max} and $K_{NH3\text{-}N}$ (i.e., "r-strategists" versus "K-strategists"); and (3) different $K_{NH3\text{-}N}$ and decay rates. Although the model-predicted results corresponded to treatment performance measured in case studies, AOB kinetic parameters were not explicitly measured, and the validity of the fundamental mechanisms assumed is uncertain. This issue is complex because a diversity of AOB having different kinetic characteristics can coexist within a given treatment plant (Park and Noguera, 2007; Siripong and Rittmann, 2007).

2.3 AEROBIC GRANULAR ACTIVATED SLUDGE

Aerobic granular activated sludge is an emerging biological wastewater treatment technology offering nutrient removal potential. In conjunction with this research, a comprehensive report on aerobic granular sludge was prepared and accepted for publication by the Water Environment and Reuse Foundation after peer review (Figdore et al., 2017). The report addressed aerobic granular sludge research and application including granule physical characteristics, granular sludge formation and selective pressures, microbial diversity, treatment performance in bench-scale and full-scale systems, modeling, resource recovery, and research needs. The entire report is provided as Appendix A, and its executive summary is excerpted in Chapter 3. Aspects of aerobic granular sludge most pertinent to this research are highlighted in this section.

Aerobic granular activated sludge can be described as an attached growth or biofilm process where carrier media is not required and rapidly-settling microbial granules containing a consortium of microorganisms are formed via application of key selective pressures. Aerobic granules are typically 0.5 to 3 mm in diameter. The higher settling velocity of granular activated sludge compared to flocculent activated sludge is attributed to its larger particle size and more compact spherical morphology resulting in a settling regime that more closely resembles discrete settling rather than hindered settling. This is made apparent by low sludge volume index (SVI) values between 30 and 50 mL/g where the ratio of the settled sludge volume after 5 minutes and 30 minutes (SVI_5/SVI_{30}) approaches 1.0 (de Kreuk et al., 2005). In comparison, well-settling flocculent activated sludge would be expected to have an SVI_{30} near 100 mL/g and SVI_5/SVI_{30} ratio from 1.6 to 2.0. A range of granule sizes and densities has been shown for laboratory and full-scale granular sludge systems, with stratification of granules of different size and density in

a settled sludge bed occurring based on settling velocity (Winkler et al., 2011). Due to these characteristics, granular activated sludge systems can have mixed liquor suspended solids (MLSS) concentrations similar those of MBRs and much higher than conventional flocculent activated sludge systems, resulting in more compact biological process designs.

Several key selective pressures and fundamental principles for aerobic granular sludge selection and growth have been identified. The liquid-solids separation is designed to allow retention of the larger and faster-settling granules over smaller and slower-settling flocs. This has typically been accomplished by applying a short settling time commonly less than 5 minutes in sequencing batch reactors (SBRs) but has also been accomplished using particle size selection by sieving or screening (Liu et al., 2014a), upflow liquid-solids separation (Tsuneda et al., 2003), and cyclonic separation in the case of anammox granules (Shi et al., 2016). When significant readily biodegradable COD (rbCOD) is present in the influent wastewater stream, the COD feeding regime should expose granular biomass to high rbCOD concentration. A feeding regime of this nature promotes substrate penetration into the granule core and helps to avoid diffusion-limited growth conditions, which are not favorable for granular sludge growth (van Loosdrecht et al., 2005). Under diffusion-limited growth conditions granular sludge is not stable and filamentous or finger-like outgrowths occur (Picioreanu et al., 1998). This detrimental growth condition can result in reactor failure by washout of the poor-settling filamentous granules (McSwain et al., 2004; Mosquera-Corral et al., 2005). Growth must also not be limited by diffusion by electron acceptor substrates such as O_2, NO_2, or NO_3. Sufficient shear must be provided in proportion to microbial growth rate, with faster-growing organisms requiring higher shear to form a smooth and compact granule biofilm morphology (van Loosdrecht et al., 2005).

Aerobic granules reported in the literature can be broadly classified into four types based on differences in growth conditions and biological processes occurring in their respective granule microbial consortia. Despite growth in an aerobic environment, granules performing deammonification and containing ammonia oxidizing bacteria (AOB) and anaerobic ammonium oxidizing bacteria (anammox) are not commonly referred to as aerobic granules. Noting the unique classification of AOB-anammox granules, aerobic granule types and their respective acronyms used in this document are given as follows:

1. Granules that perform enhanced biological phosphorus removal (EBPR) and nitrification-denitrification. For conditions favoring selection of phosphorus accumulating organisms (PAOs), glycogen accumulating organisms (GAOs) may also grow. These are referred to as *NDN-PAO granules*.
2. Granules that perform nitrification and denitrification but without anaerobic contacting for PAO/GAO growth. Heterotrophic organisms using carbon substrates in this case are termed ordinary heterotrophic organisms (OHOs). These are referred to as *NDN-OHO granules*.
3. Granules grown with aerobic feeding and where denitrification is limited. Nitrogen removal is dominated by assimilation for growth on carbon substrates. These are referred to as *OHO granules*.
4. Granules that are fed mainly ammonia or nitrite and perform ammonia and/or nitrite oxidation with no significant nitrogen removal. These are referred to as *NIT granules*.

These aerobic granule types are described in greater detail below along with feeding conditions and characteristic performance.

For NDN-PAO granular sludge systems denitrification or denitritation is done mainly by PAOs and/or GAOs. Influent rbCOD is assimilated and stored by PAOs and or GAOs under anaerobic contacting with the influent wastewater, and nitrogen removal occurs primarily by simultaneous nitrification-denitrification in a subsequent aerobic period at a dissolved oxygen concentration that is typically 2 mg/L or less. PAOs are typically present at greater abundance than GAOs, and a high degree of EBPR is achieved. The use of biodegradable chemical oxygen demand (bCOD) for both EBPR and denitrification by denitrifying PAOs (DPAOs) has been shown in NDN-PAO granules (Bassin et al., 2012a) and can result in an optimal use of influent bCOD for biological nutrient removal. NDN-PAO granules have been grown in laboratory-scale reactors (de Kreuk and Van Loosdrecht, 2004; Kishida et al., 2006) as well as in the full-scale Nereda® SBR process (Giesen et al., 2013; Pronk et al., 2015a).

NDN-OHO granules perform nitrification-denitrification or nitritation-denitritation with carbon conversions mediated by OHOs. EBPR does not occur in processes with NDN-OHO granules because of an absence of anaerobic contacting with wastewater feed. Instead, influent is fed under anoxic conditions, and a high degree of nitrogen removal occurs by alternating nitrification-denitrification in sequential aerobic and anoxic periods. To date, NDN-OHO granules have only been grown in laboratory-scale reactors (Chen et al., 2013; Wang et al., 2012).

For OHO granules carbon consumption is by OHOs, and EBPR does not occur. Influent is typically introduced in a slug feed of less than 5 minutes into an aerated reactor or quiescent

reactor followed immediately by aeration. Ammonia oxidation may occur, and nitrogen removal occurs primarily by assimilation. Although OHO granules have been primarily grown at the lab scale (Moy et al., 2002; Tay et al., 2002a), the successful growth of OHO granules in a pilot scale reactor treating municipal primary effluent has been reported (Ni et al., 2009).

NIT granules perform ammonia and/or nitrite oxidation with minimal total nitrogen removal. Influent is fed under aerobic conditions. NIT granules have been grown with autotrophic synthetic media without organic carbon or with carbon-poor streams such as anaerobic digester dewatering centrate. The relative abundance of nitrifying organisms is higher in these granules compared to the other types of granule described above. NIT granules have only been grown in laboratory-scale reactors in systems performing ammonia oxidation to nitrate (Shi et al., 2010; Tay et al., 2002b; Tsuneda et al., 2003; Vázquez-Padín et al., 2010), ammonia oxidation to nitrite (López-Palau et al., 2011; Vázquez-Padín et al., 2010; Wan et al., 2013), and nitrite oxidation to nitrate (Ni et al., 2011; Vázquez-Padín et al., 2009).

When the influent wastewater contains a significant amount of particulate biodegradable COD (pbCOD) as is the case for domestic wastewaters, an anaerobic feeding regime and NDN-PAO granular sludge is preferred. Particulate matter in the influent wastewater is sorbed to the granule surface (de Kreuk et al., 2010), where it may be hydrolyzed to rbCOD and fermented to acetate and propionate. These volatile fatty acids (VFAs) are then consumed by PAOs. The rbCOD generated from hydrolysis can diffuse to support PAO growth in the granule core as the COD assimilation capacity of PAOs located closer to the granule exterior with first access to substrate is exhausted. Conversely, if anaerobic hydrolysis is incomplete or pbCOD-containing wastewater

is fed aerobically, pbCOD will be present at the granule surface during aerobic conditions, leading to slow release of readily biodegradable COD (rbCOD) by aerobic hydrolysis. In this case, the presence of non-degraded pbCOD sorbed to the granule surface and slow production of rbCOD from hydrolysis leads to diffusion-limited growth conditions and filamentous outgrowths from the granule that are detrimental to granule morphology, settling velocity and stability.

To date, full-scale application of aerobic granular sludge has been limited to the Nereda® SBR process which selects for NDN-PAO granule growth (Pronk et al., 2015a). This process has been used to treat domestic wastewaters and industrial wastewaters from food processing operations. The first full-scale Nereda® reactors were commissioned circa 2010, and as of July 2016 there were approximately 20 Nereda® installations in operation or under construction worldwide.

2.4 SIDESTREAM CENTRATE TREATMENT USING AEROBIC GRANULAR SLUDGE

Aerobic granular sludge growth on centrate from anaerobic digestion dewatering has not been reported in the literature. A related study by Lopez-Palau et al. (2011) reported NIT granule growth in a laboratory-scale SBR on wastewater described as anaerobic digester supernatant. The supernatant was characterized by an average bicarbonate to ammonium molar ratio of 1.1 and average NH_3-N, TSS, and sCOD concentrations of 810, 690, and 340 mg/L, respectively. Approximately 50% NH_3-N removal was achieved without alkalinity addition at an NH_3-N loading rate of 2.4 g/L/d, MLVSS concentration of 9200 mg/L, process temperature of 30°C, and DO concentration near 8 mg/L. Ammonia oxidation was to NO_2-N only. Because the wastewater was digester supernatant and not from anaerobic digestion sludge with dewatering, it was not representative of that from dewatering with polymer addition.

High polymer dose is frequently used in anaerobic digestion sludge dewatering, and the residual polymer in the dewatering centrate is of concern regarding its effect on aerobic granular sludge formation. Two related studies reported contrasting results concerning the impact of polymer coagulants in aerobic granular sludge systems. An 8-d period of polyaluminum chloride dosing at a concentration of 400 mg/L successfully reformed and restabilized crushed granules (Liu et al., 2014b). This granule reformation procedure was performed on a pulse-fed aerobic SBR after 90 days of operation when granules began to disintegrate. Granule disintegration was attributed to inner core decay and lysis associated with large 3.2 mm granule size and lack of substrate penetration to the granule core. In a different study at much lower polymer dosing, the continuous inclusion of 2.5 mg/L polyaluminum chloride and 1.5 mg/L Chemifloc® polyelectrolyte flocculating agent were shown to have adverse impacts on the granular sludge quality (Val del Río et al., 2012). Granules grown in the control pulse-fed aerobic SBR without coagulant addition had lower SVI (40 mL/g vs. 80 mL/g), smaller diameter (2.3 mm vs. 5.0 mm), and possessed a more ideal smooth granule surface and morphology. A higher MLVSS concentration was maintained in the control reactor (7.9 g/L vs. 4.5 g/L) due to a lower effluent suspended solids concentration. The granules grown with coagulant addition had a lower maximum specific oxygen utilization rate normalized to VSS, which was contributed to the adsorption of the coagulants on the granule surface and greater DO diffusion limitation caused by the larger size. Despite the lower specific oxygen utilization rate in the reactor with coagulant addition, the reactor systems had similar COD and N removal efficiencies.

The results of Val del Río et al. (2012) imply that residual polymer in anaerobic digestion dewatering centrate may adversely impact aerobic granular sludge physicochemical properties.

However, the widespread use of AOB-anammox granular sludge in full-scale sidestream treatment deammonification processes (Lackner et al., 2014) suggests that granules, in general, can be formed for a variety of dewatering reject streams with upstream polymer addition.

2.5 RESEARCH CONCEPT

The overarching research concept and core thesis postulated is that bioaugmentation of nitrifying aerobic granular sludge from sidestream centrate treatment enables nitrification and nitrogen removal in non-nitrifying low-SRT mainstream flocculent activated sludge systems.

The motivating factor for use of granular sludge for nitrification bioaugmentation is the potential to selectively retain the nitrifying granules over flocs in the mainstream treatment, thereby accumulating a high concentration of the nitrifying microorganisms. This approach is expected to have significant advantages over using flocculent activated sludge from sidestream treatment because of the differences in the retention of the bioaugmented nitrifiers in the mainstream treatment reactors. The SRT of the flocculent nitrifying biomass is limited to that of the mainstream flocculent sludge SRT, whereas the SRT of the granular nitrifying biomass can be much greater due to the ability to separate it from the flocculent sludge and keep it in the mainstream system. In the case of flocculent sludge bioaugmentation, the SRT needed for complete nitrification may only be about 20% less than the value needed without bioaugmentation. However, with nitrifying granules, the granular sludge SRT can be decoupled from the low SRT of the flocs by selective granule retention due to its larger size and faster settling velocity using sieves, hydrocyclones, or possibly gravity separation. In this manner, the critical nitrifying biomass concentration in the mainstream treatment system can be greatly increased. It is hypothesized that the proposed sidestream granular sludge bioaugmentation

process will increase the nitrification capacity of existing low-SRT mainstream flocculent sludge systems, such that it may be possible to increase the nitrogen removal capability of full-scale treatment systems without additional mainstream tank construction.

The types of aerobic nitrifying granule types for mainstream nitrification bioaugmentation considered in this research include NIT, NDN-OHO, NDN-PAO granules. The sidestream AOB-anammox granular sludge that has been demonstrated in full-scale centrate treatment is not preferred for this application, which is to dramatically increase the nitrification ability of existing mainstream treatment systems. A disadvantage of using AOB-anammox granules in this case is that the AOB-anammox deammonification sidestream process can only provide about half as much nitrifying biomass because ammonia is used in the process to reduce nitrite produced by AOB.

A schematic of the granular sludge bioaugmentation concept is shown in Figure 2.5 with selective granule retention occurring on the WAS stream. Although other locations for granule retention are possible, the WAS stream presents a flow rate to the granule-floc separation process that is much lower than that of that of the forward flow or RAS streams. Key aspects of the process concept are discussed below.

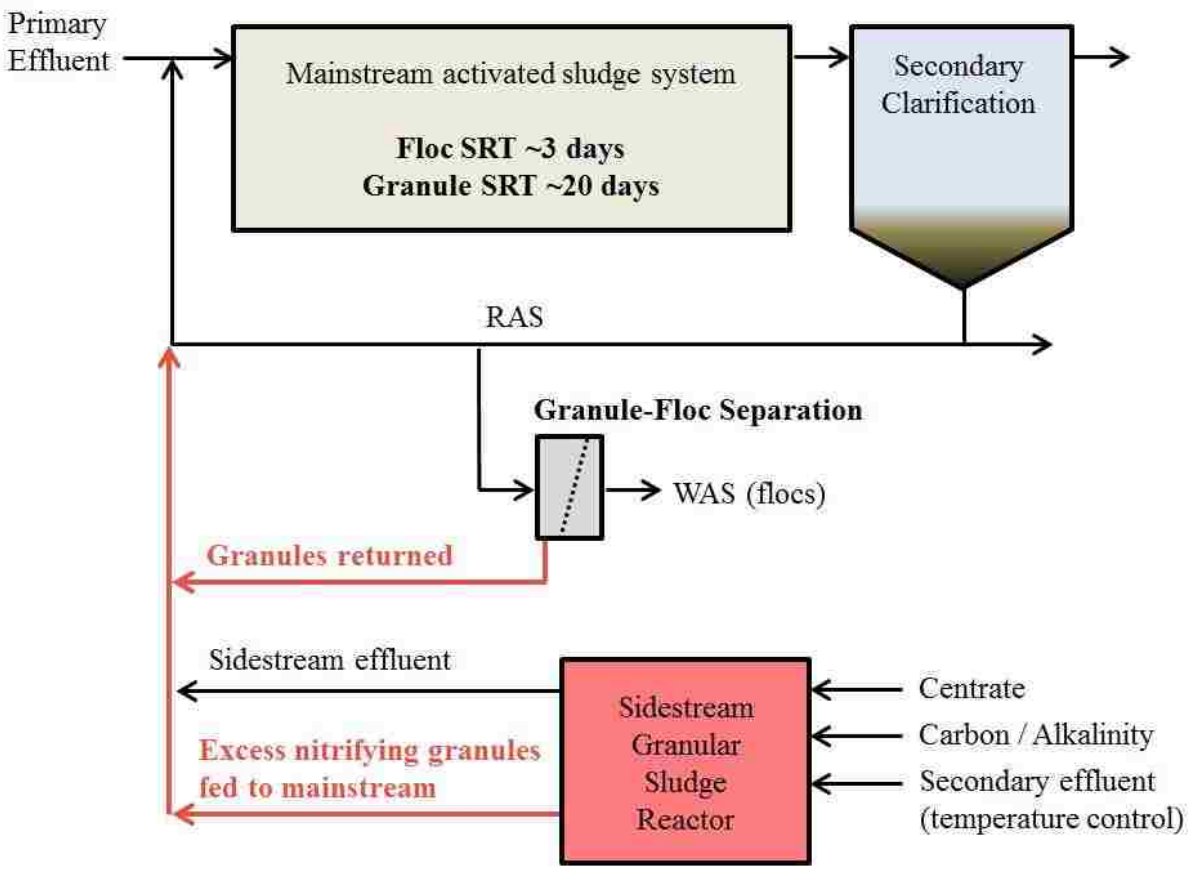

Figure 2.5. Nitrifying granular sludge bioaugmentation process schematic.

Nitrifying granular sludge generation would be in the sidestream centrate treatment reactor will require supplemental alkalinity or carbon depending on the centrate treatment process type. A nitrification-only treatment processes using NIT granules would require supplemental alkalinity to offset alkalinity consumed in nitrification, whereas a nitrification-denitrification process using NDN-OHO or NDN-PAO granules would require supplemental carbon for denitrification and its associated alkalinity production.

Granular sludge SBRs can possess a high fill ratio and therefore accommodate dilution water addition as shown in Figure 2.5. It is anticipated that a sidestream granular sludge SBR with fill ratio of 0.50 will be able to accommodate approximately a 5:1 dilution of influent centrate. Inclusion of a dilution water stream such as secondary effluent is favorable for bioaugmentation because it reduces the temperature difference between the mainstream and sidestream processes, which provides a sidestream temperature that reduces potential issues with temperature shock to the bioaugmented nitrifying biomass in the mainstream treatment. A lower process temperature also increases the nitrifying biomass yield in the sidestream centrate treatment.

Nitrifying granular sludge would be bioaugmented to the mainstream treatment by pumping waste granular sludge from the sidestream treatment. Sidestream treatment effluent would also be discharged to the mainstream secondary treatment process. Sidestream effluent solids are anticipated to contain some nitrifying biomass but be more floc-like than granule-like in morphology, size, and settling characteristics. Therefore, generation of sidestream nitrifying biomass as waste granular sludge in lieu effluent solids is important to the process concept.

Nitrifying granules bioaugmented to the mainstream treatment will be selectively retained such that the mainstream granular sludge SRT is much longer than the mainstream flocculent sludge SRT. For example, the mainstream granular sludge SRT might be 20 days while the mainstream flocculent sludge SRT may only be 3 days. In Figure 2.5, selective granule retention and separation from flocs occurs by sieving the waste sludge stream and returning granules to mainstream treatment while flocs continue to waste solids handling as normally practiced. Other granule separation approaches involving cyclonic or gravimetric separation are also possible.

The ultimate objective of the proposed granular sludge bioaugmentation process is not only limited to nitrification but to also achieve nitrogen removal. Two potential approaches for achieving mainstream nitrogen removal with granular sludge bioaugmentation are shown in Figures 2.6 and 2.7. In Figure 2.6, an anoxic-aerobic Modified Ludzack-Ettinger (MLE) process is used for nitrogen removal only. In this case, the granular sludge is expected to be the primary contributor to nitrification, while the flocculent sludge accomplishes the denitrification. NIT or NDN-OHO granules may be preferred for bioaugmentation in these anoxic-aerobic processes where EBPR is not a treatment objective and an anaerobic contact zone is not present. In Figure 2.7, an anaerobic-aerobic process is used for nitrogen removal and EBPR. In this case, granular sludge could contribute to nitrification, denitrification and EBPR with NDN-PAO granules being preferred for bioaugmentation. Influent biodegradable COD can potentially sustain bioaugmented granule PAOs with simultaneous nitrification-denitrification and EBPR occurring in the aerobic zone at low DO concentrations.

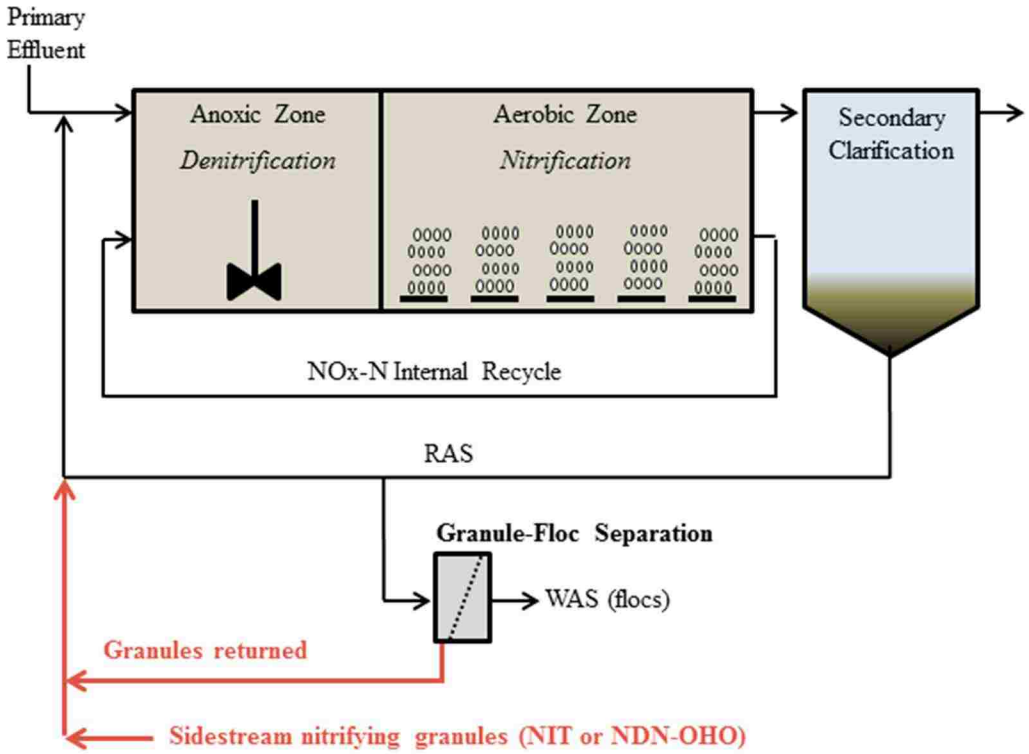

Figure 2.6. Bioaugmentation of sidestream NIT or NDN-OHO granules for nitrogen removal in an anoxic-aerobic Modified Ludzack-Ettinger process.

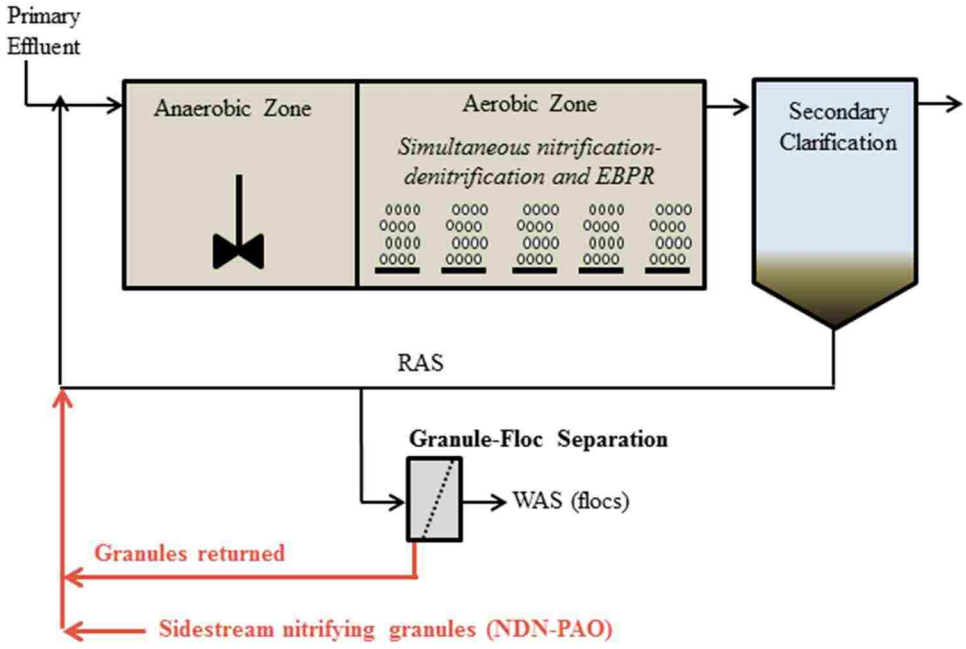

Figure 2.7. Bioaugmentation of sidestream NDN-PAO granules for nitrogen removal in an anaerobic-aerobic process.

A spreadsheet model was developed to assess the conceptual viability and important factors influencing the sidestream granular sludge nitrification bioaugmentation concept. Because ammonia oxidation is the rate-limiting step for biological nitrogen removal, the model focused on the ability of sidestream granular sludge bioaugmentation to achieve a given mainstream effluent NH_3-N concentration goal. For simplicity, the model did not use diffusion-mass transport kinetics as commonly used for biofilm processes, but did consider AOB rate reductions for temperature and bulk liquid dissolved oxygen and NH_3-N concentrations in a CSTR process. Conventional kinetic and stoichiometric parameters for AOB were assumed (Tchobanoglous et al. 2014). The following variables were considered: 1) relative nitrogen loads in sidestream and mainstream treatment, 2) NH_3-N removal efficiency in sidestream treatment, 3) the fraction of bioaugmented sidestream nitrifying biomass as granules versus flocs, 4) mainstream granule separation and recycle efficiency, 5) the rate of potential granule breakup in mainstream treatment, 6) temperature and SRT in sidestream and mainstream treatment, and 7) mainstream effluent NH_3-N concentration goal. Because the model did not include a specific term for mainstream loss of granule nitrifying activity, the assumption for granule breakup in mainstream treatment can be considered a lumped term for loss of actual granule biomass to flocs and loss of granule nitrifier activity due to heterotrophic growth on granules resulting in a decrease of granule nitrification activity. The model allowed for mainstream nitrifier growth in both granular and flocculent biomass. Nitrifier growth was assumed to be proportionate to the ratio of granule-associated and floc-associated nitrifying biomass. The mainstream treatment system was assumed to have no nitrifying biomass at the start of bioaugmentation. Nitrifier decay rates were uniformly applied to granule and floc-associated nitrifying biomass. In the case of mainstream

granule breakup, the related nitrifying mass was transferred to the floc-associated nitrifying mass. The NH_3-N oxidation capacity (mg N/L-d) of mainstream granular and flocculent biomass fractions was determined and compared to the NH_3-N oxidation required to meet a specified effluent NH_3-N concentration.

Several model scenarios are summarized and discussed below to show how these factors might influence mainstream ammonia removal performance from sidestream granule bioaugmentation. Key parameters assumed in four model Scenarios (A to D) are summarized in Table 2.1. All Scenarios assumed the following: 1) a sidestream nitrogen load of 25% of the mainstream nitrogen load, 2) 85% NH_3-N removal in sidestream treatment, 3) process temperatures of 18°C and 10°C in sidestream and mainstream treatment, respectively, and 4) SRTs of 15 and 3 days in sidestream and mainstream treatment, respectively. Different assumptions were made for the fraction of bioaugmented sidestream nitrifying biomass as granules versus flocs, mainstream granule capture efficiency, granule breakup in mainstream treatment, and mainstream effluent NH_3-N concentration goal.

Model outputs for Scenarios A to D are shown in Figure 2.8. The primary model output is a plot of the ratio of total mainstream NH_3-N oxidation capacity to NH_3-N oxidation required to meet the specified effluent NH_3-N limit. If this ratio (NOx capacity / NOx required) is greater than 1, the user-specified effluent NH_3-N concentration goal can be met, while the converse would hold for a ratio less than 1. Assumptions and results for Scenarios A to D are discussed below.

Table 2.1. Summary of key parameters in granular sludge bioaugmentation model scenarios.

Parameter	Scenario			
	A	**B**	**C**	**D**
COMMON				
Sidestream N load (% of mainstream)	25			
Sidestream NH_3-N removal (%)	85			
Sidestream temperature (°C)	18			
Sidestream SRT (d)	15			
Mainstream temperature (°C)	10			
Mainstream SRT (d)	3.0			
VARIABLE				
Bioaugmented nitrifying mass as granules (%)	80	20	80	50
Bioaugmented nitrifying mass as flocs (%)	20	80	20	50
Mainstream granule capture efficiency (%)	95	95	100	95
Mainstream granule breakup (% per day)	5	5	15	10
Mainstream effluent NH_3-N goal (mg/L)	1.5	1.5	1.5	3.0

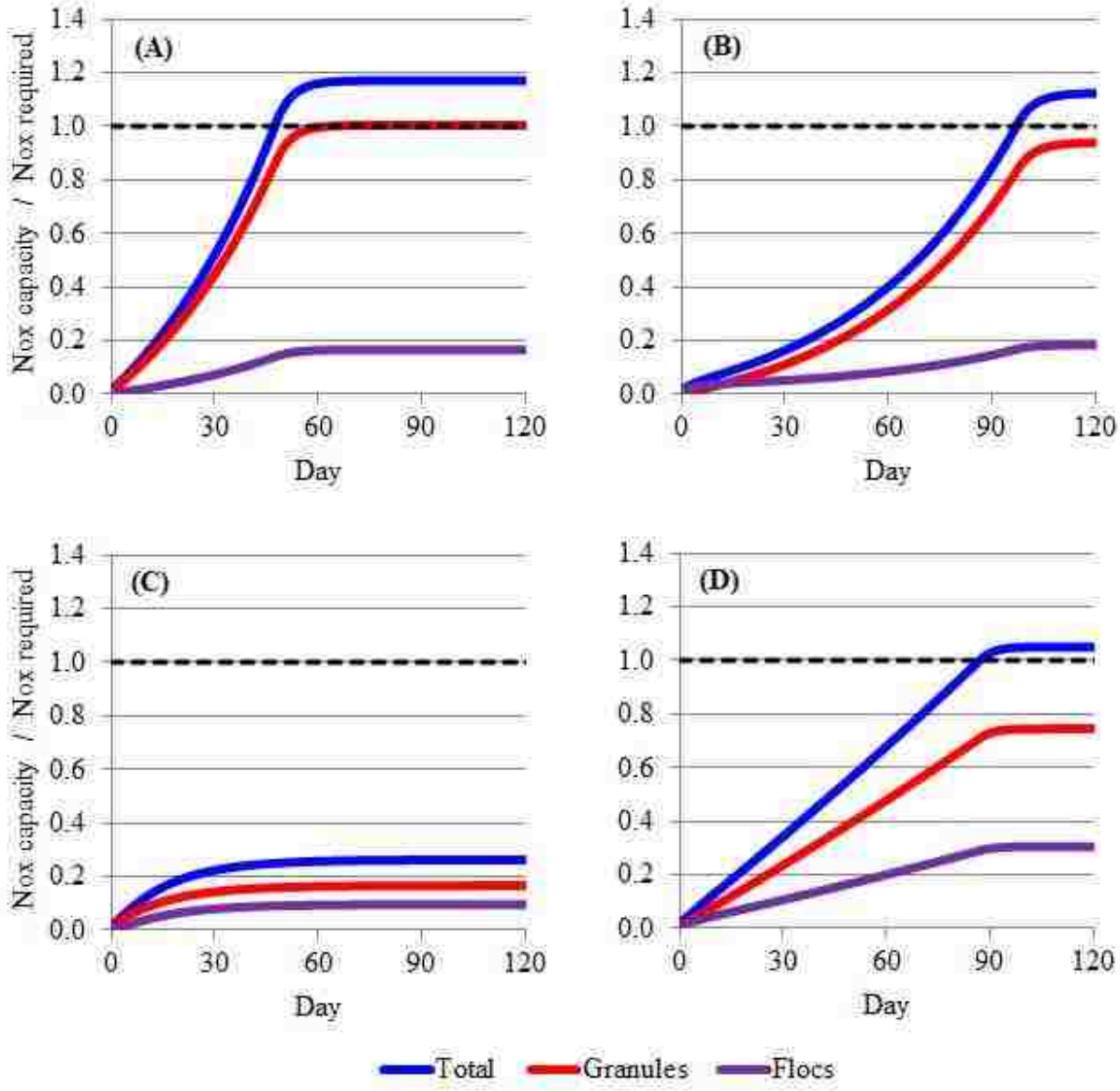

Figure 2.8. Results of granular sludge bioaugmentation model Scenarios A to D.

Scenario A assumed a high percentage of bioaugmented sidestream nitrifying biomass as granules (80%), good granule capture efficiency (95%), and low level of mainstream granule breakup (5% per day). The mainstream effluent NH_3-N concentration target was 1.5 mg/L. The model suggests that this concentration would be met after approximately 45 days of bioaugmentation, as indicated by time at which the NOx capacity / NOx required ratio reaches 1. The higher NOx capacity / NOx required ratio with continued bioaugmentation, suggests lower NH_3-N effluent concentration could possibly be achieved for this Scenario.

Scenario B assumed a low percentage of bioaugmented sidestream nitrifying biomass as granules (20%), and the same granule capture efficiency, degree of mainstream granule breakup, and mainstream effluent NH_3-N concentration target as Scenario A. The model suggests that effluent NH_3-N of 1.5 mg/L would be achieved after approximately 95 days of bioaugmentation. This outcome is expected given the lower nitrifying granule yield for bioaugmentation in Scenario B and suggests that relatively low sidestream nitrifying granule yield may still allow low mainstream NH3-N concentrations to be achieved if granule integrity and retention in mainstream treatment is favorable.

Scenario C assumed the same high sidestream granule yield (80%) and low effluent NH_3-N concentration (1.5 mg/L) as Scenario A. However, a higher rate of granule breakup (15% per day) was assumed. The model suggests that the target mainstream NH_3-N cannot be achieved despite 100% granule capture efficiency, but that a higher effluent NH_3-N concentration of 5.0 mg/L could be met (output not shown). This scenario illustrates that the ability of granules to

maintain integrity in mainstream treatment is essential to meet low effluent NH_3-N concentrations at cold temperatures and short SRTs.

Scenario D assumed an equal percentage of sidestream nitrifying biomass yield as granules and flocs, 95% granule capture efficiency, and moderate level of mainstream granule breakup (10% per day). The model suggests that a mainstream effluent NH_3-N concentration of 3.0 mg/L can be achieved, which would still represent a considerable improvement in ammonia removal performance compared to flocculent sludge bioaugmentation processes at similar mainstream temperature and SRT.

Based on the preceding scenarios and others evaluated with the model but not presented here, several inferences can be made pertaining to key factors impacting the process viability and effluent NH_3-N concentrations that can be achieved with granular sludge bioaugmentation. Assuming high mainstream granule separation efficiency, high sidestream NH_3-N removal efficiency, and typical relative sidestream and mainstream nitrogen loads, model results were most sensitive to mainstream granule breakup, sidestream nitrifying biomass produced as granules, mainstream conditions for SRT and temperature, and effluent NH_3-N concentration goals. Model outputs suggested that a moderate degree of mainstream NH_3-N removal with effluent NH_3-N concentrations between 2 and 5 mg/L could be achieved even under relatively unfavorable conditions for sidestream nitrifying granule production and longevity in mainstream treatment. However, under less favorable mainstream conditions of short granular sludge SRT and cold temperature, the loss of granule biomass and nitrification activity in the mainstream treatment must be minimal to meet low effluent NH_3-N concentration goals. Higher sidestream

nitrifying granule production shortens the duration to achieve mainstream effluent NH_3-N targets. Therefore, these key factors impacting the bioaugmentation process were considered in the research.

2.6 PRIOR WORK ON NITRIFICATION BIOAUGMENTATION USING AEROBIC GRANULAR SLUDGE

Although nitrification bioaugmentation using sidestream granular sludge appears promising, previous work investigating this general concept has been limited in quantity and scope in only two studies, both of which used ammonia-oxidizing NIT granules grown on an autotrophic synthetic wastewater feed in a continuously-fed aerobic upflow sparged reactor with solid-liquid separator and external settling tank as depicted in Figure 2.9 and originally described by Tsuneda et al. (2003). Findings and deficiencies of these studies are summarized below.

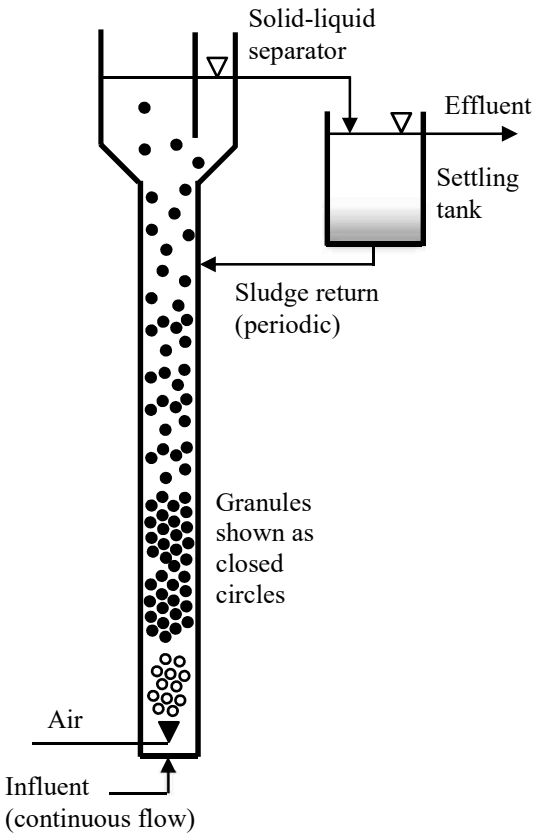

Figure 2.9. Schematic of continuously-fed aerobic upflow sparged reactor with solid-liquid separator and external settling tank.

In the first study (Tsuneda et al., 2006), granules were mixed with flocculent sludge for treatment of a wastewater containing a complex synthetic organic carbon composition in a continuously-fed reactor that was not described in detail but likely similar to the baffled completely-mixed reactor described in a subsequent study by the same research group (Kishida et al., 2011) and depicted in Figure 2.10. The inoculated granular and flocculent sludge concentrations were 1200 and 1500 mg/L, respectively. The complex synthetic organic carbon-containing wastewater was characterized by DOC, TN, and NH_3-N concentrations of 180, 100, and 60 mg/L, respectively. Reactor pH was controlled at 7.0 to 7.1 using sodium bicarbonate. A 100-200 um thick heterotrophic biofilm developed on the nitrifying granules, and an increase in the gas sparging

rate aeration rate was necessary to provide sufficient oxygen penetration to maintain nitrifying activity (Tsuneda et al., 2006). For the initial gas sparging condition of 0.5 Lpm at 20% oxygen concentration, the product of ammonia oxidation shifted from nitrate to nitrite over the first 20 days of exposure with flocculent sludge and the organic wastewater. Full ammonia oxidation to nitrate was recovered after doubling the aeration rate while maintaining the 20% oxygen condition. The dissolved oxygen concentrations at these gas sparging conditions were not reported. During this operation there were no negative changes in granule morphology and integrity.

In a similar second study by the same research group (Kishida et al., 2011), nitrifying granules and flocculent sludge were added to a continuously-fed continuously-aerated baffled completely-mixed reactor (Figure 2.10) at initial reactor MLSS concentrations of 1200 and 1500 mg/L, respectively. Parallel baffled completely-mixed reactors were operated at various COD/N ratios of 0, 2.6, and 13.2 using acetate while NH_3-N was fixed at 50 mg/L. pH in the reactors was controlled between 7.0 and 7.2 using sodium bicarbonate. The initial aeration rate of 1.0 Lpm was increased on day 12 to maintain DO concentration greater than 2 mg/L. An NH_3-N removal efficiency of approximately 85%, 80% and 65% were achieved in the reactors with COD/N ratios of 0, 2.6, and 13.2, respectively. The decrease in NH_3-N removal efficiency with increasing influent COD/N ratio was attributed to heterotrophic growth on the seeded granules. It was noted that heterotrophic growth on the granule reduced granule settling velocity.

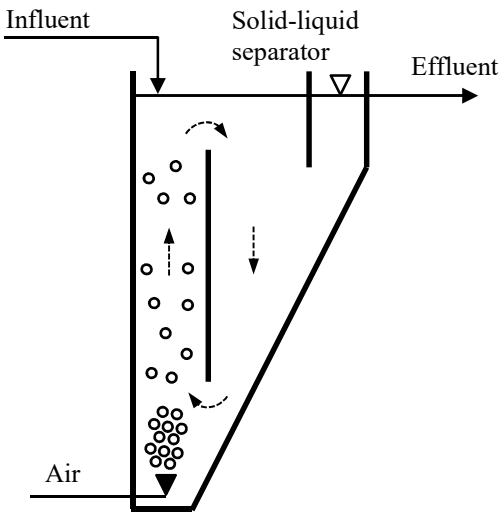

Figure 2.10. Schematic of continuously-fed baffled complete-mix reactor with solid-liquid separator.

The studies described above using NIT granules to provide nitrification in flocculent activated sludge systems were deficient in several aspects including the following: 1) Changes in granular and flocculent solids concentration over time were not reported, and the relative extent of granule-floc separation and granule retention was not characterized. 2) The SRT of the flocculent sludge fraction was not specified, and the contribution of the flocculent sludge fraction to ammonia removal was not clear. The potential wash-out of flocculent seed sludge could have occurred due to upflow solid-liquid separator zone design, which was not described in detail. Potential floc wash-out would be expected to skew heterotrophic growth towards granules rather than growth as flocs. Conversely, it is possible that flocs were retained and that nitrifying activity in the flocculent sludge fraction increased over the course of the experiments, contributing to ammonia removal along with the granular sludge fraction. 3) Temperature of the bioaugmented reactors was not specified. Therefore, nitrification kinetics and minimum SRT for nitrifier growth could not be estimated. 4) The specific nitrification activity of the bioagumented granules was not indicated. Therefore, the nitrification capacity associated with the applied dose of

nitrifying granules cannot be compared to the ammonia load applied to the bioaugmented reactors. 5) Granules were bioaugmented into completely aerobic systems. Though this experimental approach is not a fatal flaw, bioaugmentation into systems with nitrogen removal would be more representative of common discharge requirements for WRRFs treating domestic wastewater.

2.7 RESEARCH NEEDS

This research investigated fundamental process and microbiological concepts that are critical for assessing the potential and applicability of using nitrifying aerobic granular sludge from sidestream centrate treatment to increase the nitrification and nitrogen removal capacity of short-SRT flocculent activated sludge systems. The fundamental process application issues important to this research were the following:

- What is the biomass yield of nitrifying granules in the growth of different types of granular sludge treating anaerobic digestion centrate?
- How do the physical characteristics of the different types of nitrifying granules compare? Of greatest interest are granule size, density, and integrity coefficient. These parameters are important to the mainstream granule-floc separation process design and assessment of the ability to sustain granules under pumping and mixing in the mainstream treatment system.
- Do characteristics of anaerobic digester centrate, such as the suspended and colloidal solids, residual polymer, and/or other centrate substances affect the ability to grow nitrifying granules?
- What centrate treatment performance can be achieved with different types of nitrifying granules? Key metrics include the sidestream reactor nitrogen loading rates, ammonia

and nitrogen removal efficiency, and the external COD dose required for the growth of NDN-OHO and NDN-PAO granules.

- Does granular sludge mass and specific ammonia oxidation activity change after bioaugmentation and long-term exposure in the mainstream system, which has different feed characteristics and operating conditions than the centrate-fed sidestream granule growth reactor?
- What minimum effluent NH_3-N is attained using sidestream granular sludge bioaugmentation to systems with a flocculent sludge SRT below the theoretical minimum nitrification SRT?

Fundamental scientific questions for this research included the following:

- What are granule biomass-specific NH_3-N utilization rates at high and low NH_3-N concentrations representative of sidestream and mainstream treatment conditions?
- What is the effect of sidestream growth condition on the nitrifying granular sludge microbial population composition?
- Will the granule microbial population composition change after bioaugmentation and long-term exposure to mainstream treatment conditions?
- What is the fate of bioaugmented granule nitrifiers in mainstream treatment? Will granule-associated nitrifiers remain on granules or transfer to flocculent activated sludge after bioaugmentation and growth in mainstream treatment?
- Will heterotrophic bacteria out-compete the nitrifiers for space on the granule outer aerobic layer in the mainstream treatment reactor to reduce the nitrification capacity of granular biomass?

Chapter 3. AEROBIC GRANULAR SLUDGE FOR BIOLOGICAL NUTRIENT REMOVAL

A product of this research is a comprehensive report on the use of aerobic granular sludge for biological nutrient removal (BNR) which had been published by the Water Environment and Reuse Foundation (WE&RF) after peer review (Figdore et al., 2017). The following topics were addressed in detail: 1) physicochemical characteristics of aerobic granular sludge, 2) selective pressures for aerobic granular sludge growth, 3) the effect of different operating conditions on granular sludge growth, 4) microbial diversity in aerobic granular sludge, 5) nutrient removal in bench-scale systems, 6) removal of micropollutants and industrial chemicals in bench-scale systems, 7) full-scale aerobic granular sludge process applications and nutrient removal performance, 8) modeling of aerobic granular sludge processes, 9) resource recovery opportunities and 10) research needs. This Chapter consists of an edited version of the report's Executive Summary, which synthesizes the key information pertaining to these aspects of biological nutrient removal and resource recovery with aerobic granular sludge. The WE&RF report is provided in its entirety in the Supplemental Information as Appendix A. The original Executive Summary contains references to report sections where greater detail and citations are provided. Thus, citations are not included in the excerpt below but can be found in the original report, which is included in this document as Appendix A.

3.1 PHYSICOCHEMICAL CHARACTERISTICS OF AEROBIC GRANULES

Aerobic granular activated sludge can be described as an attached growth or biofilm process where carrier media is not required and rapidly-settling microbial granules are formed via application of key selective pressures. Aerobic granules are typically 0.5 to 3 mm in diameter. The higher settling velocity of granular activated sludge compared to flocculent activated sludge is attributed to its larger particle size and more compact spherical morphology resulting in a settling regime that more closely resembles discrete settling rather than hindered settling. This is made apparent by low SVI values between 30 and 50 mL/g where the ratio of the settled sludge volume at 5 minutes and 30 minutes (SVI_5/SVI_{30}) is approaching 1.0. In comparison, well-settling flocculent activated sludge would be expected to have an SVI_{30} near 100 mL/g and SVI_5/SVI_{30} ratio from 1.6 to 2.0. A range of granule sizes and densities has been shown for full-scale granular sludge systems, with stratification of granules of different size and density in a settled sludge bed based on settling velocity.

A channelized protein-polysaccharide composite matrix distinguishes granular sludge from flocculent sludge, and polysaccharides appear to be the main structural element of this matrix that allows aerobic granules to share characteristics of hydrogels including viscoelasticity and capacity for swelling and deswelling by absorption and desorption of water. In aerobic granular sludge BNR systems treating domestic and synthetic wastewaters, the key structural extracellular polymeric substance (EPS) resembles alginate and contains charged moieties. However, other systems may contain a different structural EPS backbone. Consistent with the alginate theory of bioflocculation, the presence of divalent cations, particularly Ca^{2+}, can facilitate granulation. EPS extracts has some feature similar to commercial alginate and consequently the term

"alginate-like exopolysaccharides" has been adopted to most accurately refer to this component of aerobic granular sludge. Harvesting this alginate-like biomaterial for industrial applications is a resource recovery option unique to aerobic granular sludge.

3.2 SELECTIVE PRESSURES FOR AEROBIC GRANULAR ACTIVATED SLUDGE

Several key selective pressures and fundamental principles for aerobic granular sludge selection and growth have been identified. The liquid-solids separation is designed to allow retention of mainly the larger and faster-settling granules over smaller and slower-settling flocs. When significant biodegradable COD is present in the influent wastewater stream, the COD feeding regime should expose granular biomass to high COD concentration. A feeding regime of this nature promotes substrate penetration into the granule core and helps to avoid diffusion-limited growth conditions, which are not favorable for granular sludge growth. Under diffusion-limited growth conditions granular sludge is not stable and filamentous or finger-like outgrowths occur. This detrimental growth condition can result in reactor failure by washout of the poor-settling filamentous granules. Growth must also not be limited by diffusion of other co-substrates such as O_2 or NO_x-N. Sufficient shear must be provided in proportion to microbial growth rate, with faster-growing organisms requiring higher shear to form a smooth and compact granule biofilm morphology.

3.3 OPERATING CONDITIONS IN GRANULAR SLUDGE REACTORS WITH N AND P REMOVAL

Aerobic granules can be grown under a wide variety of operating conditions. At the bench scale, a variety of granule types including N and P removing granules, nitrifying-denitrifying granules, and aerobic ammonia and/or nitrite-oxidizing granules have been grown under different

combinations and degrees of these selective pressures depending on operating conditions. Thus far, granules performing nitrogen removal and enhanced biological phosphorus removal (EBPR) have been used in the full-scale application of aerobic granular sludge at practical operating conditions. An essential element of this operation, which was first investigated at the bench-scale, is an anaerobic period where influent wastewater is fed through the settled sludge bed in an upflow regime. This feeding mode facilitates granulation by exposing the biomass to higher substrate concentrations, minimizing diffusion-limited growth conditions, and selecting for slower-growing phosphorus-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in lieu of ordinary heterotrophic organisms (OHOs) which reduces the shear necessary to form smooth and compact granular biofilm structures. Particulate matter in the influent is sorbed to the granule surface, and it is important that a high degree of anaerobic hydrolysis occurs for particulate biodegradable COD (pbCOD) in the influent allowing for COD assimilation by PAOs and/or GAOs. Incomplete anaerobic hydrolysis of pbCOD will result in the presence of pbCOD at the granule surface during the subsequent aerobic period, leading to aerobic hydrolysis and slow release of readily biodegradable COD (rbCOD). During aerobic conditions, the presence of residual rbCOD in the bulk liquid or non-degraded pbCOD sorbed to the granule surface promotes filamentous outgrowths from the granule that are detrimental to granule morphology, settling velocity and stability and cause higher effluent TSS concentrations.

Although PAOs and GAOs both have denitrification capability, selection mainly for denitrifying PAO granules is preferred for more efficient use of influent carbon for nutrient removal, because influent bCOD removed by PAOs can be used for both nitrogen and phosphorus removal, whereas GAOs do not provide EBPR. A strategy for selecting PAOs over GAOs in granular

sludge has been developed based on differences in settling velocity between GAO- and PAO-enriched granules. GAO-enriched granules have a slower settling velocity than PAO-enriched granules of equivalent size due to the lower GAO bacterial density. Consequently, GAO-enriched granules tend to be located at the top of the settled sludge bed while PAO-enriched granules tend to be located at that bottom of the settled sludge bed. Wasting the slower-settling top portion of the sludge bed has been demonstrated to improve EBPR performance by selectively removing GAO-enriched granules and retaining PAO-enriched granules.

3.4 MICROBIAL DIVERSITY AND SPATIAL DISTRIBUTION IN AEROBIC GRANULAR ACTIVATED SLUDGE FOR N AND P REMOVAL

Operation conditions in aerobic granular sludge systems with anaerobic feeding followed by aeration for simultaneous nitrification-denitrification and EBPR results in the selection and spatial distribution of key microbial groups. PAOs and GAOs are the dominant members of the granule microbial community and together have accounted for as much as 85% of the granule microbial population. Both *Accumulibacter* and *Tetrasphaera* PAOs have been detected in these granules, with the former being more abundant than the latter, while *Competibacter* has been the only GAO detected. The participation of both PAOs and GAOs in the reduction of NO_3-N to N_2 has been observed. Results of independent studies indicate GAOs are primarily responsible for reduction of NO_3-N to NO_2-N while PAOs are primarily responsible for reduction of NO_2-N to N_2. With respect to ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), granules treating domestic wastewaters have been shown to contain both *Nitrosomonas* and *Nitrosospira* as well as *Nitrobacter* and *Nitrospira*. The concomitant presence of these genera has been attributed to the availability of suitable niches within the granular biofilm and dynamic process cycle. The ratio of NOB to AOB in granular sludge has been found to be higher than

otherwise expected based on conventional stoichiometry of lithotrophic oxidation of nitrite. Phenomena that may contribute to this observation include the mixotrophic growth of *Nitrobacter* and the existence of a nitrite oxidation-nitrate reduction loop between NOB and heterotrophic denitrifiers that reduce NO_3-N to only NO_2-N. OHOs are also present and may contribute to nitrogen removal in PAO/GAO-dominated anaerobic-aerobic granular sludge. These key microbial groups are distributed spatially in the granule in following manner. PAOs and GAOs are located in both the oxygen-penetrated outer layer and inner anoxic region, where their previously stored internal carbon obtained during anaerobic feeding is oxidized with oxygen or NO_x-N as the electron acceptors according to the redox conditions in the granule. Because their metabolisms require aerobic conditions, AOB and NOB must inherently be located on the oxygen-penetrated outer layer of the granule. OHOs are present in the oxygen-penetrated outer granule layer where they can grow on endogenous decay products and residual influent bCOD that may remain following anaerobic feeding. Similarly, OHOs in the inner anoxic can use endogenous decay products for NO_x-N reduction. Depending on bulk liquid DO, NO_x-N concentration and the rate of mass transport and biological reactions inside the granule, an innermost anaerobic region may permanently or transiently exist.

3.5 FULL-SCALE AEROBIC GRANULAR SLUDGE PROCESS APPLICATIONS

Fundamental research on aerobic granular sludge growth has led to the development of the innovative full-scale Nereda® sequencing batch reactor process for biological nitrogen and phosphorus removal. In this process, influent wastewater is fed slowly through an anaerobic settled sludge bed in an upflow regime, and simultaneous treated effluent overflows at the top of the reactor. After the feeding period, an aeration period is used for simultaneous nitrification-denitrification and EBPR. After NH_3-N is reduced to target levels, DO concentration may be

lowered from approximately 2.0 mg/L to 0.5 mg/L. Anoxic recycle periods may also be introduced to increase N removal. Though the majority of P removal is achieved biologically under normal flows and loads, metal salts may be added to supplement EBPR during wet weather to reduce the required cycle time to meet effluent limits. MLSS in Nereda® reactors is dominated by granular sludge, but the slow upflow feeding regime allows some flocs to remain present in the system. Particles less than 212 um may represent approximately 10 to 15% of the MLSS mass. These particles do not appear detrimental to the process and may contribute to TSS removal by sludge blanket filtration during the period of simultaneous and effluent overflow. Cultivation of mature granular sludge from flocculent seed sludge may take approximately one year, but this duration can be affected by temperature and wastewater characteristics and can be reduced if a significant amount of granular seed sludge is available.

Nereda® facilities have demonstrated high N and P removal efficiencies and the ability to meet different levels of effluent nutrient removal requirements as applicable to the operating facility. Effluent discharge limits at the Garmerwolde and Vroomshoop facilities are similar to what would be considered BNR-level requirements in the United States. Effluent concentrations for sustained operational periods at these facilities were as follows: 6.9 and 7.2 mg/L TN, 1.1 and 2.2 mg/L NH_3-N, approximately 0.9 mg/L TP at both facilities, 0.4 and 0.6 mg/L PO_4-P, and 20 and 10 mg/L TSS, respectively. These effluent concentrations were achieved without effluent filtration and with wet-weather addition of metal salts for chemical phosphorus removal. Effluent NH_3-N limits at these facilities allowed for a modest NH_3-N residual to remain present. The Epe facility is operated to meet more stringent effluent limits. During a three-month performance verification period at process temperature of 14 to 16°C, average effluent this facility was <4.0

mg/L TN, 0.1 mg/L NH_3-N, 0.3 mg/L TP, 0.1 mg/L PO_4-P, and <5.0 mg/L TSS. These effluent concentrations were achieved with effluent filtration and wet weather metal salts addition. Influent wastewater characteristics were favorable for nutrient removal at these facilities, with influent BOD/N ratios between 4.0 and 4.5 and BOD/P ratios between 30 and 36. A pilot facility treating municipal primary effluent where BOD concentration was less than 100 mg/L demonstrated excellent cold-weather nitrification and EBPR performance. During a two-week period where temperature was 3.0 to 4.5°C, NH_3-N concentration was less than 0.6 mg/L. The effluent PO_4-P concentration was less than 0.4 mg/L and often near 0.1 mg/L without supplemental VFA or metal salts addition. However, concentrations of the influent and effluent N species were not reported, therefore a complete picture of nutrient removal performance is not available for this system treating a low-strength primary effluent.

Compared to conventional flocculent activated sludge processes, attractive aspects of the Nereda® granular activated sludge process include reductions in space and energy requirements. A lower space requirement is achieved due the elimination of secondary clarifiers, preferential utilization of deep SBR tanks, high MLSS concentrations (8-12 g/L) and short settling times that can be employed with granular sludge and an upflow feeding regime. Energy savings in the Nereda® process can be expected due to the elimination of mechanical equipment such as mixers, internal recycle pumps, and return sludge pumps and efficiencies in oxygen transfer gained through the preferential use of deep tanks. It is also likely that aeration energy savings are achieved via periods of low DO concentration that may be introduced in the reactor cycle after the target NH_3-N concentration has been achieved. Advantages of the Nereda® process must be weighed against limitations or special considerations related to 1) the ease of retrofitting existing

facilities, 2) process start-up and granule development, 3) appropriate waste sludge management to ensure wasting of PAOs and adequate P removal, and 4) instrumentation needs.

3.6 PROCESS MODELS FOR AEROBIC GRANULAR SLUDGE

Mechanistic models for aerobic granular sludge processes have been developed and applied for different laboratory-scale aerobic granular sludge systems. Well-established one-dimensional biofilm models including mass-transport and biological reaction processes using stoichiometric and kinetic parameters, similar to those included as examples in the International Water Association Activated Sludge Model (ASM) series, have been appropriate to simulate reactor nutrient removal performance. Simulation of combined EBPR and nitrogen removal has been limited, and no models have been developed and applied for calibration and validation to the performance of full-scale facilities treating municipal wastewater using either a uniform granule size or including flocs and distribution of granule sizes in the model formulation.

3.7 RESOURCE RECOVERY WITH AEROBIC GRANULAR SLUDGE

Resource recovery opportunities with aerobic granular sludge include those that are shared with flocculent activated sludge as well as a unique opportunity to harvest alginate-like biomaterial from waste granular sludge. A self-evident resource recovery opportunity is water reuse. Other resource recovery opportunities utilize the granular activated sludge solids stream. Methane production from anaerobic digestion of waste sludge is another resource recovery option. Waste granular activated sludge from the full-scale Nereda® Epe facility exhibited 42% VS destruction in lab-scale mesophilic digestion at 20-d SRT, which is within the range that would be expected for anaerobic digestion of waste activated sludge. Lab-scale thermal pressure hydrolysis pretreatment at 160°C for 2 hours and 6-8 bar pressure improved the VS destruction from 42 to

48%. Nutrient recovery via land application of biosolids or biochar is an additional resource recovery option, along with the potential to produce crystalline phosphorus fertilizers. Alginate-like biomaterial has potential to be a newly-realized marketable resource recovery product for WRRFs not otherwise found with conventional flocculent sludge. Initial investigations of the recovery of alginate-like biomaterial from Nereda® excess sludge have been promising, but also showed that extracts contained as much as 40% protein. Specific suitable commercial and industrial outlets for this material have yet to be defined. One possible use is as a thin-film water-resistant barrier in surface coating applications for paper, paper products, and gypsum board.

3.8 RESEARCH NEEDS

This review revealed numerous aspects of wastewater treatment and intensification of existing infrastructure using aerobic granular activated sludge that have not been addressed. The following topics should be addressed in future research:

- The effect of influent wastewater characteristics on granular sludge growth, particularly the strength and relative fractions of readily biodegradable and particulate biodegradable COD.
- The ability to achieve effluent TN and TP concentrations $\leq$ 3.0 and 0.1 mg/L, respectively, and operational strategies necessary to achieve these low nutrient concentrations with different wastewater characteristics.
- The effect of variable loads on granular sludge SBR performance, design, and process control.
- Expansion of existing aerobic granular sludge process models to simulate treatment of domestic wastewaters and pH and other physical-chemical phenomena observed in lab systems such as granule-mediated phosphorus precipitation and ammonium ion

exchange. If research shows hybrid systems containing flocs and granules to be viable, competition of bacteria in flocs and granules should be included in hybrid system models.

- Characterization of oxygen transfer and alpha factors in aerobic granular sludge systems.
- The impact of granular activated sludge on downstream processes including filtration and disinfection.
- The fate of micropollutants in aerobic granular sludge systems.
- New process applications for aerobic granular sludge including granule growth in continuous flow systems, granular sludge bioaugmentation to increase nutrient removal capacity in existing flocculent activated sludge systems, and the use of granules in separate wet weather treatment biosorption processes to improve water quality.
- Thickening and dewatering properties of granular sludge before and after digestion.
- Resource recovery, including the impact of granular sludge on hydrolysis and methane yield in anaerobic digestion, phosphorus recovery from granular WAS, and processing and commercialization of alginate-like EPS extracts from waste granular activated sludge.

3.9 OUTLOOK

The outlook for aerobic granular sludge is positive and its potential for use in WRRFs with nutrient removal requirements, seeking to maximize the treatment capacity of existing infrastructure or small footprint, and/or implement resource recovery is promising. With greater awareness of aerobic granulation mechanisms and broadened understanding of performance capabilities, research needs, and potential applications, the increased use of aerobic granular sludge-based wastewater treatment and resource recovery is anticipated.

Chapter 4. COMPARISON OF DIFFERENT AEROBIC GRANULAR SLUDGE TYPES FOR ACTIVATED SLUDGE NITRIFICATION BIOAUGMENTATION POTENTIAL

Chapter 4 is the manuscript that has been accepted in October 2017 after peer review for publication in the Bioresource Technology Journal.

4.1 ABSTRACT

Three types of nitrifying granules were grown on media simulating anaerobic digestion dewatering reject water and compared for their potential to increase nitrification capacity when added to mainstream flocculent activated sludge treatment. An advantage of nitrification bioaugmentation with sidestream granules instead of flocculent biomass is that the granules can be selectively maintained at longer retention times than flocs and thus provide higher nitrification capacity from bioaugmentation. The three granule types and feeding conditions were: nitrifying granules with aerobic feeding, nitrifying-denitrifying granules with anoxic feeding, and nitrifying-denitrifying/phosphate-accumulating (NDN-PAO) granules with anaerobic feeding. NDN-PAO granular sludge showed the highest potential for nitrification bioaugmentation due to its better treatment performance, granule physical characteristics, and much greater production of granular mass and nitrification capacity. *Dechloromonas*-associated organisms were dominant in these granules; *Candidatus* Accumulibacter-related organisms were also present. *Nitrosomonas* was the dominant ammonia-oxidizing bacteria, while *Candidatus* Nitrotoga was an abundant nitrite-oxidizer in all granule types.

4.2 INTRODUCTION

Increasing the nitrification capacity of activated sludge processes to meet lower effluent ammonia-nitrogen (NH_3-N) concentrations, handle higher loadings, and or increase nitrogen removal can require major changes to existing water resource recovery facilities (WRRFs). Limited space and economic considerations may favor alternatives that retrofit existing infrastructure without adding aeration tank volume. One approach to intensify nitrification capacity without increasing the aeration volume is by bioaugmentation of nitrifying organisms grown in a sidestream treatment system (Kos, 1998). For WRRFs with anaerobic digestion, the ammonia loading from untreated centrate return to the mainstream secondary system is typically 20 to 30 percent of the total ammonia loading. Flocculent nitrifying activated sludge grown on ammonia-rich anaerobic digestion dewatering streams (1000-1800 mg/L NH_3-N), commonly referred to as centrate, have been added to mainstream activated sludge systems to increase nitrification rates in laboratory reactors (Head and Oleszkiewicz, 2004) and in over 25 full-scale systems with different sidestream treatment and bioaugmentation configurations (Bowden et al., 2015). However, the improvement in nitrification by floc bioaugmentation is limited by the relative ammonia loading and amount of nitrifier biomass growth in the sidestream because the solids retention time (SRT) of the bioaugmented biomass is the same as the mainstream activated sludge. The impact of bioaugmentation with sidestream nitrifier biomass would be further enhanced if the SRT of the added sidestream biomass could be increased without increasing aeration tank volume. Therefore, it would be beneficial to bioaugment with a biofilm structure and to increase the solids inventory by uncoupling their SRT from the flocculent biomass.

Aerobic granular sludge is a type of biofilm that is grown without carrier material hence offering an excellent solution for nitrifier bioaugmentation without additional waste activated sludge handling issues associated with plastic carriers. The larger size and higher settling velocity of granules would allow for their selective retention using screens (Liu et al., 2014a), cyclones (Shi et al., 2016), or possibly gravity separation. Returning the separated bioaugmented nitrifying granules to the mainstream system can result in building a higher bioaugmented nitrifying biomass and thus a greater increase in nitrification intensification than that with sidestream flocculent nitrifying sludge. Therefore, the proposed new biotechnology leverages treatment of centrate reject water to much greater benefit.

One potential issue with biomass grown in and adapted to sidestream conditions is the higher temperature, which can lead to a temperature shock of the nitrifier population once they enter the cold mainline. Prior work suggests that a lower sidestream growth temperature, closer to that in the mainstream, may select for nitrifying organisms that are more effective in the bioaugmented mainstream process (Wett et al., 2011). Lowering the sidestream reactor temperature from the initial centrate temperature of 30-35°C has been accomplished in flocculent activated sludge sidestream processes by adding a portion or all the return activated sludge (RAS) flow (Leu and Stenstrom, 2010) to the sidestream nitrification reactor. An alternative temperature dilution source would be needed for sidestream growth of granular activated sludge to limit the nitrifying biomass growth to granules instead of flocs.

Different growth conditions may be used to grow distinct types of sidestream nitrifying aerobic granules depending on if and how supplemental organic carbon is utilized (Figdore et al., 2017).

The granule types and their growth conditions and descriptors are: 1) strictly aerobic with only nitrification (NIT granules), 2) anoxic-aerobic with nitrification and denitrification (NDN-OHO granules), and 3) anaerobic-aerobic with nitrification, denitrification and enhanced biological phosphorus removal (NDN-PAO granules). NIT granules were investigated in earlier research (Tay et al., 2002b) and require supplemental alkalinity as centrate typically only has about half the alkalinity needed to offset acidification for complete NH_3-N oxidation. These granules contain ammonia-oxidizing bacteria (AOB) and possibly nitrite-oxidizing bacteria (NOB), depending on the reactor operating conditions. Growth of NDN-OHO granules has also been studied by other researchers (Chen et al., 2013; Wang et al., 2012), and the operational scheme involves adding NH_3-N with an external carbon source under anoxic conditions. External organic carbon is consumed by ordinary heterotrophic organisms (OHOs) to denitrify the oxidized nitrogen from a prior aerobic step. Denitrification provides the alkalinity needed for full nitrification. While NIT and NDN-OHO granules are studied to a lesser extent, NDN-PAO granules are more widely studied (Bassin et al., 2012b; de Kreuk et al., 2007; Kishida et al., 2006) and have also been applied in full-scale reactors (Figdore et al., 2017). NDN-PAO granules are fed an external carbon source under anaerobic conditions, which is assimilated into storage products by phosphorus-accumulating organisms (PAOs) for use in a subsequent aeration step with simultaneous nitrification-denitrification and enhanced biological phosphorus removal (EBPR). The PAOs use the stored carbon to reduce nitrate/nitrite produced by nitrifying organisms on the aerobic outer layer of the granules with alkalinity production from denitrification (Bassin et al., 2012b).

Although growth of NIT, NDN-OHO, and NDN-PAO granules has been demonstrated under a variety of operating conditions, key information important to the design and long-term performance for sustained granular sludge bioaugmentation has not been reported. These include the sidestream growth reactor granular sludge yield, specific nitrification activity, and physical characteristics of the granules. Excess solids produced in a sidestream reactor treating centrate is the sum of intentionally-wasted granular sludge for addition to the mainstream treatment system and effluent flocculent sludge also containing nitrifying bacteria. A high sidestream granular sludge yield with good nitrification activity is desired to maximize the effectiveness of the granular sludge bioaugmentation process. However, the process that produces the greatest granule nitrification capacity is not known, and information for a direct determination or estimation of nitrifying granular sludge yield and their specific nitrifying activity is insufficient.

Granular sludge physical characteristics including size, density, and integrity affect the efficiency of granular/floc separation and ability to sustain granules long term in mainstream treatment. Further investigation is needed to determine how the granule growth conditions affect its physical characteristics. Granule integrity has been quantified through an integrity coefficient, which is equal to the fraction of granule mass remaining after exposure to mechanical perturbation. Integrity coefficients above 0.95 have been measured for NDN-PAO granules (Nor-Anuar et al., 2012) and OHO granules grown under aerobic slug feeding conditions without ammonia and nitrogen removal performance reported (Xiao et al., 2008). The integrity of NIT granules has not been measured, and the integrity coefficient of different granule types has not been compared using a uniform integrity testing protocol.

4.3 MATERIAL AND METHODS

4.3.1 *Granular sludge reactors*

Three sequencing batch reactors (SBRs) were operated at 18 to 20°C with a synthetic feed containing an NH_3-N concentration similar to digester dewatering centrate, and with growth conditions selecting for NIT, NDN-OHO, and NDN-PAO granular sludge. The SBRs had a height to diameter ratio of 4.9 at the full working volume of 2.0 L, and the decant/feed volume was 50% of the total volume for each cycle. SBR cycle operating conditions are summarized in Table 4.1 showing the necessary feeding and react conditions for selecting the distinct types of nitrifying granules. The three SBR systems were operated with 4 cycles per day at 6 hours per cycle and similar volumetric NH_3-N loadings, ranging from 0.20 to 0.25 g NH_3-N/L-d. Notable aspects of the reactor feeds and operation are discussed below. Additional details are in the Supplemental Information.

Table 4.1. Summary of operating conditions for granular sludge SBR systems

Parameter	Units	NIT	NDN-OHO	NDN-PAO
Feed stream condition	-	Aerobic	Anoxic	Anaerobic
Dilution stream				
Sequence in cycle	-	Before feed stream	Before feed stream	With feed stream
Time	min	6	6	See [a]
Feed/reaction times				
Aerobic feed	min	See [b]	-	-
Anaerobic feed	min	-	-	3[a]
Anaerobic react	min	-	-	37
Deoxygenation	min	-	(5)	-
Anoxic feed/react	min	-	(20)[c]	-
Aeration	min	345[b]	(60)	260
Repeat ( ) for anoxic		-	4 times	-
Post aeration	min	-	5	-
Settling	min	4	4	1
Effluent discharge	min	2	2	2
Idle	min	3	3	22
Post anoxic/N_2 mix	min	-	-	35
Total cycle time	min	360	360	360
NH_3-N loading	g/L-d	0.24	0.20	0.25
pH	-	7.1-7.5	7.1-7.5	7.6-8.3
Operating duration	days	90	130	165

(a) Feed and dilution streams added concurrently.
(b) Feed stream added semi-continuously at 10-minute intervals during aeration period.
(c) Feed stream added semi-continuously at 4-minute intervals during anoxic period.

The SBRs were fed two streams: 1) feed stream containing NH_3-N or NH_3-N and COD, and 2) dilution stream simulating that for temperature control and containing PO_4-P and other inorganics. In the NIT and NDN-OHO reactors, 0.8 L of the dilution stream was added under quiescent conditions at the beginning of the cycle and 0.2 L of the feed stream was added during the reaction period. In the NDN-PAO reactor, 0.5 L of each stream was added during the anaerobic feed period.

The feed stream NH_3-N concentrations, added as NH_4HCO_3, for the NIT, NDN-OHO, and NDN-PAO reactors were 600 mg/L, 500 mg/L, and 250 mg/L, respectively, and the net influent NH_3-N concentrations, when accounting for the dilution steams, were 120 mg/L, 100 mg/L, and 125 mg/L, respectively. The net influent COD concentrations were 20, 200, and 650 mg/L, respectively. The small amount of COD in the NIT reactor influent was included to represent a minimal residual acetate and other biodegradable COD (bCOD) present in centrate. Acetate was the COD source for NIT and NDN-OHO reactors, and the COD for the NDN-PAO reactor was 70% acetate and 30% propionate. Influent COD concentration in the NDN-OHO reactor was lower than planned for complete denitrification because of the development of organisms that resulted in poor granule growth as discussed later, and thus supplemental alkalinity was added to compensate for nitrification requirements. Additional alkalinity as $NaHCO_3$ was added to the NIT and NDN-OHO reactors and the total alkalinity addition in terms of g as CaCO3/g NH_3-N for the NIT, NDN-OHO, and NDN-PAO reactors were 7.2, 5.0, and 3.6, respectively. The NIT reactor dilution stream included 1800 mg/L of $MgCl_2$ to achieve a monovalent-to-divalent cation equivalent ratio of 0.3 in the blended feed stream to represent the use of $Mg(OH)_2$ for supplemental alkalinity in a full-scale process.

Gasses were sparged through an aeration stone at the reactor bottom. Dissolved oxygen (DO) concentration was not automatically controlled in NIT and NDN-OHO reactors, but was consistently near 4 mg/L from co-sparging of air and N_2. CO_2 was co-sparged with N_2 in the NDN-OHO reactor during deoxygenation and anoxic periods to limit CO_2 stripping and pH increase. DO concentration in the NDN-PAO reactor was controlled at 1.5 mg/L by on-off aeration using a DO controller (Eutech alphaDO2000W) and probe (Atlas Scientific). Magnetic stirring provided supplemental mixing in the NDN-PAO reactor during all reaction periods.

The 4-min settling time in the NIT and NDN-OHO reactors would remove particles with settling velocity less than 3.0 m/hr, and the 1 min settling time in the NDN-PAO reactor would remove particles with settling velocity less than 12 m/hr. Longer settling times were used in the NIT and NDN-OHO reactors to avoid higher effluent TSS concentrations and solids washout.

4.3.2 *Granules maximum specific ammonium oxidation rates*

Granular sludge retained on a 212-um sieve was used in batch activity tests with high NH_3-N concentration to determine the maximum ammonia oxidation rate. The temperature ranged from 20 to 24°C, the MLSS concentrations from 1000 to 1800 mg/L, DO concentration from 7 to 8 mg/L, and pH from 7.4 to 7.8 in the batch tests. NH_4HCO_3 and $NaHCO_3$ were added to the test reactor to provide an initial NH_3-N concentration of approximately 25 mg/L and 7 g alkalinity as $CaCO_3$/g NH_3-N. Measured activities were normalized to 20°C using a temperature-activity (θ) coefficient of 1.072 for AOB (Tchobanoglous et al., 2014).

4.3.3 *Analytical methods*

NH_3-N and NO_3-N were measured by ion-sensing electrode probes (Hach IntelliCAL Models ISENH4181 and ISENO3181, respectively). NO_2-N and PO_4-P were measured spectrophotometrically using standard test cuvettes (Hach Methods 10019 and 8048, respectively). Alkalinity, pH, suspended solids, and soluble COD (sCOD, 0.45 um-filtered) were determined by Standard Methods (APHA, 2012). Dissolved oxygen (DO) concentration was measured with an optical probe (YSI ProODO).

4.3.4 *Granular sludge physical characteristics*

Granular sludge physical characteristics determined were size, sludge volume index, wet density, and integrity coefficient. Granule morphology was evaluated by stereomicroscopy and image analysis of a minimum sample of 200 granules. Individual granule sizes were taken to be the diameter of a circle possessing the same area as the granule plan view area. The MLSS sludge volume index was routinely measured after 5 and 25 minutes of settling (SVI_{5min} and SVI_{25min}). Granule wet density and integrity coefficient were measured in triplicate. Density was determined by pycnometry according to Winkler et al. (2012). Integrity coefficient was determined using a modified method after Xiao et al. (2008). Mixed liquor samples were sieved at 212 um, rinsed with deionized water, transferred to a full 50 mL centrifuge tube, and vortexed at maximum speed for three minutes using a 3600-W benchtop vortexer. The vortexed sample was sieved again to fractionate granules from solids released from vortexing. The integrity coefficient is equal to the mass of remaining granules divided by the sum of remaining granules and solids evolved.

4.3.5 *High-throughput sequencing of 16S rRNA amplicons*

DNA was isolated from reactor MLSS samples using the UltraClean Microbial DNA Isolation Kit (MO-BIO, Inc.) with 10 min of thermal pretreatment at 65°C and bead beating at 4 m/s for 20 s. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). Triplicate samples were normalized to equal concentrations using nuclease-free water and stored at -80°C.

DNA samples were submitted to a service facility (www.mrdnalab.com) for sequencing of 16S rRNA gene amplicons. 16S rRNA gene primers 341F and 805R were used in a single-step PCR due to their low primer bias (Hugerth et al., 2014). PCR was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Inc.) under the following conditions: 3 min at 94°C, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 60 s, followed by the final elongation step at 72°C for 5 min. Amplicons were mixed in equimolar concentrations and purified using AMPure XP beads (Agencourt Bioscience Corp.). Sequencing was performed on a MiSeq system (Illumina, Inc.) per the manufacturer's protocol. Raw paired-end Illumina data were joined and reverse complimented to read in the 5'-3' orientation.

4.3.6 *Bioinformatics*

The UPARSE method (Edgar, 2013) was used for amplicon sequence processing and operational taxonomic unit (OTU) clustering with USEARCH version 7.0.1090 (Edgar, 2010). Sequences were truncated at 400 bases, and those less than 220 bases or with greater than 0.5 expected errors were filtered. Sequences were clustered at 97%, and UCHIME was used to identify and filter chimeric sequences based on the RDP Classifier (v9) "gold" training database. Taxonomy for representative sequences was assigned using USEARCH based on the SILVA119 reference

database. Taxa were summarized by percent abundance normalized to the total number of reads using QIIME version 1.9.1. Representative sequences that did not receive a specific genus-level taxonomy assignment based on the above methods were searched against their family-level taxonomy assignment using BLASTn and associated NCBI database on August 21, 2015. Hits resulting in a single genus-level identification at >95% coverage, >95% identity, and $<10^{-150}$ E-value were taken as the genus-level taxonomy assignment. Those not meeting these criteria were assigned taxonomy at the family-level only.

4.4 Results and Discussion

The results on the granular sludge growth reactors treatment performance on simulated reject water, and the physical characteristics, nitrification capacity production, and microbial structure for the three types of granules is presented in the following.

4.4.1 *Treatment performance*

A summary of NIT, NDN-OHO, and NDN-PAO reactor performance, mixed liquor suspended solids (MLSS) concentrations, SRTs, and the solids production is shown in Table 4.2 for the final 30 days of operation with relatively constant loadings and treatment performance. The minimum SRT target for the granular sludge systems was 20 days. The SRT target was met in the NIT and NDN-PAO systems but not in the NDN-OHO system due to the production of granules with a composition and morphology that led to poor settling characteristics and high effluent solids loss. A higher SRT was allowed in the NDN-PAO reactor to achieve MLSS concentrations near that reported for mature granular sludge suspensions in full-scale reactors of greater than 8 g/L (Pronk et al., 2015a). Differences between granular reactor types in NH_3-N

removal, inorganic nitrogen removal, solids production as effluent TSS, and solids production as manually wasted MLSS were statistically significant at an alpha of 0.05.

Table 4.2. Summary of average NIT, NDN-OHO, and NDN-PAO reactor performance, MLSS concentration, SRT, and solids production during the final 30 days of operation. (± is one standard deviation)

Parameters	**Units**	**NIT**	**NDN-OHO**	**NDN-PAO**
Granule diameter	mm	0.5 ±0.1	1.4 ±0.6	1.1 ±0.5
MLSS	mg/L	753 ±58	1125 ±111	9289 ±655
MVLSS	mg/L	672 ±47	1052 ±101	6628 ±493
SRT[a]	d	20.3 ±2.8	9.4 ±1.2	28.2 ±5.2
Influent NH_3-N	mg/L	120 ±0	100 ±0	125 ±0
Effluent				
NH_3-N	mg/L	4.5 ±3.0	19.5 ±7.9	2.4 ±0.8
NO_2-N	mg/L	4.3 ±2.8	8.6 ±4.5	0.3 ±0.3
NO_3-N	mg/L	106.8 ±5.3	41.8 ±5.2	3.2 ±2.1
PO_4-P	mg/L	18.8 ±0.4	18.2 ±0.3	3.1 ±2.8
TSS	mg/L	18 ±4	58 ±7	90 ±21
VSS	mg/L	16 ±4	54 ±6	62 ±14
pH	-	7.3 ±0.2	7.3 ±0.3	8.1 ±0.2
COD fed/TIN[b] removed	g COD/g TIN	4.6[c]	6.7 ±0.7	5.5 ±0.1
Nutrient Removal Efficiency				
NH_3-N removal	%	96 ±2	81 ±7.9	98 ±1
Inorganic N removal	%	4 ±2	30 ±2.8	95 ±2
PO_4-P removal	%	6 ±2	9 ±1	90 ±9
Solids production				
In effluent TSS	mg/d	73 ±11	229 ±17	341 ±29
Reactor manual wasting	mg/d	3 ±2	20 ±8	955 ±111
Portion from reactor	%	4.5 ±2.2	7.9 ±3.3	74 ±3

Reactor performance, MLSS concentration, SRT, and solids production based on 20 daily samples.
(a) Based on total reactor solids mass divided by solids in effluent and manual waste per day.
(b) Total inorganic nitrogen removed = Feed NH_3-N (g/d) minus effluent g/d of NH_3-N + NO_2-N + NO_3-N.
(c) Ratio is a consequence of small amount of COD added and very little inorganic N removal, not to denitrification.

NH_3-N removal efficiency was 96% and 98% in NIT and NDN-PAO reactors, respectively, but only 81% in the NDN-OHO reactor. NH_3-N removal in the NDN-OHO system was likely limited by the lower MLSS concentration and out-competition of nitrifiers by OHOs for space in the outer aerobic granule layer. In addition to lower effluent NH_3-N concentration, the NDN-PAO reactor had much lower effluent NO_2-N and NO_3-N concentrations, which is an important benefit with respect to the impact of this recycle stream to the mainstream treatment system.

Bioaugmentation of a mainstream reactor would be done by intentional manual wasting of nitrifying granules from the sidestream treatment reactor. For a given SRT, the granular solids manually wasted is equal to the total solids produced minus the flocculent and other lighter solids in the effluent. The manual solids wasting from the NIT and NDN-OHO reactors was only 4.5% and 7.9% of the average daily solids produced (Table 4.2). In addition to the solids loss in the effluent, the low autotrophic yield coefficient and minimal amount of heterotrophic growth limited the amount of granular sludge production from the NIT reactor. In contrast, the average daily manual solids wasting from the NDN-PAO reactor accounted for 74% of the solids production. The NDN-PAO reactor had a higher granular biomass production as most of its growth was as heterotrophic bacteria growth due to its higher biomass yield on the organic carbon added in addition to about the same amount of autotrophic growth as the NIT reactor. The granular sludge mass fraction in the mixed liquor suspended solids (MLSS) wasted from the NIT, NDN-OHO, and NDN-PAO reactors were 89%, 85%, and 98%, respectively.

The total inorganic nitrogen (TIN) removal for the NDN-OHO and NDN-PAO reactors averaged 30% and 95%, respectively (Table 4.2) and the COD fed to TIN removal ratio was higher for the

NDN-OHO reactor at 6.7 g COD/g TIN versus 5.5 g COD/g TIN for the NDN-PAO reactor. The NDN-PAO reactor exhibited high EBPR activity. PO_4-P release in the anaerobic feed/contact period was 0.55 ±0.03 mol P/mol C, indicating a PAO-rich microbial community (Oehmen et al., 2005). NDN-PAO cycle profiles (Supporting Information) showed concurrent PO_4-P uptake, NH_3-N oxidation and NO_x-N (NO_2-N + NO_3-N) removal during the aerobic period to indicate simultaneous nitrification-denitrification during the aerobic period. The 5.5 g COD fed/g TIN removed ratio is the lowest reported in this type of granular sludge reactor (Bassin et al., 2012b; Kishida et al., 2006), which suggest the possibility of *short-cut* nitrogen removal. In short-cut nitrogen removal, the main nitrogen removal pathway is NH_3-N oxidation to NO_2-N by AOBs and NO_2-N reduction by denitrifying PAOs (dPAOs), which use their intercellular stored carbon from the anaerobic feed period. The use of NO_2-N by the dPAOs helps suppress the amount of NO_2-N oxidation to NO_3-N, resulting in lower oxygen and carbon requirements.

Under short-cut nitrogen removal the NOB:AOB population ratio should be much lower than that for a system with complete nitrification to NO_2-N and to NO_3-N. The relative population abundance of AOB and NOB found in the high-throughput sequencing results can be used to obtain a qualitative comparison of AOB and NOB abundance in the NIT reactor with complete nitrification to NO_3-N to the NDN-PAO reactor. The NDN-PAO relative abundance ratio of NOB:AOB was much lower than for the NIT reactor to suggest short-cut nitrogen removal; the ratios are 0.10 and 3.2, respectively.

High TIN removal efficiency and the possibility of short-cut nitrogen removal in NDN-PAO granular sludge could be beneficial to overall bioaugmentation process performance. Removal of

the sidestream NH_3-N load by nitrification and denitrification eliminates a significant NO_x-N return load, which is attractive for mainstream systems with nitrogen removal requirements and insufficient influent COD to denitrify a sidestream return NO_x-N load from nitrification only. Nitrification bioaugmentation with primarily AOB instead of NOB (and AOB) could also promote short-cut nitrogen removal in mainstream treatment, thus reducing oxygen demand and denitrification carbon requirements for improved nitrogen removal and energy efficiency.

Lower TIN removal efficiency in the NDN-OHO reactor occurred because the amount of influent COD was insufficient for complete denitrification with NO_3-N as electron acceptor; the influent COD/N ratio was 2.0 g/g. Influent COD was purposely limited due to unfavorable granule growth characteristics and performance at higher influent COD concentration used in earlier operation. As discussed further in Section 4.4.2, NDN-OHO granules had poor settling characteristics with low density, high SVI, and profuse extracellular polymeric substances (EPS) production. During earlier transient operation with influent COD concentration as high as 500 mg/L, the poorly-settling solids were washing out of the reactor, and NH_3-N removal efficiency was as low as 50%. Influent COD concentration was then reduced and ultimately held at 200 mg/L to decrease the solids washout. Results for the NDN-OHO granular sludge system showed poor settling characteristics, fingered outgrowths, and low nitrogen removal capacity (Tables 4.2 and 4.3 and Supplemental Information), which is consistent with the reactor growth conditions. Semi-continuous acetate feeding during anoxic steps selected for fast-growing OHOs on the granule outer layer with fingered outgrowths that out-competed nitrifiers for space on the granule outer aerobic layer (Pronk et al., 2015b).

The NDN-OHO reactor had a different nitrification and denitrification pattern than that for the NDN-PAO reactor, which contributed to its higher COD fed/g TIN removed ratio and lower TIN removal efficiency. NO_3-N reduction proceeded faster than NO_2-N reduction with a significant portion of NO_2-N remaining after the anoxic/feed step, which was then oxidized to NO_3-N in the subsequent aerobic period (Supplemental Information). A separate denitrification test was conducted to further investigate the denitrification pattern of NDN-OHO sludge with COD feeding similar to the anoxic/step feeding. Both NO_2-N and NO_3-N were available at the start of the test, which was run until NO_x-N removal was complete. In the first 80-min period of the test, NO_3-N was reduced while NO_2-N accumulated, and then NO_2-N was reduced to complete NO_x-N removal in the final 70-min period. Observed ratios of COD fed to NO_x-N removed in the two periods were compared to their theoretical values. The observed ratio of COD fed to NO_x-N removed was approximately 40% higher than the theoretical ratio in the first period, while the observed ratio matched the theoretical ratio in the second period. The higher observed ratio in the first period with NO_2-N accumulation suggests COD was being utilized for processes other than denitrification and cell synthesis, such as assimilation and production of EPS or intracellular carbon storage compounds. The observed denitrification pattern and COD utilization was likely due to the high abundance of *Zoogloea* and *Thauera* bacteria found in the microbial population analysis (Section 4.4.4). These genera may partially denitrify NO_3-N to NO_2-N with high EPS production as discussed later in the microbial population analysis.

NDN-OHO granular sludge with SVIs less than 25 mL/g and good nitrogen removal capacity has been reported in other studies with different growth conditions (Chen et al., 2013; Wang et al., 2012). Wang et al. (2012) used higher DO concentrations from 6.0 to 7.5 mg/L, which would

tend to increase nitrification rates compared to the DO concentration of 4.0 mg/L in this study, and Chen et al. (2013) used a high superficial aeration velocity of 2.5 cm/s, which suggests a high DO concentration. These studies did not indicate whether feeding in anoxic step periods was slug, semi-continuous, or continuous. If slug feeding was used, a higher substrate gradient was created hence reducing diffusion limitations and favoring denitrification deeper in the granules. However, in this study an abundance of translucent poorly-settling growth resembling globular *Zoogloea* was observed during earlier operation with slug feeding and higher influent COD concentration in the anoxic steps. Therefore, persistence of problematic growth in NDN-OHO granules in this study appears to be linked to the microbial community with high abundance of *Zoogloea* and *Thaurea*. Other studies with NDN-OHO granule growth had favorable granule SVIs but no testing on *Zoogloea* and *Thaurea* was conducted (Chen et al., 2013; Wang et al., 2012). Therefore, it remains unclear if their presence can always be expected with NDN-OHO type granules.

4.4.2 *Granule physical characteristics*

Granule physical characteristics are of utmost importance in assessing bioaugmentation potential of sidestream nitrifying granules grown in this study. Desirable physical characteristics are: 1) larger granule size, which settle faster, 2) granules with lower SVI, which thicken better, 3) granules with higher density, which settle faster, and 4) granules with a higher integrity coefficient, which suggests a better resistance to granular loss under repeated exposure to mixing, pumping and recycling in the bioaugmented mainstream treatment.

Granule physical characteristics are compared in Table 4.3. Differences in physical characteristics in Table 4.3 between granule types were statistically significant at an alpha level

of 0.05. As shown in Table 4.3, the NDN-PAO and NDN-OHO granules are much larger than the NIT granules. The NDN–PAO granules had a higher density than the others, which is due to the inorganic phosphate deposits in the PAOs and the compact morphology. The NDN–OHO granules had a much lower density, which is likely due to its high extracellular polymeric substances (EPS) content (Supplemental Information) showing an EPS content of 586 mg/g VSS (standard deviation of ±29 mg/g VSS for 3 samples). The EPS characteristics of these granules was different than that for other OHO granules grown with slug feeding followed by aeration in which the EPS was identified as having an alginate-like composition (Lin et al., 2008). The high EPS production in the NDN-OHO granules may have been a result of the high abundance of *Zoogloea* and *Thaurea* bacteria (Allen, 2002) observed in the population composition (Section 4.4.4). The NIT granule density was similar to that measured by water displacement for NIT granules described by Tay et al. (2002b).

Table 4.3. Summary of granule physical characteristics and reactor SVI values.

Parameter	**Units**	**Average ±Std. Dev.**		
		NIT	**NDN-OHO**	**NDN-PAO**
Diameter[a]	mm	0.50 ±0.1	1.4 ±0.6	1.1 ±0.5
Density[b]	g/cm^3	1.019 ±0.001	1.004 ±0.003	1.036 ±0.001
SVI_{5min}[c]	mL/g	59 ±10	106 ±15	26 ±2
SVI_{5min}/SVI_{30min}[c]	---	1.05 ±0.02	1.02 ±0.01	1.02 ±0.01
Integrity coefficient[b]	---	0.82 ±0.01	0.78 ±0.01	0.96 ±0.01

(a) Minimum sample size of 200 granules.
(b) Measured in triplicate.
(c) Based on 20 daily samples during final 30 days of operation.

Granule morphology is also an important physical attribute because it impacts granule settling characteristics and can be indicative of granule structural integrity. For example, granules with

smooth and compact biofilm morphology would be expected to have faster settling rates and higher structural integrity than granules with an irregular biofilm morphology and an abundance of finger-like outgrowths from the granule surface. Morphology of the different granule types is shown in the Supplemental Information. The NDN-PAO granules had ideal morphology with a smooth surface and more compact biomass growth. The NIT granules had an abundance of stalked ciliates on the surface, likely grazing on suspended nitrifiers or other organisms. NDN–OHO granules had irregular finger-like outgrowth showing similarities to fingered outgrowth of *Zoogloea*-like organisms (Allen, 2002), and the NDN–OHO population analysis (Section 4.4.4) indicates a high abundance of *Zoogloea* organisms.

These differences in size, morphology, and granular content account for the differences in the reactor SVI values shown in Table 4.3. The NDN-PAO granular reactor mixed liquor had the lowest SVI_5 of 26 mL/g, which is characteristic of good granular sludge (Figdore et al., 2017). The higher SVI for the NIT reactor may be due to the outer stalked ciliate growth, which would interfere with sludge compaction, or the presence of flocculent growth in the reactor as suggested by the low amount of granular sludge production (Table 4.2) and in the higher SVI_5/SVI_{30} ratio value of 1.05. The high SVI of the NDN-OHO reactor is a very unfavorable result, which shows a low potential for pursuing this type of reactor operation and granular growth for nitrification bioaugmentation.

The integrity coefficient test results were also more favorable for the NDN-PAO granules. After the high vortexing energy for 3 minutes in the bench test method the mass reduction for the NDN-PAO granules was only 4% compared to 18% and 22% for the NIT and NDN-OHO

granules, respectively. The lower integrity coefficients of the NIT and NDN-OHO granules may have been due to stripping off the lighter outer layers of these granules associated with growth of stalked ciliates on the NIT granules and the finger-like high EPS content growth for the NDN-OHO granules. Unlike NDN-OHO granules in this study, other OHO granules grown with slug feeding followed by aeration had high integrity coefficients where a similar protocol was used (Xiao et al., 2008).

4.4.3 *Nitrification capacity production*

The potential of sidestream granular sludge bioaugmentation also depends on how well the ammonia fed to the sidestream granule growth reactor is used to grow nitrifying bacteria within granules that can then be fed to the mainstream treatment. The effectiveness of the NIT and NDN-PAO granular sludge systems for mainstream bioaugmentation was quantified based on the granule nitrification capacity generated in each system. The NDN-OHO system is not included here because of its lower ammonia removal efficiency and unfavorable granular growth characteristics. Granule nitrification capacity production (g NH_3-N/d) was calculated by multiplying the average daily waste granular sludge mass (g VSS/d) by the granular sludge - specific maximum NH_3-N oxidation activity (g NH_3-N/g VSS-d) determined in the batch tests with DO and initial NH_3-N concentrations of 8 mg/L and 25 mg/L, respectively. The reactor granule solids used in the test were retained on a 212-um sieve. The granule nitrification capacity generated in the systems was divided by the daily amount of NH_3-N fed (g NH_3-N/d) to obtain a normalized unitless value referred to as the nitrification capacity factor (NCF). Systems with higher NH_3-N removal and high capture of nitrifying bacteria into granule production are more favorable and would have a higher NCF value. The NCF values for the granular sludge reactors

and AOB flocculent sludge grown at a similar process temperature, SRT, and NH_3-N loading are compared in Table 4.4. The flocculent sludge calculation is based on using standard AOB kinetic and stoichiometric coefficient values (Tchobanoglous et al., 2014) and assuming the same amount of feed NH_3-N and NH_3-N removal efficiency as the NDN-PAO reactor. In this comparison, all the flocculent AOB produced would be added to the mainstream system and would have the same SRT as the mainstream system, whereas the nitrifying granules added to the mainstream system would have a longer SRT.

Table 4.4. Comparison of the nitrification capacity factor (NCF) for NIT and NDN-PAO granular sludge growth (>212um) and theoretical estimate for AOB flocs at 20°C.

Parameter	Units	NIT granules	NDN-PAO granules	AOB flocs[(a)]
Average feed NH_3-N	mg NH_3-N/d	480	500	500
Daily waste granules or floc production	mg VSS/d	3	644	21
Maximum specific NH_3-N oxidation rate	g NH_3-N/ g VSS-d	0.75	0.14	6.0
Maximum NH_3-N oxidation capacity	mg NH_3-N/d	2.3	90.2	126
NCF[(b)]	---	0.004	0.180	0.252
Efficiency of waste granular sludge NCF versus flocs	%	2	71	---

[(a)] Theoretical values for AOB growth on autotrophic media at 20°C and 15-d SRT assuming Y = 0.15 g VSS/g N, u_{max} = 0.9 d^{-1}, and b = 0.17 d^{-1} (Tchobanoglous et al., 2014). NH_3-N removal for cell synthesis assumed to be negligible.
[(b)] Nitrification capacity factor (NCF) is maximum NH_3-N oxidation capacity of waste granules or flocs divided by NH_3-N fed to the system.

The maximum specific NH_3-N oxidation rate was much lower for the NDN-PAO granules due to the larger granule size dominated by heterotrophic bacteria growth and smaller fraction of nitrifiers compared to the NIT granules. Despite having the lowest specific maximum NH_3-N oxidation rate, the NDN-PAO system had a much higher maximum NH_3-N oxidation capacity than the NIT system due to the higher daily granular sludge production. The NDN-PAO granular system NCF value was 45 times greater than for the NIT granular system, which suggests much better capture of nitrifiers grown on the feed NH_3-N into the granular matrix.

Even if all the nitrifiers were captured into waste granules, NCF value for granular sludge cannot equal the NCF of flocculent growth due to greater DO and NH_3-N mass transfer diffusion limitations on NH_3-N oxidation rates for granules versus floc. The fact that the NCF value of the NDN-PAO system granules was 71% of that for the AOB floc suggests very good capture of nitrifiers growth within the granules. This growth is most likely on the outer granule layers based on the operating conditions used. Based on the NDN-PAO and AOB floc NCF values, the bioaugmentation impact using granules would be higher than using flocs at granule/flocculent sludge SRT ratios greater than 1.4 in the mainstream reactor.

The lower nitrification bioaugmentation effectiveness of the granules from the NIT reactor compared to the NDN-PAO reactor is due to the lower granular sludge production in the NIT reactor as indicated by the differences in manual solids wasting from the reactors as shown in Table 4.2. Only 4.5% of the solids produced were manually wasted for the NIT reactor compared to 74% for the NDN-PAO reactor. The lower granular solids production is likely due to the limited amount of biodegradable COD in the feed to the NIT reactor of only 20 mg/L compared

to 650 mg/L for the NDN-PAO reactor. Studies on the characterization of aerobic granular sludge have indicated the importance of feed biodegradable COD for granular growth. Seviour et al. (2009) classified aerobic granular sludge as a gel-like biomass or hydrogel with extracellular polysaccharides being the key contributor to the gelatinous character and granule strength. Other studies have characterized aerobic granules as having two distinct zones: 1) a higher-density outer region primarily composed of active biomass and EPS and 2) a lower-density inner region containing dead biomass and primarily composed of noncellular proteins associated with cell decay (McSwain et al., 2005; Wang et al., 2005).

Bioaugmentation with NIT and NDN-PAO granules appears feasible based on the above results. However, NDN-PAO granules have proven to be preferred due to better granule formation, yield, and nitrification capacity. Much more effective NIT granular formation was demonstrated in a lab study by López-Palau et al. (2011) when treating anaerobic digestion supernatant with average NH_3-N, TSS and VSS concentrations of 810, 690, and 480 mg/L, respectively. Based on average influent and effluent sCOD concentrations, their study had a higher influent biodegradable sCOD concentration of about 100 mg/L versus 20 mg/L in this study. The higher COD would increase the granule sludge yield and result in different characteristics. Their reactor MLSS concentration was 11,000 mg/L, and an average granule diameter of 4.3 mm was reported. The average effluent TSS concentration was 25 mg/L, which shows much better solids capture than for the NIT reactor in this study. These results suggest improved NIT granular sludge growth is possible for sidestream treatment of centrate with sufficient biodegradable COD.

4.4.4 *Effect of granule growth reactor operation on microbial community structure*

Granule microbial community analysis by high-throughput sequencing was performed in triplicate for each granule type, where average abundance, standard deviation, and taxonomy assignments for OTUs present at greater than 2% average relative abundance were determined (Supplemental Information). Key findings are discussed below.

Zoogloea and *Thauera* dominated in NDN-OHO granules and were present at 24% and 16% abundance, respectively. The predominance of *Zoogloea* and *Thauera* in the NDN-OHO system is consistent with reactor nitrification and denitrification performance and granule physical characteristics. Both these genera are reported to grow on acetate, denitrify, exhibit clustered or fingered growth morphology, and produce prolific amounts of EPS, adversely impacting sludge settleability and dewatering characteristics (Lajoie et al., 2000; Thomsen et al., 2007). Strains of both genera have shown NO_2-N accumulation during denitrification (Liu et al., 2013; Shao et al., 2009) as observed in the NDN-OHO reactor.

Nitrosomonas was the sole dominant AOB genera detected in all reactors and present at >99% abundance relative to other AOB detected. The predominance of *Nitrosomonas* at high DO and NH_3-N concentrations is consistent with biokinetic characteristics of AOB (Limpiyakorn et al., 2013). *Nitrosospira* was not present with *Nitrosomonas* as was found for a pilot-scale NDN-PAO reactor treating domestic wastewater (Winkler et al., 2013).

NOB associating with *Candidatus* Nitrotoga (hereafter *Nitrotoga*) were detected in all reactors and was a key organism for the NO_2-N oxidation based on the relative abundance compared to

other NOB. *Nitrotoga* was the sole dominant NOB in the NDN-OHO and NDN-PAO reactors and present with *Nitrospira* in the NIT reactor. *Nitrobacter*-associated NOB were not detected in the reactors. The *Nitrotoga* abundance in NDN-OHO granules was 9.9%. *Nitrotoga* abundance in NDN-PAO granules was 0.4% and approximately 100 times greater than that of *Nitrospira*. The presence of both *Nitrotoga* and *Nitrospira* in NIT granules at 12% and 26% abundance, respectively, was likely a consequence of seeding with a mixture of crushed NDN-OHO and anammox granules. Alawi et al. (2007) discovered *Nitrotoga* in Arctic soils, and this is the first study where these NOB have been detected in aerobic granular sludge. The presence of NO_2-N in all reactors at concentrations greater than 0.3 mg/L was favorable for growth of *Nitrotoga*, which was reported to have a substrate affinity coefficient higher than that of *Nitrospira* and within the range reported for *Nitrobacter* (Nowka et al., 2015). The full metabolic capability of *Nitrotoga* has yet to be determined (Lucker et al., 2015).

Organisms associated with the genus *Dechloromonas*, which contains putative PAOs, were dominant in NDN-PAO granules at 28% abundance while organisms associated with the more traditional PAO *Candidatus* Accumulibacter (hereafter *Accumulibacter*) were present at only 6.7% abundance. Though *Dechloromonas*-related organisms may express the PAO phenotype (Kong et al., 2007) as well as the GAO phenotype (Ahn et al., 2007), anaerobic P release in this study suggests that *Dechloromonas*-related organisms were functioning as PAOs. Other studies have shown that growth of *Dechloromonas* is favored in flocculent activated sludge EBPR systems where an anoxic period follows anaerobic feeding. High-throughput sequencing by Lv et al. (2014) found that *Dechloromonas* dominated in an anaerobic-anoxic EBPR SBR while *Accumulibacter* dominated in a parallel anaerobic-aerobic EBPR SBR. Kim et al. (2013) used

oligonucleotide FISH probes for *Accumulibacter* and *Dechloromonas* and found that *Dechloromonas* abundance increased from 1.2% to 19.2% and *Accumulibacter* abundance decreased from 55.1% to 29.2% when an EBPR SBR was changed from anaerobic-aerobic to anaerobic-anoxic-aerobic operation. These findings suggest that *Dechloromonas* may have a competitive advantage over *Accumulibacter* in anaerobic-anoxic-type flocculent sludge EBPR systems or granular sludge systems where NO_x-N removal is mediated by denitrifying PAOs.

Accumulibacter has been implicated as the dominant PAO in other laboratory NDN-PAO granular sludge systems including anaerobic-aerobic (Bassin et al., 2012b; Lemaire et al., 2008; Yilmaz et al., 2008) and anaerobic-anoxic-aerobic operation (Wang et al., 2013) with acetate and/or propionate as the primary COD source(s). A common method in these studies was the use of the PAO mix of FISH oligonucleotide probes to quantify the abundance of *Accumulibacter*-related PAOs and the relative ratio of PAOs to GAOs. Though some of these studies also used probes for *Actinobacteria* PAOs, none used probes for *Dechloromonas*. Because PAO mix probes may hybridize with *Dechloromonas*-related organisms (Kong et al., 2007) and probes specifically targeting *Dechloromonas* were not used in these studies, it is possible that *Dechloromonas*-related PAOs were present and participating in P and N conversions in these granular sludge systems but not detected due to the selection and specificity of FISH probes employed. Molecular methods in future studies should account for the potential presence of *Dechloromonas*-related PAOs in NDN-PAO granule systems.

4.5 CONCLUSIONS

NIT, NDN-OHO, and NDN-PAO aerobic granules were compared for their mainstream nitrification bioaugmentation potential. NIT and NDN-PAO granules appear feasible for

bioaugmentation. NDN-PAO granules were preferred for all criteria: ammonia and nitrogen removal performance, granule physical characteristics, and nitrification capacity production. *Dechloromonas* instead of *Accumulibacter* was the key contributor to denitrification and phosphorus removal. *Zoogloea* and *Thauera* in the NDN-OHO reactor adversely impacted granule physical characteristics and treatment performance. Low organic carbon concentration in the NIT reactor feed limited granule yield, nitrification capacity production, and bioaugmentation potential. *Nitrotoga* NOB played a significant role in nitrite oxidation in all granule types.

4.6 SUPPLEMENTAL INFORMATION AVAILABLE

The supplemental information for Chapter 4 is included in this document as Appendix B.

Chapter 5. BIOAUGMENTATION WITH NITRIFYING GRANULES IN LOW-SRT FLOCCULENT ACTIVATED SLUDGE AT LOW TEMPERATURE

Chapter 5 is the manuscript that was accepted in October 2017 for publication after peer review in the Water Environment Research Journal.

5.1 ABSTRACT

Nitrifying granules were grown in a sidestream reactor fed municipal anaerobic digestion centrate and added in an initial slug dose and subsequent smaller daily doses to a non-nitrifying mainstream activated sludge system at 12°C and 2.5-day aerobic solids retention time (SRT) to increase its nitrification capacity. Effluent NH_3-N concentrations less than 1 mg/L were achieved with bioaugmentation, and nitrification was immediately lost when granules were removed after 30 days of bioaugmentation. Molecular microbial analyses indicated that nitrifying organisms remained attached to granules in the mainstream system with little loss to the flocculent sludge. Maximum specific nitrification activity of the bioaugmented granules decreased in mainstream treatment but the nitrification capacity remained due to new granule growth in the mainstream. This study demonstrated that bioaugmentation with sidestream nitrifying granules can intensify nitrification capacity in low-SRT, low-temperature flocculent activated sludge systems to achieve low effluent NH_3-N concentrations and nitrogen removal.

5.2 INTRODUCTION

Increasing the nitrification capacity in existing water resource recovery facilities (WRRFs) with activated sludge processes may be required to meet lower effluent ammonia-nitrogen (NH_3-N) concentration limits, to handle higher influent flows, and/or for conversion to nitrogen removal. Nitrification capacity has been increased without adding aeration volume by bioaugmentation with nitrifying flocculent activated sludge from sidestream treatment of anaerobic digestion dewatering reject water (Krhutková et al., 2006; Salem et al., 2004). The reject water, referred to as centrate when centrifugation is used for dewatering, can have NH_3-N concentration in the range of 700 to 1800 mg/L and account for 15 to 30% of the mainstream secondary treatment ammonia load when returned untreated (Tchobanoglous et al., 2014).

Nitrifier bioaugmentation with flocculent sludge from sidestream treatment has been demonstrated in over 25 WRRFs using various flow schemes (Bowden et al., 2015). The improvement in mainstream nitrification performance by sidestream bioaugmentation is due to the resulting increase in the mainstream nitrifying bacteria concentration. For systems with low or non-nitrifying SRTs and/or operated at low temperature, the increase in nitrification capacity may not be sufficient to meet the effluent NH_3-N concentration goals. If instead the sidestream bioaugmenting nitrifying biomass is contained in suspended biofilms, such as granular sludge, the mainstream nitrification capacity can be further increased because the granular sludge can be maintained at a longer SRT than for the flocculent sludge. This decoupling of SRTs is possible due to the larger size and more compact morphology for the granules. Separation of granules from flocs has been done using hydrocyclones (Shi et al., 2016) and screens (Liu et al., 2014a).

Sidestream growth of nitrifying (NIT) granules on municipal WRRF centrate has not been reported, but Lopez-Palau et al. (2011) have shown growth of 4.3-mm NIT granules on anaerobic digester supernatant in a bench-scale sequencing batch reactor (SBR) at 30°C and dissolved oxygen (DO) concentration near saturation. Supplemental alkalinity was not added and thus only 50% nitrification occurred due to pH suppression.

Kishida et al. (2011) grew 1.5-mm NIT granules on synthetic feed and demonstrated granular sludge nitrification bioaugmentation for a mainstream reactor continuously fed synthetic wastewater. A slug addition of NIT granules lowered the effluent NH_3-N concentration of non-nitrifying activated sludge from 40 mg/L to an average of about 10 mg/L during the subsequent 140 days of operation. Their study showed that the bioaugmenting NIT granules could improve nitrification and be sustained in a different reactor environment. However, the study lacked information on nitrifying populations and process biokinetics, SRT, temperature, and mass balances needed to assess possible design implications and extent of enhanced nitrification performance from bioaugmentation.

The objectives of this research were to evaluate the growth of NIT granules in sidestream treatment of municipal anaerobic digester dewatering centrate and the nitrification bioaugmentation performance of these granules in a low-SRT anoxic-aerobic flocculent activated sludge mainstream treatment system at low temperature. Specific aims of this evaluation included: 1) changes in the NIT granule physical characteristics between the sidestream and mainstream reactors, 2) nitrification kinetics and capacity of the sidestream NIT granules and

mainstream granules and flocculent sludge, and 3) microbial composition of the sidestream NIT granules and mainstream granules and flocculent sludge.

5.3 METHODOLOGY

5.3.1 *Sidestream nitrifying granular sludge reactor*

The NIT granular sludge sequencing batch reactor (SBR) had a height to diameter (H/D) ratio of 4.9 at the full working volume of 2.0 L and was operated at 18°C with 4-hr cycles. Each cycle consisted of the following steps: 8 min dilution stream addition under quiescent conditions, 4 min centrate stream addition under aerated conditions, 211 min aeration, 4 min settling, 2 min to pump settled supernatant to the 1.0 L level, and 11 min idle. The effluent decant/feed volume was 50% of the reactor volume. The 1.0-L feed volume was added from the top of the reactor in two feed streams. A 0.2-L stream contained centrate and supplemental alkalinity as $NaHCO_3$ to provide a total of 7.25 mg alkalinity as $CaCO_3$/mg total nitrogen (TN). A 0.8 L tap water dilution stream was added to represent addition of secondary effluent to centrate so that the sidestream reactor temperature could be much closer to the mainstream reactor temperature. Wett et al. (2011) have suggested that a lower temperature difference between sidestream and mainstream reactors may improve bioaugmentation effectiveness.

Air was sparged at 2.5 L/min from an aeration stone at the bottom of the reactor, and a small dose of silicone antifoamant was added for foam control. DO concentration was not controlled and minimum, average and maximum DO concentrations were 2.8, 3.5 and 4.3 mg/L, respectively, during long-term operation at the highest centrate TN loading used before and during bioaugmentation.

The reactor was seeded with 2500 mg/L of flocculent activated sludge from a full-scale anoxic-aerobic process. Prior to centrate feeding, granules were initially grown on synthetic wastewater simulating centrate and consisting of NH_4HCO_3, K_2HPO_4, $NaH_2PO_4 \cdot H_2O$, $NaCH_3COO$, and tap water such that NH_3-N, PO_4-P, and COD concentrations of the simulated centrate stream were 500, 10, and 100 mg/L, respectively. Stable granule growth and treatment performance was obtained after 135 days. After this initial synthetic wastewater treatment and granule formation period, influent was changed to centrate. Initial centrate feed operation involved gradual changes in TN loading. After 190 days of centrate feeding, the maximum target TN loading near 0.6 g/L-d was reached and maintained for the balance of reactor operation. The maximum target loading was based on having a nitrification volumetric oxygen demand near the limiting oxygen transfer capability of fine bubble aeration systems in full-scale reactors. The centrate was from a local municipal wastewater treatment plant with mesophilic anaerobic digestion of primary and waste activated sludge from a high-purity oxygen activated sludge process, and was pretreated by approximately 10 minutes settling and supernatant screening (53 um sieve). Pretreated centrate was stored at 4°C and replaced every two to three weeks during the 340-day centrate treatment period. Pretreated centrate characteristics are shown in Table 5.1.

Table 5.1. Pretreated centrate characteristics (average of 20 batches).

Parameter	Units	Average (Std. Dev.)	Minimum	Maximum
NH_3-N	mg/L	1126 (156)	864	1463
TN	mg/L	1315 (190)	952	1680
NH_3-N / TN	---	0.86 (0.04)	0.81	0.96
PO_4-P	mg/L	149 (57)	91	385
TSS	mg/L	149 (48)	72	270
VSS	mg/L	120 (33)	63	210
VSS/TSS	---	0.83 (0.06)	0.73	0.93
COD	mg/L	610 (126)	389	830
sCOD	mg/L	575 (73)	350	730
Alkalinity	mg/L as $CaCO_3$	4875 (783)	3250	6250
Alkalinity / NH_3-N	mol as HCO_3 / mol N	1.21 (0.06)	1.05	1.31

5.3.2 *Mainstream reactor*

A 2.0-L mainstream treatment SBR with a H/D ratio of 1.8 was operated at 12°C, non-nitrifying flocculent sludge aerobic SRT (2.5 d) and total flocculent sludge SRT of 5 days. 6-hr cycles consisted of the following steps: 10 min deoxygenation, 10 min anoxic feeding, 130 min anoxic/anaerobic reaction, 180 min aeration, 24 min settling, 5 min effluent discharge, and 1 min idle. Mixing during deoxygenation, feeding, and reaction periods was achieved by continuous sparging of N_2 at 700 Lpm and CO_2 at <5 Lpm. DO concentration during the aerobic period was controlled between 3.5 and 4.0 mg/L by on-off aeration at 1000 Lpm using a DO controller (Eutech alphaDO2000W) and galvanic probe (Atlas Scientific). All gasses were sparged from an aeration stone at the bottom perimeter wall.

1.0-L of complex synthetic wastewater was fed per cycle. Constituents contributing to COD were based on Xin et al. (2008) and adjusted to provide influent total and readily-biodegradable COD concentrations of 210 and 78 mg/L, respectively, including 39 mg/L of acetate COD. Readily-biodegradable COD constituents in 1L of feed were: 50 mg sodium acetate, 19.8 mg glucose, 9.0 mg lactose, 0.4 mg maltose, 1.0 mg sodium succinate, 3.1 mg trisodium citrate dehydrate, 2.3 mg lactic acid, 0.8 mg ethanol, and 0.4 mg/L butanol. Slowly-biodegradable COD constituents in 1L of feed were: 55.9 mg dextrin, 39.6 mg yeast extract, 15.7 mg peptone, 11.2 mg starch, 5.4 mg casein, 7.7 mg gelatin, and 0.4 mg methionine. NH_4Cl was fed at an influent concentration of 26.5 mg N/L, and the organic constituents added 5.5 mg/L of influent organic N. Influent PO_4-P concentration was 32 mg/L from equal masses of K_2HPO_4 and $NaH_2PO_4 \cdot H_2O$ to provide moderate pH buffering capacity. $MgSO_4$ and $CaCl_2$ provided influent Mg^{2+} and Ca^{2+} concentrations of 10 and 18 mg/L, respectively. Trace elements were also provided according to Xin et al. (2008).

Pre-Bioaugmentation Period

The reactor was seeded with 800 mg/L of flocculent sludge from a full-scale non-nitrifying high-purity oxygen activated sludge process and operated for four days prior to bioaugmentation. The objective of the pre-bioaugmentation period was to develop background flocculent sludge lacking nitrification activity but possessing denitrification activity and good settleability. Thus, NO_3-N was also included in the feed described in Section 5.3.2 at a concentration of 26.5 mg/L using $NaNO_3$ to provide anoxic selector conditions in absence of biological nitrification during the pre-bioaugmentation period. $NaNO_3$ was included in the feed during the pre-bioaugmentation period only and removed from the feed for subsequent bioaugmentation testing.

Bioaugmentation Period

The objective of the 30-d bioaugmentation period was to evaluate the ability of sidestream nitrifying granules to sustain nitrification and enable nitrogen removal by denitrification in a flocculent activated sludge process with an SRT below the theoretical minimum SRT for nitrifier growth. NO_3-N was removed from the feed for bioaugmentation testing and the rest of the experiment. Thus, the feed was as described in Section 5.3.2.

After establishing the absence of nitrification in the pre-bioaugmentation period, an initial seeding of 1.2 g of sidestream granules (600 mL at 2000 mg/L) was added with sufficient capacity to nitrify the NH_3-N available in the daily feed for the mainstream SBR. The mass added was based on assuming the same NH_3-N oxidation activity as that observed in centrate treatment with excess NH_3-N and similar DO concentration and correcting for the lower mainstream temperature using a temperature-activity coefficient (Θ) of 1.072.

Following the initial seeding of sidestream granules, bioaugmentation continued daily in smaller doses. Granules were bioaugmented in proportion to full-scale WRRF applications where the untreated sidestream TN load is about 25% of the TN load to the secondary process. Though the sidestream reactor TN load averaged 1.13 g/d during bioaugmentation, the mainstream TN load was much lower at 0.13 g/d. Therefore, only 2.9% of the daily sidestream reactor waste granular activated sludge was added to the mainstream reactor, thus reflecting a daily sidestream granule addition rate proportionate to common full-scale WRRF sidestream and mainstream TN loading conditions. The granule mass added during daily bioaugmentation was 0.1 g TSS (2 mL/d at 1850 mg/L for 30 days).

Selective retention of granular particles >425 um was a distinguishing feature of operation during bioaugmentation. The SRTs of granules and flocs in the mainstream reactor were uncoupled by incorporating a sieving procedure in sludge wasting. The SRT of flocs, effectively defined as particles smaller than 425 um, was controlled by wasting solids passing a 425-um sieve. The flocculent sludge mixed liquor suspended solids (MLSS) concentration was measured after sieving, and the MLSS volume directed to the sieving wasting procedure was determined based on the flocculent MLSS concentration, reactor volume, and target flocculent sludge 2.5-day aerobic SRT. Conversely, granules >425 um retained in the sieving procedure were returned to the reactor.

Post-Bioaugmentation Period

At the end of the bioaugmentation period, granules were removed by sieving the entire reactor contents. Operation continued with flocculent activated sludge only to evaluate the contribution of the flocculent sludge fraction to nitrification and nitrogen removal in the bioaugmented process.

5.3.3 *Ammonium oxidation activity testing*

Ammonium oxidation activity tests were conducted at room temperature (20-22°C) for flocculent and granular sludge fractions. Test reactors were mixed by aeration and had MLSS concentrations less than 1800 mg/L. NH_4Cl and $NaHCO_3$ stock solutions were added to the test reactor to provide an initial NH_3-N concentration of 30 mg/L and alkalinity of 300 mg/L as $CaCO_3$. DO concentration was greater than 7 mg/L and pH was approximately 7.8 during

activity tests. Measured activities were normalized to 20°C using a temperature-activity (θ) coefficient of 1.072 (Tchobanoglous et al., 2014).

5.3.4 *Apparent ammonium half saturation concentration*

Data to determine the apparent half saturation concentration for sidestream nitrifying granular sludge were obtained under conditions similar to NH_3-N oxidation activity testing with non-limiting alkalinity and DO concentrations. The test was started an initial NH_3-N concentration of 15 mg/L and proceeded until NH_3-N oxidation was complete. Data were fitted to a fourth-degree polynomial regression ($R^2 > 0.9999$). The apparent half saturation concentration was calculated based on the NH_3-N concentration where the first derivative of the regression was half of that at the start of the test under non-limiting NH_3-N concentration.

5.3.5 *Analytical methods*

Influent wastewater characteristics and reactor effluent values were measured in the following manner. NH_3-N and NO_3-N were measured by ion-sensing electrode probes (Hach IntelliCAL Models ISENH4181 and ISENO3181, respectively). TN was determined by persulfate digestion and quantification of digestate NO_3-N with an ion-sensing electrode probe. NO_2-N and PO_4-P were measured spectrophotometrically using standard test cuvettes (Hach Methods 10019 and 8048, respectively). Alkalinity, pH, TSS, VSS, SVI, COD, and sCOD (0.45 um-filtered) were determined by Standard Methods (APHA, 2012). DO concentration was measured with an optical probe (YSI ProODO). For reactor cycle profiles and ammonium oxidation activity and apparent half saturation tests, inorganic nitrogen species of interest were measured in triplicate on a Gallery™ Automated Photometric Analyzer (Thermo Fisher Scientific).

5.3.6 *Granule morphology, size, and density*

Granule morphology was evaluated by stereomicroscopy and image analysis of a minimum sample of 250 granules. Individual granule sizes were taken to be the diameter of a circle possessing the same area as the granule plan view area. Granule wet density was determined by pycnometry according to Winkler et al. (2012) for triplicate samples.

5.3.7 *Isolation and quantification of genomic DNA*

Samples of sidestream granules at the start of bioaugmentation, mainstream granules at the end of bioaugmentation, and mainstream flocs at the midpoint and end of bioaugmentation were obtained by sieving the respective mixed liquors at 425 um. The obtained granule/floc samples were stored at -80°C prior to DNA isolation. For each granule/floc type, DNA was isolated in triplicate using the UltraClean Microbial DNA Isolation Kit (MO-BIO, Inc.) according to the manufacturer's instructions with 10 min of thermal pretreatment at 65°C and bead beating at 4 m/s for 20 s to enhance DNA yield. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.).

5.3.8 *High-throughput sequencing of 16S rRNA gene amplicons*

DNA samples were normalized to equal concentrations using nuclease-free water and submitted to a service facility (www.mrdnalab.com) for sequencing of 16S rRNA gene amplicons.16S rRNA gene primers 341F and 805R were used in a single-step PCR due to their low bias compared to other primer pairs (Hugerth et al., 2014). PCR was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Inc.) under the following conditions: 3 min at 94°C, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 60 s, followed by the final elongation step at 72°C for 5 min. Amplicons were mixed in equimolar concentrations and purified using

AMPure XP beads (Agencourt Bioscience Corp.). Sequencing was performed on a MiSeq system (Illumina, Inc.) according to the manufacturer's protocol. Raw paired-end data were joined and reverse complimented to read in the 5'-3' orientation.

5.3.9 *Bioinformatics*

The UPARSE method (Edgar, 2013) was used for amplicon sequence processing and operational taxonomic unit (OTU) clustering with USEARCH version 7.0.1090 (Edgar, 2010). Sequences were truncated at 400 bases, and those less than 220 bases or with greater than 0.5 expected errors were filtered. Sequences were clustered at 97%, and UCHIME was used to identify and filter chimeric sequences based on the RDP Classifier (v9) "gold" training database. Taxonomy for representative sequences was assigned using USEARCH based on the Silva 119 reference database (Quast et al., 2013). Taxa were summarized by percent abundance normalized to the total number of reads using QIIME version 1.9.1 (Caporaso et al., 2010a). Representative sequences that did not receive a named genus-level taxonomy assignment using the methods above were queried in BLASTn version 2.7.0 (Zhang et al., 2000). Hits resulting in a single genus-level identification at >95% coverage, >95% identity, and $<10^{-150}$ E-value were taken as a genus-level taxonomy assignment. Otherwise, sequence identification was assigned at higher taxonomic ranks.

5.3.10 *Quantitative PCR (qPCR)*

The concentration of ammonia-oxidizing bacteria (AOB) in sidestream granules, mainstream granules at the end of bioaugmentation, and mainstream flocs at different points of bioaugmentation was measured by qPCR using *amoA*-1F and *amoA*-2R primers targeting the ammonia monooxygenase (*amoA*) gene (Rotthauwe et al., 1997). qPCR was performed in an

Eppendorf Mastercycler® realplex using EvaGreen® intercalating dye and associated master mix (Biotium, Inc.). The following program was used: a) 2 min denaturation at 95°C; b) 40 cycles of 30 sec denaturation at 95°C, 45 sec annealing at 57°C, and 60 sec extension at 72°C; c) 10 min extension at 72°C; d) holding temperature of 12°C. Double-stranded DNA fragments (Integrated DNA Technologies, Inc.) consisting of the 491 bp region bound by the primers in addition to the adjacent 20 bp (McTavish et al., 1993) were used as standards. Results were normalized to the amount of template DNA.

5.4 RESULTS

5.4.1 *Sidestream centrate treatment*

Growth of sidestream NIT granules (Figure 5.1a) with average diameter of 0.9 mm (±0.2 mm standard deviation) and average density of 1.026 g/cm^3 (±0.007 g/cm^3 standard deviation) was achieved with consistent excess granule production to support bioaugmentation. Treatment performance of the sidestream reactor for operating days 260 to 309 prior to bioaugmentation and days 310 to 340 during bioaugmentation is summarized in Table 5.2. Data for the period prior to bioaugmentation cover four centrate feed batches and approximately three SRTs of relatively stable performance, and data for the bioaugmentation period cover three centrate feed batches and approximately two SRTs under a similar TN loading rate.

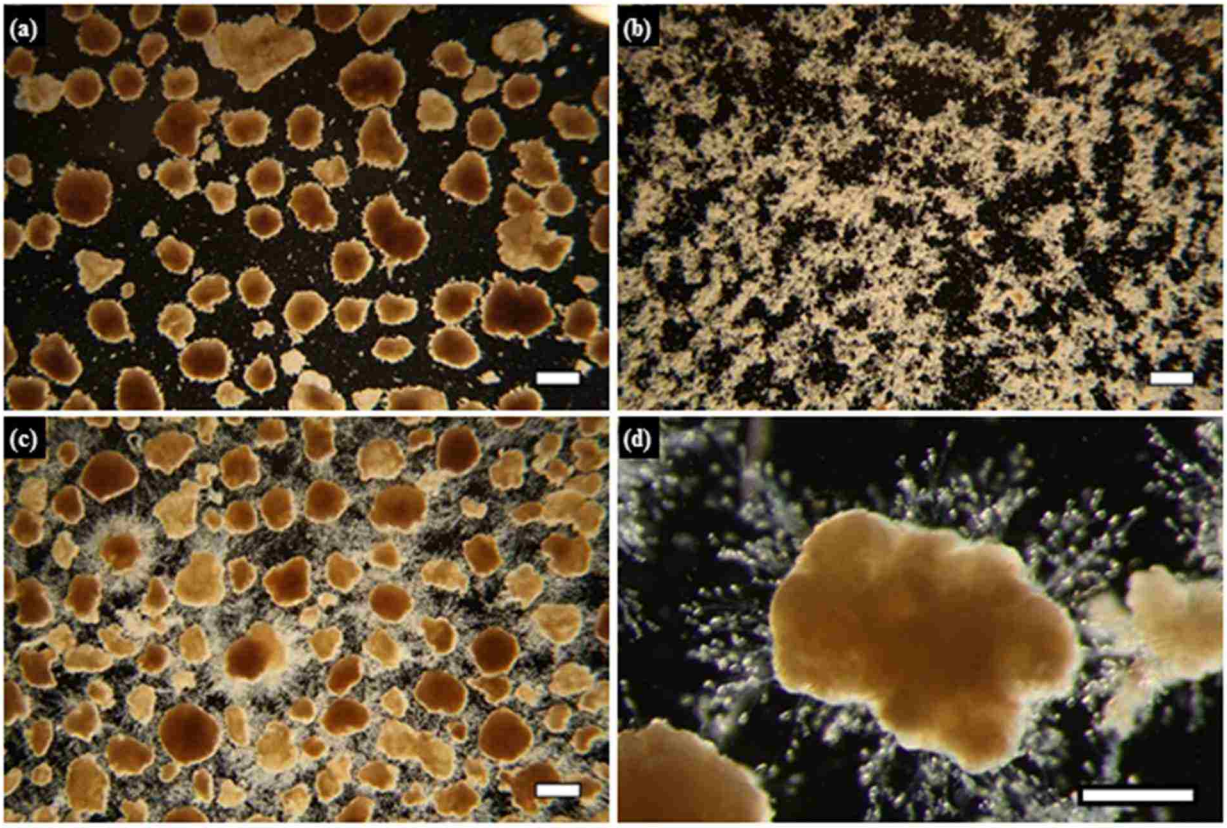

Figure 5.1. Photomicrographs of (a) sidestream granules, (b) mainstream flocs at the end of bioaugmentation, and (c, d) mainstream granules at the end of bioaugmentation. Magnification and scale bars are (a-c) 6x and 1.0 mm and (d) 32x and 0.5 mm, respectively.

Table 5.2. Average performance of sidestream nitrifying granular sludge reactor at similar centrate nitrogen loadings before and during bioaugmentation. Parentheses indicate one standard deviation.

Parameter	Units	Before bioaugmentation Day 260 to 309	During bioaugmentation Day 310 to 340
Influent[a]			
NH_3-N	mg/L	161 (19)	167 (20)
TN	mg/L	193 (21)	191 (22)
PO_4-P	mg/L	19 (2)	22 (3)
TSS	mg/L	32 (8)	23 (3)
VSS/TSS	-	0.87 (0.05)	0.88 (0.01)
COD	mg/L	108 (5)	90 (18)
NH_3-N loading rate	g/L-d	0.48 (0.06)	0.49 (0.02)
TN loading rate	g/L-d	0.58 (0.07)	0.56 (0.02)
Centrate dilution factor	-	6.0 (0.6)	6.0 (0.5)
Effluent			
NH_3-N	mg/L	32 (11)	19 (7)
NO_2-N	mg/L	23 (10)	80 (27)
NO_3-N	mg/L	126 (18)	81 (34)
TSS	mg/L	29 (7)	26 (6)
VSS/TSS	-	0.87 (0.05)	0.88 (0.06)
pH	-	7.7 (0.1)	7.4 (0.1)
Alkalinity	mg/L as $CaCO_3$	325 (92)	201 (59)
NH_3-N removal	%	80 (6)	89 (5)
SRT, MLSS, SVI, and waste solids			
SRT total, 7d average	d	18 (3)	15 (2)
MLSS	mg/L	2184 (240)	1851 (145)
MLVSS/MLSS	-	0.93 (0.01)	0.93 (0.01)
5-min SVI	mL/g	33 (3)	36 (3)
Daily effluent TSS	mg/d	171 (42)	159 (25)
Daily reactor MLSS wasted	mg/d	100 (49)	102 (16)[b]

[a] Includes diltion water addition.
[b] Excludes 1.3 g MLSS removed for initial slug bioaugmentation.

During the period prior to bioaugmentation the average nitrogen loading rate was 0.58 g TN/L-d with 77% converted to oxidized NO_3-N plus NO_2-N (NO_x-N), and the average NH_3-N loading rate was 0.48 g/L-d with 80% NH_3-N removal. The effluent total inorganic nitrogen (TIN) concentration was higher than the influent NH_3-N concentration (181 versus 161 mg/L), which indicates that some of the organic nitrogen in the centrate was converted. NO_3-N comprised 83% of effluent NO_x-N indicating substantial nitrite-oxidizing bacteria (NOB) activity. Cycle inorganic nitrogen profiles showed constant NH_3-N removal and NO_3-N production rates during the aerobic period. Ex-situ activity tests at higher DO concentration showed that the maximum specific NH_3-N oxidation activity of sidestream granules was 29.8 mg N/g VSS-hr, and their apparent NH_3-N half saturation concentration was 2.3 mg/L.

Data in Table 5.2 show that similar nitrogen loading and NH_3-N removal was maintained in the sidestream reactor during bioaugmentation, but that the completeness of NH_3-N oxidation varied. Sidestream effluent NO_2-N concentration increased to 70% of the effluent NO_x-N after about 30% of the sidestream reactor MLSS was removed for the initial slug bioaugmentation of the mainstream reactor. The increase in the NO_2-N concentration after the slug granule removal suggests that the NO_2-N oxidation capacity in the granules was lower than the NH_3-N oxidation capacity. The lower capacity is likely due to the incomplete oxidation of NH_3-N to NO_3-N in the sidestream reactor prior to bioaugmentation as shown in Table 5.2 with 15% of the NH_3-N oxidized remaining as NO_2-N. After that, the sidestream reactor effluent NO_3-N concentration increased to concentrations near the pre-bioaugmentation period, with effluent NO_2-N decreasing to only 40% of effluent NO_x-N by day 340. The high standard deviations for average effluent NO_2-N and NO_3-N concentrations reflect this variability in NO_2-N oxidation.

Granules larger than 425 um were 90% of the sidestream reactor MLSS mass. Effluent TSS did not contain granules and averaged between 25 and 30 mg/L. After accounting for solids loss in the sidestream reactor effluent, manual MLSS wasting was done to maintain the target SRT. Manual granular sludge wasting averaged 37 and 39% of the total daily solids production prior to and during bioaugmentation, respectively.

5.4.2 *Mainstream treatment*

Mainstream effluent inorganic nitrogen species concentrations during pre-bioaugmentation, bioaugmentation, and post-bioaugmentation periods are shown in Figure 5.2. Prior to bioaugmentation, removal of influent NH_3-N and ammonified influent organic nitrogen was by assimilation for cell synthesis. Bioaugmentation immediately increased mainstream nitrification, and effluent NH_3-N concentrations were less than 1 mg/L throughout the 30-day bioaugmentation period. Effluent NO_2-N concentrations were 0.1 to 0.4 mg/L indicating nearly complete nitrification to NO_3-N. The effluent NO_3-N concentration during bioaugmentation was 13 to 15 mg/L, as a result of full nitrification and denitrification during the anoxic feeding and reaction period. After the granules were removed from the reactor mixed liquor by sieving, the effluent NH_3-N concentration increased to 19.8 mg/L and then to 23.4 mg/L after 3 days during the post-bioaugmentation period. As in the pre-bioaugmentation period, the NH_3-N was removed for cell synthesis by heterotrophic organisms.

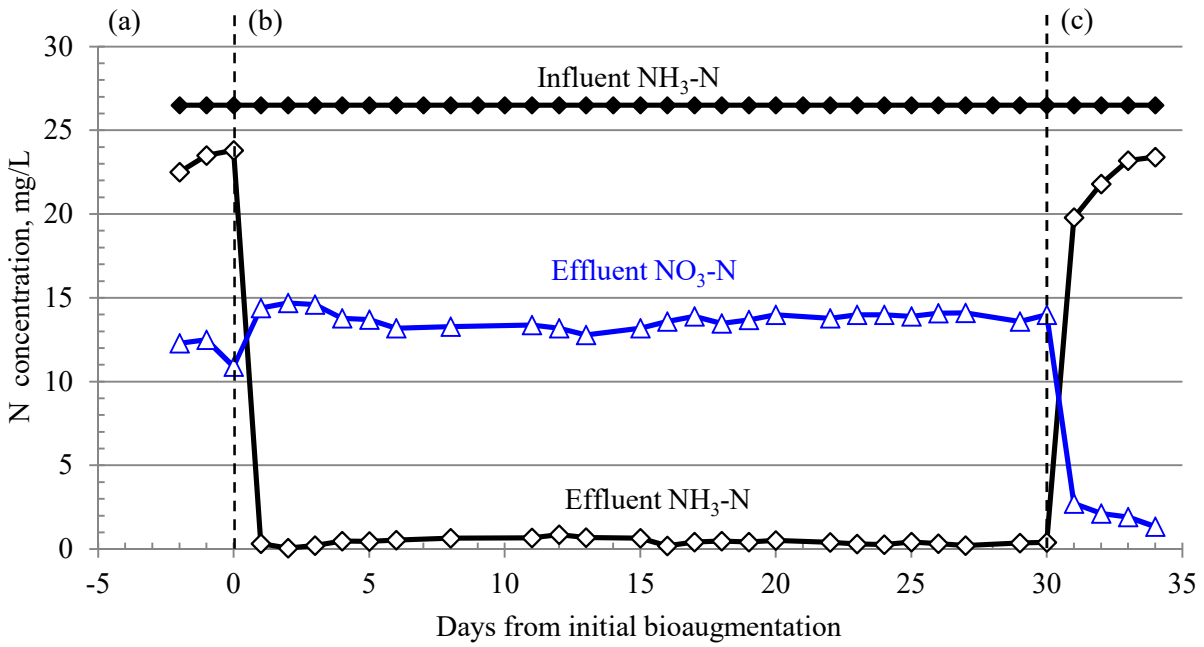

Figure 5.2. Mainstream reactor influent NH_3-N and effluent NH_3-N and NO_3-N concentrations during pre-bioaugmentation (a), bioaugmentation with granule addition (b), and post-bioaugmentation after granule removal (c). Influent included 5.5 mg/L of organic nitrogen. Effluent NO_2-N concentration averaged 0.2 mg/L with a maximum of 0.4 mg/L over all periods.

A profile of inorganic nitrogen species concentrations during a mainstream SBR cycle on day 27 of bioaugmentation is shown in Figure 5.3. Based on the prior cycle effluent NO_3-N concentration, the 50% feed volume is estimated to have diluted the NO_3-N concentration to about 7.0 mg/L. Denitrification during the deoxygenation and feed period reduced the NO_3-N concentration to 3.0 mg/L, and NO_3-N removal was near-complete at only 50 min into the 150-min anoxic period. No denitrification occurred during the aerobic period as indicated by the amount of NH_3-N removal and NO_3-N production. The NH_3-N oxidation rate decreased as the NH_3-N concentration approached 2 mg/L, which was consistent with the apparent half-saturation concentration measured for sidestream granules.

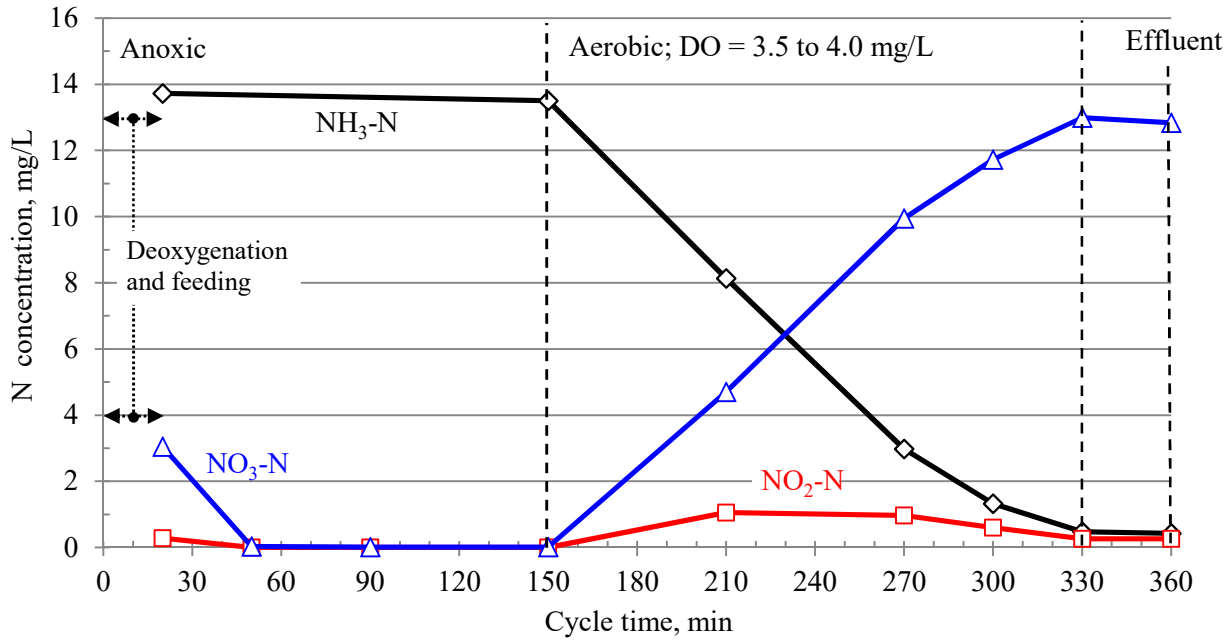

Figure 5.3. Changes in inorganic nitrogen species during the mainstream SBR cycle at 12°C on day 27 of bioaugmentation. Error bars for triplicate measurements are shown but hidden by markers for average values.

Despite anaerobic conditions due to the absence of NO_x-N during the last approximately two-thirds of the unaerated period, enhanced biological phosphorus removal (EBPR) was not apparent. The effluent PO_4-P concentration ranged from 30.0 to 30.7 mg/L for 1.5 to 2.2 mg/L PO_4-P removal, which agrees with that expected for biomass synthesis from the COD and NH_3-N removed. Assuming net yields the of 0.35 g VSS/g COD and 0.10 g VSS/g N for heterotrophs and nitrifiers, respectively, and a biomass phosphorus content of 0.025 g P /g VSS representative of non-EPBR sludge, the estimated PO_4-P removal for cell synthesis is 1.9 mg/L, which is in the range of observed PO_4-P removal.

A mixture of flocs and granules were maintained in the mainstream reactor (Figures 5.1b and 5.1c). Flocculent sludge MLSS concentration averaged 740 mg/L during bioaugmentation testing, and effluent TSS concentration averaged 10 mg/L. The granular sludge MLSS concentration was 600 mg/L at the initial seeding and 1150 mg/L at the end of bioaugmentation. Although the core granule morphology persisted under mainstream growth conditions, outgrowth of stalked ciliates became common by the end of bioaugmentation (Figures 5.1c and 5.1d). Excluding this outgrowth, the average granule diameter was 0.8 mm (±0.2 mm standard deviation), similar to sidestream granules. The particle size distribution of mainstream granules at the end of bioaugmentation was also similar to that of sidestream granules as shown in Figure 5.4.

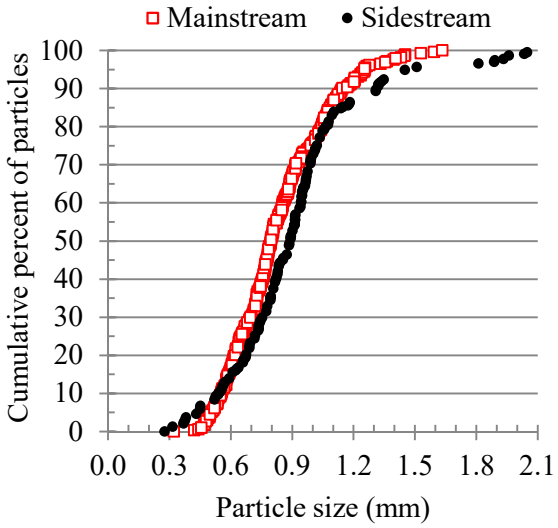

Figure 5.4. Cumulative particle size distribution for sidestream granules and mainstream granules at the end of bioaugmentation. Particles greater than 200 um with granular morphology were included in the analysis. Minimum sample size was 250 particles.

Granule mass increased and maximum specific NH_3-N oxidation activity decreased during the bioaugmentation period. The granule mass added to the mainstream reactor was about 1.3 grams (g) TSS, with 1.2 g TSS added in the initial seeding and 0.1 g TSS added during subsequent daily bioaugmentation. At the end of the bioaugmentation period the total mainstream granular mass was 2.3 g TSS, and the granule maximum specific NH_3-N oxidation activity was 14.5 g N/g VSS-hr, which was 49% of that for the sidestream granules.

5.4.3 *Molecular analyses of granules and flocs*

Microbial populations were evaluated by high-throughput sequencing and bioinformatics analyses for the following granule/floc types: a) sidestream granules at the start of bioaugmentation, b) mainstream granules at the end of bioaugmentation, and c) mainstream flocs at the end of bioaugmentation. Average abundances of microbial taxa are shown in Figure 5.5 for triplicate analyses of each granule/floc type. All nitrifiers that were detected are shown, and heterotrophs with greater 2% abundance in any granule/floc type are shown separately. The total abundance of all other heterotrophs with less than 2% abundance in any granule/floc type ranged from 22.1 to 25.1%.

Sidestream Granules	Mainstream Granules	Mainstream Flocs	Taxonomy Assignment
Nitrifiers			
17.5 ±1.1	3.5 ±0.2	1.0 ±0.1	Ca. Nitrotoga
9.8 ±0.6	3.1 ±0.1	0.4 ±0.0	Nitrosomonas
Heterotrophs			
25.4 ±1.7	4.2 ±0.1	1.7 ±0.2	Haliscomenobacter
6.5 ±0.4	1.3 ±0.1	0.1 ±0.0	Fluviicola
4.1 ±0.1	15.3 ±0.7	4.5 ±0.6	Cytophaga
3.0 ±0.0	8.7 ±1.1	11.3 ±1.2	Flavobacterium
2.8 ±0.4	2.3 ±0.2	1.6 ±0.2	Comamonas
2.8 ±0.1	3.9 ±0.5	0.7 ±0.1	Thauera
2.1 ±0.3	3.4 ±0.2	1.6 ±0.2	Ferruginibacter
1.8 ±0.3	10.1 ±0.3	11.4 ±1.4	Dechloromonas
0.9 ±0.1	2.4 ±0.2	3.2 ±0.7	Zoogloea
0.3 ±0.0	9.7 ±0.6	1.6 ±0.7	Gemmobacter
0.2 ±0.0	3.7 ±0.3	8.2 ±1.4	Persicitalea
0.1 ±0.0	1.4 ±0.1	2.0 ±1.4	Rhodobacter
0.1 ±0.0	1.5 ±0.0	2.4 ±0.5	Alphaproteobacteria bacterium
0.1 ±0.0	0.6 ±0.0	4.4 ±0.7	Ca. Saccaribacteria bacterium
0.1 ±0.0	0.1 ±0.0	8.2 ±1.0	Mangroviflexus
0.1 ±0.0	0.5 ±0.0	8.7 ±1.4	Acinetobacter
0.0 ±0.0	0.1 ±0.0	2.0 ±0.3	Prosthecobacter
22.4 ±0.7	23.9 ±1.2	25.1 ±0.7	Sum of all others < 2% not above

Figure 5.5. Average percent abundance heatmap of microbial taxa in sidestream granules at the start of bioaugmentation and mainstream granules and flocs on the final day of bioaugmentation. All nitrifying taxa detected and heterotrophic taxa with greater than 2% abundance in any granule or floc sample are shown. Taxa are listed in order of decreasing abundance in sidestream granules. Percent abundance is based on total amplicon reads with one standard deviation shown for triplicate analyses.

As shown in Figure 5.5, the sole AOB was *Nitrosomonas*, and the sole NOB was *Candidatus* Nitrotoga (hereafter *Nitrotoga*). The relative abundance of these nitrifying organisms was highest in sidestream granules and decreasingly lower in mainstream granules and flocs, respectively. A similar trend was observed in qPCR quantification of AOB amoA gene copies per unit of DNA extracted. Sidestream granules had the highest normalized amoA gene copy concentration of $8.4x10^4$ $\pm 0.4x10^4$ copies/ng DNA template. Slightly over one order of magnitude decrease was successively observed for mainstream granules and flocs, which had amoA gene copy concentrations of $4.5x10^3$ $\pm 0.4x10^3$ and $1.5x10^2$ $\pm 0.3x10^2$ copies/ng, respectively. Differences in flocculent sludge amoA gene copy concentration at the midpoint and end of bioaugmentation were not statistically significant at an alpha level of 0.05. BLAST queries of *Nitrosomonas*-associated OTUs against *N. europaea*, *N. oligotropha*, and *N. eutropha* showed that the *Nitrosomonas*-associated OTUs most closely aligned with *N. europaea* (Accession No. NR_117649.1) at 98% query coverage with 96% identity.

Figure 5.5 shows that the most abundant heterotrophic genera in sidestream granules were *Haliscomenobacter*, *Fluviicola*, *Cytophaga*, and *Flavobacterium*. Together, these accounted for 39.0% of DNA amplicons from the sidestream granules, with *Haliscomenobacter* accounting for 25.4% of DNA amplicons. The *Haliscomenobacter*-associated OTU sequence aligned with *Haliscomenobacter* (Accession No. KJ023560.1) at 98% query coverage with 98% identity and with *Haliscomenobacter hydrossis* (Accession No. NR_042316.1) at 97% query coverage with 89% identity. *Haliscomenobacter*, *Fluviicola*, *Cytophaga*, and *Flavobacterium* remained in mainstream granules, but the abundance of *Haliscomenobacter* and *Fluviicola* decreased and the

abundance of *Cytophaga* and *Flavobacterium* increased. The most abundant heterotrophic genera in mainstream granules were *Cytophaga*, *Dechloromonas*, *Gemmobacter*, and *Flavobacterium*, which had abundances between 8.7% and 15.3% and together accounted for 43.8% of DNA amplicons. Heterotrophs amongst those with high abundance in mainstream granules, *Flavobacterium* and *Dechloromonas*, were also the two most abundant genera in mainstream flocs. Other highly abundant genera in mainstream granules, *Gemmobacter* and *Cytophaga*, were present in flocs but at lower abundance. Genera with greater than 8% abundance in mainstream flocs in addition to *Flavobacterium* and *Dechloromonas* were *Persicitalea*, *Mangroviflexus*, and *Acinetobacter*.

5.5 DISCUSSION

Important aspects of this study concern sidestream growth of NIT granules on centrate, the effectiveness of nitrification bioaugmentation with these granules, and nitrifying and heterotrophic microbial populations characteristics.

5.5.1 *Sidestream nitrifying granules*

This study showed that NIT granules can be grown on anaerobic digester dewatering centrate at a high nitrogen loading rate with routine granule wasting, which is the first key to viability of the bioaugmentation process. Selective pressures for granular sludge growth have been reviewed by Figdore et al. (2017), and the key elements for NIT granule growth were the short settling time, aeration shear, and ammonia-rich feed for autotrophic slow-growing bacteria. The short 4-min settling time washed out slower-settling floc giving an advantage to the faster-settling granules. The selective pressures resulted in granules with high nitrifier abundance. Some organic carbon is necessary to grow granules as granular sludge is known to contain extracellular

exopolysaccharides, which are the key structural element of the granule matrix (Figdore et al., 2017). The carbon source for NIT granules had to be from the small amount of acetate in the centrate feed and biological endogenous decay substrate release. The superficial air velocity at about 0.8 cm/s is within a range considered to provide sufficient shear forces to form compact granules (Figdore et al., 2017).

The thickening capacity of granules at short settling times (average SVI_{5min} of 33 mL/g) allowed a high-volume exchange ratio, with the feed volume comprising 50% of the full reactor volume. The high fill fraction allowed dilution of the centrate for temperature reduction by a factor of about 6 with centrate comprising approximately 15% of the feed volume. This centrate dilution is practical because using a higher centrate fraction in the feed would lead to a reactor volumetric oxygen demand well above the oxygen transfer capability of full-scale aeration devices. The average combined feed influent NH_3-N concentration during the bioaugmentation period (Table 5.2) was 167 mg/L, which required an average oxygen demand of 102 mg/L-h based on the 3.5-hour aeration time per cycle and 4.3 g O_2/g NH_3-N oxidized (Tchobanoglous et al., 2014). This oxygen demand is close to the maximum volumetric oxygen transfer capability of fine bubble aeration (Parker and Wanner 2007). Thus, the need for centrate feed dilution can result in the sidestream reactor having a temperature closer to the mainstream temperature, which may improve bioaugmentation effectiveness (Wett et al., 2011). Some nitrification bioaugmentation processes using flocculent sludge have included mainstream return activated sludge (RAS) feed to the centrate treatment system (Bowden et al., 2015), which would also lower the centrate treatment reactor temperature.

5.5.2 *Nitrification bioaugmentation with granules*

Bioaugmentation with sidestream NIT granules in this study achieved full nitrification and lower effluent NH_3-N concentrations than reported for bioaugmentation with sidestream nitrifying flocculent sludge in full-scale WRRFs with much longer mainstream flocculent sludge aerobic SRTs and similar temperature. For example, bioaugmentation with flocs in a WRRF with aerobic and total solids retention times (SRTs) near 5 and 7 days lowered the average effluent NH_3-N concentration in consecutive 2-month periods from 15 to 7 mg/L after bioaugmentation (Krhutková et al., 2006). Corresponding temperature ranges in the periods were 13 to 16°C and 10 to 15°C. Another example is a WRRF with flocculent nitrifier bioaugmentation in a completely aerobic mainstream activated sludge process, where an average monthly effluent NH_3-N concentration near 5 mg/L was achieved at 12°C with a 5-d SRT (Parker and Wanner, 2007). The longer SRT of nitrifiers in the mainstream system bioaugmented with NIT granules in this study improved nitrification performance compared to the above systems with flocculent sludge bioaugmentation, despite having a flocculent sludge aerobic SRT of only 2.5 days.

Results of bioaugmentation testing and molecular analyses provided insight into the fate of granule nitrifiers in mainstream activated sludge treatment. Near-complete loss of nitrification occurred in the mainstream reactor after granule removal, and molecular analyses showed much lower AOB gene copy numbers and nitrifier relative abundances in mainstream flocs versus mainstream granules. Based on these results, granules were the dominant contributor to nitrification in mainstream treatment and sustained nitrification during the 30-d testing period. Nitrifiers primarily remained on granules, with little loss to the flocculent sludge.

The high level of nitrification in the granule-bioaugmented SBR allowed nitrogen removal by denitrification of NO_x-N during anoxic feeding, and the nitrogen removal efficiency was determined by the SBR operation. Based on the high exchange volume used, 50% of the NO_x-N produced in the aerobic step was in the effluent. A post-anoxic period after aeration, possibly with carbon addition, could result in a lower effluent NO_x-N concentration.

Sustained exposure in mainstream treatment with flocculent sludge wasting and selective granule retention resulted in changes in granule mass and maximum specific NH_3-N oxidation activity. Although the granule maximum specific NH_3-N oxidation activity at the end of bioaugmentation was 49% of that for fresh sidestream granules, the sidestream granule mass added to the mainstream reactor was only 56% of the final granule mass due to new granule growth. Therefore, the decrease in granule biomass-specific NH_3-N oxidation activity in mainstream treatment was roughly proportionate to the increase in granule biomass.

The decrease in specific nitrification activity is related to heterotrophic growth in the mainstream treatment system as indicated by the increase in heterotroph relative abundance and decrease in nitrifier relative abundance in mainstream granules (Figure 5.5). The much higher COD/N ratio and carbon substrate concentration in the mainstream feed, intended to simulate wastewater treatment, led to heterotrophic growth and increased heterotroph abundance in the mainstream granules. A possible explanation is that heterotrophs grew on top of nitrifiers on the outer layer of granules. The specific nitrification activity would thus be reduced because of the higher fraction of heterotrophic bacteria in the granular mass and less oxygen diffusion hence minimizing the aerobic volume fraction available to nitrifiers. However, mainstream nitrification

capacity was maintained during bioaugmentation (Figure 5.2). Another possibility for the decrease in specific nitrification activity is that the bioaugmented granules largely maintained their biofilm composition with nitrifiers on the outer layer, and that newly-grown granules had a different microbial community composition and spatial distribution with higher heterotrophic abundance. In this case, the increase in total granule mass would reduce specific nitrification activity but not nitrification capacity, as observed in this study.

The increase in mainstream granule mass during bioaugmentation indicates that granule growth occurred and suggests that the sidestream granules added were sustained. Selective pressures for growth of heterotrophic granules (Figdore et al., 2017) include 1) a short settling time to favor capture of fast-settling biomass/granules, 2) high food/mass ratio (F/M) feeding conditions with readily biodegradable substrate, and 3) sufficient shear to form the smooth and compact granular biofilm with little outgrowth. Liu et al. (2014a) showed that particle-size based selection by sieving MLSS and returning only the larger solids, as done in this study, selected for granules in absence of settling pressure. The 10-min unaerated feeding period in this study provided an instantaneous high F/M feeding condition. The mainstream gas sparging rate with N_2 and air was 1700 cm^3/min or about 70% of the sidestream aeration rate, and hence gas-driven shear in the mainstream reactor may have been sufficient to form new granules. Therefore, mainstream reactor operation included several elements favorable for new granule formation.

5.5.3 *Nitrifying populations*

The AOB in NIT granules were most closely associated with *Nitrosomonas europaea* , which has higher ammonia and oxygen half saturation concentrations than other AOB (Limpiyakorn et al., 2013). The dominance of *Nitrosomonas* was also consistent with identifications of AOB in

granular sludge, where it has been the dominant AOB genera at elevated NH_3-N and DO concentrations (López-Palau et al., 2011; Matsumoto et al., 2010). Unlike other granules grown at lower volumetric nitrogen loading rate and DO concentration (Winkler et al., 2013), an equal abundance of *Nitrosospira* and *Nitrosomonas* was not found in the sidestream NIT granules.

The presence of the relatively newly-discovered NOB of the genus *Nitrotoga* in NIT granules instead of the more familiar *Nitrobacter* or *Nitrospira* was a novel finding. *Nitrotoga* were discovered by Alawi et al. (2007) in Arctic soils and have been detected at significant abundance compared to other NOB in full-scale WRRFs with flocculent activated sludge processes under 16°C (Lucker et al., 2015). The growth kinetics of NOB are related to their maximum specific growth rate, half-saturation coefficients for NO_2-N and O_2, and temperature-activity coefficients (Tchobanoglous et al., 2014). Based on the average effluent NO_2-N concentration of 23 mg/L during the 49-day period prior to bioaugmentation (Table 5.2), NO_2-N concentrations throughout the NIT reactor cycle would not pose significant rate limitations to NOB (Nowka et al., 2015). The maximum activity of *Nitrotoga* has been studied in enrichment culture at 17°C and compared to pure cultures of *Nitrospira* and *Nitrotoga* at 28°C (Nowka et al., 2015), but oxygen half-saturation and temperature-activity coefficients were not evaluated. Therefore, a more conclusive determination on conditions resulting in *Nitrotoga* dominance in NIT granules cannot be made.

5.5.4 *Heterotrophic populations*

Haliscomenobacter-related organisms were the most abundant heterotrophs in the sidestream granules. *H. hydrossis*, an obligate aerobe, is the only isolated species in the *Haliscomenobacter* genus, and is a bacterium that has been found in bulking activated sludge, due to its growth in

straight filamentous chains of rod-shaped cells (Mulder and Deinema, 2006). However, the *Haliscomenobacter*-associated representative OTU from NIT granules shared only 89% identity with the sole sequenced *H. hydrossis* isolate and thus it cannot be concluded that *H. hydrossis* was the *Haliscomenobacter*-associated organism.

The composition of granule heterotrophs changed in mainstream treatment, which can be expected due to different growth environments. Sidestream granules were grown with aerobic feeding of a low COD/N centrate stream, whereas the mainstream growth environment involved anoxic feeding of a higher COD/N complex synthetic wastewater feed with readily-biodegradable organic substrates including acetate, glucose, lactose and ethanol as well as polymeric polysaccharide and proteinaceous substrates including dextrin, yeast extract, peptone, starch, casein and gelatin. Near-complete removal of NO_x-N prior to the midpoint of the unaerated period also allowed for the possibility of anaerobic conditions for 100 minutes prior to aeration. Anoxic/anaerobic conditions may have been detrimental to *Haliscomenobacter*-associated organisms if they were obligate aerobes as in the case of *H. hydrossis*, leading to their decline in abundance in mainstream granules. *Cytophaga*, *Dechloromonas*, *Gemmobacter*, and *Flavobacterium*-associated organisms were at high abundance in mainstream granules, which is agreement with their metabolic capabilities and mainstream growth conditions and feed composition. *Flavobacterium* can denitrify and degrade glucose, casein, gelatin, and starch (Bernardet and Bowman, 2006). *Cytophaga* are known to degrade cellulose (Nakagawa, 2011), and *Gemmobacter* can degrade sugars, amino acids and ethanol and also denitrify (Hirsch and Schlesner, 2011). *Dechloromonas*-related organisms can denitrify, utilize acetate in addition to other substrates (Achenbach et al., 2001), and have been shown to express both GAO (Ahn et al.,

2007) and PAO (Kong et al., 2007) phenotypes. It is more likely that *Dechloromonas* was expressing a GAO phenotype than a PAO phenotype because EBPR was not apparent.

While the abundance of *Dechloromonas* and *Flavobacterium*-related organisms were similar in mainstream granules and flocs, high abundance of *Mangroviflexus*, *Acinetobacter*, and *Persicitalea* was unique to flocs. These organisms may have been present in the flocculent sludge seed and not well-incorporated into granules, possibly due to factors related to their ability to flocculate or adhere to surfaces. *Mangroviflexus* has a strictly fermentative metabolism (Zhao et al., 2012) and, therefore, was likely mediating organic acid production during the anaerobic time after NO_x-N was removed in the unaerated feeding period. Organic acids, if produced by *Mangroviflexus*, may have been taken up by *Dechloromonas* if expressing the GAO phenotype.

5.6 CONCLUSIONS

- Sidestream nitrifying granules can be grown on anaerobic digester dewatering centrate with a high nitrogen loading rate and granule wasting to support bioaugmentation.
- Addition of sidestream nitrifying granules grown on centrate sustained nitrification and enabled nitrogen removal in a bioaugmented mainstream reactor with selective granule retention and a flocculent sludge SRT below the theoretical minimum nitrification SRT.
- Granules were responsible for nitrification in mainstream treatment. Loss of nitrifiers from granules to flocs and waste activated sludge was minimal.
- Bioaugmentation with nitrifying granular sludge can intensify the nitrification treatment capacity and nitrogen removal capability in flocculent activated sludge systems.

- Different growth conditions in mainstream treatment led to increased relative abundance of heterotrophs in granules and changes in the granule heterotrophic microbial community composition. Nitrification capacity and biougmentation effectiveness was not adversely impacted by granule heterototrophic growth in mainstream treatment.

Chapter 6. BIOAUGMENTATION OF SIDESTREAM NITRIFYING-DENITRIFYING PHOSPHORUS-ACCUMULATING GRANULES IN A LOW-SRT ACTIVATED SLUDGE SYSTEM AT LOW TEMPERATURE

Chapter 6 is presented as a manuscript that was submitted in September 2017 to the Water Research Journal for consideration for publication.

6.1 ABSTRACT

Sidestream granular activated sludge grown on anaerobic digester dewatering centrate was bioaugmented and selectively retained to increase the nitrification capacity of an activated sludge system at 12°C and flocculent sludge aerobic SRT of 2.5 days. Sidestream-grown granules performed enhanced biological phosphorus removal (EBPR) and short-cut nitrogen removal via nitrite. After bioaugmentation EBPR continued in the mainstream, but ammonia oxidation was eventually to nitrate. Low effluent NH_3-N concentrations from 0.6 to 1.7 mg/L were achieved with nitrification solely by granules, thus enabling denitrification and nitrogen removal. Molecular microbial analyses of flocs and granules also suggested that nitrifying organisms persisted on granules with minimal nitrifier loss to flocs. Mainstream granule mass at the end of bioaugmentation testing was 1.7 times the amount of sidestream granules added, indicating mainstream granular growth. Nitrite and nitrate availability during the unaerated feeding period encouraged significant growth of ordinary heterotrophs in mainstream granules, but nevertheless mainstream nitrification capacity was sustained.

6.2 INTRODUCTION

Increased nitrification capacity in water resource recovery facilities (WRRFs) with activated sludge processes may be needed to treat higher influent loads, meet lower effluent ammonia-nitrogen (NH_3-N) concentration limits, and/or for conversion to nitrogen removal. Bioaugmentation with nitrifying biomass produced in sidestream treatment of anaerobic digestion dewatering reject water increases the nitrifier concentration in flocculent activated sludge and intensifies the nitrification capacity without increasing the reactor volume (Kos, 1998). The reject water, referred to as centrate when centrifugation is used for dewatering, is normally returned to mainstream treatment and in the range of 700 to 1800 mg/L, which can be 15 to 30% of the total nitrogen load to the mainstream secondary treatment process (Tchobanoglous et al., 2014). Thus, sidestream centrate treatment processes can be used to reduce the return NH_3-N load and also add nitrifying bacteria to the mainstream process for nitrification bioaugmentation.

Nitrifier bioaugmentation with flocculent sludge from sidestream treatment has been widely demonstrated in full-scale WRRFs using various process configurations (Bowden et al., 2015; Parker and Wanner, 2007). At a full-scale facility with aerobic and total solids retention times (SRTs) near 5 and 7 days, respectively, bioaugmentation improved average effluent NH_3-N concentration from 15 to 7 mg/L at temperatures of 10 to 16°C (Krhutková et al., 2006). The improvement in mainstream nitrification performance by sidestream bioaugmentation was due to the increase in the mainstream nitrifying bacteria concentration, but the performance was still limited by the overall SRT of the mainstream flocculent sludge. For systems with low or non-nitrifying SRTs and/or low temperatures, the increase in nitrification capacity from sidestream

bioaugmentation may not be sufficient to reduce effluent NH_3-N concentrations to meet discharge requirements.

A more optimal solution to increase the nitrification capacity from sidestream bioaugmentation would be to uncouple the SRT of the bioaugmented population from the floc SRT, which could be accomplished by growing sidestream nitrifiers in a biofilm that can be selectively retained in the mainstream system. Granular activated sludge is a good candidate for such bioaugmentation because granular biofilms are grown without a carrier material, allowing them to be more easily pumped from sidestream to mainstream treatment and within the mainstream process. Aerobic granules are a type of biofilm structure in which bacteria are grown in dense, fast settling biomass typically 1 to 2 mm in diameter (Figdore et al., 2017). The SRT of granular sludge bioaugmented can be decoupled from the lower SRT of the flocculent sludge by selective retention due to their larger size and faster settling velocity. Granule-floc separation using hydrocyclones (Shi et al., 2016) and screens (Liu et al., 2014a) has been demonstrated. Longer retention and accumulation of granular biomass is expected to result in a higher volumetric nitrification capacity without building additional tank volume to a mainstream activated sludge system.

Growth of aerobic granules performing nitrification-denitrification (NDN) with phosphorus accumulating organisms (PAOs) mediating enhanced biological phosphorus removal (EBPR) and denitrification has been demonstrated in lab sequencing batch reactors (SBRs) treating synthetic wastewater (Bassin et al., 2012a) and in full-scale SBRs at WRRFs treating domestic wastewater (Pronk et al., 2015a). A typical operating sequence for these reactors is anaerobic

feeding, aeration with simultaneous nitrification-denitrification (SND), and a short settling time of less than 10 min. Neither the growth of NDN-PAO granules in sidestream treatment of anaerobic digestion centrate from WRRFs nor the ability to use them for nitrification bioaugmentation in a low-SRT flocculent activated sludge system has been reported. The ability to sustain NDN-PAO granule nitrification activity in a flocculent activated sludge system and for the granules to grow under potential adverse impacts of centrate solids and residual dewatering polymer is of interest for developing this novel bioaugmentation scheme.

The objective of this study was to evaluate the bioaugmentation process with NDN-PAO granules from sidestream centrate treatment to a low-temperature mainstream flocculent activated sludge system with selective granule retention and a flocculent sludge SRT below the theoretical minimum nitrification SRT. Important process information included in this evaluation are changes in granule physical characteristics and nitrification capacity between the sidestream and mainstream reactors and microbial community composition of the sidestream granules, mainstream granules, and mainstream flocculent sludge. The mainstream system was an SBR operated at 12°C with conditions to support EBPR and SND by the bioaugmented NDN-PAO granules.

6.3 Material and Methods

6.3.1 *Sidestream reactor*

A 2.0-L sidestream NDN-PAO granular sludge sequencing batch reactor (SBR) with a height-to-diameter (H/D) ratio of 4.9 was operated at 18°C with 50% decant/fill volume exchange per cycle. The high volume exchange ratio was possible due to the low sludge volume index (SVI)

and rapid thickening of granules. At the target volumetric NH_3-N loading of about 0.50 g NH_3-N/L-d, the amount of centrate feed needed per cycle was only about 10% of the reactor volume. Thus, the other portion of the fill volume was provided by dilution water to represent dilution of mesophilic digestion centrate with secondary effluent in full-scale application to reduce the sidestream reactor temperature. Wett et al. (2011) have suggested that a lower temperature difference between sidestream and mainstream reactors may improve bioaugmentation efficacy. Centrate and external COD streams were simultaneously added to the reactor in equal volumes using deionized water. External COD sources were sodium acetate and sodium propionate with acetate and propionate contributing 70% and 30%, respectively, to total external COD. Additional details on influent composition are in the Supplemental Information.

6-hr cycles consisted of the following steps: 3 min anaerobic feeding, 42 min anaerobic reaction, 270 min aeration, 1.17 min settling, 2 min pumped effluent discharge, 11.83 min idle, and a 30 min post-anoxic period. The settling time resulted in removing particles with less than 10.1 m/hr settling velocity in the discharged supernatant. The post-anoxic period, with the remaining 1.0-L reactor liquid volume, was used to minimize the NO_x-N concentration prior to the subsequent feed period. Magnetic stirring at 200 rpm occurred during feeding, aeration, and the post-anoxic period. Intermittent N_2 sparging at 600 cm^3/min for 3 seconds at 20-second intervals was applied during anaerobic and post-anoxic periods for additional mixing. A dissolved oxygen (DO) controller (Eutech alphaDO2000W) and galvanic probe (Atlas Scientific) were used to control DO between 1.8 and 2.2 mg/L by on-off aeration at 1500 cm^3/min, corresponding to a superficial gas velocity of 0.5 cm/s. All gasses were sparged from an aeration stone at the reactor bottom.

After preliminary granule formation on synthetic wastewater simulating centrate treatment (see Supplemental Information for media composition), the influent feed and nitrogen source was changed to mesophilic anaerobic digestion centrate from three of Seattle, King County municipal WRRFs processing waste primary and secondary sludge. Secondary treatment processes at these facilities are described in the Supplemental Information.

Raw centrate was settled for 10 minutes, and the supernatant was screened with a 53-um sieve. The purpose of pretreatment was: 1) to represent effluent from centrate equalization tanks at full-scale WRRFs, and 2) to ensure that granule growth in centrate treatment was from microbial self-aggregation and not biofilm growth on large particles in the raw centrate. Pretreated centrate was stored at 4°C and replaced biweekly during the 140-d centrate treatment period. Pretreated centrate characteristics are shown in Table 6.1.

The centrate reactor total nitrogen (TN) loading rate was gradually increased to a practical target value between 0.50 and 0.60 g TN/L-d based on estimated oxygen mass transfer limitations with fine bubble aeration in full-scale reactors. External COD concentration was manually adjusted in feed stream batches based on reactor performance to provide at least 80% TN removal while minimizing the external COD dose.

Table 6.1. Pretreated centrate characteristics (average of 11 batches from 3 different plants)

Parameter	Units	Average ±Std. Dev.	Minimum	Maximum
NH_3-N	mg/L	1038 ±127	864	1317
TN	mg/L	1263 ±258	952	1920
NH_3-N/TN	---	0.83 ±0.06	0.69	0.91
PO_4-P	mg/L	177 ±114	91	422
TSS	mg/L	181 ±80	93	355
VSS	mg/L	152 ±64	83	290
VSS/TSS	---	0.85 ±0.05	0.78	0.92
COD	mg/L	648 ±193	404	1140
sCOD	mg/L	596 ±225	350	1125
Alkalinity	mg/L as $CaCO_3$	4284 ±599	3250	5500
Alkalinity/NH_3-N	mol as HCO_3/ mol N	1.15 ±0.05	1.05	1.25

6.3.2 *Mainstream reactor*

The 2.0-L mainstream SBR with H/D ratio of 1.8 was operated at 12°C and a 2.5-d flocculent sludge aerobic SRT to suppress nitrification. The total SRT was 4.0 d, including the preaeration anoxic/anaerobic time. The SBR cycle time was 4 hr consisting of: 10 min deoxygenation with N_2 gas sparging, 10 min anaerobic feeding, 40 min anaerobic reaction, 150 min aerobic reaction, 24 min settling, 5 min effluent discharge, and 1 min idle. 1.0 L of complex synthetic wastewater (Table C1, Supplemental Information) was fed per cycle containing total COD, readily-biodegradable COD (rbCOD), NH_3-N, TN, and PO_4-P concentrations of 210, 78, 26.5, 32, and 32 mg/L, respectively. The influent COD and TN concentrations were set to represent municipal wastewater. Though the feeding and mixed reactor period was intended to be anaerobic, an initial anoxic period occurred due to residual NO_x-N after decanting because of limited SND during the

aeration period. Mixing during deoxygenation, feeding and reaction periods was achieved by continuous sparging of N_2 at 700 cm^3/min and CO_2 at <5 cm^3/min. DO concentration during the aerobic period was controlled between 1.8 and 2.2 mg/L by on-off aeration at 1000 cm^3/min using equipment specified in Section 6.3.1. Gasses were sparged from an aeration stone at the bottom perimeter wall.

Pre-Bioaugmentation Period

Prior to sidestream NDN-PAO granule bioaugmentation, the mainstream reactor was operated for 3 days with only flocculent activated sludge to verify lack of nitrification. The initial activated sludge was from an earlier experiment and were thus acclimated to the influent synthetic wastewater under anoxic feeding.

Bioaugmentation Period

The bioaugmentation period consisted of two phases: a 15-d phase with an initial slug addition of granules followed by a 25-d phase with continuous daily addition of granules beginning on day 15. Flocs were defined as particles smaller than 425 um, and their SRT was controlled by wasting solids passing a 425-um sieve. The granular solids retained on the sieve were returned to the reactor.

The initial slug seeding of sidestream granules was added with an estimated NH_3-N oxidation capacity sufficient to nitrify the daily amount of NH_3-N fed to the mainstream reactor after accounting for assimilatory NH_3-N removal. The estimate assumed the same specific NH_3-N oxidation rate measured in the granular sludge reactor treating centrate with excess NH_3-N and

similar DO concentration and correcting for the lower mainstream temperature using a temperature-activity coefficient of 1.072 (Tchobanoglous et al., 2014).

After the slug bioaugmentation phase, a small dose of granules was added daily during the continuous bioaugmentation phase. Granule addition was based on the observed sidestream granule yield for treating 25% of the mainstream nitrogen load as centrate nitrogen, which is a typical sidestream nitrogen return load to full-scale WRRFs. The Supplemental Information provides more details on granule addition in this phase.

Post-Bioaugmentation Period

At the end of the bioaugmentation period, granules were removed by sieving the entire reactor contents. Operation continued with flocculent activated sludge to evaluate the contribution of the flocculent sludge fraction to nitrification and nitrogen removal in the bioaugmented process. MLSS was then wasted in a conventional manner for SRT control without sieving.

6.3.3 *Ammonia oxidation kinetics*

Granular sludge maximum specific NH_3-N oxidation activity was measured at 20-22°C with excess NH_3-N and alkalinity and DO concentration greater than 7 mg/L. Activities were normalized to 20°C as above. The apparent NH_3-N half saturation concentration for sidestream granules was measured in a similar test until NH_3-N removal was complete. Data were fitted to a fourth-degree polynomial regression with $R^2 > 0.999$. The apparent half saturation concentration was calculated from the first derivative of the regression equation. Additional details for these tests are in the Supplemental Information.

6.3.4 *Analytical methods*

NH_3-N and NO_3-N were measured by ion-sensing electrode probes (Hach IntelliCAL ISENH4181 and ISENO3181). TN was determined by persulfate digestion and quantification of digestate NO_3-N as above. NO_2-N and PO_4-P were measured photometrically using standard test cuvettes (Hach Methods 10019 and 8048). Alkalinity, pH, TSS, VSS, SVI, COD, and sCOD (0.45 um-filtered) were determined by Standard Methods (APHA 2012). Outside of control systems, DO concentration was measured with an optical probe (YSI ProODO). For reactor cycle profiles and ammonium oxidation kinetic tests, inorganic nitrogen species and PO_4-P were measured in triplicate on a Gallery^{TM} Automated Photometric Analyzer (Thermo Fisher).

Granule morphology was evaluated by stereomicroscopy and image analysis of a minimum sample of 250 granules. Individual granule sizes were quantified as the equivalent circular diameter of the measured plan view area.

6.3.5 *Molecular microbial analyses*

Isolation and quantification of genomic DNA

Sludge samples were stored at -80°C prior to DNA isolation. DNA was isolated in triplicate for each sample type using the UltraClean Microbial DNA Isolation Kit (MO-BIO, Inc.) with 10 min of thermal pretreatment at 65°C and bead beating at 4 m/s for 20 s. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher).

High-throughput sequencing of 16S rRNA gene amplicons

DNA samples were normalized to equal concentrations using nuclease-free water and submitted to a service facility (www.mrdnalab.com) for sequencing of 16S rRNA gene amplicons using the

MiSeq system (Illumina, Inc.). 16S rRNA gene primers 341F and 805R were used in a single-step PCR due to their low bias compared to other primer pairs (Hugerth et al. 2014). Additional details are provided in the Supplemental Information.

Bioinformatics

The UPARSE method (Edgar, 2013) was used for amplicon sequence processing and operational taxonomic unit (OTU) clustering with USEARCH (Edgar, 2010) version 7.0.1090. Sequences were truncated at 400 bases, and those less than 220 bases or with greater than 0.5 expected errors were filtered. Sequences were clustered at 97%, and UCHIME was used to identify and filter chimeric sequences based on the RDP Classifier (v9) "gold" training database. Taxonomy for representative sequences was assigned using USEARCH based on the Silva 119 reference database (Quast et al., 2013). Taxa were summarized by percent abundance of the total reads.

Representative sequences for OTUs at >2% abundance in any sample and that did not receive a specific genus-level taxonomy assignment with the approach above were queried in GenBank with BLAST (Zhang et al., 2000). Hits resulting in a single genus-level identification at >95% coverage, >95% identity and $<10^{-150}$ E-value were taken as the genus-level taxonomy assignment. Otherwise, higher taxonomic ranks were assigned. For one OTU, taxonomy was assigned using RDP Classifier (Wang et al., 2007) at the family-level with 95% confidence threshold and cross-verified in GenBank using the hit-generating sequence's accession number.

Representative sequences were aligned to the Greengenes core reference alignment (DeSantis et al., 2006) with PyNAST (Caporaso et al., 2010b) and rarefied based on the lowest number of

sequences observed in a single sample. Beta diversity was determined by Bray-Curtis (Bray and Curtis, 1957) and weighted UniFrac (Lozupone and Knight, 2005) distances between samples. Beta diversity distance is a measure of dissimilarity between samples and ranges from 0 to 1, with 0 corresponding to identical samples and 1 corresponding to samples with no common species. Both metrics account for abundance of OTUs within samples. The weighted Unifrac metric accounts for phylogenetic relatedness of OTUs, while Bray-Curtis does not.

Quantitative PCR (qPCR)

Gene copies of ammonia-oxidizing bacteria (AOB) and *Candidatus* Nitrotoga (hereafter, *Nitrotoga*) nitrite-oxidizing bacteria (NOB) in sidestream granules and mainstream granules at the end of bioaugmentation were measured by qPCR using *amoA*-1F/*amoA*-2R (Rotthauwe et al., 1997) and NTG200F/NTG840R (Alawi et al., 2007) primers, respectively. AOB in mainstream flocs were also measured at different points of bioaugmentation. Results were normalized to template DNA mass. Additional details and PCR conditions are in the Supplemental Information.

6.4 RESULTS

6.4.1 *Sidestream centrate treatment*

Growth of NDN-PAO granules was achieved in the centrate treatment reactor with consistent production of excess granules to support bioaugmentation. The average net influent concentrations from the centrate/dilution water feed are shown in Table 6.2 along with average treatment performance for operating days 60 to 140. Daily performance data for this period are shown in Figure C1 (Supplemental Information). The 40-d bioaugmentation period corresponded to sidestream centrate treatment days 86 to 126.

Table 6.2. Sidestream granular sludge reactor influent concentrations and treatment performance at maximum centrate loading during days 60 to 140. Bioaugmentation occurred during days 86 to 126.

Parameter	Units	Average ±Std. Dev.
Influent		
NH_3-N	mg/L	227 ±14
TN	mg/L	272 ±11
PO_4-P	mg/L	55 ±16
TSS	mg/L	35 ±13
External COD	mg/L	782 ±29
NH_3-N loading rate	g/L-d	0.45 ±0.03
TN loading rate	g/L-d	0.54 ±0.02
Centrate dilution factor	-	4.8 ±0.7
Effluent		
NH_3-N	mg/L	11.2 ±14.5
NO_2-N	mg/L	9.5 ±5.5
NO_3-N	mg/L	1.8 ±05
TN	mg/L	31.3 ±16.0
PO_4-P	mg/L	12.8 ±17.6
TSS	mg/L	173 ±51
VSS/TSS	-	0.66 ±0.03
pH	-	8.0 ±0.1
Alkalinity	mg/L as $CaCO_3$	535±94
NH_3-N removal	%	95 ±6
TN removal	%	88 ±6
SRT, MLSS, SVI, and waste solids		
SRT total, 7d average	d	16 ±2
MLSS	mg/L	11,000 ±1,400
MLVSS/MLSS	-	0.70 ±0.05
5-min SVI	mL/g	22 ±4
Effluent TSS	mg/d	701 ±156
Waste MLSS	mg/d	916 ±244
MLSS mass >425 um	%	94.8 ±0.5

Effluent TN removal averaged 88% at a loading rate of 0.54 g TN/L-d. Nitrogen removal occurred primarily via nitritation-denitritation in the aerobic period as indicated by the high effluent NO_2-N concentration, average feed COD:TN removed ratio, and lack of granule nitrite oxidation activity in batch tests (see Supplemental Information for methods). The average feed COD:TN removed ratio was 3.3, including values as low as 3.0 during optimal performance periods. In comparison, the COD/N requirement for denitrification of NO_3-N is approximately 5.0 (Tchobanoglous et al., 2014) due to its higher oxidation state. Granule nitrite oxidation activity was nearly zero in batch tests. Anaerobic PO_4-P release averaged 0.58 mol P/mol C indicating that granules were enriched with PAOs (Oehmen et al., 2005). Cycle profiles (Figures C2 and C3, Supplemental Information) showed high degrees of SND and EBPR.

The average diameter of sidestream granules (Figure 6.1a) was 1.3 mm (±0.6 mm standard deviation) according to the granular definition in this study of solids retained on a 425-um sieve. Manual wasting of MLSS accounted for 57% of the average daily solids production. About 95% of the reactor MLSS mass and 15% of effluent TSS could be retained on a 425-um sieve. The maximum specific NH_3-N oxidation activity of sidestream granules was 10.0 mg N/g VSS-hr, and their apparent NH_3-N half saturation concentration was 2.3 mg/L (Figure C4, Supplemental Information).

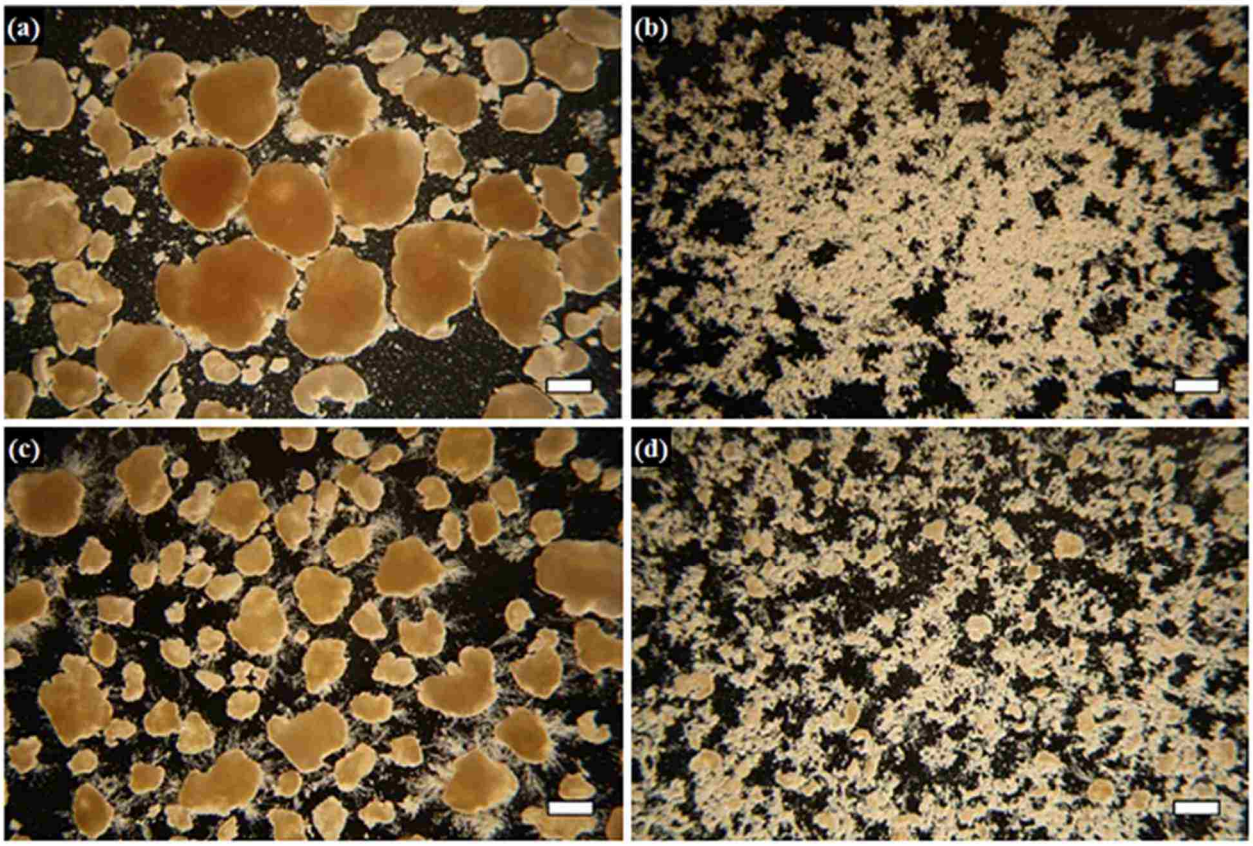

Figure 6.1. Photomicrographs of (a) sidestream granules, (b) mainstream flocs at the start of bioaugmentation, in addition to (c) mainstream granules and (d) mainstream flocs at the end of bioaugmentation (6x magnification and 1mm scale bars).

6.4.2 *Mainstream treatment*

Mainstream effluent inorganic nitrogen species concentrations were measured prior to bioaugmentation, during bioaugmentation, and after granule removal (Figure 6.2). NH_3-N removal beyond assimilation for cell synthesis was minimal before bioaugmentation, and nitrification was immediately intensified after the initial charge of granules. Effluent pH and alkalinity during bioaugmentation were at non-inhibitory levels for nitrification and averaged 7.6 and 330 mg/L as $CaCO_3$, respectively. Effluent NH_3-N concentrations between 0.6 and 1.7 mg/L were sustained during the continuous bioaugmentation period with lower values in the last 15

days. The rate of NH_3-N removal decreased at the end of the aeration period as NH_3-N concentration approached 2 mg/L, which was consistent with the apparent half-saturation concentration of sidestream granules (Section 6.4.1). Effluent NH_3-N rapidly increased to pre-bioaugmentation concentrations when the reactor granules were removed (Figure 6.2).

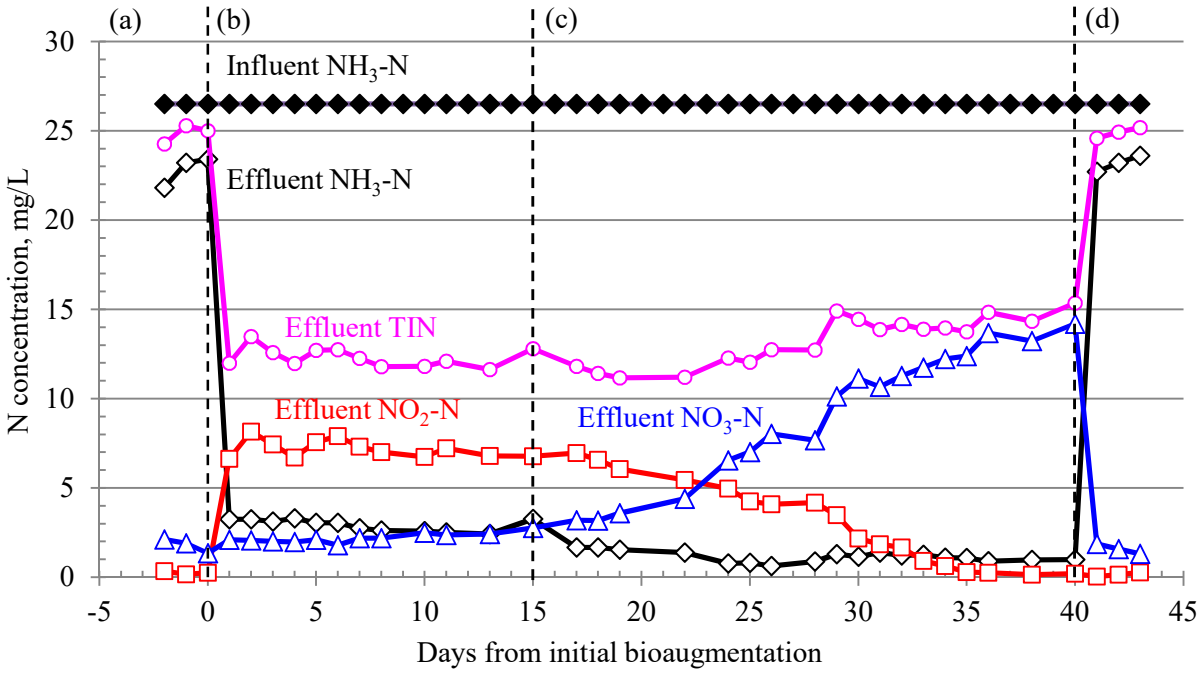

Figure 6.2. Mainstream reactor influent NH_3-N and effluent NH_3-N, NO_2-N, NO_3-N, and TIN concentrations during pre-bioaugmentation (a), slug granule bioaugmentation (b), continuous granule bioaugmentation (c) and post-bioaugmentation after granule removal (d). Influent included 5.5 mg/L of organic nitrogen.

The dominant NH_3-N oxidation product shifted from NO_2-N to NO_3-N over time. The effluent NO_x-N concentration increased from 8.7 to 14.2 mg/L during bioaugmentation, and most of the removal occurred during the feed/react period. A cycle profile of inorganic nitrogen species on the final day of bioaugmentation (Figure C5, Supplemental Information) showed rapid and

nearly complete denitrification by the end of influent feeding. The slightly lower effluent TIN concentration during the first 20 days of bioaugmentation (Figure 6.2) suggests that some SND occurred until the NO_2-N to NO_3-N conversion efficiency increased.

The bioaugmented granules continued EBPR in mainstream treatment; the average PO_4-P concentrations (± standard deviations) were 43.1 ±2.6 mg/L at the end of the anaerobic react time and decreased to 27.5 ±1.1 mg/L in the aeration period. Accounting for estimated denitrification COD demand, the observed PO_4-P release was 0.19 mol P/mol C as detailed in the Supplemental Information. This indicates that some EBPR activity was sustained but it was much lower than that of sidestream granules. PO_4-P removal averaged 4.7 mg/L, but the extent of anaerobic P release and P removal decreased over the bioaugmentation period (Figure C6, Supplemental Information). Toward the end of the bioaugmentation period, the 14 mg/L of NO_3-N remaining from the previous cycle would require 70 mg/L of influent COD for denitrification at a COD/N ratio of 5, which is about equal to the influent feed rbCOD.

A mixture of flocs and granules were maintained in the mainstream reactor (Figures 6.1b-d and Figure C7, Supplemental Information), and the sieving protocol for selective wasting of flocculent sludge during bioaugmentation provided good separation of flocs and granules (Figure C8, Supplemental Information). Mainstream reactor settled sludge bed was stratified with granules and flocs located at the bottom and top, respectively (Figure C9, Supplemental Information). Flocculent sludge MLSS and effluent TSS concentrations during bioaugmentation averaged 900 and 8 mg/L, respectively (Figure C10, Supplemental Information). The granular sludge MLSS concentration increased from an initial concentration of 3400 mg/L to 5800 mg/L

at the end of the bioaugmentation period. The average mainstream granule size decreased from 1.3 mm to 0.8 mm (±0.2 mm standard deviation) at the end of the bioaugmentation test. The particle size distribution was also different from the sidestream reactor granules (Figure C11, Supplemental Information). Granule-like particles of less than 425 um were observed at the end of bioaugmentation (Figure 6.1d), which were not present at the start of bioaugmentation (Figure 6.1b).

The total granule mass added to the mainstream was 6.8 g TSS, with 5.9 g added in the initial slug seeding and 0.9 g added during the continuous bioaugmentation phase. The mainstream reactor granule mass at the end of bioaugmentation was 11.6 g TSS indicating granule growth in the mainstream system.

6.4.3 *Molecular analysis of microbial populations*

A decreasing trend in normalized amoA gene copy concentration was observed for the sidestream granules, mainstream granules and mainstream flocs. Normalized amoA gene copy concentration in sidestream granules was of $2.3x10^4$ $\pm0.3x10^4$ copies/ng template DNA. Values were successively about one order of magnitude lower in mainstream granules ($3.1x10^3$ $\pm0.3x10^3$ copies/ng) and flocs at the end of bioaugmentation. Flocculent sludge amoA copies increased marginally during bioaugmentation from $2.5x10^2$ $\pm0.2x10^2$ copies/ng to $4.1x10^2$ $\pm0.2x10^2$ copies/ng.

Normalized *Nitrotoga* gene copy concentrations in sidestream and mainstream granules had an opposite trend than those of AOB. *Nitrotoga* gene copy concentration in sidestream granules was only $6.9x10^2$ $\pm1.1x10^2$ copies/ng and hence 1.5 orders of magnitude less than AOB. *Nitrotoga*

increased to 6.9 x10^3 ±0.5x10^3 copies/ng in mainstream granules at the end of bioaugmentation, which was slightly greater than AOB.

Taxonomy assignments for OTUs present at greater than 2% relative abundance in any sample are summarized in Figure 6.3 for all sludge types. Taxa are presented in order of descending abundance in sidestream granules. The dominant nitrifying AOB and NOB were associated with *Nitrosomonas* and *Nitrotoga*, respectively. In sidestream granules, average abundance of *Nitrotoga* was 0.5% and much lower than that of *Nitrosomonas* (3.2%). The ratio of *Nitrotoga* to *Nitrosomonas* abundance was low in sidestream granules (0.16) and higher in mainstream granules (2.0) and flocs (6.5), which agrees with mainstream reactor performance where nitrification was proceeded primarily to NO_2-N in the sidestream reactor (Section 6.4.1) and to NO_3-N in the mainstream reactor by the end of bioaugmentation testing (Figure 6.2). qPCR measurements and the sequencing-based relative abundances of AOB and NOB showed similar trends between sidestream granules and mainstream granules at the end of bioaugmentation.

Figure 6.3 shows that distinct heterotrophic populations were associated with each sample. Sidestream granules were dominated by *Haliscomenobacter*, *Desulfobulbaceae*, *Ca.* Accumulibacter (hereafter, *Accumulibacter*), *Cytophaga*, and *Ca.* Competibacter (hereafter, *Competibacter*) at similar abundances between 11% and 15%. *Flavobacterium* was the dominant genera in bioaugmented mainstream sludge and comprised 20% and 32% of mainstream granules and flocs, respectively. *Flavobacterium* was also present at 10% abundance in mainstream flocs before bioaugmentation and 4% abundance in sidestream granules. The abundances of all leading sidestream granule taxa were lower in mainstream granules and even lower in final

mainstream flocs with the exception of *Accumulibacter*, which had similar abundance near 4% in both mainstream granules and flocs. Other taxa including *Dechloromonas*, *Sphaerotilus*, *Halaingium*, and *Cryseobacterium* were present at relatively similar abundances between about 2% and 5% in mainstream granules and flocs. The most significant changes in taxa abundance for flocs before and after bioaugmentation were a decrease in *Mangroviflexus* from 13.2% to 0.1% and an increase in *Flavobacterium* from 10% to 32% as noted above.

Sidestream Granules	Mainstream Granules	Initial Flocs	Final Flocs	Taxonomy Assignment
Nitrifiers				
3.2 ±0.3	0.8 ±0.1	0.2 ±0.0	0.2 ±0.0	Nitrosomonas
0.5 ±0.0	1.6 ±0.1	0.8 ±0.0	1.3 ±0.1	Ca. Nitrotoga
Heterotrophs				
15.0 ±1.6	6.4 ±0.3	0.4 ±0.0	1.2 ±0.1	Haliscomenobacter
14.4 ±1.3	1.7 ±0.2	0.3 ±0.0	0.2 ±0.1	Desulfobulbaceae sp.
13.4 ±1.2	4.1 ±0.3	1.2 ±0.1	3.6 ±0.1	Ca. Accumulibacter
12.3 ±3.3	10.4 ±1.0.	2.3 ±0.2	2.9 ±0.2	Cytophaga
10.8 ±0.9	2.6 ±0.3	0.1 ±0.0	0.4 ±0.0	Ca. Competibacter
3.9 ±0.1	20.0 ±2.2	9.5 ±0.1	32.0 ±1.2	Flavobacterium
2.7 ±0.1	3.1 ±0.5	0.1 ±0.0	0.4 ±0.0	Arenimonas
2.3 ±0.2	0.7 ±0.1	0.5 ±0.0	0.2 ±0.0	Hydrotalea
2.1 ±0.1	1.9 ±0.2	0.1 ±0.0	1.0 ±0.1	Aquimonas
1.5 ±0.1	0.9 ±0.2	2.2 ±0.1	1.8 ±0.2	Verrucomicrobium
0.9 ±0.1	5.3 ±0.4	10.5 ±0.4	5.6 ±0.2	Dechloromonas
0.9 ±0.1	5.8 ±0.2	0.3 ±0.0	3.1 ±0.2	Sphaerotilus
0.9 ±0.1	1.9 ±0.1	0.9 ±0.1	2.1 ±0.2	Saprospiraceae sp.
0.4 ±0.0	1.9 ±0.0	8.6 ±0.5	10.1 ±0.3	Persicitalea
0.3 ±0.1	1.7 ±0.1	3.6 ±0.2	2.1 ±0.1	Zoogloea
0.3 ±0.0	0.5 ±0.0	2.0 ±0.1	0.2 ±0.0	Pedobacter
0.1 ±0.0	0.1 ±0.0	13.2 ±1.1	0.1 ±0.0	Mangroviflexus
0.1 ±0.0	0.4 ±0.0	4.6 ±0.1	1.0 ±0.1	Acinetobacter
0.1 ±0.0	0.5 ±0.0	2.2 ±0.1	0.6 ±0.0	Alphaproteobacteria sp.
0.1 ±0.0	0.2 ±0.0	2.6 ±0.3	7.8 ±0.5	Cloacibacterium
0.1 ±0.0	3.3 ±0.1	0.5 ±0.0	3.8 ±0.1	Haliangium
0.1 ±0.0	0.9 ±0.1	5.3 ±0.1	3.0 ±0.2	Ca. Saccaribacteria sp.
0.0 ±0.0	2.6 ±0.0	8.8 ±0.3	1.8 ±0.0	Cryseobacterium
13.1 ±0.6	20.6 ±0.9	19.0 ±0.5	13.6 ±0.3	Sum of all others <2%

Figure 6.3. Average percent abundance heatmap of microbial taxa in sidestream granules at the start of bioaugmentation, mainstream granules at the end of bioaugmentation, and mainstream flocs at the start and end of bioaugmentation. All nitrifying taxa detected and heterotrophic taxa with greater than 2% abundance in any granule or floc sample are shown. Taxa are listed in order of decreasing abundance in sidestream granules. Percent abundance is based on total amplicon reads with one standard deviation shown for triplicate analyses.

Beta diversity within and between granule/floc types were compared for sidestream granules and mainstream granules and flocs at the end of bioaugmentation. Average beta diversity distances between granule/floc types are shown in Figure 6.4. Distances for comparisons between granule/floc types were significantly higher than control comparisons within granule/floc types, and weighted UniFrac distances were consistently less than Bray-Curtis distances for all comparisons. Beta diversity distances between sidestream and mainstream granules were greater than distances between mainstream granules and flocs.

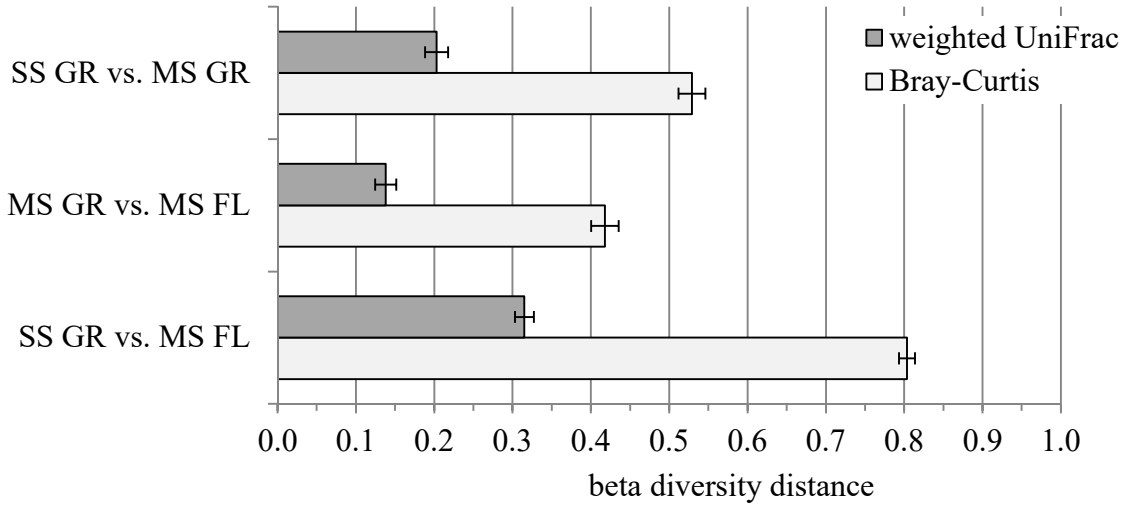

Figure 6.4. Weighted UniFrac and Bray-Curtis distances between sidestream granules (SS GR) and mainstream granules (MS GR) and mainstream flocs (MS FL). Average beta diversity distances between granule/floc types are shown with their standard deviation and based on nine comparison pairs for triplicate samples of each granule/floc type. For control comparisons within samples of the same granule/floc type, average weighted UniFrac and Bray-Curtis distances were less than 0.04 and 0.16, respectively.

6.5 DISCUSSION

6.5.1 *Granule growth in mainstream treatment*

The increase in mainstream granule mass during bioaugmentation indicates that granules added were sustained and that granular growth occurred. Selection pressures claimed for growth of NDN-PAO granules (van Loosdrecht et al., 2005) are 1) a short settling time to favor capture of fast-settling biomass/granules, 2) a high food/mass (F/M) ratio of readily-biodegradable substrate in an anaerobic contact period to drive diffusion into the granular biofilm and intracellular substrate storage, and 3) sufficient shear to form smooth and compact granular biofilms with little outgrowth. Similar selection pressures are used for growth of granules dominated by ordinary heterotrophic organisms (OHOs), but with anoxic or aerobic feeding and higher aeration intensity required to provide sufficient oxygen and shear to form granular biofilms with the faster-growing OHOs.

Considering these granular sludge selective pressures, aspects of mainstream reactor operation included elements favorable for new granule growth. In lieu of a short settling time, selection granular particles was by sieving, which has been previously shown to be effective for granule selection in absence of settling pressure (Liu et al., 2014a). The short 10-min unaerated feed period in this study provided high F/M feeding conditions to encourage substrate uptake by OHOs and storage by PAOs to some extent. Substrate uptake by OHOs would be favored during feeding with residual NO_x-N available from the preceding cycle, while uptake by PAOs or possibly glycogen-accumulating organisms (GAOs) would occur for substrate remaining in the anaerobic time after NO_x-N was removed. The total gas sparging rate with N_2 and air on was slightly higher than in the sidestream reactor (1700 versus 1500 cm^3/min). Thus, mainstream gas

sparging may have provided sufficient shear to promote granule biofilm formation and discourage unwanted outgrowth.

The granules added were sustained in mainstream treatment and capable of being separated from flocs, but the reduction in granule size by about 38% suggests some granule disruption, which was likely due to lower rbCOD concentration in the mainstream feed, longer feed period, competition from OHO in flocs for influent rbCOD, and a decline in PAOs in granules due to the lack of a strictly anaerobic feeding period. Collectively, these factors reduced substrate diffusion to the granule inner core, resulting in starvation and fragmentation. Therefore, granule growth could have occurred on smaller granular particles after fragmentation of larger granules. Granulation from flocs is also a possibility due to the operating conditions discussed above. Differences in the microbial composition between the sidestream and mainstream granules (Figures 6.3 and 6.4) supports the contention of new granule growth with primarily OHOs as affected by the operating conditions in the mainstream reactor.

6.5.2 *Nitrification performance*

Mainstream effluent NH_3-N concentration during the final 20 days of bioaugmentation averaged 1.1 mg/L, which is significantly lower than that achieved in full-scale systems with flocculent sludge bioaugmentation (Krhutková et al., 2006; Parker and Wanner, 2007; Salem et al., 2004) and longer SRTs. A cycle profile on the final day of bioaugmentation (Figure C5, Supplemental Information) showed that nitrification occurred throughout the aerobic period, with nitrification rate slowing as the granule apparent NH_3-N half-saturation concentration was approached at the end of the cycle. Methods to provide lower effluent NH_3-N concentration include aeration at higher DO concentration and using a longer aeration time. It is also possible that with continued

operation and addition of sidestream granules, more nitrifying biomass could accumulate in mainstream treatment allowing lower effluent NH_3-N concentrations.

Although granular mass increased, granule size decreased in mainstream treatment. A smaller granule allows a higher aerobic surface area for nitrifier growth and thus a greater nitrification rate if the granules have the same fraction of nitrifiers for a given bulk liquid DO concentration. For a given bulk liquid DO concentration the smaller granule will also result in a decreased anoxic volume fraction to reduce the denitrification capacity of internal PAOs (Winkler et al., 2015). Increased NH_3-N oxidation capacity with mainstream granule growth was seen after the initial slug bioaugmentation period, with effluent NH_3-N concentrations decreasing from 3.3 to 2.4 mg/L without further granule addition. However, the mainstream nitrification capacity did not appear to increase proportionately to the granule mass increase, otherwise effluent NH_3-N concentrations would have been lower.

Heterotrophs were the primary contributor to mainstream granule growth as indicated by sequencing and qPCR results showing lower AOB abundance in mainstream granules than sidestream granules. The higher mainstream feed COD/N and availability of NO_x-N during unaerated feeding likely allowed growth of heterotrophs on top of nitrifiers in the granule outer aerobic layer, which would reduce the granule specific nitrification rate and total nitrification capacity. However, the effluent NH_3-N concentration decreased and completeness of nitrification increased with time and daily addition of sidestream granules indicating increasing rather than decreasing nitrification capacity. Therefore, outer heterotrophic growth on bioaugmented granules was not high enough to critically impact nitrification bioaugmentation effectiveness.

Another possibility is that sidestream granules or fragments thereof largely maintained their biofilm composition with nitrifiers on the outer layer, and that newly-grown granules were formed primarily from flocs and had a different microbial community composition and spatial distribution with higher heterotrophic abundance, particularly in the outer aerobic layer.

NH_3-N oxidation in sidestream granules was primarily limited to nitritation with little NO_2-N oxidation to NO_3-N, which facilitated short-cut nitrogen removal. Sidestream treatment conditions favoring AOB and hence out-competition of NOB for space on the outer aerobic layer of sidestream granules may have included 1) free ammonia inhibition of NOB due to elevated pH and NH_3-N concentrations from slug feeding of centrate, and 2) high NH_3-N and alkalinity concentrations to maintain maximum AOB growth rates (Tchobanoglous et al., 2014). The fact that NO_2-N was the predominant NO_x-N species in mainstream effluent at the start of bioaugmentation further showed that sidestream granules had little NOB activity. However, nitrification in mainstream treatment shifted towards full nitrification and hence to the accumulation of NO_3-N over time. Reduced free ammonia inhibition and greater aerobic surface area from new granule growth and smaller granules in mainstream treatment likely allowed greater NOB activity and a transition to complete nitrification.

6.5.3 *EBPR and denitrification performance*

The sidestream reactor showed high levels of concurrent EBPR and SND, implicating enrichment of denitrifying PAOs (dPAOs). However, mainstream reactor performance was characterized by modest EBPR and little SND. Several factors may have contributed to reducing COD utilization by dPAOs and thus EBPR and SND in mainstream treatment. NO_x-N from the preceding cycle disrupted anaerobic feeding conditions, which are needed to promote the growth

of PAOs. The influent rbCOD concentration was 78 mg/L and approximately equal to the COD requirement for denitrification of NO_3-N remaining from prior cycles. Rapid denitrification during feeding (Figure C5) suggests OHOs rather than PAOs were consuming influent rbCOD. Additionally, nitrogen sparging for mixing during deoxygenation and feeding may have induced surface oxygen transfer and microaerobic conditions during these periods. Another factor concerns competition between granules and flocs for influent COD, where flocs have an advantage over granules because of higher specific surface area and shorter substrate diffusion distances.

Although SND changed over time due to a decrease in dPAO activity, it has to be emphasized that nitrification capacity was sustained and granules largely remained intact during bioaugmentation suggesting that granules with dPAOs can persist in mainstream treatment and serve as a carrier for nitrifying organisms even if mainstream conditions do not sustain dPAO activity. However, a greater degree of granule-mediated EBPR and SND would be preferred to enhance nutrient removal performance. A post-anoxic period after effluent withdrawal, as used for the centrate granule growth reactor, might have decreased anoxic heterotrophic growth after feeding to allow better substrate uptake by dPAOs and possibly improved SND during the aerobic period.

6.5.4 *Microbial characterization of the different reactor mixed liquors*

The results of sequencing-based bioinformatics analyses are valid to use for approximate microbial distributions in a sample and comparisons between samples, but should be considered semi-quantitative because they are subject to biases from primer selection, PCR efficiency, and

differences in the number of 16S rRNA genes copies in bacteria (Hugerth et al., 2014; Lee et al., 2009).

Two beta diversity metrics were used to assess the similarity of the microbial community composition in sidestream granules and mainstream granules and flocs at the end of bioaugmentation. The weighted UniFrac and Bray-Curtis distances (Figure 6.4) for comparisons showed similar trends but different absolute values with UniFrac distances consistently lower than Bray-Curtis distances, which is likely because the former accounts for phylogenetic distance in the beta diversity calculation whereas the latter only considers binary absence/presence of OTUs. Distances between granule/floc types were significantly higher than control comparisons within granule/floc types, indicating that unique microbial communities existed in each granule/floc type. The higher beta diversity distance for the comparison of sidestream and mainstream granules than that of for mainstream granules and flocs indicates that the microbial community composition of mainstream granules was more similar to that of the flocs than sidestream granules. The dominance of *Flavobacterium* and similar abundances of several other heterotrophic taxa in mainstream granules and flocs (Figure 6.3) were likely drivers for this outcome. Additional aspects of microbial populations in the sludge samples are discussed below.

6.5.4.1 Nitrifying organisms

While AOB were detected in mainstream flocs by molecular microbial analyses, AOB gene copies and relative abundance in flocs were much lower than in granules. Additionally, near-complete loss of nitrification occurred after granule removal in the mainstream reactor. Together, these results suggest that granules were the dominant contributor to nitrification in mainstream

treatment and that loss of granule nitrifiers was minimal and did not present significant adverse impacts to achieving nitrification in the bioaugmented process.

A notable aspect of the microbial composition of all sludge samples involved the NOB present, which were identified as *Nitrotoga* instead of the more familiar *Nitrospira* or *Nitrobacter*. *Nitrotoga* was recently identified (Alawi et al., 2007) and has been detected at significant abundance compared to other NOB in full-scale WRRFs at process temperatures less than 16°C (Lucker et al., 2015). Its substrate affinity coefficient was higher than *Nitrospira* and within the range of *Nitrobacter* (Nowka et al., 2015). Therefore, transient NO_2-N concentrations near 10 mg/L were favorable for maintaining a small *Nitrotoga* population in the sidestream biomass, which allowed for their mainstream growth with bioaugmentation.

6.5.4.2 Sidestream biomass

Accumulibacter PAOs and possibly *Competibacter* GAOs were anticipated to be the leading genera in sidestream granules, but other heterotrophs were present at similar or higher abundances than these PAOs and GAOs. *Haliscomenobacter*-associated organisms were most abundant. *H. hydrossis*, the only species isolated in the genus, has been found to cause poor settling and thickening characteristic in some flocculent activated sludge systems due to its linear filamentous growth structure extending out of flocs (Mulder and Deinema, 2006). *H. hydrossis*-like filaments produce extracellular saccharolytic enzymes, which may support a growth niche on cellular decay products (Kragelund et al., 2008). Thus, *Haliscomenobacter* may have an advantage for growth on complex substrates from slowly biodegradable centrate COD or endogenous decay within granules. If the *Haliscomenobacter*-associated genus possessed

filamentous morphology of *H. hydrossis*, it was neither apparent in microscopic observation nor from granule settling characteristics.

Sulfate-reducing bacteria (SRB) associated with the family *Desulfobulbaceae* were second most abundant in sidestream granules. The presence of 13 mg SO_4-S/L from influent $MgSO_4$ addition could have provided an electron acceptor for *Desulfobulbaceae*, which were likely continuously seeded by centrate solids where the upstream anaerobic digester provided ideal growth conditions. Reduced sulfur compounds were likely present in the centrate. Sulfur cycle-associated EBPR with NO_3-N as electron acceptor has been demonstrated in an anaerobic-anoxic reactor where known PAOs were not detected and SRB comprised 21% of the microbial community based on 16S rRNA gene sequencing (Wu et al. 2014). EBPR activity in the reactor in absence of known PAOs and in the presence of a significant SRB population raised the possibility that SRB may possess the PAO phenotype. Therefore, *Desulfobulbaceae*-related bacteria in sidestream granules may have been mediating denitrifying sulfur cycle-associated EBPR. Absence of sulfur-oxidizing partners such as *Thiobacillus* in sidestream granules further points to this possibility.

6.5.4.3 Mainstream biomass

The dominance of organisms associating with the genus *Flavobacterium* in mainstream flocs and granules suggested that these bacteria were playing a major role in bioconversions in the mainstream reactor. Physiological capabilities for strains of *Flavobacterium* corroborate its suitability for mainstream growth. These capabilities include: a) nitrate reduction, b) degradation of glucose, gelatin, casein and starch, c) ability to grow anaerobically by fermentation of carbohydrates or yeast extract (Bernardet and Bowman, 2006). All of these substrates were

present in the mainstream feed (Table C1, Supplemental Information). Additionally, genome sequencing of *F. johnsoniae* (McBride et al., 2009) revealed genes encoding for glycogen synthase (UniProtKB Accession No. A5FG96), indicating that at least one species of *Flavobacterium* possesses ability to convert influent COD to internal glycogen reserves similar to the GAO metabolism. *Dechloromonas*, which was present between 5 and 6% abundance in mainstream flocs and granules, possesses similar carbon storage capability and may express the PAO (Kong et al., 2007) or GAO (Ahn et al., 2007) phenotypes. Growth of PAOs and GAOs has been shown to enhance granule formation (van Loosdrecht et al., 2005), and mainstream granule formation may have been promoted if the more abundant *Flavobacterium* organisms, in particular, were functioning as GAOs.

6.6 CONCLUSIONS

- Sidestream nitrifying-denitrifying phosphorus-accumulating granules can be grown on anaerobic digester dewatering centrate with a high nitrogen loading rate to promote short-cut nitrogen removal and granule wasting to support bioaugmentation.
- Bioaugmentation with granular sludge (as opposed to flocs) has a much greater impact on nitrification intensification and performance due to the ability to decouple the granular sludge SRT from the lower flocculent sludge SRT.
- Bioaugmentation with granules can achieve low effluent NH_3-N concentrations in a system with low temperature and non-nitrifying flocculent sludge SRT.
- After bioaugmentation, granules remained primarily responsible for mainstream nitrification, and little loss of ammonia-oxidizing bacteria from the bioaugmenting granules in mainstream treatment occurred.

- Residual NO_x-N present during feeding in the mainstream system resulted in a decrease in PAO activity from the sidestream seed sludge and limited the dPAO-driven denitrification capacity.

6.7 SUPPLEMENTAL INFORMATION AVAILABLE

The supplemental information for Chapter 6 is included in this document as Appendix C.

Chapter 7. CONCLUSIONS AND FUTURE OUTLOOK

Aerobic granular sludge presents a new horizon for activated sludge-based treatment of wastewaters. To advance the state of knowledge within the industry and provide guidance for future research, a comprehensive report covering the fundamentals of aerobic granular sludge growth and its use or biological nutrient removal was prepared for publication by the United States' preeminent wastewater research organization. Key concepts, demonstrated treatment applications and research needs were summarized in Chapter 3. One of the research needs identified in the report was the subject of this study and involved a novel intensified treatment process with nitrification bioaugmentation using sidestream aerobic granular sludge. In Chapter 4, three types of nitrifying granules were grown on simulated centrate and evaluated for mainstream bioaugmentation potential based on granule nitrification capacity yield and physical characteristics favorable for separation from flocs and selective retention in mainstream treatment. NIT and NDN-PAO granules showed potential for bioaugmentation, and Chapters 5 and 6, respectively, focused on their use for sidestream centrate treatment and mainstream bioaugmentation. Collectively, the results indicated that nitrification bioaugmentation with sidestream granular sludge could sustain nitrification and thus enable nitrogen removal when combined with biological denitrification in non-nitrifying flocculent activated sludge. Molecular microbiology analyses provided greater insight into relationships between the granule microbial community, granule physical characteristics, and the fate of granule biopopulations with prolonged exposure to mainstream treatment growth conditions. The major findings of this study along with their engineering significance are presented in more detail below.

7.1 MAJOR FINDINGS AND ENGINEERING SIGNIFICANCE

This body of research investigated and demonstrated the viability of a novel wastewater treatment process using different types of nitrifying granular sludge from sidestream centrate treatment to bioaugment nitrification in flocculent activated sludge and enable nitrogen removal and low effluent ammonia concentrations at challenging mainstream activated sludge treatment conditions of low temperature and short flocculent sludge SRT. Effluent NH_3-N concentrations near 1 mg/L were achieved under non-nitrifying flocculent sludge conditions. High sidestream NH_3-N and TN removal efficiencies were achieved with NIT and NDN-PAO granules, respectively, at similar sidestream TN loading rates near 0.6 kg N/m^3-d. These highly-loaded sidestream reactors would consume minimal site space and installed footprint. The estimated footprint for sidestream treatment is only 5% of non-nitrifying flocculent sludge mainstream aeration tanks and much less than the 3- to 4-fold increase that may be required if upgrading to biological nitrogen removal with flocculent activated sludge and no increase in design MLSS concentration. Although process development and demonstration at larger scales is required, sidestream granular sludge bioaugmentation appears to be a promising option to upgrade non-nitrifying activated sludge treatment plants to nitrogen removal or increase the treatment capacity at existing nitrogen removal plants without the need to build additional tanks for the mainstream treatment system. Therefore, sidestream granular sludge bioaugmentation would be an attractive option for many existing WRRFs lacking nitrification and nitrogen removal or seeking to increase existing nitrification and nitrogen removal capacity.

The granular sludge bioaugmentation process offers advantages over established technologies to increase biological nitrogen removal capacity in existing treatment systems. Compared to MBRs,

the bioaugmentation process eliminates the high operating cost for membrane replacement and chemical cleaning as well as energy costs to compensate for headloss across the membranes and membrane air scour. Compared to IFAS, the bioaugmentation process avoids biocarrier capital costs and higher aeration costs associated with operation at higher DO concentrations in the range of 4 to 6 mg/L necessary to achieve nitrification in the attached growth biofilm (Tchobanoglous et al., 2014). Process DO concentrations in laboratory systems were 1.8 to 2.2 and 3.5 to 4.0 mg/L for bioaugmentation with NDN-PAO and NIT granules, respectively.

In both bioaugmentation tests, granule mass at the end of bioaugmentation was greater than that added as sidestream granules, indicating that the granules added were sustained and that new granule growth occurred in mainstream treatment. Additionally, granule microbial community composition changed in mainstream treatment. The mainstream granule microbial community composition at the end of bioaugmentation testing was unique compared to sidestream granules and mainstream flocs but more similar to that of the flocculent sludge than the original sidestream granules. Therefore, bioaugmentation with granular sludge and selective retention of granules may not only be an approach to enhance nitrification and nitrogen removal without increasing mainstream reactor tank volume, but also a means of seeding and promoting granular sludge growth in mainstream treatment.

The ability of the granular sludge to not only persist but also grow in mainstream treatment and retain suitable nitrification activity to provide low mainstream effluent NH_3-N concentrations raises the question whether bioaugmentation will need to be continued once the mainstream system is sufficiently charged with granules. This research did not specifically address this

question because granule bioaugmentation was not ceased in the mainstream system. However, there is no perceived practical detriment to continuing bioaugmentation at a rate allowed by granule wasting from sidestream treatment, since ongoing bioaugmentation would provide greater process safety factor and possibly allow even lower effluent NH_3-N concentrations to be achieved.

The success of these laboratory studies using NIT and NDN-PAO granules suggests that both granule types are potentially viable candidates for use in mainstream bioaugmentation. Sidestream NIT granules treating centrate showed higher granule yield and load-normalized nitrification capacity production compared to NIT granules grown on synthetic wastewater, thus allowing for mainstream bioaugmentation. However, the sidestream NDN-PAO system had higher production of granular sludge mass and nitrification capacity and, therefore, might be favored for implementation at full-scale. The daily maximum NH_3-N oxidation capacity for waste granular sludge is the product of average daily sidestream granule yield and granule maximum specific NH_3-N oxidation activity and was 0.06 and 0.14 g NH_3-N/d for waste NIT and NDN-PAO granules, respectively. Thus, granule nitrification capacity production was approximately 2.3 times greater in the NDN-PAO system with a NH_3-N loading rate similar to the NIT system.

The cost of providing supplemental alkalinity for NIT granules or external carbon for NDN-PAO granules could be an additional driver in deciding which granule type to use. Preliminary evaluations suggest that the cost of using 50% sodium hydroxide for supplemental alkalinity for sidestream NIT granules is comparable to using undiluted acetic acid for supplemental carbon

for NDN-PAO granule growth if nitrification is limited to NO_2-N as observed in this study. The cost of acetic acid for NDN-PAO granules would be approximately 60% higher if complete nitrification occurs in NDN-PAO granules and NO_3-N must be denitrified. Average chemical costs from several municipal procurement bid tabulations across the United States were used in the evaluation. Chemical costs can vary based on local supply chains, and site-specific costs need to be evaluated on a plant-by-plant basis. However, the initial evaluation suggested that supplemental chemical costs for NIT and NDN-PAO granules are roughly cost-competitive with each other. The ability to beneficially use a zero-cost or low-cost high-COD fermentable waste stream for NDN-PAO granule growth would be highly attractive. Industrial wastewaters from food and beverage processing applications could be targeted for this use. Other considerations related to mainstream phosphorus removal goals would also be important to the selection of preferred sidestream granule type.

NDN-PAO granules showed a high degree of NOB repression in the sidestream treatment reactor with NH_3-N oxidation primarily to NO_2-N only. However, NH_3-N oxidation in the bioaugmented mainstream treatment system gradually shifted to NO_3-N over the course of bioaugmentation testing. This finding suggests that a recirculation of granules between mainstream and sidestream treatment conditions may be an approach to obtain long-term NOB repression in mainstream treatment by continually exposing granules to sidestream treatment conditions favoring NOB repression. Limiting NH_3-N oxidation to NO_2-N in mainstream treatment would lower the COD required for nitrogen removal and either allow greater levels of mainstream nitrogen removal at a fixed influent COD concentration or allow a greater portion of influent COD to be diverted to anaerobic digestion to maximize methane production and energy recovery.

The granular sludge bioaugmentation concept described in this work utilizes a sidestream centrate treatment reactor for nitrifying granule generation. However, the solids handling processes used at some plants may not produce a high-strength ammonia-laden stream such as centrate. Examples include incineration, gasification, pyrolysis, and thermal drying. In these plants where sidestream centrate treatment is not an option for nitrifying granule generation, a potential alternative could be to generate nitrifying granular sludge on a slipstream of the main plant flow. This works suggests that a typical centrate nitrogen load near 25% of the total nitrogen load is suitable to provide granular sludge for mainstream nitrification bioaugmentation. By extension, a possible approach for plants without centrate would be to treat about 25% of the main flow in a parallel granular sludge process such as the Nereda® SBR process and bioaugment granular sludge generated from slipstream treatment to main flow treatment.

7.2 FUTURE OUTLOOK

Based on the success of laboratory bioaugmentation studies, a key thrust of future work involves scaling-up of the bioaugmentation process. In particular, the following issues should be addressed: 1) sidestream centrate treatment performance under more variable loadings; 2) the ability of granules to persist under full-scale pumping and mixing forces, with attention to changes in granule mass, size, and nitrification oxidation activity; 3) evaluation of different approaches to separate flocs and granules such as screening, hydrocyclones, or gravimetric separation; and 4) treatment performance of a bioaugmented mainstream fed municipal primary effluent or screened and de-gritted wastewater, particularly in a continuous-flow mainstream system with variable influent wastewater composition.

A consideration relevant to scaling-up the bioaugmentation process not specifically addressed in this research concerns the settling behavior of granules in a granule-floc suspension such as that in the bioaugmented mainstream system. Settling behavior of granules in this suspension may be different than that in a suspension without a significant flocculent sludge fraction. Typical flocculent activated sludge MLSS concentrations are in the range of 1000 to 4000 mg/L, and the presence and concentration of flocculent sludge MLSS could impact the effective settling velocity of granules in the overall granule-floc suspension. Specifically, the flocculent sludge could retard the granule settling velocity otherwise observed in absence of flocs by acting as a physical barrier that must be displaced by the faster-settling granules or by increasing drag forces through temporary attachment to the settling granules. If granule settling velocity is reduced in granule-floc suspensions, higher flocculent sludge MLSS concentrations would be expected to retard granule settling velocity to a greater extent. This potential effect should also be evaluated with different granule size categories. For example, smaller granules might be impacted to a greater degree than larger granules. A better understanding of settling behavior in granule-floc suspensions will inform and enable more effective design and performance predictions for gravimetric separation of flocs and granules.

Aspects of bioaugmentation using sidestream NIT granules require further research to better understand the process and optimize its performance. In the current work, sidestream NIT granule growth and mainstream bioaugmentation reactors were operated at DO concentrations between 3 and 4 mg/L. Lower process DO concentrations at or below 2 mg/L would be preferred to increase oxygen transfer efficiency and lower aeration energy demand. Operation at lower DO concentration is anticipated to especially impact sidestream treatment. On one hand, sidestream

operation at lower DO concentration may promote NOB repression and out-competition of NOB by AOB for space in the granule outer aerobic layer due to the AOB's higher affinity for oxygen (Tchobanoglous et al., 2014), which would reduce total oxygen demand and aeration energy by reducing or eliminating NO_2-N oxidation. On the other hand, lower process DO concentration is anticipated to reduce the NH_3-N oxidation rate and possibly reduce the allowable NH_3-N loading rate to the sidestream reactor or otherwise require a higher granule biofilm surface area (i.e., higher MLSS concentration and SRT) and lower the allowable sidestream granule wasting rate to support bioaugmentation. Pairing sidestream NIT operation at lower DO concentration with a sidestream process model would provide insight into tradeoffs of these competing interests.

Another approach to promote greater NOB repression in sidestream NIT granules in combination with or in absence of lower DO concentration would be operating the sidestream reactor at the same loading rate but with longer cycle times. In this manner, the NH_3-N concentration at the start of the cycle would be higher and promote NOB repression by free ammonia inhibition (Tchobanoglous et al., 2014). A tradeoff of this operation is that the influent centrate would occupy a greater portion of the total influent feed volume and dilution water addition would be reduced, resulting in a higher sidestream process temperature. The anticipated impacts of this operation on overall bioaugmentation efficiency are unclear because the effect of sidestream temperature on bioaugmentation efficiency is not well-understood. However, even if the sidestream NIT reactor cycle time was increased from 4 hours (as in Chapter 5) to 8 hours, the dilution water:centrate ratio would be approximately 2:1, and a significant amount of dilution and cooling would be provided.

Efforts operate the sidestream NIT reactor with NOB repression and lower DO concentrations should be considered with awareness of the unintended consequence of promoting anammox growth and transition to deammonification granules instead of granules performing aerobic ammonia oxidation only. This transition could adversely impact bioaugmentation due to the much lower AOB yield in the deammonification process. Sidestream operation at lower temperatures with dilution water addition and at lower SRTs with granule wasting for bioaugmentation would ward against anammox growth potential but must be weighed against potential impacts to allowable sidestream loading rates.

Aspects of bioaugmentation using sidestream NDN-PAO granules also warrant additional research and were alluded to in the preceding section on engineering implications. Recirculation of granules between sidestream and mainstream treatment systems to promote sustained mainstream NOB repression and lower COD requirements for denitrification in the mainstream treatment system should be evaluated in future work. The use of concentrated high-COD industrial wastewaters as alternative external carbon sources for sidestream NDN-PAO granule growth and denitrification was also suggested. The treatment performance, COD/N requirements, and sidestream granule characteristics with feeding of these elevated COD streams should be investigated. The high-COD waste stream could be used in two manners: 1) with preliminary volatile fatty acid production in a separate fermentation process and feeding of fermentate to the sidestream reactor or 2) with direct addition of the high-COD waste stream to the sidestream NDN-PAO reactor and fermentation and uptake of COD by PAOs during the anaerobic feeding step. Readily-hydrolyzable high-COD waste streams such as many food processing wastewaters could be particularly suitable for direct anaerobic feeding.

Other approaches could be used to promote NOB repression in mainstream treatment in lieu of granule circulation between sidestream and mainstream treatment. One approach would be to operate the mainstream system at lower DO concentrations in continuously-aerated aerobic zones to favor out-competition of NOB by AOB for space in the granule outer aerobic layer as discussed earlier in this section. Based on this research, mainstream DO concentration at a process temperature of 12°C would need to be less than 2.0 mg/L and likely in the range of 0.3 to 1.0 mg/L for possible NOB repression in mainstream granules at DO concentrations that can be reliably controlled in full-scale systems. Aeration control strategies using oxygen reduction potential and real-time effluent NH_3-N concentration could also be used. Another approach would be to apply high-frequency cyclic aerobic-anoxic/anaerobic conditions in mainstream treatment. NOB activity has shown greater lag time compared to AOB activity in response to aerobic conditions after anoxia (Kornaros et al., 2010), and cyclic aerobic-anoxic conditions have been associated with NOB repression in both flocculent (Regmi et al., 2014) and granular sludge (Lochmatter et al., 2014). Operation at lower DO concentrations and/or cyclic aeration would be expected to improve denitrification potential, but also reduce NH_3-N oxidation rates. Based on this research where nitrification occurred throughout the mainstream aerobic period, higher effluent NH_3-N concentrations would be anticipated at lower DO concentrations in absence of an increase in granule aerobic SRT. Therefore, these potential approaches to promote NOB repression in mainstream treatment must be evaluated against reductions in NH_3-N removal and/or increases in bioreactor volume required to achieve effluent NH_3-N limits.

Although the mainstream system with NDN-PAO granule bioaugmentation was envisioned as an anaerobic-aerobic process with a high degree of EBPR and SND, mainstream EBPR activity was moderate, and SND was negligible when nitrification proceeded to NO_3-N and limited when nitrification proceeded to NO_2-N. These processes are interrelated because anaerobic feeding conditions were disturbed by residual NO_x-N from preceding cycles with limited SND. While mainstream wastewater composition and DO concentration may have contributed to this outcome, another key consideration is that flocs and granules had equal access to influent COD in the mixed feeding period. In this case, flocs had easier access to COD due to much lower diffusion limitations. Also, with much lower diffusional distances and biomass density, the potential for internal anoxic zones during aeration is limited in flocs compared to granules, and thus the SND potential of flocs is much lower than that of granules. Therefore, mainstream EBPR and SND performance may be improved by providing granules preferential access over flocs to influent COD, which could be accomplished in a mainstream process with granule-floc separation by returning granules to an influent feed zone and flocs to a different zone downstream of the influent feed zone. This approach is currently being studied by the University of Washington Department of Civil and Environmental Engineering in a pilot-scale system with sidestream granule addition.

Development of a comprehensive mechanistic model for the total bioaugmentation process that includes and links sidestream and mainstream treatment systems would provide greater insight into performance capabilities and sensitivity to critical parameters including SRT, DO concentration, and granule capture efficiency. However, a comprehensive model with

appropriate mechanistic accuracy presents a high degree of complexity and computational intensity. Considerations in this regard are discussed below.

As covered in greater detail in Appendix A, granular sludge process models to date are based on user-specified values for a fixed number of granules and a maximum granule radius. In these models, all solids production occurs as solids detachment from granules when the outward radial growth dimension exceeds the maximum user-specified value. Thus, established granular sludge models do not have a waste granular sludge stream, per se, as would be needed for integration into a comprehensive bioaugmentation process model. One way to model the bioaugmentation process would be to segregate the sidestream and mainstream models. The sidestream model could be used to simulate sidestream treatment performance and arrive at a quasi-steady-state sidestream granule biopopulation spatial distribution. The user would need to adjust the number of granules in the sidestream model based on the rate of granule wasting for bioaugmentation. A separate mainstream process model could include a granule addition stream based on the predicted sidestream granule biopopulation spatial distribution. A dynamic simulation is necessary for the sidestream granular sludge process due to the changing phases of SBR operation, and these dynamic simulations may require approximately one year of simulated operation to reach steady-state conditions (de Kreuk et al., 2007), which can result in significant computational times on the order of days for typical personal computers.

The separate mainstream process model would need to account for the presence of granules and flocs. Hubaux et al. (2015) developed a model structure with granules and flocs to simulate a sidestream deammonification process with anammox granules and retention of flocculent solids

resulting from detachment from granules as described above. Though a similar structure could be used to model the mainstream system, the bioaugmentation process introduces additional complexity not accounted for in the model of Hubaux et al. One is that addition of fresh sidestream granules must be included. Preferential exposure of granules over flocs to influent wastewater, as discussed earlier, would be another new aspect to the model. Biofilm models such as those used to date for granular sludge utilize static biocarrier elements that are fixed within reactor zones. New modeling approaches would need to be developed to simulate the segregation of flocs and granules and their conveyance to different mainstream reactor zones. A dynamic process model would also be required to account for different biopopulation compositions and associated treatment performance for fresh sidestream granules recently added to the mainstream treatment system and older granules retained in mainstream treatment. A steady-state model would ultimately arrive at a uniform mainstream granule microbial spatial distribution, which may cause inaccurate simulation of mainstream treatment performance.

In light of the complexities and anticipated computational demands of bioaugmentation process model structures envisioned and discussed above, empirical approaches may provide more accessible and practical design criteria. For example, nitrification curves have been developed for MBBRs as a function of BOD loading and DO concentration (Hem et al., 1994; McQuarrie and Boltz, 2011). Similar curves could be developed for mainstream systems with long-term granular sludge bioaugmentation. These design curves would comprehensively provide guidance on the granule biofilm area, MLSS concentration, and aerobic contact time required to meet NH_3-N removal goals. With respect to nitrogen removal, one design approach would be to utilize empirical curves for granular nitrification bioaugmentation coupled with conventional flocculent

activated sludge denitrification design (Tchobanoglous et al., 2014) and the assumption of negligible SND by mainstream granules. This would provide the highest level of conservatism for denitrification design. Another approach would be to develop empirical design curves for SND or overall nitrogen removal as a function of BOD loading and DO concentration, particularly in anaerobic-aerobic granule-bioaugmented processes. This would only be relevant if a greater degree of SND is observed in future studies of granular sludge, unlike this research where SND was limited in laboratory mainstream reactors.

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APPENDIX A

Supplemental Information
for Chapter 3:

Aerobic Granular Sludge for Biological Nutrient Removal

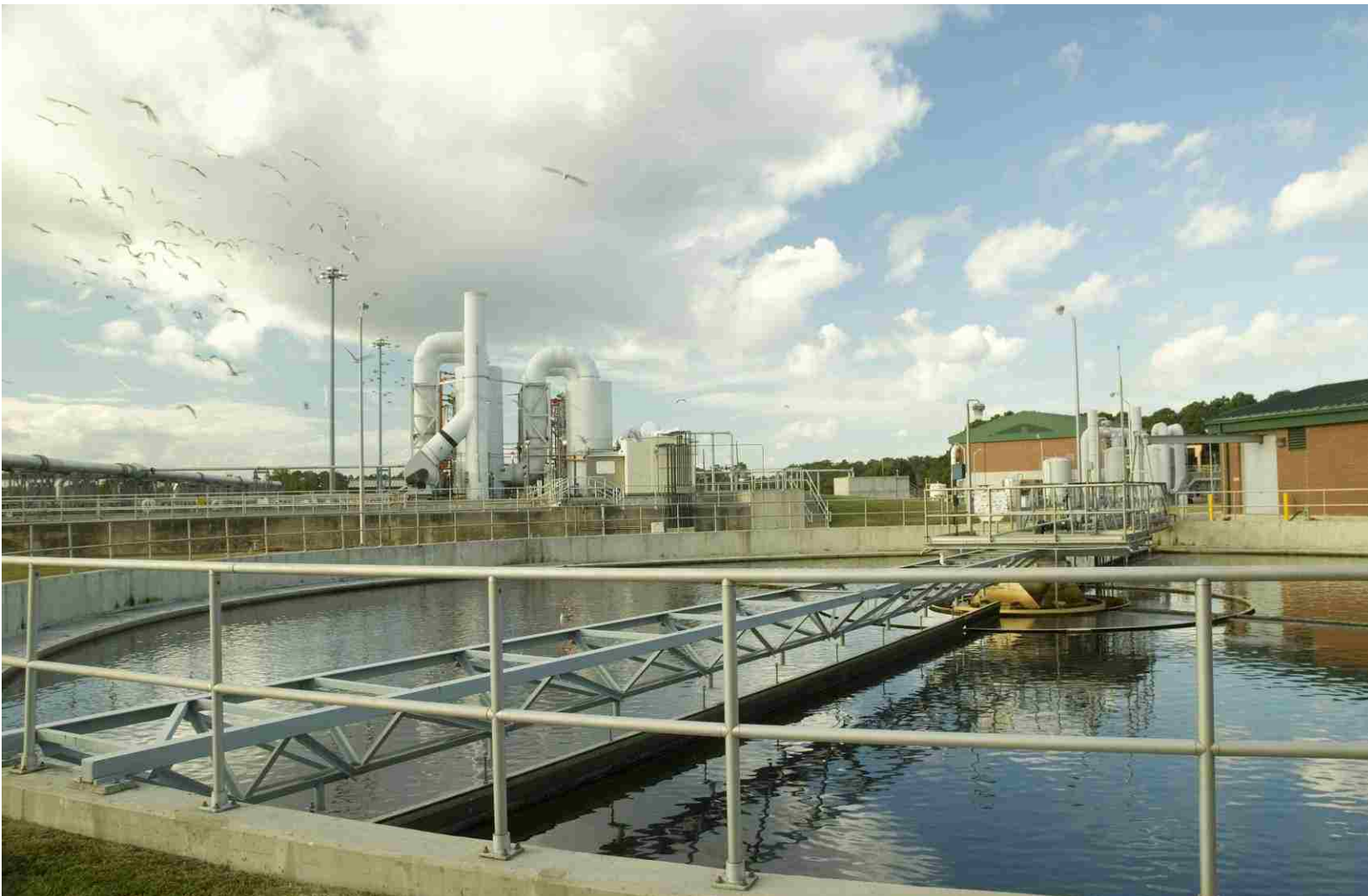
The publication associated with Water Environment and Resuse Foundation Project No. NUTR5R14h is presented in its published format as Appendix A. The 180-page document contains 10 chapters. A table of contents can be found in the introductory section following the cover page.

WATER ENVIRONMENT & REUSE FOUNDATION

Final **Report**

Aerobic Granular Sludge for Biological Nutrient Removal

White Paper



Aerobic Granular Sludge for Biological Nutrient Removal

WHITE PAPER

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WATER ENVIRONMENT & REUSE FOUNDATION
WE&RF

The Water Environment & Reuse Foundation (WE&RF) is a nonprofit (501c3) organization officially formed in July 2016 as the result of the merger of Water Environment Research Foundation and the WateReuse Research Foundation. The merged research foundation, with a combined research portfolio representing over $200 million, conducts research to treat and recover beneficial materials from wastewater, stormwater, and seawater including water, nutrients, energy, and biosolids.

The Foundation also plays an important role in the translation and dissemination of applied research, technology demonstration, and education, through creation of research-based educational tools and technology exchange opportunities. WE&RF materials can be used to inform policymakers and the public on the science, economic value, and environmental benefits of wastewater and recovering its resources, as well as the feasibility of new technologies.

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About WE&RF

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WE&RF operates with funding from subscribers, donors, state agencies, and the federal government. Our supporters include wastewater treatment facilities, stormwater utilities, state and federal government agencies, technology vendors and equipment companies, engineers, and environmental consultants. WE&RF takes a progressive approach to research, stressing collaboration among teams of researchers, environmental professionals, scientists, and staff. All research is peer reviewed by leading experts.

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Abstract and Benefits

Abstract:

Aerobic granular sludge is generally described as approximately spherical self-formed biofilms of 0.50 to 2.0 mm diameter with settling velocities at least 10 times that of flocculent activated sludge. Granular sludge systems can be operated with much higher mixed liquor suspended solids (MLSS) concentrations than flocculent activated sludge systems and can provide biological nutrient removal (BNR) in simpler process configurations. This document provides a comprehensive review of fundamental aspects of selector mechanisms for granular versus flocculent sludge growth, types of aerobic granular sludge and microbial compositions, and experience with aerobic granular sludge BNR processes performing simultaneous nitrification, denitrification, and enhanced biological phosphorus removal (EBPR).

Bench-scale and full-scale experiences of various aerobic granular sludge process configurations, operating conditions, and BNR performance are summarized. High nitrogen and phosphorus removal efficiencies have been shown for full-scale aerobic granular sludge facilities treating municipal wastewaters. Full-scale plant performance has demonstrated the ability to meet a summer effluent permit limit at the Epe Sewage Treatment Plant located in the Netherlands for total nitrogen (TN) and total phosphorus (TP) of less than 5.0 mg/L and 0.3 mg/L, respectively. The winter permit limits of less than 12.0 mg/L and 0.5 mg/L for TN and TP, respectively, were also met. Other facilities showed a similar range of performance for nutrient removal. These facilities had favorable influent wastewater characteristics for biological nitrogen removal with BOD/total Kjeldahl nitrogen (TKN) ratios of 4.0 to 4.5 and BOD/TP ratios of 30 to 36. The effluent permit requirements for these plants were not as low as the more common stringent effluent nutrient concentration limits set in the United States of 3.0 mg/L TN and 0.1 mg/L TP. Even lower TN and TP effluent concentration limits have been set for some water resources recovery facilities (WRRFs) in the United States. The ability to meet these lower TN and TP concentrations is yet to be tested and demonstrated for aerobic granular activated sludge systems.

The current full-scale aerobic granular sludge BNR facilities in operation are upflow fed, sequencing batch reactor (SBR) processes. Energy savings of approximately 25% are expected for these aerobic granular sludge SBRs compared to continuous flow flocculent activated sludge systems designed for similar effluent concentrations. Other granular sludge process configurations are being considered, including methods to advance granular sludge growth in existing continuous-flow flocculent activated sludge systems and bioaugmentation processes.

Granular sludge BNR systems offer similar resource recovery opportunities of energy, nutrients, water, and biosolids as that of flocculent activated sludge BNR systems. In addition, granular sludge offers the potential to recover alginate-like biomaterial for use in commercial and industrial applications.

This document also presents a number of research needs for this relatively recent activated sludge process including impacts of wastewater characteristics, aeration design issues, other process configurations and applications, micropollutant removal, solids handling, and resource recovery.

Benefits:

- Summarizes physicochemical characteristics and microbial diversity of aerobic granules.
- Describes fundamental selective pressures for aerobic granular sludge growth and the influence of common operation parameters on aerobic granular sludge growth.
- Identifies different types of aerobic granules and provides an overview of prior laboratory research with regard to nutrient removal performance.
- Describes the only full-scale aerobic granular sludge process for nutrient removal and performance.
- Describes the resource recovery potential for aerobic granular sludge systems.
- Summarizes process models that have been used for aerobic granular sludge systems.

Keywords: Aerobic granular sludge, biological nutrient removal, enhanced biological phosphorus removal, nitrogen, phosphorus, nitrification and denitrification.

Contents

Tables

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Acronyms and Abbreviations

A2O	Anaerobic-anoxic-oxic
ALE	Alginate-like exopolysaccharides
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
AOR	Actual oxygen requirement
ASM	Activated sludge model
ATU	Allythiourea
bCOD	Biodegradable COD
BF-anaerobic	Anaerobic bottom feeding through settled sludge bed
BNR	Biological nutrient removal
BOD	Biochemical oxygen demand
BODr	BOD removed
COD	Chemical oxygen demand
CT	Computed tomography
DO	Dissolved oxygen
EBPR	Enhanced biological phosphorus removal
EDC	Endocrine disrupting compound
EGSB	Expanded granular sludge bed reactor
EPS	Extracellular polymeric substance(s)
FA	Free ammonia
FAS	Flocculent activated sludge
FISH	Fluorescent in-situ hybridization
F/M	Food to microorganism ratio
FNA	Free nitrous acid
G	α-L-guluronate, a monomeric unit of alginate
GAOs	Glycogen accumulating organisms
H/D	Height-to-diameter ratio of biological reactor
Hac	Acetate
HRT	Hydraulic retention time
IFT	Influent feed tank
IWA	International Water Association

MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MTBE	Methyl tert-butyl ether
N	Nitrogen
NDN	Nitrification-denitrification and/or nitritation-denitritation
NH_3-N	Ammonia-nitrogen, which is used to represent the total ammonical nitrogen
NIT	Nitrification-only or ammonia and/or nitrite oxidation-only
NMR	Nuclear magnetic resonance
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
NO_x-N	Sum of NO_2-N and NO_3-N
NOB	Nitrite oxidizing bacteria
NTU	Nephelometric turbidity units
OHOs	Ordinary heterotrophic organisms
OLR	Organic loading rate
P	Phosphorus
pbCOD	Particulate biodegradable COD
PAOs	Phosphorus accumulating organisms
PF	Pulse-fed aerobic SBR
PHA	Polyhydroxyalkanaote
PHB	Poly-beta-hydroxybutyrate
PHV	Poly-beta-hydroxyvalerate
PH2MV	Poly-beta-hydroxy-2methylvalerate
PLC	Programmable logic controller
PO_4-P	Phosphate phosphorus
PPCP	Pharmaceuticals and personal care products
qPCR	Quantitative polymerase chain reaction
RAS	Return activated sludge
rbCOD	Readily biodegradable COD
SBBGR	Hybrid attached-growth/granular sludge reactor
sbCOD	Soluble biodegradable COD
SBR	Sequencing batch reactor

SCADA	Supervisory control and data acquisition system
scfm/sf	Standard cubic feet per minute per square foot
SCOD	Soluble COD
SON	Soluble organic nitrogen
SOR	Standard oxygen requirement
SOTE	Standard oxygen transfer efficiency
SOUR	Specific oxygen utilization rate
SRT	Solids retention time
STOWA	Foundation for Applied Water Research (Netherlands)
SVI	Sludge volume index
SVI_5	Sludge volume index at 5 minutes of settling
SVI_{30}	Sludge volume index at 30 minutes of settling
TDH	Total dynamic head
TIN	Total inorganic nitrogen
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket reactor
U.S.	United States
UV	Ultraviolet
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WE&RF	Water Environment and Reuse Foundation
WGAS	Waste granular activated sludge
WRRF	Water resource recovery facility
WWTP	Wastewater treatment plant

Executive Summary

The purpose of this document is to provide the reader with a comprehensive review of aerobic granular sludge for biological nutrient removal (BNR). The following topics were addressed in detail: 1) physicochemical characteristics of aerobic granular sludge; 2) selective pressures for aerobic granular sludge growth; 3) the effect of different operating conditions on granular sludge growth; 4) microbial diversity in aerobic granular sludge; 5) nutrient removal in bench-scale systems; 6) removal of micropollutants and industrial chemicals in bench-scale systems; 7) full-scale aerobic granular sludge process applications and nutrient removal performance; 8) modeling of aerobic granular sludge processes; 9) resource recovery opportunities; and 10) research needs. This section summarizes the key information pertaining to these aspects of BNR and resource recovery with aerobic granular sludge.

Physicochemical Characteristics of Aerobic Granules

Aerobic granular activated sludge can be described as an attached growth or biofilm process where carrier media is not required and rapidly settling microbial granules are formed via the application of key selective pressures. Aerobic granules are typically 0.5 to 3 mm in diameter. The higher settling velocity of granular activated sludge compared to flocculent activated sludge is attributed to its larger particle size and more compact spherical morphology resulting in a settling regime that more closely resembles discrete settling rather than hindered settling (Section 2.3). This is made apparent by low sludge volume index (SVI) values between 30 and 50 mL/g, where the ratio of the settled sludge volume at five minutes to that at 30 minutes (SVI_5/SVI_{30}) is approaching 1.0. In comparison, well-settling flocculent activated sludge would be expected to have an SVI_{30} near 100 mL/g and SVI_5/SVI_{30} ratio from 1.6 to 2.0. A range of granule sizes and densities has been shown for full-scale granular sludge systems, with stratification of granules of different size and density in a settled sludge bed based on settling velocity.

A channelized protein-polysaccharide composite matrix distinguishes granular sludge from flocculent sludge, and polysaccharides appear to be the main structural element of this matrix that allows aerobic granules to share characteristics of hydrogels including viscoelasticity and capacity for swelling and deswelling by absorption and desorption of water (Section 2.7). In aerobic granular sludge BNR systems treating domestic and synthetic wastewaters, the key structural extracellular polymeric substance (EPS) resembles alginate and contains charged moieties. However, other systems may contain a different structural EPS backbone. Consistent with the alginate theory of bioflocculation, the presence of divalent cations, particularly Ca^{2+}, can facilitate granulation. EPS extracts have some features similar to commercial alginate and consequently the term “alginate-like exopolysaccharides” has been adopted to most accurately refer to this component of aerobic granular sludge. Harvesting this alginate-like biomaterial for industrial applications is a resource recovery option unique to aerobic granular sludge.

Selective Pressures for Aerobic Granular Activated Sludge

Several key selective pressures and fundamental principles for aerobic granular sludge selection and growth have been identified (Section 3.2). The liquid-solids separation is designed to allow retention of mainly the larger and faster-settling granules over smaller and slower-settling flocs. When significant biodegradable chemical oxygen demand (COD) is present in the influent wastewater stream, the COD feeding regime should expose granular biomass to a high COD concentration. A feeding regime of this nature promotes substrate penetration into the granule core and helps to avoid diffusion-limited growth conditions, which are not favorable for granular sludge growth. Under diffusion-limited growth conditions, granular sludge is not stable and filamentous or finger-like outgrowths occur. This

detrimental growth condition can result in reactor failure by washout of the poor-settling filamentous granules. Growth must also not be limited by diffusion of other co-substrates such as O_2 or NO_x-N. Sufficient shear must be provided in proportion to microbial growth rate, with faster-growing organisms requiring higher shear to form a smooth and compact granule biofilm morphology.

Operating Conditions in Granular Sludge Reactors with N and P Removal

Aerobic granules can be grown under a wide variety of operating conditions (Section 3.4). At the bench scale, a variety of granule types, including N and P removing granules, nitrifying-denitrifying granules, and aerobic ammonia- and/or nitrite-oxidizing granules, have been grown under different combinations and degrees of elective pressures depending on operating conditions. Thus far, granules performing nitrogen removal and enhanced biological phosphorus removal (EBPR) have been used in the full-scale application of aerobic granular sludge at practical operating conditions. An essential element of this operation, which was first investigated at the bench-scale, is an anaerobic period where influent wastewater is fed through the settled sludge bed in an upflow regime. This feeding mode facilitates granulation by exposing the biomass to higher substrate concentrations, minimizing diffusion-limited growth conditions, and selecting for slower-growing phosphorus-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in lieu of ordinary heterotrophic organisms (OHOs), which reduces the shear necessary to form smooth and compact granular biofilm structures. Particulate matter in the influent is sorbed to the granule surface (Section 3.4.1), and it is important that a high degree of anaerobic hydrolysis occurs for particulate biodegradable COD (pbCOD) in the influent, allowing for COD assimilation by PAOs and/or GAOs. Incomplete anaerobic hydrolysis of pbCOD will result in the presence of pbCOD at the granule surface during the subsequent aerobic period, leading to aerobic hydrolysis and the slow release of readily biodegradable COD (rbCOD). During aerobic conditions, the presence of residual rbCOD in the bulk liquid or non-degraded pbCOD sorbed to the granule surface promotes filamentous outgrowths from the granule that are detrimental to granule morphology, settling velocity, and stability, and that cause higher effluent total suspended solids (TSS) concentrations (Section 3.6).

Although PAOs and GAOs both have denitrification capability, selection mainly for denitrifying PAO granules is preferred for more efficient use of influent carbon for nutrient removal, because influent bCOD removed by PAOs can be used for both nitrogen and phosphorus removal, whereas GAOs do not provide EBPR. A strategy for selecting PAOs over GAOs in granular sludge has been developed based on differences in settling velocity between GAO- and PAO-enriched granules. GAO-enriched granules have a slower settling velocity than PAO-enriched granules of equivalent size due to the lower GAO bacterial density. Consequently, GAO-enriched granules tend to be located at the top of the settled sludge bed while PAO-enriched granules tend to be located at that bottom of the settled sludge bed. Wasting the slower-settling top portion of the sludge bed has been demonstrated to improve EBPR performance by selectively removing GAO-enriched granules and retaining PAO-enriched granules (Section 3.4.8).

Microbial Diversity and Spatial Distribution in Aerobic Granular Activated Sludge for N and P Removal

Operation conditions in aerobic granular sludge systems with anaerobic feeding followed by aeration for simultaneous nitrification-denitrification and EBPR results in the selection and spatial distribution of key microbial groups. PAOs and GAOs are the dominant members of the granule microbial community and together have accounted for as much as 85% of the granule microbial population (Section 4.1). Both *Accumulibacter* and *Tetrasphaera* PAOs have been detected in these granules, with the former being more abundant than the latter, while *Competibacter* has been the only GAO detected. The participation of both PAOs and GAOs in the reduction of NO_3-N to N_2 has been observed. Results of independent studies indicate GAOs are primarily responsible for the reduction of NO_3-N to NO_2-N while PAOs are primarily responsible for the reduction of NO_2-N to N_2. With respect to ammonia oxidizing bacteria

(AOB) and nitrite oxidizing bacteria (NOB), granules treating domestic wastewaters have been shown to contain both *Nitrosomonas* and *Nitrosospira* as well as *Nitrobacter* and *Nitrospira* (Section 4.2). The concomitant presence of these genera has been attributed to the availability of suitable niches within the granular biofilm and dynamic process cycle. The ratio of NOB to AOB in granular sludge has been found to be higher than otherwise expected based on the conventional stoichiometry of the lithotrophic oxidation of nitrite. Phenomena that may contribute to this observation include the mixotrophic growth of *Nitrobacter* and the existence of a nitrite oxidation–nitrate reduction loop between NOB and heterotrophic denitrifiers that reduce NO_3-N to only NO_2-N. OHOs are also present and may contribute to nitrogen removal in PAO/GAO-dominated anaerobic-aerobic granular sludge (Section 4.3). These key microbial groups are distributed spatially in the granule (Section 4.5). PAOs and GAOs are located in both the oxygen-penetrated outer layer and inner anoxic region, where their previously stored internal carbon, obtained during anaerobic feeding, is oxidized with oxygen or NO_x-N as the electron acceptors according to the redox conditions in the granule. Because their metabolisms require aerobic conditions, AOB and NOB must inherently be located on the oxygen-penetrated outer layer of the granule. OHOs are present in the oxygen-penetrated outer granule layer where they can grow on endogenous decay products and residual influent bCOD that may remain following anaerobic feeding. Similarly, OHOs in the inner anoxic region can use endogenous decay products for NO_x-N reduction. Depending on bulk liquid dissolved oxygen (DO), NO_x-N concentration, and the rate of mass transport and biological reactions inside the granule, an innermost anaerobic region may permanently or transiently exist.

Full-Scale Aerobic Granular Sludge Process Applications

Fundamental research on aerobic granular sludge growth has led to the development of the innovative full-scale Nereda® sequencing batch reactor process for biological nitrogen and phosphorus removal (Chapter 7). In this process, influent wastewater is fed slowly through an anaerobic settled sludge bed in an upflow regime, and simultaneously, treated effluent overflows at the top of the reactor. After the feeding period, an aeration period is used for simultaneous nitrification-denitrification and EBPR. After NH_3-N is reduced to target levels, DO concentration may be lowered from approximately 2.0 mg/L to 0.5 mg/L. Anoxic recycle periods may also be introduced to increase N removal. Though the majority of P removal is achieved biologically under normal flows and loads, metal salts may be added to supplement EBPR during wet weather in order to reduce the required cycle time to meet effluent limits (Section 7.1). Mixed liquor suspended solids (MLSS) in Nereda® reactors are dominated by granular sludge, but the slow upflow feeding regime allows some flocs to remain present in the system. Particles less than 212 µm may represent approximately 10 to 15% of the MLSS mass (Section 7.2.2). These particles do not appear detrimental to the process and may contribute to TSS removal by sludge blanket filtration during the period of simultaneous and effluent overflow. Cultivation of mature granular sludge from flocculent seed sludge may take approximately one year, but this duration can be affected by temperature and wastewater characteristics and can be reduced if a significant amount of granular seed sludge is available.

Nereda® facilities have demonstrated high N and P removal efficiencies and the ability to meet different levels of effluent nutrient removal requirements as applicable to the operating facility (Section 7.2.4). Effluent discharge limits at the Garmerwolde and Vroomshoop facilities (Netherlands) are similar to what would be considered BNR-level requirements in the United States. Effluent concentrations for sustained operational periods at these facilities were as follows: 6.9 and 7.2 mg/L total nitrogen (TN), 1.1 and 2.2 mg/L NH_3-N, approximately 0.9 mg/L total phosphorus (TP) at both facilities, 0.4 and 0.6 mg/L PO_4-P, and 20 and 10 mg/L TSS, respectively. These effluent concentrations were achieved without effluent filtration and with wet-weather addition of metal salts for chemical phosphorus removal. Effluent NH_3-N limits at these facilities allowed for a modest NH_3-N residual to remain present.

The Epe Sewage Treatment Plant (Netherlands) is operated to meet more stringent effluent limits. During a three-month performance verification period at a process temperature of 14 to 16°C, the average effluent at this facility was <4.0 mg/L TN, 0.1 mg/L NH_3-N, 0.3 mg/L TP, 0.1 mg/L PO_4-P, and <5.0 mg/L TSS. These effluent concentrations were achieved with effluent filtration and wet-weather metal salts addition. Influent wastewater characteristics were favorable for nutrient removal at these facilities, with influent five-day biochemical oxygen demand (BOD)/N ratios between 4.0 and 4.5 and BOD/P ratios between 30 and 36. A pilot facility treating municipal primary effluent where the BOD concentration was less than 100 mg/L demonstrated excellent cold-weather nitrification and EBPR performance. During a two-week period in which temperature was 3.0 to 4.5°C, NH_3-N concentration was less than 0.6 mg/L. The effluent PO_4-P concentration was less than 0.4 mg/L and often near 0.1 mg/L without supplemental volatile fatty acid (VFA) or metal salts addition. However, concentrations of the influent and effluent N species were not reported; therefore, a complete picture of nutrient removal performance is not available for this system treating a low-strength primary effluent.

Compared to conventional flocculent activated sludge processes, attractive aspects of the Nereda® granular activated sludge process include reductions in space and energy requirements (Section 7.4). A lower space requirement is achieved due to the elimination of secondary clarifiers, preferential utilization of deep sequencing batch reactor (SBR) tanks, high MLSS concentrations (8–12 g/L), and short settling times that can be employed with granular sludge and an upflow feeding regime. Energy savings in the Nereda® process can be expected due to the elimination of mechanical equipment such as mixers, internal recycle pumps, and return sludge pumps and efficiencies in oxygen transfer gained through the preferential use of deep tanks. It is also likely that aeration energy savings are achieved via periods of low DO concentration that may be introduced in the reactor cycle after the target NH_3-N concentration has been achieved. Advantages of the Nereda® process must be weighed against limitations or special considerations (Section 7.5) related to: 1) the ease of retrofitting existing facilities, 2) process start-up and granule development, 3) appropriate waste sludge management to ensure the wasting of PAOs and adequate P removal, and 4) instrumentation needs.

Process Models for Aerobic Granular Sludge

Mechanistic models for aerobic granular sludge processes have been developed and applied for different laboratory-scale aerobic granular sludge systems (Chapter 8). Well-established one-dimensional biofilm models, including mass-transport and biological reaction processes using stoichiometric and kinetic parameters similar to those included as examples in the activated sludge model (ASM) series, have been appropriate to simulate reactor nutrient removal performance. Simulation of combined EBPR and nitrogen removal has been limited, and no models have been developed and applied for calibration and validation to the performance of full-scale facilities treating municipal wastewater using either a uniform granule size or including flocs and a distribution of granule sizes in the model formulation.

Resource Recovery with Aerobic Granular Sludge

Resource recovery opportunities with aerobic granular sludge include those that are shared with flocculent activated sludge as well as a unique opportunity to harvest alginate-like biomaterial from waste granular sludge. A self-evident resource recovery opportunity is water reuse. Other resource recovery opportunities utilize the granular activated sludge solids stream (Chapter 9). Methane production from the anaerobic digestion of waste sludge is another resource recovery option. Waste granular activated sludge from the full-scale Nereda® Epe facility exhibited 42% VS destruction in lab-scale mesophilic digestion at a 20-day solids retention time (SRT), which is within the range that would be expected for the anaerobic digestion of waste activated sludge (WAS). Lab-scale thermal pressure

hydrolysis pretreatment at 160°C for 2 hours and 6–8 bar pressure improved the VS destruction from 42 to 48%. Nutrient recovery via land application of biosolids or biochar is an additional resource recovery option, along with the potential for the production of crystalline phosphorus fertilizers. Alginate-like biomaterial has the potential to be a newly realized marketable resource recovery product for WRRFs not otherwise found with conventional flocculent sludge. Initial investigations of the recovery of alginate-like biomaterial from Nereda® excess sludge have been promising, but also showed that extracts contained as much as 40% protein. Specific suitable commercial and industrial outlets for this material have yet to be defined. One possible use is as a thin-film water-resistant barrier in surface coating applications for paper, paper products, and gypsum board.

Research Needs

This review revealed numerous aspects of wastewater treatment and the intensification of the existing infrastructure using aerobic granular activated sludge that have not been addressed (Chapter 10). The following topics are recommended for future research:

- The effect of influent wastewater characteristics on granular sludge growth, particularly the strength and relative fractions of readily biodegradable and particulate biodegradable COD.
- The ability to achieve effluent TN and TP concentrations less than or equal to 3.0 and 0.1 mg/L, respectively, and the operational strategies necessary to achieve these low nutrient concentrations with different wastewater characteristics.
- The effect of variable loads on granular sludge SBR performance, design, and process control.
- The expansion of existing aerobic granular sludge process models to simulate the treatment of domestic wastewaters and pH and other physical-chemical phenomena observed in lab systems such as granule-mediated phosphorus precipitation and ammonium ion exchange. If research shows hybrid systems containing flocs and granules to be viable, the competition of bacteria in flocs and granules should be included in hybrid system models.
- The characterization of oxygen transfer and alpha factors in aerobic granular sludge systems.
- The impact of granular activated sludge on downstream processes including filtration and disinfection.
- The fate of micropollutants in aerobic granular sludge systems.
- New process applications for aerobic granular sludge including granule growth in continuous flow systems, granular sludge bioaugmentation to increase the nutrient removal capacity in existing flocculent activated sludge systems, and the use of granules in separate wet-weather treatment biosorption processes to improve water quality.
- The thickening and dewatering properties of granular sludge before and after digestion.
- Resource recovery, including the impact of granular sludge on hydrolysis and the methane yield in anaerobic digestion, phosphorus recovery from granular WAS, and the processing and commercialization of alginate-like EPS extracts from waste granular activated sludge.

Outlook

The outlook for aerobic granular sludge is positive, and its potential for use in WRRFs with nutrient removal requirements, seeking to maximize the treatment capacity of existing infrastructure or a small footprint and/or to implement resource recovery, is promising. With greater awareness of aerobic granulation mechanisms and broadened understanding of performance capabilities, research needs, and potential applications, the increased use of aerobic granular sludge-based wastewater treatment and resource recovery is anticipated.

CHAPTER 1

Introduction

Granular activated sludge presents a major change from the flocculent activated sludge that has been used for over 100 years since its introduction by Ardern and Lockett (1914) on April 3, 1914, to the Society for Chemical Industry in Manchester, England. Interest in this century in using granular activated sludge for biological nutrient removal (BNR) has been growing since the first full-scale aerobic granular activated sludge system was commissioned in 2011 in Epe, the Netherlands, for biological nitrogen and phosphorus removal. In contrast to "flocs," which are defined as loose and permeable aggregates composed of bacteria colonies enmeshed in extracellular polymeric substances (EPS), granular activated sludge contains bacteria and EPS in a more compact dense structure approaching a more spherical morphology (Hubaux et al., 2015). Because of the differences in morphological and physicochemical properties, granular sludge exhibits much faster discrete settling rates compared to the hindered settling rates of flocculent activated sludge. As a result, granular sludge systems can operate at much higher mixed liquor suspended solids (MLSS) concentrations and require more compact biological process designs. In addition, the phosphorus uptake for enhanced biological phosphorus removal (EBPR) and nitrification/denitrification for nitrogen removal can occur within granules in a single aerobic tank without separate reactor zones and internal recycle streams.

The first application of granular sludge occurred in anaerobic treatment. In 1969, Young and McCarty initially reported the presence of sludge granules in an upflow attached-growth anaerobic filter. During the 1970s, Lettinga and colleagues exploited anaerobic sludge granulation to develop the upflow anaerobic sludge blanket reactor (UASB; Lettinga et al., 1980). Subsequent modifications to the UASB aimed to improve treatment for lower strength wastes and lower process temperatures. These modifications included hydraulic distribution improvements, effluent recirculation, and an increase in the reactor height-to-diameter ratio, thus creating the expanded granular sludge bed reactor (EGSB), which operates at higher upflow velocities (>6 m/hr) and organic loading rates (10-30 kg/m^3/d) than the UASB. Unlike the UASB, flocculent sludge is washed out of the EGSB (Schmidt and Ahring, 1996; Seghezzo et al., 1998). These anaerobic granular sludge processes have been successfully applied to treat high strength industrial and agricultural wastes [>1000 mg chemical oxygen demand (COD)/L] and municipal wastewater in warm climates. As of 2013, there are reportedly about 2000 UASB and 1500 EGSB installations worldwide (Tchobanoglous et al., 2014). A more comprehensive review of anaerobic granular sludge can be found in other sources (Lim and Kim, 2014; Seghezzo et al., 1998).

During the latter half of the 1990s a new anaerobic granular sludge application was developed when it was found that anammox bacteria could grow and be maintained in systems with anammox granular sludge. In 1995 came the first reports of biological anaerobic ammonia oxidation with nitrite (Mulder et al., 1995; van de Graaf et al., 1995) by what are now commonly referred to as *anammox* bacteria involved the growth of anammox bacteria in fixed-film reactors. By 1998, the growth of anammox granules in a sequencing batch reactor (SBR) was achieved (Strous et al., 1998). Between 2004 and 2010, a number of anammox granular sludge processes were implemented at full scale for the treatment of warm, ammonium-rich anaerobic digestion dewatering reject streams (Bowden et al., 2015; van der Star et al., 2007; Wett, 2006). These processes have become common, and current research efforts are focused on applying anammox processes for nitrogen removal in colder, low-strength wastewaters (Hu et al., 2013a; Wett et al., 2013). A greater understanding of anammox organisms and their metabolism and kinetics has occurred, along with an understanding of engineering for full-scale applications, including findings that growth doubling time can be as low as 3.3 days instead of the 14 days originally

thought to be the growth doubling time (Lotti et al., 2014) and that anammox is capable of dissimilatory nitrate reduction by using propionate or acetate as the electron donor (Güven et al., 2005; Kartal et al., 2007a; Kartal et al., 2007b). Selective pressures for the development of anammox granular sludge include (a) maintenance of granules over flocs by short settling times, size separation by screening, or size and density separation by cyclones; (b) a low growth rate [long solids retention time (SRT)] for the anammox bacteria; (c) sufficient concentration of calcium and/or magnesium; and (d) exposure to low concentrations of dissolved oxygen (DO; Lotti et al., 2014; Oshiki et al., 2013; van der Star et al., 2008). The reader is referred to additional sources for more comprehensive information on anammox granules (Hu et al., 2013b; Jetten et al., 2009; Jin et al., 2012; Kuenen, 2008; Lackner et al., 2014; Van Hulle et al., 2010) and to a WE&RF report summarizing anaerobic digestion sidestream anammox treatment processes for nitrogen removal (Bowden et al., 2015).

Though the first report on granular sludge grown in an aerobic environment was by Mishima and Nakamura in 1991, in which they observed 2-8 mm diameter granules in a pilot-scale UASB, the cultivation of aerobic granular sludge in a lab-scale SBR by Morgenroth et al. in 1997 appears to be the launching point for the development of aerobic granular activated sludge processes. Early stages of research on aerobic granular sludge were done by groups at Delft University of Technology, the Netherlands (Beun et al., 1999, 2000a, 2001, 2002a) and Nanyang Technological University, Singapore (Jiang et al., 2002; Liu and Tay, 2002; Liu et al., 2002a; Moy et al., 2002; Tay et al., 2001a, 2001b, 2002a, 2002b). A key step in the development of the aerobic granular sludge SBR process for simultaneous BOD and nitrogen removal, as well as enhanced biological phosphorus removal (EBPR), was the progression from lab studies to a pilot plant demonstration led by de Kreuk, van Loosdrecht, and colleagues (de Kreuk, 2006) at a water resource recovery facility (WRRF) in Ede, the Netherlands in 2005. This was eventually followed by the development of the commercially available Nereda® process (Giesen et al., 2013), with the first full-scale demonstration facility, in Gansbaai, South Africa, commissioned in 2009. The first European installation was in 2011 at a WRRF in Epe, the Netherlands, which is not far from the location of the initial pilot plant demonstration in Ede, the Netherlands. Two major advantages shown in the current full-scale aerobic granular sludge system installations include 1) less energy and 2) less space and tankage due to having a higher biomass concentration and greatly reduced settling time for liquid-solids separation. These and other advantages of aerobic granular sludge specific to the Nereda® process are discussed in Section 7.4.

Although the Nereda® aerobic granular sludge process involves combined biological nitrogen and phosphorus removal with their associated specialized microorganisms present in the granule microbial community, a variety of other aerobic granule types have been cultivated at the bench scale. These include granules performing nitrification-denitrification in the absence of EBPR (Beun et al., 2001; Chen et al., 2013), ammonia oxidation to nitrate (Tay et al., 2002c; Tsuneda et al., 2003), ammonia oxidation to nitrite (Lopez-Palau et al., 2011; Vázquez-Padín et al., 2010b), and nitrite oxidation to nitrate (Ni et al., 2011; Vázquez-Padín et al., 2009). Early aerobic granular sludge studies only reported COD removal (Moy et al., 2002; Tay et al., 2002b), but it is likely that both nitrification and denitrification also occurred in those studies due to the thickness of the activated sludge granules and the effects of a decreasing DO concentration profile from the bulk liquid to the granular sludge interior.

The development and application of granular sludge technology is less mature compared to other aerobic biological processes, and there are many features with regard to granule selection, physical characteristics, and microbial composition that are unique to granular sludge systems. The purpose of this document is to provide information on aerobic granular sludge with regard to the fundamentals of growth conditions and its physical, chemical, and biological characteristics, types of applications employed, current state-of-art process designs for BNR, the potential for resource recovery, and

research needs. Chapter 2 discusses physicochemical characteristics of aerobic granular sludge. Chapter 3 discusses fundamental conditions leading to the selection of aerobic granular sludge and the effect of environmental factors on granule characteristics. Chapter 4 describes the microbial populations observed in aerobic granular sludge. Chapters 5 and 6 review results of bench-scale studies with aerobic granular sludge. Chapter 7 presents an overview of full-scale applications of aerobic granular sludge in a SBR process. Aerobic granular sludge process models are discussed in Chapter 8. Resource recovery opportunities and research needs are discussed in Chapters 9 and 10, respectively. The key findings of this report are summarized in Chapter 11.

CHAPTER 2

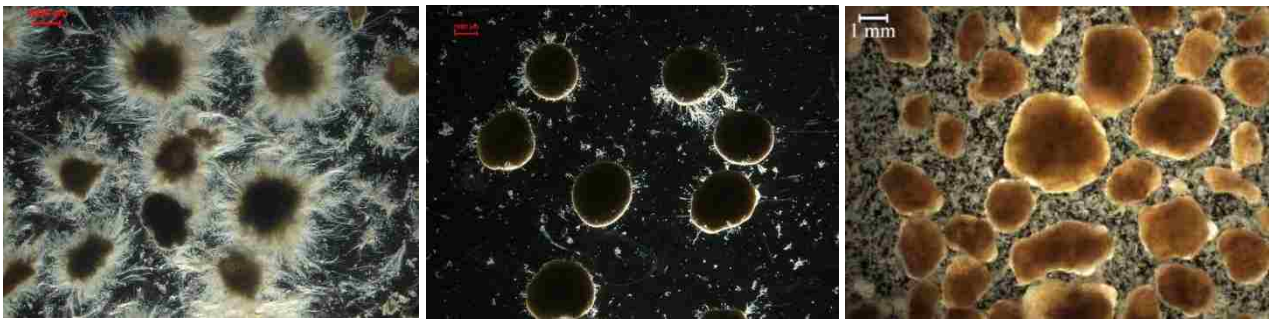
Physicochemical Characteristics of Aerobic Granular Sludge

In this section, distinguishing physicochemical characteristics of aerobic granular sludge are reviewed. These characteristics include size, morphology, structure, settling velocity, density, strength, composition, and the role of EPS, hydrophobicity, starvation and reactivation capability, and sorption capacity. Key methods that have been used to characterize granules are summarized.

2.1 Size and Morphology of Aerobic Granular Sludge

Aerobic granules are roughly spherical with a typical shape factor and aspect ratio of 0.7-0.8 (Beun et al., 2000a, 2002a). These dimensiononless parameters are used to describe the plain view shape of particles, independent of their size. For both parameters, lines have a value of 0 and perfect circles have a value of 1. A range of granule diameters, typically less than 5 mm, can be found in aerobic granular activated sludge systems (Liu, 2007). No consensus was reached on the minimum size for an aerobic granule at the 1st International Water Association (IWA) Aerobic Granular Sludge Workshop (de Kreuk et al., 2005a), but approximately 0.2 mm has been commonly used as a minimum in sieving procedures to determine reactor granular size distribution by weight (de Kreuk, 2006; Li et al., 2009). Laser particle size analysis has been occasionally used to determine granular sludge size distribution (Liu et al., 2005a), but image analysis of a representative sample of granular sludge has been most commonly used to determine granule size and morphology (Beun et al., 2000a) and can be accomplished with basic microscopes and open-source image processing software packages.

A variety of granule colors and morphologies have been observed. Typical colors for aerobic granules are white to yellow-brown, while anammox granules are red-tinted (de Graaff et al., 2011) and anaerobic granular sludge is black (Gonzalez-Gil et al., 2001). The surface of aerobic granules may be smooth, noded with microcolony structures, or may contain filamentous outgrowths. For example, light-colored, smooth spherical granules have been cultivated in acetate-fed reactors, whereas in glucose-fed reactors the spherical granules had white filamentous growth on the surface, or there were irregular granules with filamentous outgrowths (Moy et al., 2002; Tay et al., 2002b). As shown in Figure 2-1, granules cultivated on domestic wastewater or other complex wastes including biodegradable particulate COD tend to be yellow-brown and more likely to have attached protozoan or filamentous outgrowths (de Kreuk et al., 2010; Schwarzenbeck et al., 2004). Ideal granular sludge possesses a smooth and compact biofilm morphology, which contributes to its settling characteristics as discussed in Section 2.3.



(a) **(b)** **(c)**

Figure 2-1. Examples of granule morphology for growth on complex wastewaters.

Scale bar = 1 mm

(a) Growth on particulate soy protein as the sole COD source.
Courtesy of Mario Pronk, Delft University of Technology Department of Biotechnology. Copyright Mario Pronk.

(b) Growth on raw wastewater at Garmerwolde Nereda® full-scale plant.
Image of granules obtained after sieving and rinsing.
Courtesy of Mario Pronk, Delft University of Technology Department of Biotechnology. Copyright Mario Pronk.

(c) Growth on primary effluent at Ede Nereda® pilot plant.
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2.2 Structure of Aerobic Granular Sludge

Aerobic granular sludge structure can be broadly described as a channelized EPS matrix where polysaccharides appear to be the key structural element (Section 2.7) and microorganisms are distributed according to redox condition and substrate availability (Chapter 4). Channelization in aerobic granular sludge (Figure 2-2) has been measured by using fluorescent microspheres (Tay et al., 2002a, 2003) and visualized microscopically (Gonzalez-Gil and Holliger, 2014). Similar channelization in attached-growth aerobic biofilms has been documented (Massol-Deyá et al., 1995).

Aerobic granules are generally characterized by two distinct zones: 1) a higher-density outer region primarily composed of active biomass and EPS and 2) a lower-density inner region containing dead biomass and primarily composed of noncellular proteins associated with cell decay (McSwain et al., 2005; Toh et al., 2003; Wang et al., 2005a). However, precipitates may also be present in the inner region to varying degrees and increase the local density of the granule core as well as the net granule density as discussed in Section 2.4. For granules with an average diameter of 0.6-3.0 mm, the outer layer has been reported to be 0.2-0.8 mm thick depending on granule size and growth conditions (McSwain et al., 2005; Tay et al., 2002a, 2003).

The granular activated sludge pore structure is indicative of a dense biofilm structure. In two different studies of aerobic granular sludge grown in an aerobic pulse feeding regime (Xiao et al., 2008; Zheng and Yu, 2007), granules exhibited a range of net porosity values from 68%, which is much lower than the values of >90% observed for flocculent activated sludge (Li et al., 2003; Li and Ganczarczyk, 1990), to net porosity values of approximately 93%, similar to flocculent activated sludge. There is limited information suggesting that porosity may vary within the granule. For granules grown with an aerobic slug feeding regime, the outer layer was found to be more porous than the inner core, as determined by fluorescence of microspheres (Tay et al., 2003) and size exclusion chromatography (Zheng and Yu, 2007).

An opposite porosity change with depth was seen in a different study with different growth conditions and a different analytical method (Gonzalez-Gil and Holliger, 2014). The granules were grown in a low-rate anaerobic feeding regime, and based on microscopic image analysis the outer layer was less porous than the inner core (Figure 2-2). Channels from the granule exterior led to large void spaces in the granule interior region. A higher porosity or degree of channelization would be expected to improve mass transfer in aerobic granules, but this parameter and its impact on treatment performance has not been studied in detail.

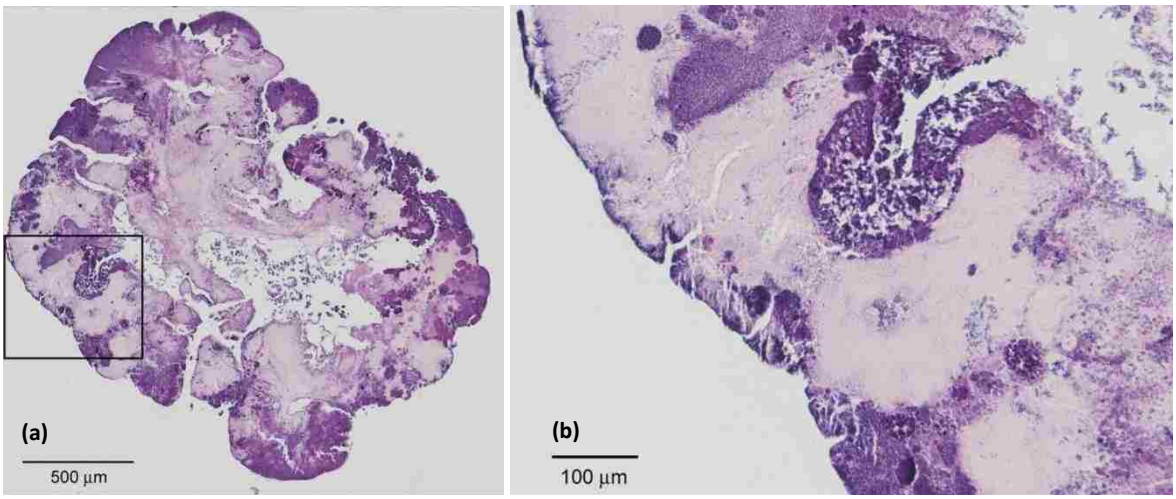


Figure 2-2. Channelization in aerobic granular sludge.

Brightfield microimages from sectioned granules stained with haematoxylin and eosin showing high levels of channelization, dense microcolonies (dark purple), and a matrix of EPS (lighter pink). (a) Section of the entire granule taken from the center of the granule. (b) Higher magnification of inset square. Reprinted with permission from Gonzalez-Gil and Holliger (2014). Copyright 2014 American Society for Microbiology.

2.3 Settling Behavior, Settling Velocity, and Density of Aerobic Granular Sludge

Granular and flocculent sludges exhibit distinctly different settling behaviors. Conventional flocculent activated sludge is characterized by hindered settling, in which the settling velocity may be 0.5-5 m/hr (Vanderhasselt and Vanrolleghem, 2000). Conversely, granular sludge is characterized by behavior more representative of discrete settling such that granules settle rapidly as separate particles and the extent of hindered settling is limited. Settling velocities for aerobic granules grown in lab-scale reactors under a variety of operating conditions have been reported to range from 30 to 100 m/hr for granules with diameters in the range of 1.1 to 2.4 (Liu, 2007; Tay et al., 2002b; Winkler et al., 2012a). Some researchers have considered 10 m/hr to be a minimum settling velocity that distinguishes granules from flocs (Tay et al., 2002b), and a settling regime in laboratory reactors is often applied such that particles with settling velocities less than 10 m/hr are washed out of the system (Beun et al., 2002a; de Kreuk et al., 2004). However, it is possible for smaller granules to have settling velocities in the range of 5 to 10 m/h.

The thickening ability and rapid settling characteristics of granular sludge have been captured in the traditional sludge volume index (SVI) test. The SVI of granular sludge is typically near 30 mL/g (de Kreuk and Van Loosdrecht, 2004; Liu et al., 2005b; Tay et al., 2004a). The ratio of settled sludge volume at five minutes and 30 minutes (SVI_5/SVI_{30}) is approximately 1.0 for a system with only granules, but higher values in the range of 1.3 are likely more common for full-scale systems dominated by granules, because flocculent sludge will also be present (van Haandel and van der Lubbe, 2012).

Two methods have been demonstrated to characterize the settling velocity of granular activated sludge and account for the fact that a granular activated sludge system can have a range of granular size and shapes with different respective settling velocities. The first method, by Winkler et al. (2012a), used a simple column settling test for laboratory systems dominated by granular sludge and containing a negligible flocculent sludge fraction. Key aspects of the protocol used are summarized here. A relatively small amount of granules (about 5 g wet mass) was released in a water column to assure observation of discrete settling behavior. The granular sludge sample was gently released at the top of a 5.6 cm-diameter column with a 1.5 m-water depth (approximately 3.7 L volume). The required time to settle a predefined distance was recorded using a chronograph, and the weighted average settling velocity of granules in the original sample was determined using data from five tests.

A second column settling test method by Mancell-Egala et al. (2014) has been used for a mixed liquor with both flocculent and granular sludge, and determines the distribution of the fraction of mixed liquor mass with different settling velocities. The settling velocity was defined in these tests as the distance from the full liquid depth of the column to the supernatant sampling port divided by the sampling time. The test method used a 4 L graduated cylinder with two supernatant sampling ports oriented 180 degrees from each other and opened at the same time for rapid, more-uniform supernatant withdrawal. The 4 L cylinder's water level was 508 mm, and the supernatant sampling ports were 50 mm (about 400 mL) from the top. The first step in the testing was to run a series of settling tests at decreasing mixed liquor concentrations by dilution to identify the MLSS concentration where discrete settling occurred. The discrete settling MLSS concentration was determined by one of two methods. The first was the MLSS concentration that resulted in the absence of a clear solids/liquid interface after about a 30 second settling time. In the second method, the supernatant total suspended solids (TSS) concentration was measured for the different initial MLSS concentrations at a supernatant sampling time that corresponded to a settling velocity of about 1.5 m/hr. The MLSS discrete settling concentration was assumed to occur when the percent TSS remaining in the supernatant versus the decreasing test MLSS concentration did not appreciably increase. For test results with the second method by Mancell-Egala et al. (2014) and Welling et al. (2015), discrete settling was deemed to occur at a test MLSS concentration of about 450 and 280 mg/L and percent TSS in the supernatant of about 90 and 95%, respectively.

After the discrete settling MLSS concentration was determined, the test was repeated at different settling/supernatant sampling times. At a given test settling velocity the supernatant solids represent particles with settling velocities less than the test settling velocity. The mass fraction is the mass of those remaining solids to the mass of the solids in the column upper supernatant volume at the start of the test. The test data was used to categorize the mass fraction of the mixed liquor according to particles with one of three settling velocity ranges: 1) <1.5 m/hr, (2) 1.5 to 10 m/hr, and 3) >10 m/hr. For example, for a settling velocity of 1.5 m/hr the mass fraction of solids in the supernatant represents the fraction of solids that are more characteristic of flocculent sludge. By definition, a settling time of 10 m/hr may be used to indicate a higher level of granular sludge development in the mixed liquor. The mass fraction of solids with settling velocities of >10 m/hr is the solids mass in the supernatant volume at the start of the test minus the solids mass in the test supernatant after the settling time, divided by

the initial solids mass. This mass fraction provides an indication of the distribution of mixed liquor solids between flocculent and smaller granules, and larger granules in a hybrid flocculent/granular activated sludge. For example, if 90% of the initial MLSS concentration remains in the supernatant at a settling velocity of 10 m/hr, the mixed liquor consists 10% of larger, more developed granules.

A more fundamental understanding of the mixed liquor characteristics associated with the settling velocities in the above test methods could be gained by relating the settling characteristics to particle size distribution, density, and morphology. The ability to determine particle size distribution for flocculent activated sludge has been demonstrated by Torfs et al. (2014).

Granular sludge density has been determined by researchers because of its importance in affecting settling velocity. Both granular and flocculent activated sludge density have typically been measured as *wet density*, where granules or flocs are immersed in an aqueous solution for which the increase in volume and mass includes water absorbed and in the saturated pore space of the granules or flocs.

The water pycnometer method, which has been widely used for measuring the wet density of soils (ASTM, 2014), has been found to be useful for determining the wet density of a granular sludge sample (Winkler et al., 2012a). This method uses a laboratory apparatus called a pycnometer that can be filled to a known volume. Typical pycnometer liquid volumes are in the range of 10 to 100 mL. The following briefly describes the procedure.

The mass of the empty pycnometer (m_o) is determined, and the granular sludge sample is added to the empty pycnometer after rinsing and sieving to remove residual flocs. Under this condition, the granules possess entrained water, but bulk liquid is absent. The mass of the pycnometer and granular sludge sample ($m_o + m_s$) is measured and the mass of the wet granular sludge sample, m_s, is determined by subtracting the empty pycnometer mass, m_o.

$$m_s = (m_o + m_s) - m_o \qquad \text{(Equation 2-1)}$$

The pycnometer is then filled with water and the total mass (m_T) is measured. The water mass added (m'_{H2O}) to fill the pycnometer is determined as follows.

$$m'_{H2O} = m_T - (m_o + m_s) \qquad \text{(Equation 2-2)}$$

The volume of water added (V'_{H2O}) is determined from the water mass added and the density of the water at the test temperature.

$$V'_{H2O} = m'_{H2O} / \rho_{H2O} \qquad \text{(Equation 2-3)}$$

The volume occupied by the granular sludge sample displaces water that would otherwise fill the pycnometer. The volume occupied by the granular sludge sample (V_S) is the difference between the known full pycnometer water volume (V) without a sample and the volume of water added (V'_{H2O}) to the granular sludge sample to fill the pycnometer according to

$$V_s = V - V'_{H2O} \qquad \text{(Equation 2-4)}$$

From the above test results the granular sludge sample density is determined.

$$\rho_s = m_s / V_s \qquad \text{(Equation 2-5)}$$

Granular sludge's wet density has also been determined by density gradient centrifugation that uses a specialized solution of known viscosity termed a Percoll solution. A Percoll solution is a low viscosity medium containing polyvinylpyrrolidone-coated silica particles of 15 to 30 nm diameter. Centrifugation at conditions of 16,000 rpm for two minutes (e.g., Winkler et al., 2013a) allows a density gradient to form due to the silica particles in the solution. In this procedure, a granular or flocculent sludge particle

migrates to a point of equilibrium density with the surrounding Percoll solution density gradient. This migration distance is recorded. Commercially available microspheres of known density are used to create a calibration curve of migration distance versus density from which sample density is determined.

Granular sludge density measurements have been obtained by pycnometry for samples at different depths for granules grown with acetate as the sole organic carbon source in a lab-scale batch-fed reactor operating at 20°C with EBPR and simultaneous nitrification/denitrification (Winkler et al., 2012a). The granules at the top of the settled sludge bed had a wet density of 1.020 g/cm^3, ash content of 15%, average effective diameter of 1.5 mm, and average settling velocity of about 45 m/hr. Granules from the bottom of the sludge bed had a higher wet density of 1.037 g/cm^3, ash content of 34%, average effective diameter of 2.3 mm, and average settling velocity of about 100 m/hr. This work also introduced the practice of selective wasting of slower-settling biomass at the top of the settled sludge bed to select for larger, faster-settling granules in the system. This is further discussed in Section 3.4.8.

Others used the density gradient centrifugation method to measure the density of granular sludge from a lab-scale aerobic pulse-fed reactor without EBPR (Etterer and Wilderer, 2001). The density of individual granules was determined on a set of 20 granules taken at four sampling events. The average density ranged from 1.038 to 1.050 g/cm^3 for four sampling events.

It appears from the limited results stated above that granular sludge density can vary within a given system and as a function of the granular sludge system operating conditions. The range of densities is also within that reported for flocculent activated sludge. Dammel and Schroeder (1991) found flocculent sludge densities in the range of 1.02 to 1.06 g/mL using the density gradient centrifugation method.

The settling velocity of granular sludge is affected by the granule size, shape, and density. Stokes's Law (Eq. 2-6) for ideal discrete settling of spherical particles can be used to illustrate the relative effects of particle size and density, shown in Figure 2-3. According to Stokes's Law, the particle diameter has a greater impact on settling velocity because it is raised to the second power. In consideration of the above wet densities, if the specific gravity of a 0.40 mm particle is increased from 1.02 to 1.06 g/mL, the settling velocity increases from about 0.63 m/hr to 1.88 m/hr or by a factor of about 3. However, if the particles size is increased by a factor of 3 from 0.40 mm to 1.20 mm, the settling velocity increases from 0.63 m/hr to 5.64 m/hr or a factor of 9. The use of Stokes's Law to convey this concept is not meant to suggest that ideal Stokian settling occurs for granular activated sludge suspensions. However, the more regular, smooth, and spherical morphology of granules likely contributes to the higher settling velocity and lower relative degree of hindered settling compared to flocculent activated sludge suspensions.

$$V_s = \frac{g(\rho_s - \rho)d^2}{18\mu} \qquad \text{(Equation 2-6)}$$

where

V_s = terminal settling velocity of the spherical particle

g = gravitational acceleration

ρ_s = density of particle

ρ = density of fluid

μ = dynamic viscosity of fluid

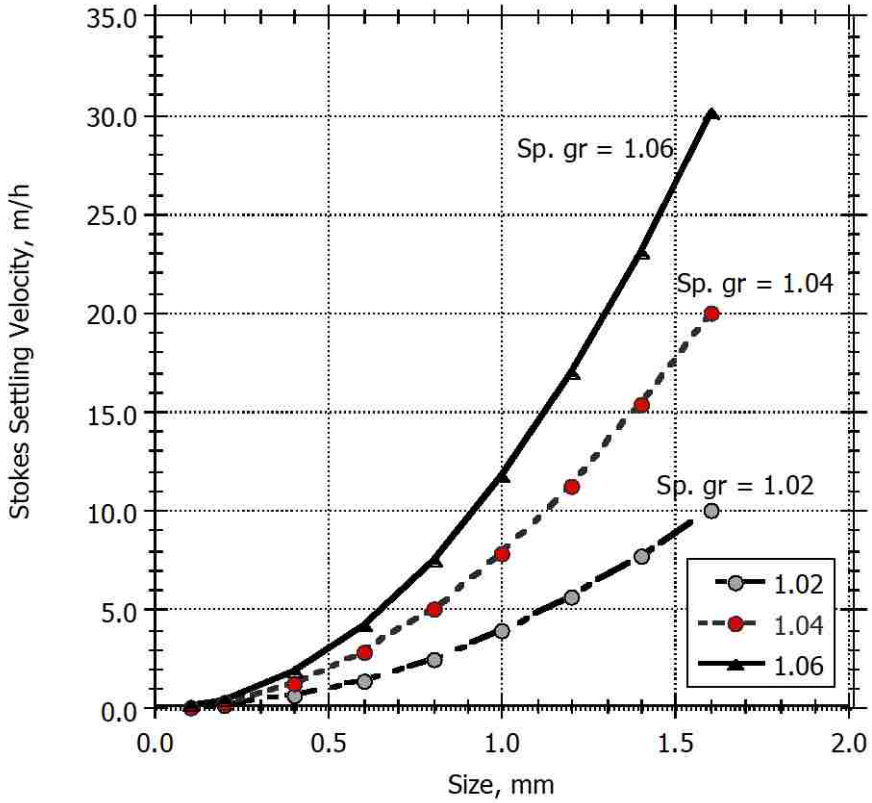


Figure 2-3. Effect of density and size for discrete settling of spherical particles in water at 20°C according to Stokes's Law.

2.4 Precipitate Formation in Aerobic Granular Sludge

The potential for chemical precipitation in biological wastewater treatment processes has long been recognized for flocculent activated sludge and most often associated with EBPR processes (Maurer et al., 1999). Similar precipitate formation potential exists for aerobic granular activated sludge, particularly in systems with EBPR. Precipitated phosphorus minerals are typically hydroxydicalciumphosphate [$Ca_2HPO_4(OH)_2$] and hydroxyapatite [$Ca_5(PO_4)_3OH$] but may also include fluoroapatite [$Ca_5(PO_4)_3F$; Maurer et al., 1999; Winkler et al., 2013a]. Precipitation becomes increasingly favorable as pH and PO_4-P and Ca concentrations increase (Winkler et al., 2013a). Elevated PO_4-P concentrations during anaerobic phosphorus release associated with the activity of phosphorus accumulating organisms (PAOs) can mediate phosphorus precipitation by significantly increasing the bulk liquid PO_4-P concentration. Additionally, acetic acid uptake by PAO during anaerobic feeding increases the bulk liquid pH, though concurrent Mg^{++}, K^{+}, and $PO4^{3-}$ release by PAO tends to buffer this effect (Winkler et al., 2013a). Precipitation can also be facilitated by denitrification occurring inside the granule, which results in a higher internal pH than that measured in the bulk liquid (Juang et al., 2010; Winkler et al., 2013a).

The presence of internal precipitates in aerobic granules grown in a bench-scale reactor with anaerobic feeding and a subsequent aeration period with simultaneous nitrification-denitrification and EBPR was shown using computed tomography (CT) scanning (Winkler et al., 2013a). The same study showed that

the presence of small amounts of internal precipitates could increase granular sludge settling velocity as a consequence of increased granular sludge density, as previously illustrated in Section 2.3.

Winkler et al. (2012a) reported that the density and ash content of 2.3 ± 0.5 mm PAO-enriched granules from the bottom of the settled sludge bed of a lab-scale reactor with simultaneous nitrogen removal and EBPR were 1.037 g/cm^3 and 34%, respectively, under operational conditions (Bassin et al., 2012a; Winkler et al., 2011) that included the feeding of synthetic waste with 400 mg/L acetate COD, 20 mg/L PO_4-P, and 18 mg Ca^{++}/L, and a bulk liquid pH range of 6.8 to 7.2.

In another study, Mañas et al. (2011) reported that biologically mediated phosphorus precipitation accounted for the removal of approximately 45% of the 30 mg/L influent PO_4-P concentration to a granular sludge system. The ability of phosphorus precipitation in this study was also encouraged by a high bulk liquid pH of up to 9.0 and a high influent acetate COD concentration of 1000 mg/L, which would encourage a higher PO_4-P concentration in the anaerobic contact step due to a greater amount of phosphorus release by PAOs than that of more typical influent domestic wastewater readily biodegradable (rbCOD) concentrations.

These studies suggest the potential for PAO-mediated precipitation in granular sludge systems with EBPR. For domestic wastewater treatment, the importance of precipitates in granular sludge is yet to be determined but should be less than that in the above studies due to the lower influent phosphorus and rbCOD concentrations than those used in the laboratory studies.

2.5 Physical Strength of Aerobic Granules

The ability to sustain granules in full-scale biological treatment systems requires that they have adequate physical strength to endure the shear and abrasive forces that may be caused by aeration or pumping environments, and thus methods to assess granule physical strength have been developed. An attempt to measure the effect of granular sludge resistance to mechanical agitation was first applied for anaerobic granules (Ghangrekar et al., 1996) and termed the *integrity coefficient* (also termed *stability coefficient*). The integrity coefficient is determined by measuring the ratio of the mass of granules retained on a given sieve size before and after a defined mechanical perturbation. Integrity coefficients above 95% based on the mass retained have been reported for granules developed under various growth conditions and subjected to perturbations ranging from more gentle platform shaking (Moy et al., 2002; Pan et al., 2004; Tay et al., 2002b) to intense vortexing (Xiao et al., 2008) and mechanical stirring (Nor-Anuar et al., 2012). Nor-Anuar et al. (2012) proposed classifying granules as “very stable,” “stable,” or “not stable” for integrity coefficient ranges of >95, 80 to 95, and <80%, respectively, with granular mass defined as that which was retained on a 0.2 mm sieve after the exposure of a 300 mL sample to mechanical stirring at 200 rpm using a 7.5 cm diameter impeller in a 13.3 cm wide cylindrical vessel.

Another approach based also on mechanical stirring effects determined a granule *abrasion coefficient*. The abrasion coefficient was obtained by fitting a first-order kinetic model to the amount of fines, defined as granules of <0.2 mm, produced over time when subjecting a granular sludge suspension to a constant rate of mechanical stirring for a defined duration (Pereboom, 1997; Ren et al., 2008).

The integrity and abrasion coefficients are qualitative parameters whose values depend on the intensity of the mechanical perturbation and the initial solids concentration, both of which influence the frequency of the particle-particle collisions contributing to granular biomass loss (Ren et al., 2009). However, high integrity coefficient and low abrasion coefficient values provide an insight into the ability of granules to survive in a full-scale operation and are useful for comparing one system to another or for monitoring changes in a granular sludge operation over time.

Although not representative of the forces in granules in an operation system, granule characteristics have also been measured by *compression strength*. Using the resistance to a downward-acting piston as an indicator of failure, granule compression strength has been quantified as the applied pressure preceding a stepwise change in measured resistance (van Hullebusch et al., 2007). A higher calcium content may increase granule compression strength and resistance to abrasion (Ren et al., 2008). Others have measured granule strength by rheological response to oscillatory vibration (Seviour et al., 2009a).

Physical testing methods to characterize granules have been applied in bench-scale research studies, but their usefulness for monitoring full-scale or pilot-scale facilities has not yet been proven. It has not been necessary for the Nereda® process, in view of the long-term sustainability of the granules and the absence of sludge pumping and mechanical mixing of the granules. However, for other conceivable aerobic granular sludge processes that may involve sludge pumping and mechanical mixing, granular sludge strength measurements could provide useful information and potentially be linked to trends in process performance. Although it may not be practical to replicate full-scale hydrodynamic conditions in a bench test, employing a standardized integrity testing protocol could facilitate correlations on process performance and granular sludge characteristics between bench-scale and full-scale systems.

2.6 Hydrophobicity of Aerobic Granules

From a thermodynamic perspective, hydrophobicity represents an attractive force such that increased hydrophobicity lowers the repulsive force between cells and therefore lowers the free energy change of cell-to-cell aggregation (Liu et al., 2004a). A greater hydrophobicity thus suggests better granule formation and better floc formation for flocculent activated sludge. The increase in surface hydrophobicity associated with microbial adhesion in biofilms and granules is well demonstrated (Liu et al., 2004b).

Methods used to quantify hydrophobicity have included adherence to hexadecane (Rosenberg et al., 1980), contact angle measurement (Duncan-Hewitt et al., 1989), and phenathrene adsorption (Kim et al., 2000). Expressed as the percentage of cells adhering to hexadecane after a partitioning period, hydrophobicity for stable aerobic granules may be 2 to 3 times that of flocculent sludge, typically 70 to 80% for granules compared to 30 to 40% for flocculent inocula (Pan et al., 2004; Tay et al., 2004a; Zhang et al., 2007). Using the contact angle as a measure of hydrophobicity, the general trend of increased hydrophobicity for aerobic granular sludge has been observed, but the values measured have been more variable (Zheng et al., 2005, 2006).

2.7 Characteristics and Role of Extracellular Polymeric Substances in Aerobic Granules

The complex gel-like matrices of biomacromolecules, including polysaccharides, proteins, nucleic acids, lipids, and humic substances located on or immediately outside the bacteria cells in flocculent and granular sludge, are referred to as extracellular polymeric substances (EPS), which play a key role in microbial adhesion and aggregation. EPS also offers protection from environmental stresses such as toxicity, starvation, and dehydration and aids in the transport of larger molecular weight compounds into the cell by sorption and catalysis of hydrolysis (Sutherland, 2001; Vu et al., 2009).

EPS extraction has been accomplished via various combinations of 1) physical processes including thermal treatment, centrifugation, sonication, and filtration and 2) chemical steps including caustic, EDTA, formamide, or cation exchange treatment (Adav et al., 2008a). Certain approaches involving

thermal and caustic treatments may result in cell lysis and increase the measured EPS content (McSwain et al., 2005).

The amount of EPS polysaccharide and protein and its location within granular sludge was a major thrust in early aerobic granular sludge research. The concentrations of EPS polysaccharides and proteins have been found to increase with granulation (during the transition from flocculent to granular sludge) and decrease with granule disintegration. In one study, the polysaccharide content was greater than the protein content (Tay et al., 2001a), but in other studies proteins have been found to be present at higher concentrations (Adav et al., 2007a; McSwain et al., 2005; Zhang et al., 2007). Utilizing fluorescently labeled probes, proteins have been generally found in the granule core, whereas cells and polysaccharides have been found in the granule shell. The location of α-polysaccharides has generally been limited to the granule shell, whereas β-polysaccharides may be distributed throughout the granule shell and core (Adav and Lee, 2008; Chen et al., 2007; McSwain et al., 2005).

Selective hydrolysis of proteins, lipids, and polysaccharides in aerobic granules has been used as an approach to elucidate the importance of various macromolecules to granule integrity. For phenol-fed granules, hydrolysis of β-polysaccharides resulted in granule disintegration, whereas hydrolysis of proteins, lipids, and α-polysaccharides had minimal effect on granule integrity (Adav et al., 2008b). For granules cultivated on abattoir wastewater, α-amylase and protease resulted in an exponential decrease of mechanical strength, whereas β-amylase, DNase, RNase, and lipase were not significantly different than the negative control (Seviour et al., 2009a). Aside from the reported differences in wastewater composition, these studies utilized different enzyme concentrations and mechanical forces (rotary shaking versus oscillatory vibration, respectively) to disrupt granule integrity.

The demonstrated viscoelasticity and capacity for swelling and deswelling by the absorption and desorption of water have led to the classification of aerobic granular sludge as a gel-like biomass or hydrogel (Seviour et al., 2009a), with extracellular polysaccharides being the key contributor to the gelatinous character and granule strength (Seviour et al., 2009b). Polysaccharide extracts from granular sludge treating abattoir wastewater exhibited gel-like properties, whereas those from flocculent sludge treating the waste stream did not. The gel-like character of these granules was maintained when subjected to an ex-situ pH of 2-9 and a temperature of 8-47°C (Seviour et al., 2009a, 2009b).

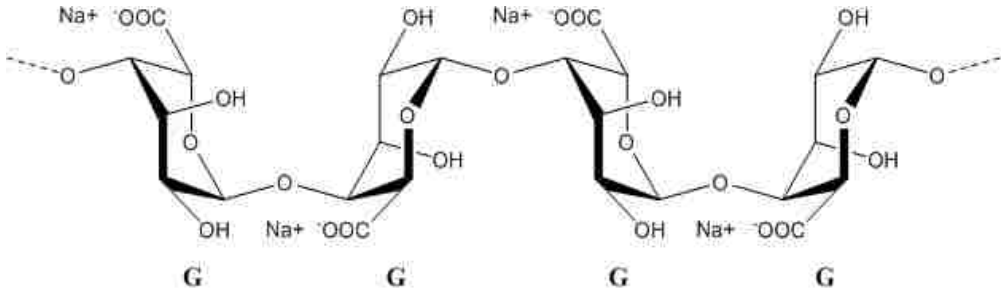
Two different types of exopolysaccharides have been identified in efforts to characterize the structural gel-forming polysaccharide moieties in aerobic granular sludge: alginate-like exopolysaccharides (ALE) (Lin et al., 2008, 2010, 2013) and an extraction product termed “Granulan” (Seviour et al., 2010a, 2010b, 2011). Their characteristics, the analytical methods used, and the growth conditions of the granules studied are summarized in Table 2-1.

The term alginate-like exopolysaccharides (ALE) has been used in the research literature and is adopted here to reflect that ALE possesses characteristics and behavior similar to commercial sodium alginate in many but not all categories (Lin et al., 2010). Bacterial alginates are a well-known family of extracellular polysaccharides that are produced by a variety of bacteria (Seviour et al., 2012a) and that are capable of forming gels with metal cations and consisting of linear polymers of the β-(1-4)-linked uronic acid residues β-D-mannuronate (M) and its C-5 epimer, α-L-guluronate (G; Lin et al., 2008; ASTM 2012). The M and G residues are distributed along the alginate polymer chain in three types of “blocks”: 1) homopolymeric blocks of repeating G residues (G-blocks), 2) homopolymeric blocks of repeating M residues (M-blocks), and 3) blocks of alternating M and G units (MG-blocks). The molecular structure of these polymeric units of alginate is shown in Figure 2-4 using sodium alginate as an example.

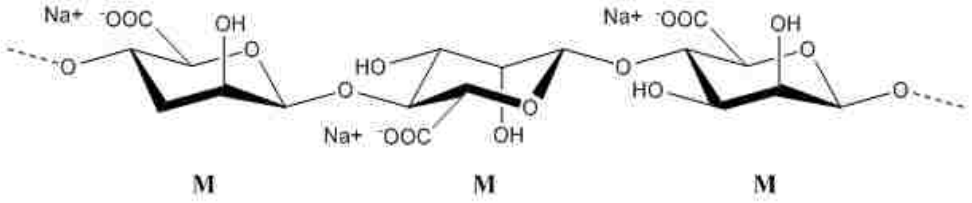
Table 2-1. Characteristics, Analytical Methods, and Growth Conditions for Gel-Forming Polysaccharide Moieties Identified in Aerobic Granular Sludge

Parameter	Alginate-like Exopolysaccharides (ALE)	Granulan
Polysaccharide characteristics:		
Polymer structure	Linear	Branched
pKa	4.5	9
Uronic acid residues	Yes	Yes
Analytical methods:		
EPS extraction	Alkaline (Na_2CO_3)	Alkaline (NaOH)
EPS precipitation	Ethanol	Methanol + chloroform
Protein removal step included prior to EPS structural identification methods below	No [(1)]	Yes – fractional precipitation
EPS structural identification methods	Alginate identification per Food Additives Organization protocol Gelation with Ca^{2+} UV spectroscopy Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy	Nuclear magnetic resonance (NMR) with high-performance anion-exchange chromatography and pulsed amperometric detection (HPAEC-PAD)
Granule growth conditions:		
	Detected in Nereda® nitrogen and phosphorus removing granules grown on domestic wastewaters Detected in granules grown on acetate with rapid-fill and aerobic feast period	Detected in enriched *Competibacter* glycogen accumulating organisms (GAOs) in granules grown on acetate Not detected in PAO-enriched (*Accumulibacter*) granules grown on acetate and propionate Not detected in denitrifying granules grown on methanol and nitrate
[(1)] UV spectroscopy confirmed protein contamination was negligible.		

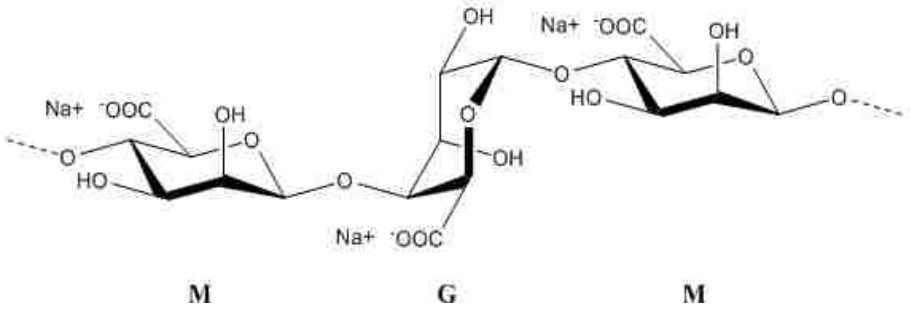
Sources: Lin et al., 2008, 2010, 2013; Seviour et al., 2010a, 2010b, 2011, 2012a.



a) G-block



b) M-block



c) MG-block

Figure 2-4. Sodium alginate structure and constitutive polymeric blocks.

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ALE has resembled sodium alginate in that (a) it formed a gelatinous precipitate in calcium chloride solution, (b) it did not form a precipitate in saturated ammonium sulfate solution, and (c) the UV-visible spectra and atomic mass spectra of soluble ALE were similar to those of soluble sodium alginate. However, ALE has not resembled sodium alginate in its reactions with acid ferric sulfate, where a brown color was observed for ALE in lieu of the cherry red color that would be expected for sodium alginate. The term “alginate” has been used in reference to ALE in aerobic granular sludge, though it is a less accurate description of the biomaterial.

In contrast to ALE, Granulan has a branched structure (Seviour et al., 2010b) and higher pKa (Seviour et al., 2009b). However, Granulan is similar to ALE in that it contains uronic acid residues (Seviour et al., 2010b). Molecular dynamics modeling (Seviour et al., 2012b) suggests that the gel-forming capacity of Granulan is associated with hydrogen-bonded antiparallel helices of the macromolecule that can be mediated and strengthened by Ca^{2+} bridging, analogous to ALE. Microbial production of Granulan

appears to be associated with the enrichment of the GAO *Candidatus "Competibacter phosphatis"* (Seviour et al., 2011).

Because analytical methods for ALE and Granulan have not been applied to the same granular sludge samples, it is not known if they both may be present in a granule matrix. In addition, the possibility of other types of polysaccharides in granules is not known as the analytical methods used in ALE and Granulan identification select for anionic polar polysaccharides that are soluble under alkaline conditions. Seviour et al. (2012a) noted that the composition of aerobic granular sludge has not been studied for all types of growth conditions, microbial populations, and feed substrates, and that the gel-forming EPS associated with other granular sludge may not be limited to only ALE and Granulan.

ALE can be present in flocculent activated sludge, but it appears to be enriched in granular activated sludge with different characteristics that provide enhanced structural properties. Lin et al. (2008) reported that ALE concentration increased during granulation (from 139 mg/g activated sludge to 310 mg/g granular sludge, respectively) and became enriched in G-blocks over M-blocks (from 0.94 to 1.18 G/M ratio). Because G-blocks may complex with metal cations, notably Ca^{2+} ions, to form insoluble regions, the higher G/M ratio facilitated granulation. Later, Lin et al. (2013) found that ALE from a pilot-scale Nereda® granular sludge reactor treating municipal wastewater contained a higher G-block content and stronger mechanical properties than ALE from a full-scale flocculent activated sludge system treating the same wastewater. Significant quantities of ALE have been found in aerobic granular sludge systems treating domestic wastewater in which the feed is first in contact with the granules under anaerobic conditions (Giesen et al., 2015; Lin et al., 2010, 2013), which favors PAO and GAO growth, but also in a system with aerobic pulse feeding of acetate (Lin et al., 2008). Thus, growth of PAOs and/or GAOs does not appear to be requisite for higher ALE content.

2.8 Effect of Storage on Integrity and Activity of Aerobic Granules

Aerobic granules have demonstrated an ability to recover their integrity and activity after extended storage periods without feeding. The extent of granule activity loss and deterioration in integrity and morphology during storage is influenced by conditions such as the storage duration, medium, temperature, and redox state (Liu et al., 2004c).

Aerobic granular sludge starvation and reactivation experiments are summarized in Table 2-2 and presented in order of increasing storage duration, which ranged from 28 to 360 days. Storage temperatures ranged from 4°C to ambient temperatures as high as 26°C. Most studies used granules dominated by ordinary heterotrophic organisms (OHOs) grown under an aerobic feeding regime with limited N and P removal occurring under process conditions applied in the reactors. Two studies (Pijuan et al., 2009; Zhu and Wilderer, 2003) used granules dominated by PAOs grown under an anaerobic feeding regime with simultaneous COD, N removal, and EBPR occurring under process conditions applied in the reactor. All studies involved wetted unaerated storage conditions except for Pijuan et al. (2009), in which both unaerated and intermittently aerated storage conditions were investigated in parallel experiments. Storage liquids used in the studies included granular growth reactor effluent, granular growth nutrient solution, and tap water.

The experiments showed that aerobic granules could maintain their morphology and structural integrity during storage without growth substrate for more than 30 days. Though some deterioration of morphology and structural integrity was observed during storage, original granule characteristics were recovered after resuming normal feeding and operation. Modest 10-20% reductions in the integrity coefficient were measured at the conclusion of storage and starvation prior to restarting normal feeding and growth conditions (Tay et al., 2002b; Yuan et al., 2012; Zhang et al., 2005). In some starvation

experiments there were no changes in morphology (Tay et al., 2002b; Zhu and Wilderer, 2003), but in other cases investigators noted the presence of cavities or rifts in the granule surface (Pijuan et al., 2009; Wang et al., 2008a) and darkening of color (Pijuan et al., 2009; Yuan et al., 2012). In all studies, granules recovered their original integrity coefficient and/or morphology within 30 days after returning to normal operation.

Granules have also exhibited the ability to recover biological activity following storage without growth substrate for more than 30 days. In the study involving PAO-dominated granules with simultaneous COD, N removal, and EBPR occurring under process conditions applied in the reactor (Pijuan et al., 2009), the original ammonia oxidation and PAO activities were recovered within 21 and 10 days, respectively, for granules that were intermittently aerated 15 minutes every six hours during storage. Results were not reported for granules exposed to unaerated storage in a parallel experiment. Other studies that involved OHO-dominated granules showed that biological activity as measured by specific oxygen utilization rate (SOUR) could be recovered within 30 days (Tay et al., 2002b; Wang et al., 2008a; Yuan et al., 2012; Zhang et al., 2005; Zhu and Wilderer, 2003). Yuan et al. (2012) reported that granules of this type recovered >95% of their original SOUR after seven days of normal operation following 360 days of storage.

The resilience of granules to recover their activity following extreme conditions has been shown. In one study, OHO-dominated granules frozen for 40 days at -20°C recovered full COD removal capacity after one day of feeding and aeration (Lv et al., 2013a). In another study, granules recovered their original COD removal capacity within 12 hours and their original appearance within five days of feeding and aeration following 40 days of storage in a wetted but dehydrated state in 100% acetone (Lv et al., 2013b).

Conversely, others reported granule disintegration between 90 and 180 days of storage under an unaerated wetted condition at 15 to 26°C (Adav et al., 2007b). These granules were grown on phenol as the sole carbon source and stored in Milli-Q water. At 90 days of storage, granule morphology deteriorated slightly, with visibly smaller granules and a significant amount of small particles presumably originating from granule decay. The change in diameter and the amount of non-granular particles were not explicitly quantified. The authors reported that granules began losing stability after 90 days of storage and completely disintegrated by 180 days of storage. While these granules disintegrated between 90 and 180 days of storage at room temperature without growth substrate, granules stored at 4°C without growth substrate maintained their integrity at 180 days of storage. After 180 days of storage at 4°C, granules exhibited 27% of their original specific phenol degradation capacity. After returning to normal feeding and operation for 180 days, these granules only recovered 56% of their original specific phenol degradation capacity, indicating a longer activity recovery period than that of the other OHO-dominated granules cited above. Results by Adav et al. (2007b) for granules stored in Milli-Q water for 180 days at room temperature and 4°C are shown in Table 2-2. Unidentified unique microbial or structural characteristics of these granules grown with a phenol substrate may have contributed to the different outcome compared to the other storage experiments summarized in Table 2-2, for which the feed substrates included glucose, acetate, and ethanol as the primary carbon sources.

A proposed fundamental mechanism of granule deterioration and potential disintegration during storage is granule core hydrolysis by the action of proteolytic (Adav et al., 2009a) and saccharolytic (Lee et al., 2009) bacteria.

Table 2-2. Summary of Laboratory Studies on the Effects of Granular Sludge Storage on Granule Integrity and Ability to Recover Activity

All storage in liquid without feeding

Storage Duration (d)	Storage Temp. ^{0}C	Storage Liquid	Granule Type	Change in Integrity Coefficient or Morphology during Storage	Recovery of Activity and Integrity or Morphology after Returning to Normal Operation	Reference
28	N/A	Growth reactor effluent	PAO-dominated granules with simultaneous N removal and EBPR	Minor morphology deterioration noted; intermittently aerated storage showed more deterioration than unaerated storage	Ammonia oxidation and PAO activities recovered within 21 and 10 days, respectively; morphology recovered within 7 days; results reported for intermittently aerated storage	Pijuan et al., 2009
49	N/A	Growth reactor effluent	PAO-dominated granules with simultaneous N removal and EBPR	No significant changes in morphology and settling velocity	SOUR (measured following anaerobic period) recovered in 7 days	Zhu and Wilderer, 2003
60	Room Temp.	Growth reactor effluent	OHO-dominated granules with limited N and P removal	Integrity coefficient decreased from 98 to 89%	Settling velocity, integrity coefficient, and 80% of original SOUR recovered in 20 days	Zhang et al., 2005
140	4°C	Growth reactor nutrient feed solution	OHO-dominated granules with limited N and P removal	Integrity coefficients decreased from 97–98 to 89–91%	Not investigated	Tay et al., 2002b
180	15–26°C	MilliQ water	OHO-dominated granules with limited N and P removal	Disintegration reported between 90 and 180 days	Not applicable	Adav et al., 2007b
180	4°C	MilliQ water	OHO-dominated granules with limited N and P removal	Morphology and integrity qualitatively maintained as determined by microscopic observation	56% of original specific phenol degradation capacity recovered after 180 days of normal feeding and operation	Adav et al., 2007b
210	4°C	Tap water	OHO-dominated granules with limited N and P removal	Minor morphology deterioration noted (rifts in granule surface)	Original SOUR for COD oxidation recovered within 14 days; original settling velocity and SVI recovered within 30 days	Wang et al., 2008a
360	20–26°C	Tap water	OHO-dominated granules with limited N and P removal	Integrity coefficient decreased from 99 to 78% at 180 days	COD removal efficiency, SOUR, and integrity coefficient >95% of original values within 7 days of normal feeding and operation	Yuan et al., 2012

Note: All studies used unaerated storage conditions except for Pijuan et al. (2009) where both unaerated and intermittently aerated storage conditions were employed in parallel experiments.

Though none of the studies on the starvation and reactivation of aerobic granular sludge summarized in Table 2-2 performed parallel tests on flocculent activated sludge, flocculent activated sludge has demonstrated a similar capacity to recover biological activity following starvation conditions. Yilmaz et al. (2007) showed that activated sludge performing biological nitrogen removal and EBPR recovered nitrogen and phosphorus removal performance within four days following a five-week starvation period where the sludge was aerated 15 minutes every six hours. In a different study (Tora et al., 2011), activated sludge performing partial nitrification recovered its original performance of 98% NH_3-N removal to NO_2-N at a NH_3-N loading rate of approximately 1.2 kg/m^3-day within 10 days following 23 days of aerobic-anoxic starvation conditions.

2.9 Sorption Capacity

For aerobic granular sludge treatment processes operated with an elevated bulk liquid ammonia (NH_3-N) concentration, adsorption and desorption of ammonium (NH_4^+) may be significant with regard to the dynamics of nitrification in batch-fed systems. Bassin et al. (2011a) studied the sorption and desorption of ammonia-N with granules grown in an anaerobic-fed SBR system that was capable of EBPR and nitrogen removal by nitrification-denitrification. Sorption/desorption tests were conducted under anaerobic conditions in nitrogen sparged flasks to prevent nitrification. At an equilibrium NH_4^+-N concentration of 30 mg/L, the granules had significantly higher NH_4^+ adsorption capacities of 1.7 and 0.9 mg N/g VSS for acetate-fed lab-scale and abattoir-fed pilot-scale granules, respectively, than for activated sludge flocs or anammox granules with a sorption capacity of approximately 0.2 mg N/g VSS. NH_4^+ was capable of being fully desorbed as governed by measured sorption isotherms. The NH_4^+ adsorption capacity of aerobic granules decreased approximately 50% at a salt concentration of 10 g NaCl/L, and NH_4^+ adsorption did not occur at 30 g NaCl/L. These results indicated that Na^+ competed with NH_4^+ for binding sites and that the observed NH_4^+ adsorption could best be described as an ion exchange process. Ion exchange equilibria and the selectivity of granular sludge to NH_4^+ and Na^+ or other cations were not studied in detail.

Aerobic granules have been shown to be effective at removing metals including Zn(II), Cu(II), and Cd(II) (Gai et al., 2008; Liu et al., 2003a; Xu et al., 2004) and the cationic dye Rhodamine B (Zheng et al., 2005) via sorption processes. Although the sorption capacity of aerobic granular sludge is comparable to other biosorbents, it offers a potential advantage in solids separation compared to other flocculent or dispersed biosorbents (Liu et al., 2003b). Additionally, aerobic granular sludge can rapidly remove up to 100 mg/L of uranium(VI) (Nancharaiah et al., 2006). Ion exchange has been demonstrated to play an important role in these sorption processes (Gai et al., 2008; Nancharaiah et al., 2006). A general model has been developed to describe biosorption with aerobic granular sludge (Liu et al., 2003b). In particular cases, the familiar Langmuir or Freundlich models have been well-suited to describe sorption behavior (Gai et al., 2008; Liu et al., 2003b; Zheng et al., 2005).

CHAPTER 3

Aerobic Granular Sludge Formation and Selective Pressures

This chapter discusses fundamental concepts and important parameters influencing aerobic granular sludge formation. First, granule types that have been grown in laboratory and full-scale systems are summarized. Selective pressures and key factors affecting the ability to grow granules are reviewed and then discussed in the context of different granule types. The effect of operating conditions on granular sludge growth and important physical mechanisms involved in granule formation are examined. Finally, start-up approaches employed at the bench scale and factors leading to granule instability and disintegration are discussed.

3.1 Aerobic Granule Types

Aerobic granules reported in the literature can be broadly classified into four types based on the biological processes occurring in their respective granule microbial consortia. These key biological functions in granules with different growth conditions and the respective acronyms used in this document are as follows:

1. Granules that perform EBPR and nitrification-denitrification. For conditions favoring selection of PAOs, GAOs may also grow—NDN-PAO granules.
2. Granules that perform nitrification and denitrification but without anaerobic contacting for PAO/GAO growth. Heterotrophic organisms using carbon substrates in this case are referred to as other heterotrophic organisms (OHOs)—NDN-OHO granules.
3. Granules grown with aerobic feeding and where denitrification is limited. Nitrogen removal is dominated by assimilation for growth on carbon substrates—OHO granules.
4. Granules that are fed mainly ammonia or nitrite and perform nitrification—NIT granules.

These granule types are described in greater detail below along with feeding conditions and characteristic performance.

For NDN-PAO granular sludge systems, denitrification or denitritation is done mainly by PAOs and/or GAOs. Influent rbCOD is assimilated and stored by PAOs and or GAOs under anaerobic contacting with the influent wastewater, and nitrogen removal occurs primarily by simultaneous nitrification-denitrification in a subsequent aerobic period. PAOs are typically present in greater abundance than GAOs, and a high degree of EBPR is achieved. NDN-PAO granules have been grown in laboratory-scale reactors (Bassin et al., 2012a; de Kreuk et al., 2005b) as well as in the full-scale Nereda® SBR process (see Chapter 7).

The use of biodegradable COD (bCOD) for both EBPR and denitrification by denitrifying PAOs (DPAOs) can result in an optimal use of influent bCOD for BNR. A theoretical minimum influent bCOD/NO_3-N removal ratio of 3.6 is estimated with the following assumptions: (a) all of the influent bCOD is readily available and assimilated only by PAOs; (b) the temperature is 20°C; (c) the SRT is 15 days; (d) all of the growth and endogenous decay activity of the PAOs after bCOD storage during anaerobic uptake is done under anoxic conditions using NO_3-N; and (e) the nitrogen needed for PAO cell growth is provided by

assimilatory NO_3-N uptake. The influent bCOD/NO_3-N ratio for high nitrogen removal efficiency would be higher for real wastewaters, because some portion of the influent bCOD may not be used by the PAOs and is thus consumed by OHOs. It would also be higher if some of the PAO growth and/or endogenous decay occurs under aerobic conditions because the synthesis cell yield is higher for aerobic growth versus anoxic growth.

On a practical basis, it is common to relate the ratio of influent bCOD removed to influent total Kjeldahl nitrogen (TKN) with biological nutrient removal performance. Lower values for this ratio are indicative of more bCOD uptake and NO_3-N reduction by PAOs/GAOs. The bCOD removed to influent TKN ratio includes many factors related to the fate of bCOD and nitrogen, including the bCOD consumed by PAOs, GAOs, and OHOs; the nitrogen used in the synthesis of PAOs, GAOs, and OHOs; and the nonbiodegradable portion of the influent TKN. An influent bCOD/TKN ratio of 6.7 was reported for a laboratory NDN-PAO granular sludge system achieving greater than 90% total inorganic nitrogen (TIN) removal with NO_3-N and no NO_2-N present in the effluent (Bassin et al., 2012a). A TIN removal efficiency greater than 95% has also been demonstrated in a full-scale granular sludge system where the influent BOD/TKN ratio averaged 4.3 (see Section 7.2.4). Assuming 1.6 g bCOD/BOD, the influent bCOD/TKN ratio would be 6.9.

NDN-OHO granules perform nitrification-denitrification or nitritation-denitritation with carbon conversions mediated by OHOs. EBPR does not occur in processes with NDN-OHO granules because of the lack of anaerobic conditions. Instead, influent is fed under anoxic conditions, and a high degree of nitrogen removal occurs by alternating nitrification-denitrification in sequential aerobic and anoxic periods. To date, NDN-OHO granules have only been grown in laboratory-scale reactors (Chen et al., 2013; Wang et al., 2012; Figdore et al., 2015).

For OHO granules, carbon consumption is by OHOs, and EBPR does not occur. Influent is typically introduced in a slug feed of less than five minutes into an aerated reactor or quiescent reactor followed immediately by aeration. Ammonia oxidation may occur, and nitrogen removal occurs primarily by assimilation. Although OHO granules have been primarily grown at the lab scale (Moy et al., 2002; Tay et al., 2002b), a study by Ni et al. (2009) reported the successful growth of OHO granules at the pilot scale on municipal primary effluent.

NIT granules perform ammonia and/or nitrite oxidation with minimal total nitrogen removal. Influent is fed under aerobic conditions. NIT granules have been grown with autotrophic synthetic media without organic carbon or with carbon-poor streams such as anaerobic digester dewatering centrate. The relative abundance of nitrifying organisms is higher in these granules compared to the other types of granule described above. To date, NIT granules have only been grown in laboratory-scale reactors in systems performing ammonia oxidation to nitrate (Tay et al., 2002c; Tsuneda et al., 2003; Figdore et al., 2015), ammonia oxidation to nitrite (Lopez-Palau et al., 2011; Vázquez-Padín et al., 2010b), and nitrite oxidation to nitrate (Ni et al., 2011; Vázquez-Padín et al., 2009).

3.2 Selective Pressures and Key Factors Affecting Granular Sludge Growth

This section discusses selective pressures and key factors affecting granular sludge growth. These include 1) liquid-solids separation design, 2) COD feeding regime, 3) shear conditions, 4) microbial growth rate, and 5) avoiding diffusion-limited growth conditions.

3.2.1 Liquid-Solids Separation Design

The selection of granules over flocs requires that liquid-solids separation favor the retention of faster-settling, larger granule particles over slower-settling, smaller floc particles. This has most commonly

been accomplished in systems with gravity separation by controlling the settling time and/or upflow velocity during settling. Therefore, this section emphasizes selective pressure applied in gravity separation processes. However, granules have also been selected in physical separation processes based on particle size discrimination, as discussed at the conclusion of this section.

Because the larger, more spherical, and potentially denser granular sludge can settle faster, a hydraulic selective pressure can be used to wash out the slower-settling flocculent sludge, which thus minimizes the flocculent sludge competition for the substrate and favors substrate utilization by granular sludge and its dominance in the activated sludge system. The settling time and/or upflow velocity in liquid-solids separation are major design and operating parameters that are used to select for granular over flocculent sludge and are referred to here as *hydraulic selective pressure,* but may also be referred to as *selective settling pressure*.

The use of short settling times in the range of four to 10 minutes, compared to the 30-60 minutes commonly used in flocculent activated sludge SBR operation, has been shown in numerous reports as a key factor in selecting for granular activated sludge. Typically, settling times in granular SBRs with decanting is five minutes or less (Arrojo et al., 2004; de Kreuk et al., 2005b; Pan et al., 2004). In a representative bench-scale granular sludge SBR with a total depth of 1.6 m and a decanting depth of 0.8 m, granules or floc with settling velocities less than 10 m/hr would not be retained. A 10 m/hr settling velocity or more is within the capability of granular activated sludge, but settling velocities for flocculent sludge are much lower. For example, the zone settling velocity of a 3500 mg/L activated sludge mixed liquor with a favorable SVI of 120 mL/g would be about 2 m/hr (Tchobanoglous et al., 2014).

This differential settling velocity is also utilized for granular sludge selection in Nereda® granular sludge reactors that have simultaneous filling and effluent overflow with an upflow feeding regime instead of decanting. Targeted average and maximum upflow velocities are approximately 2.5 and 5.0 m/hr, respectively (van Haandel and van der Lubbe, 2012) as later shown in Section 7.1.1.

During a granular sludge reactor start-up phase, longer settling times or lower upflow velocities may initially be used to avoid washing out small granules that may grow to larger granules and to avoid excessive biomass loss (Lochmatter and Holliger, 2014). At longer settling times in the range of 10-20 minutes it is possible to maintain a mixture of flocculent and granular sludge (Qin et al., 2004). For example, in laboratory aerobic pulse-fed SBR reactors fed a synthetic wastewater composed of 800 mg COD/L of a glucose-peptone blend and operated with a 50% fill ratio, a 10-minute settling time resulted in a mixture of flocculent sludge and small granules, whereas much larger granules were maintained using only a two-minute settling time. For the reactor decant depth of 0.4 m, these settling times corresponded to capturing particles with settling velocities of 2.4 m/hr or more and 11.8 m/hr or more, respectively (McSwain et al., 2004b). In a similar laboratory, for an aerobic pulse-fed SBR operated with a 50% fill ratio, fed an acetate-based synthetic wastewater, and with a 10-minute settling time, the MLSS granule fraction was 22% with an average granule size of 1.1 mm, whereas at a five-minute settling time, the MLSS granule fraction was 81% with an average granule size of 2.2 mm (Adav et al., 2009b). The MLSS granule fraction was measured by laser particle size analysis using a granule cutoff size of 0.5 mm. In addition, microbial community analysis showed a greater washout of non-flocculating bacteria strains with shorter settling times.

Though a hydraulic selective pressure with short settling times in SBR activated sludge systems appears necessary to develop a granular sludge system, there are examples in which unexpected granular sludge dominance occurred with conventional flocculent sludge settling times. In the first case (Barr et al., 2010), an SBR had a one to six-minute anaerobic feeding period followed by anaerobic and aerobic react times of varying duration and a settling period of 40 or 65 minutes. When sludge wasting was changed

from wasting during the mixed/react period to wasting from the top portion during the first four minutes of the settling period, a granular-dominant sludge (1.0 mm average diameter) was selected. However, prior to this change in wasting protocol, average the particle size was approximately 0.5 mm, indicating the presence of large aggregates despite no hydraulic selective pressure. In this case, even though the settling time period was long, the selective wasting did provide something equivalent to a hydraulic selection pressure by wasting the more slowly settling floc from the top of the reactor during the first few minutes of settling.

The second report of granular sludge growth in a system lacking a high degree of hydraulic selective pressure noted an MLSS containing a mixture of well-settling flocs and granules with SVI_{30} as low as 50 mL/g (de Villiers and Pretorius, 2001). The system consisted of a pilot-scale SBR (60 m^3) with an external Imhoff-type secondary clarifier treating concentrated abattoir wastewater (4000–7000 mg COD/L and 1100-2100 mg TSS/L). The batch bioreactor was operated with 24-hour cycles, and the external settler overflow rate was 0.7 m/hr. Coarse bubble aeration at a constant gas supply rate was used for mixing and aeration. Feeding occurred over a coarse-bubble-mixed 4 hr time period during which no DO was detected. Denitrification and the development of PAOs from the high strength wastewater could be possible under this aeration condition and high strength wastewater feed, but no nutrient removal performance data were reported. During the subsequent react period of approximately 12 hours, the DO increased from zero to no greater than 3 mg/L. Additional cycle time included decanting, settling, and idle periods of unspecified durations. An average granule diameter of 1.5 mm was reported, but the underlying granular sludge selection factors are not known due to the lack of sufficient operating and performance information.

A variation of hydraulic selective pressure to obtain aerobic granular sludge has been used in continuously fed aerobic upflow sparged reactors with a solid-liquid separator. These reactors contain a lower fluidized bed section with a smaller diameter and higher upflow velocity than the upper liquid-solids separation section, which has a larger diameter and lower upflow velocity. Slower-settling particles are washed out of the system based on the critical upflow velocity in the liquid-solids separation section, resulting in the retention of larger and faster-settling particles in the lower fluidized bed section. Laboratory studies with these reactors are discussed in Section 5.6. Reactors of this type were also used in first-generation anammox granular sludge systems.

An alternative to using a granular selective settling pressure is a physical size-based selective pressure by the use of a screen (Liu et al., 2012, 2014a) as illustrated in Figure 3-1. The smaller particles passing through the screen are wasted from the system, and the larger particles retained by the screen are returned to the activated sludge reactor. This size-based selective pressure has been employed at the laboratory scale in continuously fed, continuously aerated reactors. In one system (Liu et al., 2012), filamentous granules of loose structure developed, as would be anticipated based on the disadvantageous continuous aerobic feeding regime as discussed in Section 3.2.2. In this system, sludge was pumped into a granule selection tank once per day. The granule selection tank contained a 0.6 mm sieve, and air was intermittently sparged to prevent excessive biological growth and blockage of the sieve. The air scour rate was not specified. In another continuously fed, continuously aerated system (Liu et al., 2014a), approximately 80% of the reactor MLSS was between 1 and 3 mm and of favorable morphology and SVI (35 mL/g). A possible explanation for this improvement is a high aeration shear rate in the bioreactor (discussed in Section 3.2.3), which could compensate for the disadvantageous continuous aerobic feeding regime. However, details on the aeration system and intensity were not given. In this study, a similar granule selection tank with air scour was used, but sieve aperture was gradually increased from 0.1 to 1.0 mm. This selective pressure may also be considered for conventional SBR reactors or activated sludge systems for waste activated sludge (WAS) management. Full-scale demonstration of screening systems for aerobic granular sludge selection has yet to occur.

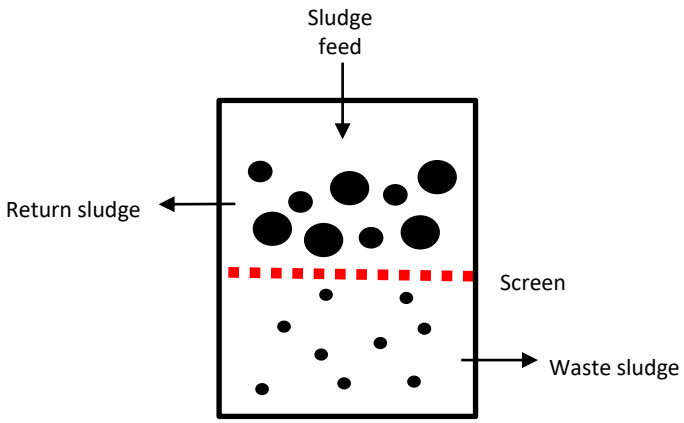

Figure 3-1. Schematic of size-based aerobic granular sludge selection by screening.

Size-based granule selection has also been used in laboratory-scale anammox systems. A size-based screening approach was reported by de Clippeleir et al. (2013) to select for anammox granule retention over flocs in a single-sludge deammonification process and by Galvagno et al. (2014) as a polishing step following the use of hydrocyclones to separate anammox granules from flocculent sludge.

3.2.2 COD Feeding Regime

The COD feeding regime plays an important role in the selection of NDN-PAO, NDN-OHO, and OHO granular sludges. The amount of biodegradable COD (bCOD) is important for granular growth in denitrification processes, and exposing the biomass to a readily biodegradable COD (rbCOD) concentration is particularly important for the growth of PAO granules, as discussed below.

Feeding regimes that expose the biomass to high readily biodegradable COD (rbCOD) concentrations are more favorable to granular sludge growth for several reasons. First, such a feeding regime promotes the diffusion of the substrate into the granule interior, which enables the center of the granule to be biologically active provided that electron acceptors such as oxygen, nitrate, or nitrite are simultaneously or later available at sufficient concentrations to avoid granule core decay by anaerobic hydrolysis. This practice also avoids prolonged low bulk liquid substrate concentrations and diffusion-limited growth conditions (discussed in Section 3.2.5) that promote the outward growth of filamentous bacteria from flocs and filamentous structures from biofilms (Martins et al., 2003; Picioreanu et al., 1998).

Exposing biomass anaerobically to high rbCOD concentrations selects for organisms capable of assimilating rbCOD as polyhydroxyalkanaotes (PHAs) for subsequent utilization when substrate is not present in the bulk liquid (Chiesa et al., 1985). Within the broader category of PHA, the key polymers include poly-beta-hydroxybutyrate (PHB), poly-beta-hydroxyvalerate (PHV), and poly-beta-hydroxy-2methylvalerate (PH2MV; Oehmen et al., 2005). PAOs or GAOs outcompete other organisms for rbCOD uptake under anaerobic conditions and store carbon as PHA, which is ultimately used as the carbon source for cell synthesis and the electron donor for phosphorus-accumulating or glycogen-accumulating metabolisms, respectively, under aerobic or anoxic conditions (Lopez-Vazquez et al., 2009; Mino et al., 1998). These polymers may be accumulated by ordinary heterotrophs under aerobic or anoxic conditions (Beun et al., 2000b, 2000c; Dionisi et al., 2001; Krasnits et al., 2013) but to a lesser extent than for PAOs.

Exposing biomass to high rbCOD concentrations has classically been accomplished in flocculent sludge SBRs with a rapid pulse feed, leading to what is commonly referred to as feast-famine conditions. In the feast phase, influent wastewater rbCOD and bCOD are present in excess. During the famine phase, influent wastewater rbCOD and bCOD are not present in the bulk liquid, and growth occurs on intracellular carbon storage products and endogenous decay products. Excellent sludge settleability for these growth conditions has long been demonstrated (Chiesa et al., 1985). Conversely, excessive filamentous growth and poor settleability has widely been reported in complete-mix activated sludge systems (Chiesa and Irvine, 1985; Chudoba, 1985) where filamentous organisms have a competitive advantage and can flourish where substrate is continuously available at low concentrations.

Two feeding approaches have been commonly employed in laboratory systems to expose biomass to high rbCOD concentrations and facilitate granular sludge growth. One is a rapid pulse feed, comparable to that used in feast-famine flocculent sludge SBRs (Figure 3-2a). This fill period is typically less than 10 minutes in duration and may be without mixing or aeration (termed static feeding), mechanically mixed, or aerated. In this pulse-fed operation, the bulk liquid rbCOD concentration is high, and rbCOD removal is rapid due to intracellular uptake at the start of the cycle. Another approach, used in laboratory reactors and at full scale, involves slow upflow feeding in a plug-flow manner through the settled granular sludge bed under anaerobic conditions (Figure 3-2b). Contacting the settled biomass directly with influent wastewater in this fashion promotes a higher diffusion gradient and diffusion of the substrate throughout the granule. Anaerobic feeding into the settled blanket promotes the metabolic selection of PAOs and/or GAOs. In both feeding approaches, a fill fraction of approximately 50% is typical in granular sludge SBRs. Though both feeding approaches have been successfully employed for aerobic granular sludge growth, only the slow feeding regime has been employed at full scale. An additional advantage of slow feeding to an SBR is the use of smaller influent pumping equipment and reduced peak power demands in full-scale systems.

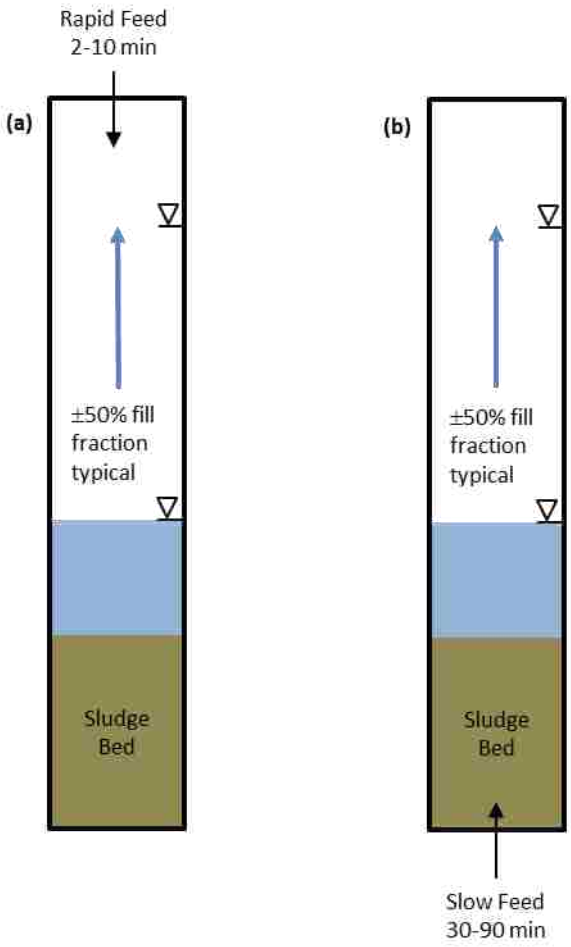

Figure 3-2. Common feeding regimes in aerobic granular sludge sequencing batch reactors used to expose biomass to high bulk liquid readily biodegradable substrate concentrations.

(a) Rapid pulse feeding (Tay et al., 2002b).
(b) Slow anaerobic bottom feeding through the settled sludge bed (de Kreuk et al., 2004).

3.2.3 Shear Conditions

Hydrodynamic shear is an important operating parameter that affects aerobic granular sludge characteristics. Higher hydrodynamic shear results in biofilms (Kwok et al., 1998; van Loosdrecht et al., 1995; Wäsche et al., 2002) and granules (Liu and Tay, 2002) that are smoother, denser, and more stable. Shear has been positively correlated to polysaccharide production, bioactivity, hydrophobicity, and specific gravity (Tay et al., 2001c). The shear necessary to create smooth, dense granules is related to the maximum growth rate of the associated bacteria. Under the same conditions, faster-growing organisms (for example, OHOs) will form less-dense structures than slower-growing organisms (for example, PAOs and nitrifiers) and require higher shear rates to form a smooth, dense granule structure (van Loosdrecht et al., 2005), which would have a higher settling velocity.

Shear forces in typical aerobic granular sludge SBRs are related to and quantified by the aeration intensity and superficial air velocity. A recirculated off-gas stream with air and nitrogen addition for DO control is often used for aeration in lieu of once-through fresh air in laboratory studies. The term *superficial gas velocity* is used in this document to refer to the reactor inlet gas sparging rate with or

without gas recirculation. Superficial gas velocities commonly applied at the laboratory scale are between approximately 1.0 cm/s (McSwain et al., 2004b; Su and Yu, 2005; Wang et al., 2004) and 2.0 cm/s (de Kreuk and van Loosdrecht, 2006; Liu et al., 2003c; Wang et al., 2009). Laboratory studies have demonstrated successful granular sludge growth or the maintenance of pre-existing seed granules using lower superficial gas velocities and intermittent aeration, as discussed in Section 3.3.

The laboratory superficial gas velocities may not be directly applicable to full-scale aerobic granular sludge reactors, where aeration intensity would likely be lower due to improved oxygen transfer efficiency with greater diffuser submergence depth and fine bubble aeration designs to meet oxygen demand. For example, a scenario representative of a relatively intensely aerated full-scale treatment system would be 25% floor coverage of conventional circular membrane diffusers operating at a membrane flux of 35 $Nm^3/hr/m^2$ (2.0 scfm/sf). For this scenario, the superficial gas velocity would be 0.25 cm/s (0.50 ft/min), which is significantly lower than the superficial gas velocities commonly applied in laboratory systems.

Granules have been cultivated in reactors with mechanical mixing by a submerged impeller rotated from 100 to 300 rpm (Ahn et al., 2009; Mosquera-Corral et al., 2011) or by a magnetic stirring bar at speeds up to 200 rpm (Yilmaz et al., 2008), showing that mechanical mixing can be used without hampering granule formation. In both cases, favorable operating conditions for the selection of granular sludge growth were used.

An energy dissipation-based model (Ren et al., 2009) provided insight towards the contributions of fluid shear, gas bubble shear, and collision shear stresses on granules in a bench-scale SBR without external mechanical mixing. Based on the analysis, the contribution of fluid shear was negligible, whereas the gas bubble and collision shears were significant. The predominance of gas bubble or collision shear depended on the aeration intensity. The reactor solids concentration strongly impacted collision shear, as would be expected due to the increased frequency of particle-particle collisions at higher MLSS. The effect of reactor height was not significant for superficial gas velocities below 1.5 cm/s.

3.2.4 Microbial Growth Rate

The influence of the microbial growth rate on aerobic granular sludge growth relates to the shear necessary to form smooth, dense granules. Under the same conditions, faster-growing organisms will form less-dense biofilm structures than slower-growing organisms (van Loosdrecht et al., 2005). For example, Tijhuis et al. (1994a, 1994b) demonstrated that under similar reactor temperature and shear conditions, nitrifying bacteria formed a denser biofilm than ordinary heterotrophs. Consequently, systems dominated by faster-growing organisms such as OHOs will require higher shear to form smooth, dense granules. Therefore, the selection of slower-growing heterotrophs such as PAOs and GAOs may be advantageous to reduce the shear necessary to form granules (de Kreuk and van Loosdrecht, 2004).

3.2.5 Avoiding Diffusion-Limited Growth Conditions

The occurrence of filamentous or finger-like outward growth in flocs (Chiesa et al., 1985; Martins et al., 2003), bacterial colonies (Ben-Jacob et al., 1994; Matsushita and Fujikawa, 1990), and biofilms (van Loosdrecht et al., 1995) where growth conditions involve low bulk liquid substrate concentrations has long been recognized. Under these conditions microbial growth is limited by substrate transport into the floc or biofilm. Consequently, the growth regime is diffusion limited, and filamentous bacteria or filamentous outgrowths from biofilms have an advantage in accessing the limited substrate in the bulk liquid. The opposite scenario would be a growth-rate-limited regime where the substrate is abundant and conversion processes are limited by the intrinsic growth rate of the microorganisms involved in the conversion processes.

Picioreanu et al. (1998) derived a growth parameter, G, to characterize the ratio of the maximum biomass growth rate to the maximum substrate transport rate in biofilms:

$$G = \frac{\text{maximum biomass growth rate}}{\text{maximum substrate transport rate}} = L_z^2 \frac{\mu_m c_{Xm}}{D_s c_{s0}} \qquad \text{(Equation 3-1)}$$

The G parameter includes factors that have been found to affect biofilm structure. Specifically, L_z is the biofilm thickness or granule radius, μ_m is the maximum specific microbial growth rate, c_{Xm} is the maximum biomass density in the biofilm, D_s is the soluble substrate diffusion coefficient, and c_{s0} is the bulk liquid soluble substrate concentration. When G is high, the growth regime is diffusion limited or transport limited. When G is low the growth regime is growth rate limited.

Two- and three-dimensional modeling by Picioreanu et al. (1998) showed that smooth and dense biofilms are formed under a growth-rate-limited regime (low G), while open and filamentous biofilms are formed under a diffusion-limited regime (high G). The observation of filamentous outgrowth from flocs and biofilms under diffusion-limited conditions corroborates these model results.

This fundamental understanding of the growth regime required for smooth and compact biofilm structure was instrumental in facilitating aerobic granular sludge development and explaining how some of the growth conditions described earlier in the section favor granular sludge growth and avoid diffusion-limited growth conditions. A growth-rate-limited condition is promoted over a diffusion-limited condition by exposing the biomass to high soluble substrate concentrations and selecting for slower growing organisms.

3.3 Application and Importance of Selective Pressures to Different Granule Types

Building on the previous section on selective pressures and key factors affecting the ability to grow granules, this section discusses these factors and other considerations in the context of how they relate to the NDN-PAO, NDN-OHO, OHO, and NIT granule types.

The need to employ a liquid-solids separation strategy that selects for granules over flocs is universal to all granule types. Examples cited in Section 3.2.1 in which granular sludge was not obtained in systems lacking appropriate hydraulic selective pressure illustrate this fact. As will be later discussed in Section 7.2.2, MLSS in Nereda® reactors is dominated by granular sludge, but the slow upflow feeding regime with simultaneous effluent overflow allows some flocs to remain present in the system. Particles less than 212 um may represent approximately 10 to 20% of the MLSS mass in these reactors.

For the typical NDN-PAO granular reactor system, upflow anaerobic feeding through the settled sludge bed accomplishes two important factors for granular sludge growth: 1) anaerobic feeding with rbCOD selects for PAO granules and 2) a high rbCOD substrate gradient drives substrate diffusion deeper to favor selection of larger granules. The upflow feeding into the sludge blanket without any mixing with the full reactor liquid exposes the settled granules to the maximum rbCOD concentration provided in the influent wastewater. The influent is fed through the settled sludge bed, leading to a substrate gradient in the settled sludge bed. The bottom biomass can consume the rbCOD first, leading to lower rbCOD concentrations in the upper sludge bed. The larger, faster-settling granules at the bottom of the sludge bed will be exposed to higher bulk liquid rbCOD concentrations than the smaller, slower-settling granules at the top of the sludge bed (Winkler et al., 2011). The anaerobic feeding regime also selects

for slow-growing PAOs and/or GAOs, which can internally store rbCOD and outselect OHOs. Unlike PAOs/GAOs, OHOs require an electron acceptor, such as oxygen, nitrate, or nitrite, to oxidize organic carbon (Beun et al., 2002b). PAOs/GAOs can use the internally stored substrates such as PHA as electron donors for denitrification purposes. While some PAOs located on outer layers will oxidize PHB aerobically, the PAOs/GAOs located in the anoxic granular core can remain metabolically active by reducing the produced nitrate and nitrite (from nitrifiers) to dinitrogen gas. Supplying nitrate or nitrite to the inner core is also necessary for granule stability and to maintain an active granular core, which will reduce the potential for granule breakup due to inner core starvation and decay (Pronk et al., 2015a).

Though NDN-PAO granular sludge reactors may be operated at the laboratory scale with relatively high shear, as quantified by superficial gas velocities near 2.0 cm/s (de Kreuk and van Loosdrecht, 2006), granules of this type have also been grown at a lower aeration shear intensity. Lemaire et al. (2008b) grew NDN-PAO granules using on/off aeration for DO control between 1.3 and 1.7 mg/L with air applied at a superficial gas velocity of 0.9 cm/s. These granules were later maintained after being seeded into a different reactor using a similar on/off aeration regime for DO control between 3.0 and 3.5 mg/L at a superficial gas velocity of 0.6 cm/s (Yilmaz et al., 2008). Successful growth of NDN-OHO or OHO granules at similar low-shear, low-DO conditions has not been widely reported. The selective settling pressure is also an important part of NDN-PAO granular sludge SBRs, as illustrated by the fact that numerous laboratory- and full-scale SBRs without granular sludge have been operated with the anaerobic fill and feast-famine conditions but with much longer settling periods (Tsuneda et al., 2006a; Zeng et al., 2003).

In the case of NDN-OHO and OHO granules, a rapid pulse feeding is essential to expose the biomass to high bCOD concentrations and drive carbon storage processes and diffusion into the granule. However, because OHOs in these processes exhibit fast growth concurrent with carbon uptake, higher shear must be applied to form a smooth biofilm. OHO carbon storage processes also require co-substrates such as oxygen, nitrate, or nitrite, and the bulk liquid co-substrate concentrations must be high in order to avoid a diffusion-limited growth regime and the resultant filamentous outgrowth. For pulse-fed reactors with aerobic feeding or static feeding immediately followed by aeration, aerobic phase DO concentrations near saturation have been necessary to achieve granular sludge growth. Commonly applied superficial gas velocities may be near 2.0 cm/s (Liu et al., 2003c; Wang et al., 2009), but lower-end superficial gas velocities are near 1.0 cm/s for both OHO (McSwain et al., 2004b; Su and Yu, 2005) and NDN-OHO (Wang et al., 2012) granule types. Of these granule types, OHO granules have been more frequently investigated, and high DO concentrations (>5 mg/L) have been required to prevent outgrowth driven by severe oxygen diffusion limitations in the granule (Mosquera-Corral et al., 2005; Pronk et al., 2015a; Sturm and Irvine, 2008).

Mosquera-Corral et al. (2005) specifically applied the same shear regime as quantified by superficial gas velocity of 2.5 cm/s to OHO reactor systems at 100% and 40% oxygen saturation, corresponding to DO concentrations of approximately 9.2 and 3.7 mg/L, respectively, at a 20°C process temperature. For otherwise equivalent loading and operating conditions, stable granules were grown at 100% oxygen saturation, but granule structure deteriorated into unstable filamentous morphology upon transition to 40% oxygen saturation, highlighting the importance of maintaining a high DO concentration to avoid diffusion-limited growth conditions for OHO granules, with oxygen being the limiting substrate. The lower DO concentration was obtained without lowering the total superficial gas velocity by recycling the reactor headspace off-gas and adding fresh air as required. Granules were also not obtained in the subsequent reactor restart at a DO concentration of 3.7 mg/L.

Though OHO granules have been primarily grown at the lab scale with high aeration intensities and DO concentrations (Moy et al., 2002; Tay et al., 2002b), a study by Ni et al. (2009) reported the successful growth of granules in a pilot-scale SBR treating municipal primary effluent with operation at a low DO concentration. A static pulse feeding regime was followed by on-off aeration with the DO concentration

controlled to approximately 2.0 mg/L using fine bubble aeration. The authors did not report the aeration sparge rates to allow a determination of the aeration intensity and superficial gas velocity applied. Phosphorus removal or microbial community analysis was not performed such that the potential growth of PAO and/or GAO could be elucidated. Limited simultaneous nitrification-denitrification, below what would be expected for NDN-PAO granules, was apparent based on an effluent NO_3-N concentration of approximately 30 mg/L for an influent NH_3-N concentration of approximately 38 mg/L. The apparent OHO granules were relatively small (average diameter 0.5 mm), which may have been due to the relatively low influent total COD concentration of 95–200 mg/L and/or the low DO concentration.

Because NIT granule systems, by definition, do not contain significant influent bCOD, there is no need for a specific feeding regime to drive rbCOD diffusion and carbon storage processes. Slow growing nitrifying organisms are inherently selected due to the nature of the influent characteristics, allowing lower shear rates to be applied to achieve granular sludge growth. For example, nitrifying granules, which were originally seeded from a granular sludge inoculum, were maintained on synthetic inorganic wastewater at a superficial gas velocity of 0.5 cm/s (Vázquez-Padín et al., 2010b). Additionally, nitrite-oxidizing granules were grown in an on/off aeration regime at unspecified superficial gas velocity to control DO between 2.8 and 3.3 mg/L (Ni et al., 2011). Though NIT granules have been grown in continuously fed SBRs (Lopez-Palau et al., 2011; Vázquez-Padín et al., 2010b), these systems were characterized by residual NH_3-N concentrations greater than 20 mg/L. Therefore, in these systems the nitrifying organisms were likely growing in a growth-rate-limited regime. However, the growth of NIT granules in a laboratory continuously fed reactor with a bulk liquid NH_3-N concentration less than 10 mg/L (Tsuneda et al., 2003) suggests that an elevated bulk liquid NH_3-N concentration is not necessary for NIT granule growth, as compared to granules dominated by heterotrophic organisms where exposure to high rbCOD concentrations appears to be a critical aspect of heterotrophic granule formation.

3.4 Effect of Operating Conditions on Granular Sludge Growth

Fundamental selective pressures for obtaining aerobic granular sludge were discussed in the previous section. Building on that foundation, this section reviews the impact that different operating conditions have had on granular sludge growth and characteristics.

3.4.1 Wastewater Characteristics

Aerobic granules have been cultivated using a variety of industrial, municipal, and synthetic wastewaters. In synthetic wastewaters using a sole carbon source, acetate has been most frequently used (Beun et al., 2000a, 2001; Liu et al., 2003c). Other sole carbon sources have included glucose, ethanol, molasses, sucrose, starch, and phenol (Adav et al., 2007a; Beun et al., 1999; de Kreuk et al., 2010; Morgenroth et al., 1997; Shi et al., 2009; Wang et al., 2005b; Yuan et al., 2012). Studies with complex synthetic wastewaters have used blends of the above carbon sources or included peptone and meat extract (Jiang et al., 2003; McSwain et al., 2005; Moy et al., 2002; Tay et al., 2004a; Zhang et al., 2005; Zheng et al., 2005). Aerobic granules have also been shown to degrade high concentrations of aniline and nitrobenzene as sole carbon and nitrogen sources (Xiang et al., 2009; Zhao et al., 2011a).

Aerobic granular sludge growth has been reported for treating municipal wastewater (de Kreuk and van Loosdrecht, 2006; Liu et al., 2007; Ni et al., 2009) and industrial wastewater from malting (Schwarzenbeck et al., 2004), dairy processing (Arrojo et al., 2004; Giesen et al., 2013; Schwarzenbeck et al., 2005), soybean processing (Su and Yu, 2005), fish canning (Figueroa et al., 2008), palm oil mill

effluent (Abdullah et al., 2011), landfill leachate (Di Iaconi et al., 2009), abattoir (Yilmaz et al., 2008), papermaking (Hailei et al., 2006), and textiles (Lotito et al., 2014).

Nitrifying granules have been cultivated on synthetic wastewaters without organic carbon in sequencing batch as well as upflow fluidized bed reactors. A longer cultivation time may be required for the development of nitrifying-only granular sludge on these waste streams lacking organic carbon (Tsuneda et al., 2003).

Biodegradation of particulate COD in aerobic granular sludge reactors occurs by sorption to the granule surface, where hydrolysis occurs to release soluble biodegradable COD. The soluble substrate released at the surface increases the substrate gradient and increases the potential for irregularly shaped granules with filamentous or finger-like outgrowths (de Kreuk et al., 2010), as illustrated in Figure 3-3. Filamentous outgrowths have also been more commonly associated with carbohydrate-laden waste streams (de Kreuk et al., 2010; Tay et al., 2002b; Wang et al., 2005b). In excess, outgrowths of this nature may result in less-dense, lighter granules of poor settleability and ultimately may lead to granular reactor failure via biomass washout (Liu and Liu, 2006). For an anaerobic feeding regime, hydrolysis products can ultimately be stored by PAO and GAO and later slowly metabolized, improving granule structure and reducing the extent of outgrowths. For an aerobic feeding regime or the continued aerobic hydrolysis that may occur after incomplete anaerobic hydrolysis, products of hydrolysis will be immediately available for surficial growth, reducing granule stability and increasing the extent of outgrowths and bulk liquid suspended solids (Pronk et al., 2015a). Granules cultivated on particulate-laden wastes have exhibited more attached protozoa, which may be beneficial by filtering dispersed solids and improving effluent quality. On the other hand, they may also decrease settleability and reduce the amount of active biomass by predation (de Kreuk and Van Loosdrecht, 2004; Schwarzenbeck et al., 2004; Winkler et al., 2012c).

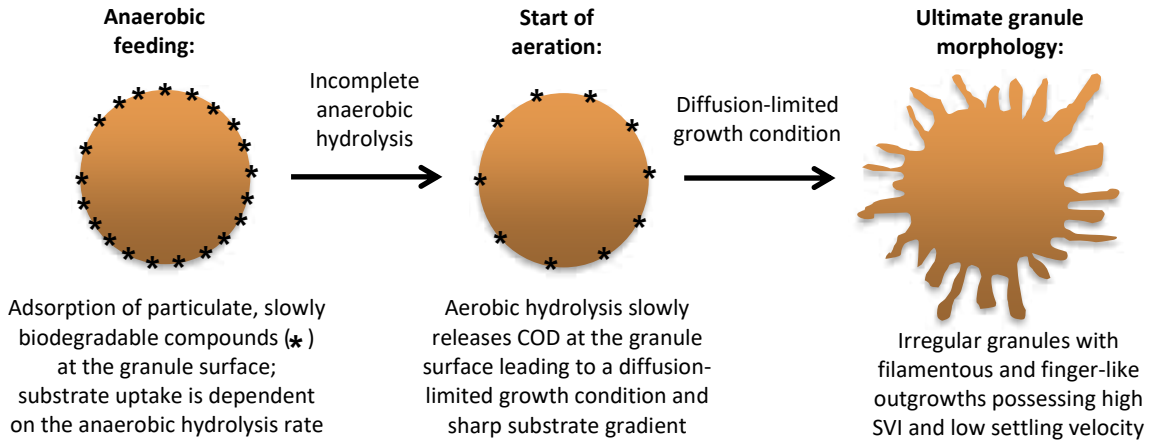

Figure 3-3. Surface adsorption and hydrolysis of particulate organic matter leading to irregular aerobic granule morphology. Adapted from Pronk et al. (2015a). Copyright Pronk et al. 2015.
Open access (http://link.springer.com/article/10.1007/s00253-014-6358-3/fulltext.html).

Multivalent cations, particularly Ca^{++}, have been shown to expedite granulation and enhance granule physical properties. Potential mechanisms by which multivalent cations provide these benefits may include 1) bridging of negatively charged functional groups, 2) enhancing the gelation of alginate, 3) charge neutralization, and 4) formation of precipitates serving as nucleation sites for microbial aggregation (Bruus et al., 1992; Gao et al., 2011a; McKinney, 1952). Compared to a control aerobic granular sludge reactor without Ca^{++}, addition of 100 mg/L Ca^{++} was shown to decrease granule cultivation time from 32 days to 16 days; increase polysaccharide content, integrity, and density; and improve granule morphology and SVI (Jiang et al., 2003). Similar findings were obtained when 10 mg/L

Mg^{++} was augmented to an aerobic granular sludge reactor influent containing Ca^{++} at only 2 mg/L (Li et al., 2009).

The impacts of metal salt and polymer coagulants have also been observed in aerobic granular sludge systems. An eight-day period of polyaluminum chloride dosing at high concentration (>400 mg/L) successfully reformed and restabilized crushed granules (Liu et al., 2014b). This granule reformation procedure was performed on a pulse-fed aerobic OHO granular sludge reactor after 90 days of operation, when granules began to disintegrate. Granule disintegration was attributed to inner core decay and lysis associated with large granule size (3.2 mm) and lack of substrate penetration to the granule core. Under a more normal granular growth condition, the addition of 2.5 mg/L polyaluminum chloride and 1.5 mg/L Chemifloc® polyelectrolyte flocculating agent were shown to have adverse impacts on the granular sludge quality (Val del Río et al., 2012). Granules grown in a control reactor without the coagulants had lower SVI values (40 mL/g vs. 80 mL/g), smaller diameters (2.3 mm vs. 5.0 mm), and smoother surfaces. A higher mixed liquor volatile suspended solids (MLVSS) concentration was maintained in the control reactor (7.9 g/L vs. 4.5 g/L) due to a lower effluent suspended solids concentration. The granules grown with coagulant addition had a lower maximum specific oxygen utilization rate normalized to VSS, which was likely due to the adsorption of the coagulants on the granule surface and greater DO diffusion limitation caused by the larger size. Despite the lower bioactivity in the reactor with coagulant addition, the reactor systems had similar COD and N removal efficiencies.

3.4.2 pH

Most aerobic granular sludge reactors have been operated in pH range of 6-8, and negative effects of pH have only been reported at extreme high and low values. Tests performed on aerobic granules and associated Granulan EPS extracts (Seviour et al., 2009b; Seviour et al., 2009a) suggest that granule disintegration via solubilization of the dominant structural exopolysaccharide would occur above pH 9. Similarly, alginate-like EPS (see Section 2.7) is stable over a wide range of pH values if adequate divalent cations, particularly Ca^{++}, are available to crosslink constitutive blocks of the alginate polymer (Lin et al., 2010). At low pH (3–5), fungal rather than bacterial granules have been observed. Fungal granules emerged more rapidly than bacterial granules and exhibited a loosely structured filamentous morphology that was prone to erosion by turbulence (Williams and de Los Reyes III, 2006; Yang et al., 2008).

3.4.3 Temperature

Process temperature may range from 8 to 30°C in most municipal and industrial wastewater treatment processes. Aerobic granules have typically been grown and studied in controlled environments near 20°C (Show et al., 2012), but have been cultivated from flocculent seed sludge between 8 and 56°C (de Kreuk et al., 2005c; Zitomer et al., 2007) and maintained at process temperatures as low as 5°C after earlier granulation (see Section 7.2.4). As shown in Table 3-1, granules cultivated in parallel laboratory synthetic wastewater-fed systems at 25, 30, and 35°C with slug feeding followed by continuous aeration showed similar granule sizes of 0.44 to 0.57 mm, settling velocities of 27 to 32 m/hr, and SVIs of 28 to 35 mL/g after 42 days from start-up with flocculent seed sludge (Song et al., 2009). Although reactors operated at higher temperatures (>30°C) may be more prone to excess filamentous growth that contributes to a weaker granule structure (Ebrahimi et al., 2010), a mixture of flocculent biomass and aerobic granules up to 1.9 mm in diameter were cultivated at a thermophilic temperature of 56°C (Zitomer et al., 2007).

Table 3-1. Size, Settling Velocity, and SVI of Granules Grown in Bench-Scale Pulse-Fed Aerobic SBR Reactors at 25, 30, and 35°C

Temperature (°C)	Granule Size (mm)	Settling Velocity (m/hr)	SVI (mL/g)
25	0.57	28	35
30	0.44	32	28
35	0.52	27	35

Source: Song et al., 2009.

Temperature does not appear to affect granule formation and stability as long as an appropriate cultivation and operation strategy is followed. As in flocculent activated sludge systems, a higher SRT is needed for granular sludge systems to account for slower biological reaction rates at lower temperatures. The longer SRT is achieved by operating at a higher MLSS concentration and/or greater volume. A granular sludge system start-up strategy at lower temperatures must assure sufficient reaction times due to reduced kinetics to avoid conditions that would be detrimental to granule formation, such as the presence of rbCOD during aerobic periods (de Kreuk et al., 2005c). The increase in water viscosity at lower temperatures can have a significant impact on liquid-solids separation. A two-fold reduction in granule settling velocity was measured for the same granules when water temperature was decreased from 40 to 5°C (Winkler et al., 2012a).

3.4.4 Dissolved Oxygen Concentration

DO concentration alone does not appear to be a controlling factor for granulation, as aerobic granules have been cultivated at DO concentrations near 1.8 mg/L (de Kreuk and Van Loosdrecht, 2004; Ni et al., 2009), similar to DO concentrations employed in full-scale bioreactors, as well as at higher DO concentrations of 5 to 9 mg/L (Liu et al., 2005c; Mosquera-Corral A. et al., 2005; Tay et al., 2004a). At a given DO concentration, other operating conditions such as aeration shear intensity and rbCOD uptake regimes are also important in affecting granular sludge growth. Filamentous outgrowth as described in Section 3.2.5 is related to the bulk liquid rbCOD concentration during aeration following the feed period and the adequacy of the bulk liquid DO concentration in preventing diffusion-limited growth conditions, as described in Section 3.2.5. The use of a greater aeration intensity to provide oxygen as a co-substrate for aerobic rbCOD uptake by OHOs and/or provide greater shear in order to combat filamentous outgrowth on granules can result in a high-DO operation. For optimum simultaneous nitrification and denitrification in granular sludge systems, low DO conditions are preferable. In this case, excess heterotrophic growth on the outer layers of the granule from available bulk liquid rbCOD is undesired due to its oxygen consumption and decreased nitrification rates as slower-growing nitrifiers are out-competed by faster-growing heterotrophs for space in the outer oxygen-penetrated layer of the granule. A sufficient anaerobic contact during feeding and a sufficient PAO/GAO population can help to minimize this possible operating condition (de Kreuk and Van Loosdrecht, 2004).

3.4.5 Salinity

Stable aerobic granular sludge performance has been demonstrated for saline, synthetic and industrial fish canning effluents in the range of 30–33 g/L NaCl, although increased salt content particularly reduced the activity of nitrite-oxidizing bacteria (NOB; Bassin et al., 2011b; Figueroa et al., 2008). An increase in salt concentration from 5 to 40 g/L NaCl corresponded to an approximately 10% decrease in granule settling velocity, primarily attributable to water density (Winkler et al., 2012a). Furthermore, granules exhibited floatation when suddenly transferred from the original low-salinity environment to high-salinity environments. Original settling behavior was recovered after a 15-minute pre-incubation period in the saline solutions.

3.4.6 Free Ammonia and Free Nitrous Acid

High concentrations of free ammonia (FA) and free nitrous acid (FNA) have been shown to inhibit ammonia-oxidizing bacteria (AOB) and NOB in flocculent activated sludge systems, and thus the effects of these compounds on aerobic granular sludge are also of interest. Inhibition due to elevated FA and FNA levels is mainly a concern in the treatment of high ammonia concentration wastewaters from anaerobic digestion solids dewatering, landfill leachate, some industrial wastewaters, and animal feeding operations. However, the operation of aerobic granular sludge reactors involving high fill fractions and rapid pulse feeding or contacting settled sludge with undiluted influent wastewater exposes biomass to higher concentrations than would otherwise be associated with typical activated sludge or SBR operations. For typical domestic wastewaters, the pH and ammonia concentration would be expected to be low enough that FA inhibition would not be expected for higher fill fractions, but for wastewaters with higher ammonia concentration, the potential for FA inhibition would be greater for aerobic granular sludge systems with a high fill fraction.

Under elevated volumetric ammonia loading rates (generally >0.8 kg NH_3-N/m^3/d) and/or rapid feeding regimes, nitrite accumulation in aerobic granular sludge reactors may occur due to NOB inhibition by FA and/or FNA (Lemaire et al., 2008a; Lopez-Palau et al., 2011; Vázquez-Padín et al., 2010b). The conditions associated with NOB inhibition in these granular sludge reactor systems are within the ranges reported for NOB inhibition in flocculent activated sludge (Tchobanoglous et al., 2014).

In some cases, the failure of granular sludge formation has been associated with a high FA concentration. In one laboratory study (Yang et al., 2004), five parallel granular sludge systems at 25°C were fed an acetate-based synthetic wastewater (1000 mg COD/L) in an aerobic pulse-feeding mode with feed NH_3-N concentrations from 50 to 300 mg/L. Reactor pH was reported to be 8.5 after COD uptake was complete, at approximately 60 minutes of the 332-minute aerobic react period. As a consequence of limited nitrification in the reactors, resultant FA concentrations during the aerobic period ranged from 2.2 to 39.6 mg N/L. Granules failed to form in reactors with FA concentrations greater than 23.5 mg N/L. Decreased hydrophobicity and polysaccharide production was observed with the higher FA conditions associated with the failed granular sludge formation. In another study (Wang et al., 2007), granules failed to form in a reactor in which the FA concentration was estimated to be as high as 42 mg N/L. This laboratory study involved parallel reactors with aerobic pulse feeding of ethanol-based synthetic waste at 500 mg COD/L concentration and influent NH_3-N of 50 or 200 mg/L. Reactor temperature was 20 to 30°C and pH was 7.5 to 8.5. Granules failed to form in the reactor fed the higher NH_3-N concentration and possessing the higher FA concentration during the aerobic period, but rapidly formed for the lower feed NH_3-N concentration. In a third reactor, the feed NH_3-N concentration was gradually increased from 50 to 200 mg/L as the corresponding nitrification capacity increased, which averted an elevated FA concentration and resulted in a stable granular sludge operation. This result shows the importance of an appropriate balance between the reactor loading and nitrification capacity, and of managed operating strategies to transition to higher loads.

3.4.7 Organic Loading Rate and Food-to-Microorganism Ratio

Aerobic granular sludge has been successfully cultivated across a wide range of conditions, as shown by the granular sludge SBR systems that are presented Table 3-2 in order of increasing nominal food to microorganism ratio (F/M ratio) based on the amount of COD fed each day. These systems were selected because they represent a range of domestic and synthetic wastewaters, reactor operating conditions, influent COD concentrations, organic loading rates (OLRs), and F/M ratios. Granular sludge was grown in these reactors at a range of nominal F/M ratios from 0.1 to 1.3 kg COD/kg VSS-day. A

common aspect to all systems was that the instantaneous F/M associated with influent feeding was higher than the nominal F/M and thus exposed biomass to the high rbCOD concentrations favorable for granule growth, as discussed in Section 3.2.2. These instantaneous F/M ratios, based on the amount of COD added and actual feeding time, are also presented in Table 3-2. In Systems A, C, and H with slow anaerobic upflow feeding through the settled sludge bed, the effective instantaneous F/M ratio for biomass contacting influent wastewater at the very bottom of the settled sludge bed will be even higher than that calculated based on the total mixed liquor.

For low to moderate strength wastewaters (Systems A–D and H), with influent COD concentrations typically less than 500 mg/L, the nominal OLR was limited to less than 2.0 kg COD/m^3-day by practical limits on the hydraulic loading rate and cycle times. In these instances, the nominal F/M ratio was generally in the range of 0.1 to 0.3 kg COD/kg VSS-day for typical granular sludge reactor MLVSS concentrations in the range of 6 to 12 g/L. However, granular sludge growth can also occur at much higher nominal F/M ratios, as illustrated by System H, which had an influent wastewater COD concentration of 330 mg/L and nominal F/M ratio of 1.3 kg COD/kg VSS-day due to a relatively low MLVSS concentration of 1.6 g/L in the system. The increasing nominal OLR and F/M ratios in Systems E–G correspond to increasing influent COD and demonstrate that aerobic granules can be grown in pulse-fed aerobic reactors at nominal OLRs and F/M ratios as high as 19.5 kg COD/m^3-day and 1.3 kg COD/kg VSS-day, respectively. It appears that the F/M ratio is not a controlling factor in granular sludge selection.

Table 3-2. Summary of Range of Organic Loading Rates and Food to Microorganism Ratios Experienced for Aerobic Granular Sludge Sequencing Batch Reactors

System	Wastewater (WW)	Feed COD (mg/L)	OLR 24 hr basis (kg COD/m^3-day)	MLVSS (g/L)	F/M Ratio 24 hr basis (kg COD/kg VSS-day)	F/M Ratio Feed time basis (kg COD/kg VSS-day)
A	Domestic WW	350–1170	0.6–3	6–10	0.1–0.3	0.4–1.2
B	Domestic WW	<200	<1	6.8	<0.15	< 7.2
C	Acetate	400	1.8	12	0.15	0.45
D	Acetate	500	1.5	8.4	0.18	8.6
E	Glucose + Peptone	800	2.4	8	0.30	0.8
F	Acetate	1000	3	9.5	0.32	15
F	Acetate	2000	6	11.2	0.54	26
F	Acetate	3000	9	12.3	0.73	35
G	Acetate	5550	16.7	13.5	1.2	58
G	Acetate	6470	19.5	15.5	1.3	62
H	Domestic WW	330	2.1	1.6	1.3	3.9

Reactor System Descriptions:
A: Full-scale Nereda® reactors. Slow anaerobic bottom feed. Aerobic react. DO = ~2.0 mg/L. 4 hr cycles with 1 hr feed assumed.
B: Pilot-scale. Pulse-fed aerobic. DO = 2.0 mg/L by on-off aeration. Temperature range not specified.
C: Lab-scale. Slow anaerobic bottom feed. Aerobic react. Temperature = 20-30°C. DO = 1.8 mg/L.
D: Lab-scale. Four reactors at different influent COD. Pulse-fed aerobic. Temperature = 25°C. DO not specified.
E: Lab-scale. Slow static fill from top. Aerobic react. Temperature and DO not specified.
F: See system D above.
G: Lab-scale. Pulse-fed aerobic, with stepwise increases in COD. Temperature and DO not specified.
H: Lab-scale. Slow anaerobic bottom feed. Aerobic react. Temperature = 20°C. DO >8 mg/L. 80% MLVSS assumed.

Sources: A (Giesen, 2015; van Haandel and van der Lubbe, 2012); B (Ni et al., 2009); C (Bassin et al., 2012a); D (Liu et al., 2003c); E (McSwain et al., 2005); F (Liu et al., 2003c); G (Adav et al., 2010); H (de Kreuk and van Loosdrecht, 2006).

In System G, an increase in influent acetate COD to 7070 mg/L resulted in granule disintegration. Experiments on bacteria isolated from granules in this system showed that isolates did not grow and lost capacity for EPS production in a critical acetate COD range of 3000 to 4000 mg/L. For the influent COD of 7070 mg/L and 50% fill fraction in this system, the start-of-cycle COD would fall within the critical range where loss of activity was observed. Therefore, depending on the substrate type and reactor design and operation, substrate inhibition may limit the maximum OLR and influent COD concentration suitable for aerobic granular sludge growth.

3.4.8 SRT Control and Solids Wasting Strategies

There is very little work on finding optimal SRTs for aerobic granular sludge, and the systems are generally operated at SRTs greater than that needed for nitrification as a result of the high reactor solids concentrations. In laboratory reactors with short settle and rapid decant periods, effluent TSS is typically high (>50 mg/L) and governs the reactor SRT in the absence of additional controlled biomass wasting, which is often not performed.

The difference in settling velocities between PAOs and GAOs has been exploited by selective wasting of the slower-settling GAO-dominant granules in the upper portion of the settled sludge bed for active SRT control to improve EBPR performance by retaining the faster-settling PAO granules (Bassin et al., 2012a; Winkler et al., 2011). The settling velocities of PAO-enriched granules are faster than those of GAO-enriched granules of similar size because of a higher density from a greater inorganic content as a result of biologically stored phosphorus and possibly calcium phosphate precipitates within the granule (see Section 2.4; Winkler et al., 2013a). The degree of inorganic accumulation in PAO-enriched granules is indicated by their lower VSS/TSS ratio. The preferential wasting of GAOs was demonstrated in an anaerobic-aerobic lab granular sludge SBR operated at a DO of 2.0 mg/L and total SRT of 30 days under conditions that would tend to favor GAO dominance (Lopez-Vazquez et al., 2009). These conditions were a process temperature of 30°C, acetate as the sole COD source, and a pH controlled between 6.8 and 7.2. A change to selective wasting from the top of the settled sludge bed improved the P removal efficiency from 60% to above 98%, with the enrichment of PAO confirmed by fluorescence in-situ hybridization (FISH). Subsequent reversal of the wasting protocol involving wasting from the bottom of the sludge bed resulted in the opposite trend of GAO enrichment and a decline in P removal efficiency.

A potential adverse impact of excessively long SRTs in EBPR granular sludge systems is a reduced phosphorus removal efficiency as a consequence of less phosphorus being removed from the system with less waste sludge production, as seen for flocculent EBPR systems. Near-complete removal of 20 mg PO_4-P/L has been achieved in aerobic granular sludge systems operated in the range of 20 to 30 days total SRT with influent acetate COD of 400 mg/L, suggesting that excellent P removal can be maintained in aerobic granular sludge systems at relatively long SRTs (Bassin et al., 2012a; Winkler et al., 2011) with sufficient COD. The extent of biologically mediated phosphorus precipitation in these systems was not determined (Winkler et al., 2013a).

3.5 Granular Sludge Reactor Start-up

Start-up of a granular sludge reactor with granular sludge seed from an active system or stored seed (see Section 2.8) is the preferred approach, but this is currently not a common occurrence due to the availability of granular seed sludge and potential transportation constraints. Start-up of a granular sludge reactor without a sufficient seed source requires time for granule aggregation, selection, and accumulation to the levels necessary to meet treatment objectives. Indications of successful progress towards establishing a granular sludge system are having 80 to 90% of the MLSS mass at a size of 0.212

mm or greater, an SVI_{30} of less than 60 mL/g, and an SVI_5/SVI_{30} of less than 1.3. Operational factors that have been appropriately adjusted to accelerate start-up include organic loading rate, calcium concentration, selective settling pressure, aeration intensity, and famine duration within the feast-famine regime (Gao et al., 2011b).

Another approach used to facilitate start-up has involved using crushed granules as part of the new reactor inoculum. The effect of using crushed granules as part of the inoculum was illustrated in lab-scale anaerobic-aerobic-anoxic reactors treating abattoir wastewater at 20-23°C (Pijuan et al., 2011). In the study, parallel reactors were inoculated with a mixture of manually crushed granules and flocculent sludge. Although the morphology of manually crushed granules was not described, it is likely that these solid particles were larger than typical activated sludge flocs. In a reactor that was inoculated with a 50% MLSS mass fraction of crushed granules, granulation time was only 18 days, whereas granulation time was 133 days for a reactor inoculated with a 5% MLSS mass fraction of crushed granules. In contrast to reactors inoculated solely with flocculent sludge, reactors inoculated with crushed granules did not experience substantial biomass loss during the granulation process. A similar study (Coma et al., 2012) using anaerobic-aerobic-anoxic lab-scale reactors to treat domestic wastewater at 20-23°C found that a 10% mass fraction of crushed granules in the seed sludge did not substantively decrease granulation time compared to the parallel reactor lacking crushed granules in the inoculum (~60 days in both systems), but did exhibit more stable nitrogen and phosphorus removal performance and less biomass loss during start-up.

Successful start-up in less than 30 days using flocculent seed sludge from a biological nitrogen and phosphorus removal system was demonstrated in a laboratory SBR treating propionate-based synthetic wastewater at 20°C with slow anaerobic bottom feeding followed by aeration (Lochmatter and Holliger, 2014). Process conditions employed by the authors in this system included 1) alternating DO control between 0.5 and 4.6 mg/L, 2) gradual decreases in settling time from 30 to three minutes to avoid excessive biomass washout, 3) adaptive feed loading increases that ensured influent bCOD was consumed in the anaerobic period prior to aeration, and 4) a pH between 7.0 and 7.3, or near neutral conditions.

At larger scale systems treating domestic wastewaters, start-up time has been as short as about six months, as it was at the Ede pilot plant, where the inoculum included a 10% MLSS mass fraction of intact, non-crushed granules. In other systems not receiving a granular sludge inoculum, start-up time may be closer to one year. Additional details for these systems are discussed in Section 7.2.2.

Process temperature is an important consideration in aerobic granular sludge reactor start-up. Although microbial growth rates are faster at higher temperatures, which is less favorable for the formation of dense biofilms, hydrolysis and substrate uptake kinetics are faster at higher temperatures. The benefit of the latter may override the former, as the greater hydrolysis and faster substrate uptake may maximize PAO or GAO internal carbon storage and thus minimize the amount of rbCOD present in the bulk liquid and slowly biodegradable particulate COD sorbed to the biofilm surface in the aerobic phase. The presence of sorbed biodegradable particulate COD on the granule surface during aerobic conditions promotes granule outgrowths and lower density and settleability (de Kreuk et al., 2005c, 2010).

3.6 Granule Instability and Disintegration

Long-term maintenance of stable aerobic granules is a requisite for successful full-scale treatment applications. Therefore, granule instability and disintegration must be avoided. Granule instability and disintegration resulting in the loss of granular sludge is primarily associated with filamentous or finger-like outgrowths leading to a large, loose, fluffy, filamentous morphology of low density (Figure 3-4). This scenario can lead to reactor failure due to solids washout from high effluent solids, poor settleability, and weakened structural integrity (McSwain et al., 2004a; Mosquera-Corral et al., 2005; Tay et al., 2002d).

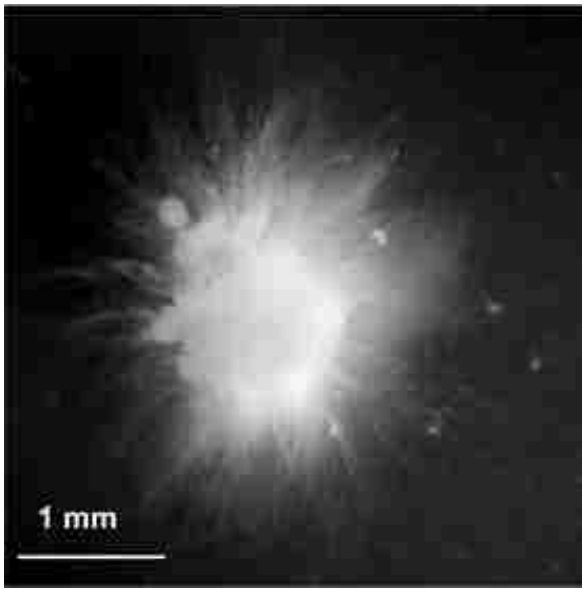

Figure 3-4. Representative morphology of a filamentous aerobic granule.
Reprinted from Weissbrodt et al. (2012). Copyright 2012 Weissbrodt et al.
Open access (http://journal.frontiersin.org/article/10.3389/fmicb.2012.00332/full).

Diffusion-limited growth conditions, as discussed in Section 3.2.5, cause detrimental filamentous outgrowths in aerobic granules and may be manifested in a variety of scenarios (Pronk et al., 2015a). Diffusion limited growth and filamentous outgrowths may occur due to 1) slow feeding of rbCOD under aerobic conditions (McSwain et al., 2004a), 2) the presence of residual rbCOD during aeration following anaerobic feeding (de Kreuk et al., 2005c), or 3) production of rbCOD from the aerobic hydrolysis of slowly biodegradable polymeric substances sorbed to the granule surface (de Kreuk et al., 2010). In these cases, low substrate concentrations result in diffusion-limited growth conditions, and only the outermost biomass layer has access to substrate. The proliferation of filamentous growth in these cases is in agreement with kinetic selection theory, whereby at low substrate concentrations, filamentous organisms have a competitive advantage and can flourish in complete-mix activated sludge systems where substrate is continuously available at low concentrations (Chiesa and Irvine, 1985; Chudoba, 1985). In pulse-fed aerobic reactors, oxygen diffusion limitations associated with operation at an insufficient bulk liquid DO concentration, well below DO saturation, have been implicated in granular sludge reactor failure by excessive filamentous outgrowths (Mosquera-Corral et al., 2005; Pronk et al., 2015a; Sturm and Irvine, 2008). Granular growth deterioration and failure was demonstrated for an initial stable, pulse-fed aerobic system by a stepwise change in DO concentration from 9.2 to 3.7 mg/L while maintaining the same system shear (Mosquera-Corral et al., 2005). The lower DO concentration was obtained without lowering the total gas sparging rate and shear by recycling the reactor headspace off-gas and adding process air as required. In addition, granules were not obtained in a subsequent reactor restart at a DO concentration of 3.7 mg/L with the same pulse-fed conditions.

Another process contributing to granule breakup is fragmentation caused by a change in operation that leads to starvation and decay at the granule core due to limited substrate penetration. The effect of granule fragmentation can be observed by a change to irregularly shaped granules and the accumulation of smaller granular debris particles in the sludge matrix (Figure 3-5). The granule integrity is weakened due to anaerobic degradation of granule core EPS when the substrate or electron acceptor (oxygen or NO_x) does not penetrate at sufficient granule depth (Adav et al., 2009a). As granule size increases, pulse-fed aerobic systems become more prone to this condition (Moy et al., 2002; Toh et al., 2003). Granule fragmentation and granule filamentous outgrowth can occur together for aerobic pulse-fed systems with large granule sizes when operated with a low bulk liquid substrate concentration. This can result in a higher SVI value and higher effluent suspended solids concentrations caused by debris from granule fragmentation (Pronk et al., 2015a). It should be noted that some amount of granule fragmentation and reformation occurs with normal granular sludge growth (Verawaty et al., 2013).

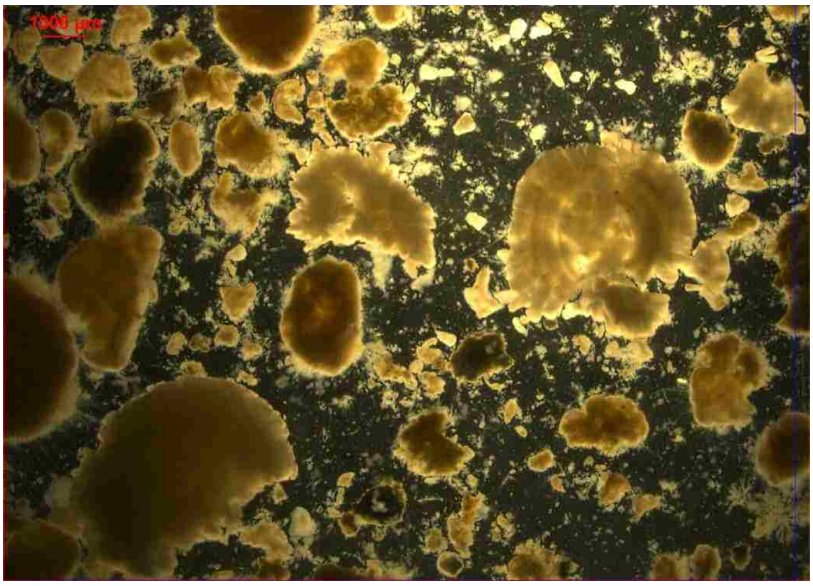

Figure 3-5. Granules grown on acetate in a pulse-fed aerobic reactor exhibiting fragmentation, weakened structure, and non-granular debris from breakup.
Scale bar = 1 mm.
Courtesy of Mario Pronk, Delft University of Technology Department of Biotechnology. Copyright 2016 Mario Pronk.

3.7 Preference for BNR Granules

For BNR requirements, the NDN-PAO granular sludge system is the system of choice. Even without the need for phosphorus removal it may also be favored over an OHO or NDN-OHO granular sludge system in view of the concerns presented above about the potential effect of bulk liquid substrate and DO concentrations on filamentous outgrowth and granular disintegration for OHO and NDN-OHO granular sludge systems. For the NDN-PAO system, all or most of the rbCOD can be taken up under a slow anaerobic feeding condition with substrate stored by slow-growing PAO/GAO in the inner portion of the granule. The intracellular carbon storage products are then oxidized by nitrite and/or nitrate produced by nitrifying organisms in the outer aerobic layer of the granule during the aerobic phase. The oxygen diffusion limitations and/or inner core decay noted with aerobic pulse-fed systems are minimized. Granular sludge growth and maintenance can be more stable for PAO-type granules under practical wastewater treatment conditions of varying influent loadings compared to OHO-type granules subject to varying bulk liquid substrate concentrations. Anaerobic hydrolysis of particulate biodegradable COD sorbed to the granule surface can also occur with the PAO-type granules so that the amount of rbCOD released from subsequent aerobic hydrolysis is minimized.

CHAPTER 4

Microbial Diversity in Aerobic Granular Sludge

Aerobic granular sludge contains a variety of microorganisms, and the predominant genera and species may vary as a function of seed source, wastewater characteristics, and selective pressures due to reactor design and operating conditions (Li et al., 2008; Liu et al., 2010). It is reasonable to assume that the predominant organisms share a common physiological function requisite for biofilm formation, which is an ability to produce exopolysaccharides and proteins that facilitate adhesion to other cells (Weissbrodt et al., 2013a). Similar bacteria found in flocculent activated sludge systems for BNR have been observed in granular activated sludge BNR systems. Experiences with PAOs, GAOs, other heterotrophs, AOB, and NOB in granular sludge are reviewed here. Section 4.5 discusses the type and spatial location of bacteria in a granular sludge accomplishing biological nitrogen removal and enhanced biological phosphorus removal.

4.1 Phosphorus Accumulating and Glycogen Accumulating Organisms

PAOs and GAOs will dominate the granule heterotrophic community in systems with an anaerobic reactor feed period, with sufficient acetate, propionate, and/or easily fermentable carbon-containing wastes, and a subsequent anoxic and/or aerobic period of sufficient duration (Lemaire et al., 2008b; Wang et al., 2015). PAOs and GAOs are able to use the carbon stored in the anaerobic contact period for denitrification (Mino et al., 1998). In a bench-scale anaerobic-aerobic reactor fed acetate as the sole organic carbon source and operated at 20 to 22°C, PAOs and GAOs accounted for up to 74% of the granule microbial population with the PAO abundance being 1.8 times the GAO abundance (Lemaire et al., 2008b). In a different bench-scale anaerobic-anoxic-aerobic reactor with propionate as the sole organic carbon source and operated at 19°C to 21°C, PAOs and GAOs accounted for up to 85% of the granule microbial population. In this case, there were three times as many PAOs as GAOs (Wang et al., 2015).

High PAO domination over GAO is desired in an NDN-PAO granular sludge system because it results in the most efficient use of available carbon for both phosphorus and nitrogen removal instead of only denitrification by the GAOs. Similar factors known to favor PAO growth over GAO growth in flocculent activated sludge systems apply to granular sludge systems (Mino et al., 1998; Lopez-Vazquez et al., 2009). These are lower temperature, higher pH, and the coexistence of acetate and propionate in the feed (Lopez-Vazquez et al., 2009; Weissbrodt et al., 2013b). An additional technique to favor PAOs over GAOs that is applicable only to granular activated sludge is the selective wasting of slower-settling GAO-dominant granules at the top of the granular sludge bed after settling with the retention of more of the faster-settling PAO-dominant granules (Winkler et al., 2011; see Section 3.4.8). A later study by Bassin et al. (2012a) showed that this selective wasting method improved EBPR performance in granular activated sludge treatment.

Much more work has been done to identify specific PAOs for EBPR in flocculent activated sludge systems. The most commonly reported PAO for flocculent activated sludge systems has been *Candidatus Accumulibacter* in the *Rhodocyclus* group of the *Betaproteobacteria*. Other PAOs include *Dechloromonas*, also in the *Rhodocyclus* group, and organisms within the *Actinobacteria* phylum including those related to *Tetrasphaera* (Seviour and Nielsen, 2010). For granular activated sludge, *Accumulibacter* dominated in microbial analyses of a bench-scale anaerobic-aerobic granular sludge reactor operated at 20 to 22°C and fed acetate as the sole organic carbon source (Lemaire et al., 2008b)

and in another bench-scale anaerobic-aerobic-anoxic granular sludge reactor operated at 18 to 22°C and fed a mixture of abattoir wastewater and supplemental acetate (Yilmaz et al., 2008). In the latter study, *Actinobacteria* was present at only 4% of the total bacteria compared to 40% for *Accumulibacter*. The presence of *Actinobacteria* was attributed to their ability to assimilate amino acids but not acetate (Kong et al., 2005). After 6 months of operation to obtain mature granular sludge in an anaerobic-aerobic reactor on a volatile fatty acid (VFA)-based synthetic feed, Weissbrodt et al. (2013b) monitored granule microbial community changes over eight successive 50-day operational periods with variable pH, temperature, and ratios of acetate and propionate in the feed. Over the course of these operational periods, *Accumulibacter* were between 7 and 53% of the microbial community. *Tetrasphaera* were between 2 and 14% of the microbial community and always less abundant than *Accumulibacter*. With respect to GAOs in aerobic granular sludge, *Competibacter*-related GAOs were the main ones observed (Lemaire et al., 2008b; Weissbrodt et al., 2014).

The PAO and GAO populations selected in an NDN-PAO granular sludge system should be affected by the denitrification capabilities of PAOs and GAOs and the availability of NO_2-N and/or NO_3-N within the granule anoxic zone from nitrification in the outer layers of the granule during aeration. As summarized in Table 4-1, the denitrification capabilities of PAOs and GAOs vary, with some groups being able to fully reduce NO_3-N to N_2, and others being able only to reduce NO_3-N to NO_2-N (denitratation) or NO_2-N to N_2 (denitritation; McIlroy et al., 2014; Oehmen et al., 2010). The participation of both PAOs and GAOs in the reduction of NO_3-N to N_2 has been observed in bench-scale anaerobic-aerobic granular sludge systems at 20 and 30°C. Batch tests suggested that GAOs were primarily responsible for the reduction of NO_3-N to NO_2-N, and that a significant portion of the GAO-produced NO_2-N was used by PAOs capable of NO_2-N reduction but not NO_3-N reduction (Bassin et al., 2012b). A similar symbiotic relationship between PAOs and GAOs was observed in a bench-scale anaerobic-anoxic-aerobic granular sludge system at 20°C that was fed propionate in the anaerobic step as the sole carbon source and fed KNO_3 in the anoxic step (Wang et al., 2015). Allylthiourea (ATU) was added to inhibit nitrification in the aerobic step. Type II *Accumulibacter* PAOs represented approximately 70% of the total *Accumulibacter* PAOs present in this system. The importance of PAOs over GAOs for NO_2-N reduction was also shown for an anaerobic-aerobic-anoxic granular sludge reactor treating abattoir anaerobic pond effluent supplemented with acetate. At the end of the aeration step prior to the start of the anoxic step, the NO_2-N and NO_3-N concentrations were approximately 45 and 6 mg/L, respectively (Yilmaz et al., 2008). *Accumulibacter* PAOs were present, but *Competibacter* GAOs were not present above detection limits (Lemaire et al., 2008a). These results suggest that even though *Competibacter* subgroup 6 is possibly capable of NO_2-N reduction, as indicated in Table 4-1, it may not be able to compete well with *Accumulibacter* PAOs for NO_2-N under anoxic conditions.

Table 4-1. Summary of PAO and GAO Denitrification Capabilities Observed in Flocculent Enrichment Cultures

	NO_3 to NO_2	NO_2 to N_2	Reference(s)
PAO			
Accumulibacter type I	Y	Y	A, B
Accumulibacter type II	N	Y	A, C, D
Tetrasphaera	Y	N	E, F
Dechloromonas	Y	Unknown	G
GAO			
Competibacter subgroups 1,4,5	Y	N	H
Competibacter subgroups 3,7	N	N	H
Competibacter subgroup 6	Y	Y(1)	H
Defulviicoccus vanus cluster I	Y	N	I, J
Defulviicoccus vanus cluster II	N	N	I

(1) Only showed acetate uptake with NO_2 addition. No denitrification products measured.

References: A (Flowers et al., 2009); B (Lanham et al., 2011); C (Kim et al., 2013); D (Martin et al., 2006); E (Kong et al., 2005); F (Kristiansen et al., 2013); G (Kong et al., 2007); H (Kong et al., 2006); I (Burow et al., 2007); J (Wang et al., 2008b).

Based on the above information, NO_2-N is a key intermediate in biological nitrogen removal by PAOs in NDN-PAO granules that also contain GAOs. Elevated NO_2-N concentrations in the range of 5 to 10 mg/L have caused inhibition of PAO activity in flocculent activated sludge systems (Saito et al., 2004; Meinhold et al., 1999), and thus the effect of NO_2-N concentration on PAOs in granular sludge is of interest. Nitrite inhibition of phosphorus uptake was observed when NO_2-N concentration spiked to greater than 5 mg/L during normal reactor operation of an anaerobic-aerobic-anoxic granular sludge system at 21°C treating domestic wastewater with acetate addition and in anaerobic-aerobic batch tests (Coma et al., 2012).

A comparison of the effect of nitrite inhibition on granular and flocculent sludge was done in batch tests using sludge from the lab-scale anaerobic-anoxic-aerobic granular sludge system described in the preceding paragraph (Wang et al., 2015). Granular and flocculent activated sludge reactors were operated with the same organic loading and at a similar SRT and MLSS concentration of approximately 20 days and 4000 $\pm$ 200 mg/L, respectively. NO_2-N was added intermittently in batch tests with the two sludges at target NO_2-N concentrations of 5.0 and 10 mg/L to observe the effect of elevated NO_2-N concentration on phosphorus uptake after carbon feeding. For the granular sludge, the anoxic phosphorus uptake rate was lower by 28% for 10 mg NO_2-N/L versus 5.0 mg NO_2-N/L: the rate was 5.2 mg P/L-hr uptake versus 7.2 mg P/L-hr, respectively. For the flocculent sludge, the anoxic phosphorus uptake rate was lower by 62% for 10 mg NO_2-N/L versus 5.0 mg NO_2-N/L: the rate was 4.0 mg P/L-hr uptake versus 10.6 mg P/L-hr, respectively. The lower phosphorus uptake rate and lower impact of a higher bulk liquid NO_2-N concentration for the granular sludge is likely due to the granular morphology and diffusion limitations into the granular biofilm.

Inhibition effects of NO_2-N should also consider the pH concentration in addition to the NO_2-N concentration, because free nitrous acid (FNA) is considered the inhibitory compound. In a lab-scale anaerobic-aerobic *Accumulibacter* PAO flocculent sludge enrichment, the aerobic phosphate uptake rate

was reduced 50% at a FNA concentration of 0.5 x 10^{-3} mg/L (2.0 mg/L NO_2-N at pH 7) and fully inhibited at 6 x 10^{-3} mg/L (24 mg/L NO_2-N at pH 7; Pijuan et al., 2010).

Though the preceding data indicates the potential for adverse impacts on EBPR at an elevated NO_2-N concentration, such elevated NO_2-N concentrations may not be reached within a granule due to NO_2-N reduction as it diffuses into the inner granule volume from the outer nitrification zone. The bulk liquid NO_2-N concentration was less than 0.6 mg/L during the operation of the lab granular sludge and flocculent sludge reactors reported in the above by Wang et al. (2015), and other lab studies have not shown elevated NO_2-N concentrations. The maximum NO_2-N accumulation was approximately 1 mg/L in a granular sludge reactor system operated at 8, 15, and 20°C during different experimental periods, and the effluent NO_x-N consisted entirely of NO_3-N (de Kreuk et al., 2005c). Similar data were observed by Bassin et al. (2012a). In related work by the same research group, anoxic batch experiments with either NO_2-N or NO_3-N addition suggested that nitrogen removal occurred primarily by complete nitrification-denitrification (Bassin et al., 2012b).

Other lab studies with NDN-PAO granular activated sludge systems have shown higher effluent NO_2-N concentrations than those of the above work without any indication of reduced EBPR performance. NOB repression was indicated for a laboratory NDN-PAO granular sludge system that used intermittent aeration for simultaneous nitrification-denitrification following anaerobic feeding (Lochmatter et al., 2014). The effluent NO_x-N was mainly NO_2-N (up to 4 mg/L) with less than 1 mg/L NO_3-N. Ammonia and nitrite oxidation activity tests and qPCR indicated the repression of NOB growth. EBPR performance did not appear to be adversely impacted, which may have been due to the highest bulk liquid NO_2-N concentration being normally less than 4 mg/L. No work was performed to identify the PAO(s) or GAO(s) in this system. Higher bulk liquid NO_2-N concentrations were observed with a different NDN-PAO granular activated sludge system operation by Yilmaz et al. (2008). The system was fed anaerobic lagoon effluent with supplemental acetate, and the anaerobic feeding period was followed by aerobic and post-anoxic periods. Prior to the post-anoxic period, peak NO_2-N and NO_3-N concentrations reached approximately 43 and 8 mg/L, respectively. High influent NH_3-N concentration (220 mg/L average) and process pH (up to 8.6) likely contributed to NOB repression by FA inhibition. Despite the elevated NO_2-N concentration, excellent EBPR occurred in this system, with average influent and effluent PO_4-P concentrations of 33.6 and 0.6 mg/L, respectively. The PO_4-P concentration at the end of the anaerobic period was approximately 335 mg/L and was reduced to less than 10 mg/L by minute 255 of the 315 minute aerobic period, at which time the NO_2-N concentration had reached only 10 mg/L. Therefore, the majority of phosphorus uptake occurred prior to the much higher peak NO_2-N concentration at the end of the aerobic period. Results from FISH indicated that *Accumulibacter* accounted for approximately 90% of the PAOs with the balance being *Actinobacteria*. The results obtained in these studies suggest that high phosphorus removal efficiencies can be achieved in granular sludge systems with elevated NO_2-N concentrations at 10 mg/L or less.

4.2 Nitrifying Organisms

In view of the changing conditions of NH_3-N and DO concentrations in the bulk liquid and within the biofilm depth of nitrifying granules, a brief review of the types of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) is provided first. Typically, AOB belonging to the genera *Nitrosospira* or species *Nitrosomonas oligotropha* are dominant in engineered systems with low NH_3-N concentrations, while *Nitrosomonas europaea* or *Nitrosococcus mobilis* are found at high NH_3-N concentrations due to their lower substrate affinity (higher half-velocity coefficient; Limpiyakorn et al., 2007). Patterns in oxygen affinity among these AOB clusters are less clear (Limpiyakorn et al., 2013), though *Nitrosospira* has been negatively correlated with higher DO concentration (Wells et al., 2009) and found at high

abundance in a simultaneous nitrification-denitrification process with the DO concentration close to zero (Park et al., 2002). With high oxygen affinity and NH_3-N affinities and with half-velocity coefficients one to four orders of magnitude lower than those of AOB, ammonia oxidizing archaea (AOA) play a significant role in global nitrogen cycling and wastewater treatment under conditions where they can outcompete AOB at very low DO and/or NH_3-N concentration (Limpiyakorn et al., 2013).

NOB of the genus *Nitrospira* typically dominate flocculent activated sludge environments, where they can outcompete *Nitrobacter* under conditions of low DO concentration and low NO_2-N concentration (Noguera and Melo, 2006; Schramm et al., 2000). More recently, NOB of the candidate genus *Nitrotoga* have been found in tundra soils (Alawi et al., 2007) and domestic wastewater treatment sequencing batch and attached growth reactors at temperatures less than 16°C, along with *Nitrospira* as the sole NOB detected (Lucker et al., 2015).

In a pilot-scale anaerobic-aerobic granular sludge SBR treating complex domestic wastewater in which DO was controlled at approximately 2.0 mg/L, sequences of ammonia monooxygenase genes equally clustered with *Nitrosomonas* and *Nitrosospira* genera, whereas *Nitrosomonas* was dominant in the parallel full-scale flocculent sludge system (Winkler et al., 2013b). The coexistence of AOB in the granular sludge system was attributed to the availability of suitable niches at different depths of the granular biofilm, particularly with respect to low DO in the case of *Nitrosospira*. *Nitrosomonas* has been the dominant AOB in lab-scale granular sludge reactors with DO concentrations greater than 8.0 mg/L (Bin et al., 2011) in addition to an elevated ammonia (Lopez-Palau et al., 2011; Matsumoto et al., 2010) or salt concentration (Bassin et al., 2011b). Thus far, ammonia oxidizing archaea (AOA) have not been detected in anaerobic-aerobic (Winkler et al., 2012b) or pulse-fed aerobic systems (Zhao et al., 2011b, 2013).

In a pilot-scale anaerobic-aerobic granular sludge SBR treating complex domestic wastewater, NOB belonging to the genera *Nitrobacter* and *Nitrospira* were present in similar abundance. However, in acetate-fed anaerobic-aerobic lab-scale reactors at 30°C and a DO concentration of 2.0 mg/L, *Nitrobacter* was the dominant NOB (Winkler et al., 2012b). In all systems, the NOB/AOB ratio was higher than would be expected from the conventional stoichiometry of lithotrophic oxidation of nitrite from AOB. It was hypothesized that *Nitrobacter* were growing mixotrophically on acetate by dissimilatory nitrate reduction and/or that the high ratio was due to heterotrophic denitrifiers reducing nitrate to only nitrite that was again oxidized by NOB, thereby leading to a nitrate reduction-nitrite oxidation loop. A model simulation study suggested that both phenomena contributed to the NOB distribution in the granules (Winkler et al., 2015).

The NOB population was characterized in a nitrifying granular sludge system continuously fed a synthetic feed with only 500 mg/L NO_2-N under aeration (Vázquez-Padín et al., 2009). The granules contained a nearly equal NOB distribution of *Nitrobacter* and *Nitrospira* after 220 days of operation following seeding with aerobic granules, where *Nitrospira* was the dominant NOB and *Nitrobacter* was not detected by FISH. It was hypothesized that the emergence of *Nitrobacter* was associated with its ability to outcompete *Nitrospira* under conditions of elevated DO and NO_2-N, which were greater than 7 and 12 mg/L, respectively, in this system.

4.3 Ordinary Heterotrophic Organisms and Protozoa

Under rapid fill operation or other scenarios with a high concentration of readily biodegradable substrate present during aeration, a variety of bacterial groups may emerge including Zoogloea, Dechloromonas, Rhodocyclus, Thauera, Flavobacterium, Pseudomonas, Acinetobacter, Sphingomonas, and Comamonadaceae-related bacteria. (De Sanctis et al., 2010; Ebrahimi et al., 2010; Li et al., 2008;

Weissbrodt et al., 2012, 2013a; Williams and de Los Reyes III, 2006). The emergence of these organisms in granular sludge is not surprising given their capacity to store PHA under aerobic feast conditions and produce EPS that facilitates aggregation and biofilm formation (Weissbrodt et al., 2012). Ordinary heterotrophic organisms (OHOs) are also present and may contribute to nitrogen removal in PAO-dominated anaerobic-aerobic granular sludge by performing denitrification on soluble microbial products (Weissbrodt et al., 2014; Zhang et al., 2011b). The filamentous bacteria *Thiothrix, Sphaerotilus natans*, and *Leptothrix* have been associated with fluffy granules of poor structural integrity (Ebrahimi et al., 2010; Weber et al., 2007).

Though granules are typically composed of bacteria, protozoa have been observed as part of mature granules (de Kreuk et al., 2010; Pronk et al., 2015b; Winkler et al., 2012c). The effects of protozoa on aerobic granular sludge may include reducing granule settling velocity, filtering fines and colloids, and grazing on and depleting the bacterial population (Pronk et al., 2015b; Winkler et al., 2012c). The presence of protozoa in full-scale anaerobic-aerobic granular sludge systems with stable granules and nutrient removal performance suggests that protozoan growth does not pose significant adverse impacts in these systems.

4.4 Changes in Community Structure with Granulation

Typically, the microbial community structure of mature aerobic granules is very different from that in the inoculum (Liu and Tay, 2008; Song et al., 2009; Weissbrodt et al., 2012, 2013a; Winkler et al., 2013b; Zhang et al., 2011b). However, a comparable community structure was observed in flocs and granules, respectively, from a reactor containing a mixed flocculent-granular sludge, suggesting that the microbial community could exist in densely aggregated or flocculated form (Liu et al., 2010). An increased hydraulic selection pressure was necessary to wash out the flocs. The microbial diversity in the transition from flocculent to granular sludge has been shown to decrease upon the imposition of hydraulic selection, increase as granulation occurs, then decrease when granules have matured under steady-state conditions (Li et al., 2008; Zhang et al., 2011b). More intense stress or selective conditions such as higher biomass-specific organic loading or extended starvation periods may reduce the diversity of the granule population (Li et al., 2008; Liu and Tay, 2008). In a long-term study comparing parallel operations of a full-scale activated sludge plant and pilot-scale granular sludge plant treating the same domestic wastewater, the microbial communities were shown to be disparate but possessing comparable statistical measures of microbial diversity including richness, entropy, and evenness. The granular sludge community made a permanent change away from the initial population, whereas the activated sludge community moved closely around the initial population (Winkler et al., 2013b).

4.5 Microbial Consortia and Organisms' Spatial Distribution in Aerobic Granular Sludge for Nutrient Removal

The above material discussed the types of microbial groups observed in granular activated sludge reactors. For BNR systems, each granule contains a consortia of nitrifying bacteria, PAOs, GAOs, and other heterotrophic bacteria that accomplish BOD removal, EBPR, and biological nitrogen removal via nitrification and denitrification. This section discusses the spatial distribution of these key microbial groups in granular activated sludge systems with an anaerobic feeding regime for substrate uptake by PAOs and possibly GAOs followed by an aerobic period during which nitrification, denitrification, and phosphorus uptake occur at various locations in the granule.

FISH images of sliced granules grown in systems with anaerobic feeding followed by aeration for simultaneous nitrification-denitrification and EBPR are shown in Figures 4-1 and 4-2. In these granules,

PAOs and GAOs are the dominant groups of the granule microbial community and are located in both the oxygen-penetrated outer layer and inner anoxic region, where their previously stored internal carbon, obtained during anaerobic feeding, is oxidized with oxygen or NO_x-N as the electron acceptors according to the redox conditions in the granule. Because their metabolisms require aerobic conditions, AOB and NOB must inherently be located on the oxygen-penetrated outer layer of the granule. However, Figure 4-1a shows that AOB tend to be located on the outermost layer of the granule and NOB tend to be located closer to the granule interior behind the AOB. Similar AOB and NOB spatial distribution has been found in attached-growth nitrifying biofilms (Okabe et al., 1999) as well as aerobic autotrophic (Shi et al., 2010) and other anaerobic-aerobic granular sludge systems (Bin et al., 2011). Figure 4-2 shows that the granule microbial community contains a significant amount of bacteria located in both the oxygen-penetrated outer layer and inner anoxic region that are not PAOs or GAOs and most likely other OHOs. Because of the molecular probes used in the study where these images were obtained, nitrifying organisms that may be present in the oxygen-penetrated outer layer of the granule could not be distinguished from OHOs that may also be present in the same region. However, it is reasonable to expect that some OHOs are present in the oxygen-penetrated outer granule layer where they can grow on endogenous decay products and residual influent soluble and particulate biodegradable COD that may remain following anaerobic feeding. Similarly, OHOs in the inner anoxic region can use endogenous decay products for NO_x-N reduction.

A schematic representation of the microbial spatial distribution in granules grown in systems with anaerobic feeding followed by aeration for simultaneous nitrification-denitrification and EBPR in the granule is shown in Figure 4-3. Key aspects conveyed in Figure 4-3 with respect to microbial spatial distribution and redox zones within these granules include 1) the presence of PAOs, GAOs, and OHOs in both the outer aerobic and inner anoxic regions of the granule, 2) the restriction of nitrifiers to the outer aerobic layer, and 3) the potential presence of an anaerobic zone at the granule core. The presence and size of this anaerobic zone depends on the bulk liquid DO and NO_x-N concentrations and the rate of mass transport and biological reactions inside the granule. Because the presence of the anaerobic zone may be transient and its size may be variable, microbial groups are not shown in the anaerobic zone in Figure 4-3 for clarity. However, this inner core space could contain PAOs, GAOs, and OHOs.

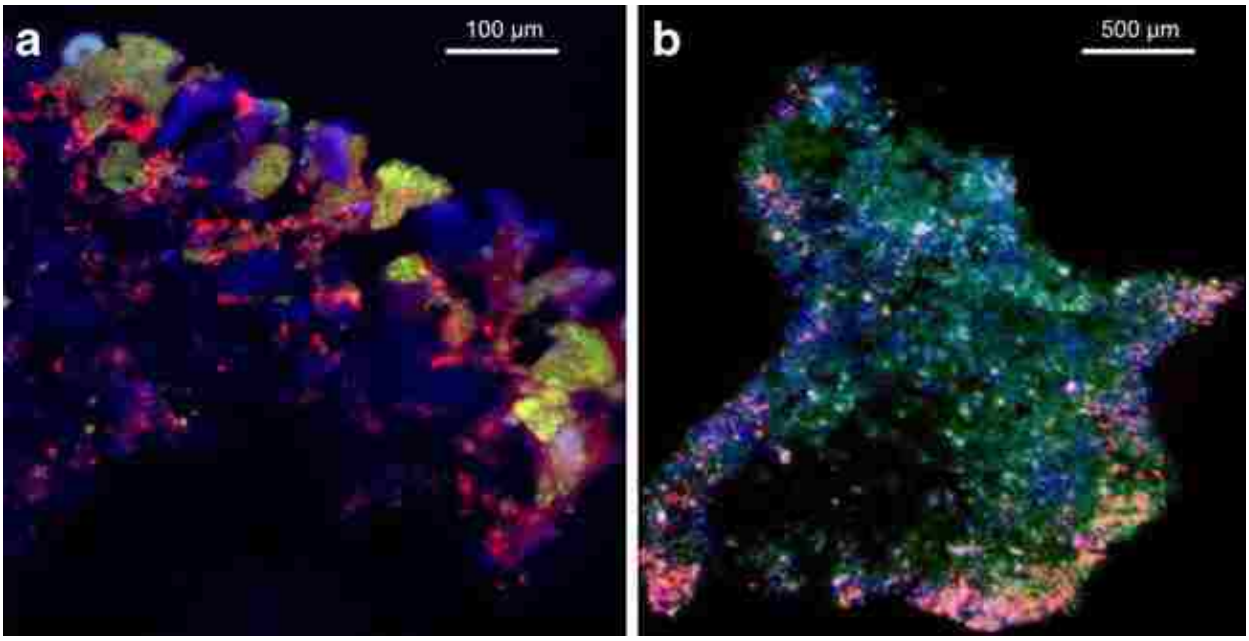

Figure 4-1. Microscopic FISH images of sliced granules from a system with anaerobic feeding and simultaneous nitrification-denitrification and enhanced biological phosphorus removal where probes for AOB, NOB, PAOs, and GAOs were used.
(a) AOB are shown in green-yellow. NOB are shown in red. PAOs are shown in blue.
(b) AOB and NOB are shown in red-pink. PAOs are shown in blue. GAOs are shown in green.
Reprinted with permission from Winkler et al. (2012b). Copyright Winkler et al., 2012.
Open access (http://link.springer.com/article/10.1007/s00253-012-4126-9/fulltext.html).

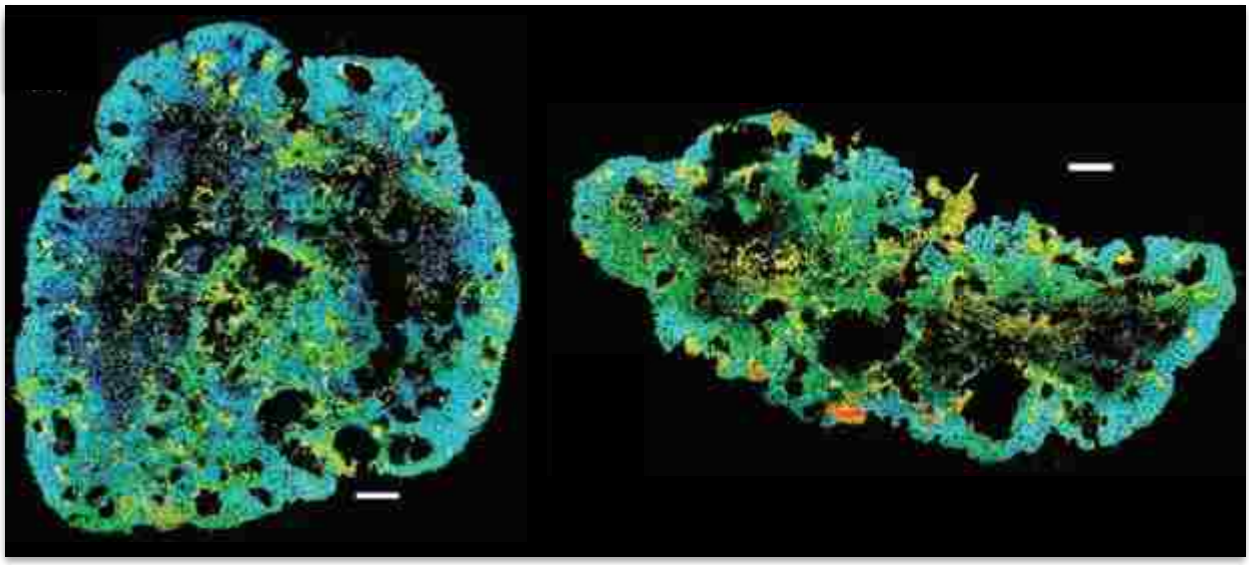

Figure 4-2. Microscopic FISH images of sliced granules from a system with anaerobic feeding and simultaneous nitrification-denitrification and enhanced biological phosphorus removal where probes for PAOs, GAOs, and other bacteria were used.
Scale bar = 100 um. PAOs are shown in cyan. GAOs are shown in yellow. Other bacteria are shown in green.
Reprinted with permission from Lemaire et al. (2008b). Copyright 2008 John Wiley & Sons.

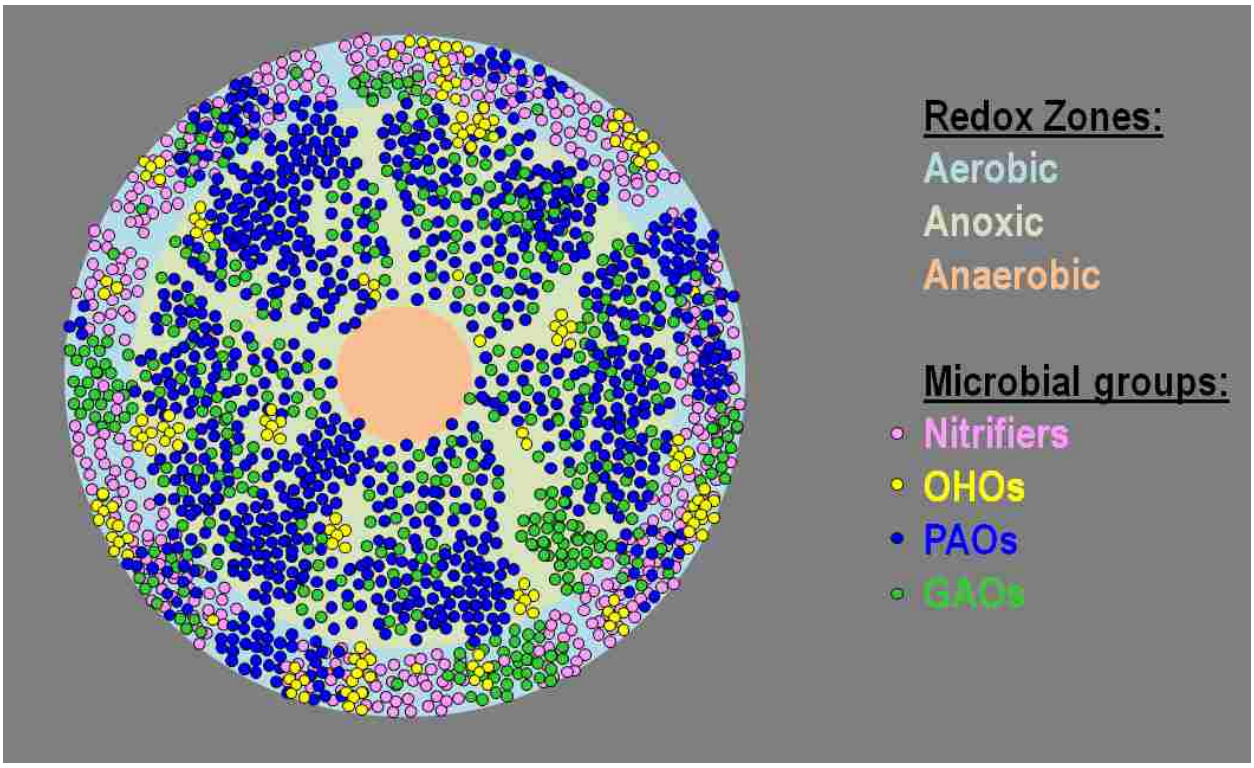

Figure 4-3. Schematic representation of microbial spatial distribution in aerobic granules with anaerobic feeding and aeration period for simultaneous nitrification-denitrification and EBPR.

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CHAPTER 5

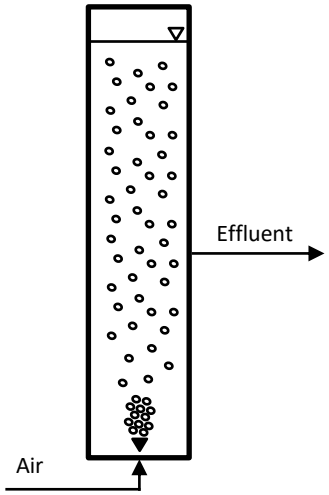
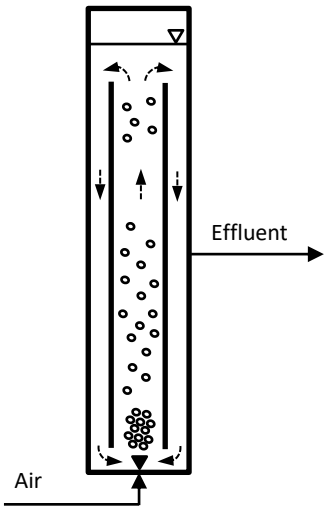
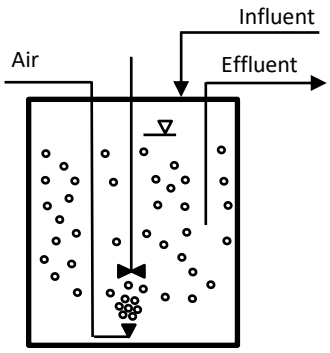
Nutrient Removal in Bench-Scale Aerobic Granular Sludge Systems

Laboratory studies on nutrient removal in aerobic granular activated sludge systems are reviewed in this chapter. The review is first divided into three process categories, categories which resulted in different granule microbial compositions: 1) nitrogen removal and EBPR, 2) nitrogen removal without EBPR, and 3) ammonia and nitrite oxidation without nitrogen removal or EBPR. Experimental results using ammonium-oxidizing granules for bioaugmentation is then presented. Observations on nitrous oxide emissions in these laboratory studies are also discussed. Most of the studies were done using SBR configurations described as *bubble column* reactors and *airlift* reactors, which are described in the following section. Other types of laboratory reactors have also been used to develop aerobic granular activated sludge, and their configurations and respective research results are summarized in the final section.

5.1 Common Laboratory Granular Sludge Reactor Configurations

Aerobic granular sludge has been cultivated in a variety of reactor configurations at the laboratory scale. The most common reactor configurations use SBRs, which can be further categorized into bubble column SBRs, airlift SBRs, and conventional SBRs as summarized in Table 5-1. Bubble column and airlift SBRs have a tall reactor geometry with height to diameter (H/D) ratios greater than 5 and frequently greater than 10 (Beun et al., 1999; de Kreuk and van Loosdrecht, 2006; Liu and Tay, 2007). The key difference between these two reactor types is that the airlift reactor contains a riser, also called a downcomer, which induces a recirculating flow pattern. Bubble column and airlift SBRs have typically been operated with aerobic pulse feeding or slow anaerobic bottom feeding as shown in Figure 3-2 in Section 3.2.2. SBRs with shorter reactor geometry and H/D ratios below 5 have also been used for granular sludge growth and are referred to as conventional SBRs. All types of granular sludge including NDN-PAO, NDN-OHO, OHO, and NIT granules have been grown in one or more of these types of SBRs.

Table 5-1. Common Aerobic Granular Sludge Reactor Configurations used in Laboratory Studies on Nutrient Removal

Reactor Configuration	Description
(a) Bubble Column SBR 	Laboratory sequencing batch reactors with a high height to diameter (H/D) ratio of >5 have been termed *bubble column sequencing batch reactors*. They have been the most commonly used laboratory reactor configuration for aerobic granular sludge studies. The high H/D ratio results in a deeper sludge blanket after settling and a higher gas sparging rate per unit of cross-sectional area. The reactor geometry also reduces the risk of short circuiting in an upflow feeding regime. A reactor H/D ratio of >10 was most common, but some studies used H/D ratios between 5 and 10. After a short settling period, typically less than 5 min, effluent is withdrawn from a side port. The typical decant depth was about 50% of the full liquid depth. An equal fill volume is used and at 50% depth is referred to as a 0.50 fill fraction. Slow upflow feeding through the settled anaerobic sludge blanket is common and selects for PAO growth. A rapid pulse feed, typically less than 10 min, from the top of the reactor is also common in granular activated sludge systems without EBPR. Rapid pulse feeing may occur under sparged mixing or static conditions without mixing.
(b) Airlift SBR 	Laboratory sequencing batch airlift reactors were used in early aerobic granular sludge research before the bubble column design became common. The distinguishing feature is a center column or "riser," which receives the sparged air to result in a recirculation flow pattern as shown. The airlift reactor induces higher shear than the bubble column reactor due to a higher superficial gas velocity inside the riser and liquid velocity in recirculation. The reactor is also designed with a high H/D ratio, and values of 10 to 20 have been used. Feeding and effluent withdrawal methods are similar to the bubble column reactor.
(c) Conventional SBR 	Granular sludge SBRs with H/D ratios less than 5 have been used in nutrient removal studies; this design is referred to here as a conventional SBR. Because of the shorter height, a shorter settling time must be used to have the same degree of selective settling pressure as with the bubble column and airlift SBRs. Effluent withdrawal typically occurs via a drop pipe. Filling occurs from the top of the reactor, and fill fractions of approximately 0.50 have been used. In reactors with EBPR, a static feed period has been followed by a stirred anaerobic period in view of the difficulty of having uniform feeding into the shallow settled sludge blanket. In reactors without EBPR, pulse feeding has been employed as described for the bubble column reactor. Mechanical or magnetic stirring has been used during the sparged air reaction phases to assure solids suspension and well-mixed conditions.

5.2 Nitrogen and Phosphorus Removal

This section summarizes the operation and performance of lab-scale aerobic granular sludge systems with combined nitrogen removal and EBPR.

5.2.1 Reactor Design and Operating Conditions

Bench-scale aerobic granular sludge reactors operated for simultaneous N and P removal often involve slow upflow feeding under anaerobic conditions through the settled granular sludge bed to assure maximum substrate availability to PAOs and/or GAOs under anaerobic conditions in reactors where the H/D ratio is greater than 10 (de Kreuk and Van Loosdrecht, 2004, 2005b). Upflow feeding through the settled sludge bed is a key aspect of the Nereda® process and is claimed in an associated patent (van Loosdrecht and de Kreuk, 2007). The feed flow is applied in a plug flow manner, which displaces treated liquid at the top of the reactor column as effluent. However, reactors with rapid feeding, not necessarily through the settled sludge bed, and with a mixed anaerobic period have also been used to successfully cultivate N- and P-removing granules (Kishida et al., 2006; Zhang et al., 2011a).

A summary of design and operating conditions for five laboratory nitrogen removal and EBPR aerobic granular sludge reactor studies reported in the literature is shown in Table 5-2. The systems shown were selected to represent different reactor configurations, feeding methods, mixing conditions, aeration regimes, DO control strategies, loading rates, and reaction phase redox conditions. These systems were also selected because of the available long-term reactor operations and performance information. The systems are organized in Table 5-2 from A to E in order of increasing NH_3-N volumetric loading rate. Aerobic granular sludge systems compared in subsequent tables in this chapter are also organized in the same manner.

Volumetric NH_3-N loading rates in Systems A to E ranged from 0.08 to 0.43 kg NH_3-N/m^3-day. Systems B, C, and E were bubble column SBRs with H/D ratios from 5.5 to 21. System D was an airlift SBR with an H/D ratio of 14.0, and System A was a conventional SBR with an H/D ratio of 1.0. Systems A and E had mechanical or magnetic mixing due to their reactor geometry and aeration method, whereas Systems B, C, and D were mixed solely by gas sparging. The mechanical/magnetic stirrer mixing in Systems A and E occurred during the anaerobic period after feeding and throughout the aerobic and post-anoxic periods. Systems B, C, D, and E were fed in an upflow manner from the bottom of the reactor. System A was fed for 20 minutes without mixing and followed by 90 minutes of mixing. System E was fed for 18 minutes and followed by 60 minutes of mixing.

Systems A, B, C, and D were fed a synthetic wastewater containing acetate or acetate and propionate for the carbon source, which are preferred substrates for EBPR. About half of the COD in the feed to System E, which was treating an abattoir anaerobic lagoon effluent, was from supplemental acetate. All systems were operated at 20°C except for System D, which was at 15°C. The average granule size ranged from 0.9 to 1.2 mm for Systems A, C, D, and E. An SVI_{30} of 30 mL/g was reported for System B to indicate a granular sludge characteristic.

A variety of DO or aeration control strategies were applied in these systems with the general intention to encourage simultaneous nitrification and denitrification for nitrogen removal. System A lacked DO control, but the aeration DO concentration was typically around 3 mg/L. System B also lacked DO control, but intermittent aeration with frequent cycling between a DO concentration of approximately 6 mg/L and near zero was used. Systems C and D included DO control at DO concentrations from 1.8 to 2.0 mg/L by employing a DO sensor and headspace off-gas recirculation, with air added when the target DO declined. In System E, a DO sensor and on/off aeration was used to control the DO concentration

between 3.0 and 3.5 mg/L. Continuous magnetic stirring was applied in System E during the aeration period to supplement mixing provided by air sparging.

Table 5-2. Operating and Design Conditions of Laboratory Aerobic Granular Sludge Systems for Nitrogen Removal and EBPR

System	A	B	C	D	E
Study duration, day	60	260	150	170	390
Avg. granule diameter, mm	1	n/r	1.2	0.9	1
Carbon feed	Acetate	Acetate + propionate	Acetate	Acetate	Abattoir anaerobic lagoon effl. + Hac
Temperature, °C	20 ± 2	20	15	20	20 ±2
Inoculum	Flocs	Flocs	Flocs + crushed granules	Granules	Granules
Reactor configuration	Conventional SBR	Bubble column SBR	Airlift SBR	Bubble column SBR	Bubble column SBR
H/D ratio	~1	21	14	14	5.5
Cycle fill fraction	0.33	0.50	0.53	0.57	0.60
Equivalent HRT (V/Q), hr	18	6.3	5.6	5.2	13.3
Total cycle time, hr	6	3.2	3	3	8
Anaerobic step Feed type Feed time, min Add'l time, min	 Static 20 90 mixed	 Upflow 60 0	 Upflow 60 0	 Upflow 60 0	 Upflow 18 60 mixed
Aeration step Sparge air Time, min Supplemental mixing	 Continuous 120 Mechanical	 Intermittent 120 None	 Continuous 112 None	 Continuous 112 None	 Intermittent 315 Magnetic
Anoxic step Time, min Mixing	 120 Mechanical	None - -	None - -	None - -	 80 Magnetic + N_2
Settling time, min	0.5	5	3	3	2
Decant time, min	9.5	5	5	5	5
DO control	No	No	Yes	Yes	Yes
DO, mg/L	1–5 mg/L range but 70% of aeration step was ~3 mg/L	20 min air ON at 5.5–6.5 mg/L; 20 min air OFF without mixing	2 gas recirculation	1.8 gas recirculation	3–3.5 air on/off
Superficial gas velocity, cm/s	0.12	2.8	5.3	2.2	0.6
Total SRT, day	15	20–25	n/r	30	15–20
Aerobic SRT, day	~5	~7–9	n/r	~20	~10–13
NH_3-N load, kg/m^3-day	0.08	0.16	0.24	0.27	~0.43

Note: n/r = not reported.

References: A (Kishida et al., 2006); B (Lochmatter et al., 2014); C (de Kreuk et al., 2005c); D (Bassin et al., 2012a); E (Yilmaz et al., 2008).

An operating strategy unique to System B was used to control the total aeration step time based on the completeness of NH_3-N oxidation. The last high-DO period was stopped when NH_3-N oxidation approached completion. This was determined based on an observed increase in DO concentration under the constant aeration rate applied as NH_3-N reached low concentrations and oxygen demand decreased under ammonia-limited conditions. As a consequence of this operation, the total aeration step time varied between 100 and 150 minutes. A total aeration step time of 120 minutes is shown in Table 5-2 based on three 40-minute periods of on-off aeration.

The total SRT in the systems ranged from 15 to 30 days. An aerobic SRT was estimated from the total SRT by multiplying the total SRT by the cycle aerobic time divided by the total cycle time, and ranged from approximately five days in System A to 20 days in System D. The SRT in System D was controlled by selective wasting from the top of the settled sludge bed. No special waste sludge location was indicated for the other systems, which suggests that it was done under aerated mixed conditions.

5.2.2 Treatment Performance for Nitrogen and Phosphorus Removal

As shown in the treatment performance summary in Table 5-3, greater than 96% EBPR occurred and the average effluent PO_4-P concentration was equal to or lower than 0.9 mg/L in all systems, and as low as 0.3 mg/L in System A. Such good EBPR is expected for Systems A, B, D, and E in view of the good anaerobic feed contacting, the very low NO_x-N concentration at the end of the operating cycle, the amount of VFA in the feed rbCOD, and the favorable rbCOD/P ratios of 20-60. The higher effluent PO_4-P concentration of 0.9 mg/L for System C was likely related to the higher NO_x-N concentration of 19 mg/L at the end of the SBR cycle and a feed rbCOD/P ratio of 21, which was favorable but at the lower end of that used for the other systems. It is emphasized that VFA was the only component of the influent bCOD in Systems A-D and most of the feed bCOD in System E, which is favorable for the selection of PAO granules and EBPR performance. EBPR performance for domestic wastewater treatment in several full-scale aerobic granular sludge systems is summarized later, in Section 7.2.4.

The PAO and GAO populations were studied for Systems A, D, and E. A mixture of PAOs and GAOs were detected by FISH in System A. For System D, enrichment of PAOs due to selective wasting of the GAOs in the top of the settled sludge bed was confirmed by FISH. The observation of denitrification concurrent with EBPR during the aerobic period after the acetate was fully consumed in the anaerobic period suggested that denitrification was done primarily by PAOs. System E was seeded with granules containing both *Accumulibacter* PAOs and *Competibacter* GAOs, but after one year of operation *Competibacter* GAOs were below the FISH detection limit and *Accumulibacter* PAOs were dominant. Based on the observation of Type II *Accumulibacter* PAOs suggested by anoxic batch tests and NO_2-N accumulation during the aerobic period, the authors suggest short-cut nitrogen removal.

The MLSS concentrations for Systems B, C, D, and E were very high, ranging from 15,000 to 33,000 mg/L, which can be attributed to the relatively high fill fractions, long SRTs, and wastewater strengths. A low MLSS volatile solids fraction is expected due to the increased inorganic content of PAO-enriched EBPR sludge, but the very low values of 60 to 70% for Systems C, D, and E raised the possibility of some phosphorus precipitates in the granules.

The effluent NH_3-N concentrations indicate that essentially complete ammonia oxidation occurred in Systems A, C, D, and E for NH_3-N loading rates up to 0.27 kg/m^3-day and aerobic SRTs ranging from five to 20 days. Effluent NH_3-N concentrations were higher for System B, which had an aerobic SRT of seven to nine days and NH_3-N loading of 0.16 kg/m^3-day. The residual NH_3-N concentration of 1-3 mg/L in this system is likely attributable to the aeration time control strategy that relied on changes in the oxygen demand rate. After feeding, the nitrification rate and oxygen demand would not be ammonia-limited,

but at 1-3 mg/L NH_3-N, the rates would be much lower and ammonia-limited based on typical half-saturation coefficients for AOB.

Effluent NO_2-N concentrations were nondetectable for Systems A, C, and D, but elevated NO_2-N concentrations were observed for Systems B and E, suggesting some repression of NOB activity. Aerobic NO_2-N accumulation in System B was confirmed by batch activity tests with NH_3-N spikes in the System B mixed liquor. The NOB activity repression in System B may be due to the high frequency aerobic-anoxic cycling. Other studies have shown a lag in NOB activity compared to AOB activity following a change from anoxic to oxic conditions (Kornaros et al., 2010; Regmi et al., 2014; Turk and Mavinic, 1986; Yang et al., 2007). For System E, NOB repression was not total, but elevated influent NH_3-N concentrations and periods of higher pH after feeding may have led to free ammonia inhibition of the NOB for at least a portion of the aerobic period.

In Systems A, C, and D, TIN removal efficiencies were >99%, 65%, and >90%, respectively, for influent wastewater fed VFA for the feed COD, with rbCOD/N ratios of 10, 6.5, and 6.7 g rbCOD/g N, respectively. The high TIN removal efficiency in System A reflects high influent rbCOD/N for denitrification and effective simultaneous nitrification and denitrification in the granular sludge. The rbCOD/N ratios for Systems C and D were closer, but System C had less efficient nitrogen removal. The lower nitrogen removal efficiency may have been due to less carbon storage by PAOs for use in denitrification during aeration, as an elevated acetate concentration of 10-20 mg COD/L was present at the start of aeration. Although System A included a post-anoxic period, most nitrogen removal was achieved by simultaneous nitrification and denitrification in the aerobic period. The NO_x-N concentration was lowered from approximately 2 mg/L at the end of aeration to near zero at the end of the post-anoxic period.

In Systems B and E where NO_2-N accumulation was observed, TIN removal efficiencies were >90% and 96%, respectively, for influent wastewater COD/N ratios of 8 and 6 g COD/g N, respectively. These COD/N ratios are higher than that expected for conventional short-cut nitrogen removal. In System E, abattoir anaerobic lagoon effluent and acetate contributed approximately equally to the total influent COD. The biodegradability of the anaerobic lagoon effluent COD was not evaluated. If the abattoir anaerobic lagoon effluent COD was primarily not biodegradable, then System E would be characterized by a lower effective COD/N ratio. The post-anoxic period in System E was instrumental in achieving enhanced TIN removal. Approximately 43 and 8 mg/L of NO_2-N and NO_3-N, respectively, remained at the end of the aerobic period but was subsequently reduced to under 10 mg NO_x-N/L during the anoxic period. The bulk liquid PO_4-P concentration was less than 1 mg/L at the end of the aerobic period, and no significant additional PO_4-P uptake occurred during the anoxic period, which suggests that denitrification in this period occurred by endogenous denitrification rather than denitrifying PAO activity on internal carbon storage products.

Table 5-3. Treatment Performance Summary of Laboratory Aerobic Granular Sludge Systems for Nitrogen Removal and EBPR

System	A	B	C	D	E
Influent					
COD (mg/L)	600	400	365	400	~1440
N load (kg/m^3-day)	0.08	0.16	0.24	0.27	~0.43
NH_3-N (mg/L)	60	50	56	60	~220 (TN ~245)
COD/N	10	8	6.5	6.7	~6
PO_4-P (mg/L)	10	20	18	20	~40
COD/P	60	20	21	20	~36
Reactor					
pH	7.5–8.1	7–7.3	6.8–7.2	6.8–7.2	7–8.6
MLSS (mg/L)	4750	~16,000	33,000	~17,000	~15,000
MLVSS (mg/L)	n/r	n/r	20,000	~12,000	~10,500
%MLVSS	n/r	n/r	60	~70	~70
Effluent					
NH_3-N (mg/L)	<0.1	1–3	<0.6	<1	0.8
NO_2-N (mg/L)	<1	<4	<0.1	<0.1	3
NO_3-N (mg/L)	<1	<1	19	<5	5
TIN rem. %	>99	>90	65	>90	96
PO_4-P (mg/L)	0.3	0.2–0.8	0.9	<0.4	0.6
PO_4-P rem. %	97	96–99	95	>98	98
TSS (mg/L)	n/r	n/r	n/r	n/r	306

Note: n/r = not reported.

References: A (Kishida et al., 2006); B (Lochmatter et al., 2014); C (de Kreuk et al., 2005c); D (Bassin et al., 2012a); E (Yilmaz et al., 2008).

5.3 Nitrogen Removal Without EBPR

This section summarizes the operation and performance of lab-scale aerobic granular sludge systems with nitrification and denitrification without concurrent EBPR.

5.3.1 Reactor Design and Operating Conditions

A summary of design and operating conditions for several lab-scale aerobic granular sludge systems achieving various degrees of nitrogen removal without EBPR is shown in Table 5-4. These systems were selected because they include a diversity of feeding and reaction period schedules, mixing conditions, reactor geometries, and nitrogen loading rates.

Nitrification and denitrification were achieved in SBR cycles with 1) pulse feeding followed by an aerobic react period (System B), 2) pulse feeding followed by anoxic and aerobic react periods (Systems C and E), and 3) anoxic step feed and react periods with aerobic periods between each step (Systems A and D). The reported anoxic mixing method in these systems included nitrogen sparging (System A), pumped liquid recirculation (System C), and stirring (System E).

Systems A and C were bubble column SBRs with an H/D ratio of 20, and System E was a bubble column SBR with an H/D ratio of 6. System B was an airlift SBR with an H/D ratio of 14. A conventional SBR with an H/D ratio of 3.5 was used in System D. Operating temperature was between 20 and 30°C for Systems B, D, and E, but not reported for Systems A and C. The average granule size ranged from 1.0 to 2.0 mm. A SRT was only reported for System B, in which the total SRT was reported at nine days.

Synthetic wastewater was used in all systems, and the influent COD/N ratio ranged from a low of 1.4 for System E to a high of 14 for System B. The reported influent carbon sources included acetate only for System B, and acetate and glucose for Systems D and E. Feeding was always done under nonaerated or anoxic conditions. The volumetric NH_3-N loading rates ranged from a low of 0.15 kg N/m^3-day for System A to 1.05 kg N/m^3-day for System E.

The DO concentration in all systems was not controlled and was greater than 6 mg/L during oxic periods for all systems except System A, in which the DO concentration in the final oxic period was controlled at 1.6 mg/L with on-off aeration and supplemental N_2 sparging for mixing. The DO concentration in the first two oxic periods in System A was likely higher than 1.6 mg/L based on the authors describing the final oxic period as a low aeration phase. The superficial gas velocity during aeration in Systems A-E ranged from 1.0 to 2.6 cm/s.

Table 5-4. Operating and Design Conditions of Laboratory Aerobic Granular Sludge Systems for Nitrogen Removal Without EBPR

System	A	B	C	D	E
Study duration, day	70	90	30	130	240
Avg. granule diameter, mm	1.3	1	1.5	1.1	2
Carbon feed	Synthetic, unspecified	Acetate	Synthetic, unspecified	Glucose + acetate	Glucose + acetate
Temperature, °C	n/r	20	n/r	28	20–30
Inoculum	Flocculant	Flocculant	Granules	Flocculant	Flocculant
Reactor configuration	Bubble column SBR	Airlift SBR	Bubble column SBR	Conventional SBR	Bubble column SBR
H/D ratio	20	14	20	3.5	6
Cycle fill fraction	0.50	0.53	0.50	0.50	0.50
Equivalent HRT (V/Q), hr	16	5.6	8	8	8
Total cycle time, hr	8	3	4	4	4
Feed type	Step feeding during three anoxic periods	Static	Static	Step feeding during two anoxic periods	Static
Sequence and duration of feeding and reaction periods	Anoxic 1 = 30 min Oxic 1 = 90 min Anoxic 2 = 30 min Oxic 2 = 90 min Anoxic 3 = 30 min Oxic 3 = 203 min	Feed = 3 min Oxic = 173 min	Feed = 10 min Anoxic = 10 min Oxic = 213 min	Feed 1 = 6 min Anoxic 1 = 42 min Oxic 1 = 79 min Feed 2 = 6 min Anoxic 2 = 42 min Oxic 2 = 60 min	Feed = 6 min Anoxic = 60 min Oxic = 168 min
Anoxic mixing method	N_2 sparging	Not applicable	Pumped recirculation	n/r	Stirring
Settling time, min	2	2	2	1	2
Decant time, min	5	5	5	4	4
DO control	Oxic 1 = No Oxic 2 = No Oxic 3 = Yes (on/off)	No	No	No	No
DO, mg/L	Oxic 1 = n/r Oxic 2 = n/r Oxic 3 = 1.6	>8	~8	6–7.5	6–8
Superficial gas velocity, cm/s	2.6	2.4	2.6	1.0	1.3
Total SRT, day	n/r	9	n/r	n/r	n/r
Aerobic SRT, day	n/r	9	n/r	n/r	n/r
N load, kg/m^3-day	0.15	0.17	0.18	0.90	1.05
Comments	Anoxic feeding time was not specified			Feed periods were not aerated	Type of stirring not specified

Note: n/r = not reported.
References: A (Chen et al., 2013); B (Beun et al., 2000a); C (Chen et al., 2011); D (Wang et al., 2012); E (Shi et al., 2011).

5.3.2 Treatment Performance for Nitrogen Removal Without EBPR

The results from Systems A-E showed that nitrifying-denitrifying granules could be grown with distinct aerobic and anoxic operating periods and without EBPR. In contrast to the nitrogen removal and EBPR granular systems described in Section 5.2, nitrification and denitrification for the granular systems reported in this section did not occur under a sustained low DO concentration of 2 mg/L or less.

As shown in the treatment performance summary in Table 5-5, high NH_3-N removal efficiencies were achieved in all systems. In Systems D and E, with higher N loading rates the effluent NH_3-N concentrations ranged from less than 5 to 7 mg/L, corresponding to greater than 98% NH_3-N removal. In these systems, NH_3-N was oxidized primarily to NO_2-N as a consequence of an elevated free ammonia concentration during the oxic periods. In the lower-loaded systems with high DO concentrations (Systems B and C), the effluent NH_3-N concentration was less than 0.1 mg/L.

The amount of denitrification depended on the influent COD/N ratio and reactor cycle operation. System A achieved the highest TIN removal efficiency (85%) as a consequence of having three anoxic step feed periods and an influent COD/N ratio of 5.0, which is sufficient for denitrification. In comparison, System D had less favorable conditions for nitrogen removal, which resulted in a lower TIN removal efficiency of only 70%. It had only two anoxic step feed periods and a limiting feed COD/N ratio of 2.67. System B had no anoxic period, and most nitrogen removal was primarily due to assimilation for biomass synthesis under the high COD/N ratio applied. A major portion of the nitrogen removal in System C was also due to nitrogen assimilation for biomass synthesis. The percent TIN removal was a little higher than that for System B (78% versus 75%) even though System C had a lower influent COD/N ratio. The slight improvement may have been due to having the short anoxic period of 10 minutes after feeding, whereas System B did not have a dedicated anoxic period. In System E, the TIN removal of only 35% can be attributed to the low influent COD/N ratio of 1.4 and the use of a single pre-anoxic period.

Though this section was intended to show research results for nitrification/denitrification granular sludge systems, it is worth noting that a granular sludge system has also been developed with growth under only nitrate reduction conditions (Li et al., 2013). The system was continuously fed nitrate and methanol at an influent COD/N ratio of 5 in an upflow column reactor with liquid recirculation from top to bottom and effluent overflow from the top of the reactor, which was operated at 30°C. Over 97% nitrogen removal was achieved at loading rates from 2 to 45 kg N/m^3-day. At a nitrogen loading rate of 55 kg N/m^3-day, biomass was lost by floatation due to the high nitrogen gas production rate, and the nitrogen removal efficiency declined to approximately 73%. Nitrogen gas-filled voids within the granule resulted in a specific gravity of 0.913 in the floating sludge. When these gasses were released by gentle agitation, the specific gravity increased to 1.025 and the granule could then settle (Li et al., 2014).

Table 5-5. Treatment Performance Summary of Laboratory Aerobic Granular Sludge Systems for Nitrogen Removal Without EBPR

System	A	B	C	D	E
Influent					
COD (mg/L)	500	560	600	800	500
N load (kg/m^3-day)	0.15	0.17	0.18	0.90	1.05
NH_3-N (mg/L)	100	39	60	300	350
COD/N	5	14	10	2.67	1.4
PO_4-P (mg/L)	4	86	4	n/r	5
COD/P	125	6.5	150	n/r	100
Reactor					
pH	n/r	6.8–7.2	n/r	7.5–8.5	7.5–8.5
MLSS (mg/L)	6000	4000	n/r	27,000	n/r
MLVSS (mg/L)	n/r	n/r	6000	n/r	12,000
%MLVSS	n/r	n/r	n/r	n/r	n/r
Effluent					
NH_3-N (mg/L)	~2	<0.1	<0.1	<5	<7
NH_3-N rem %	~95	>99	>99	>98	>98
NO_2-N (mg/L)	3	3	0	100	225
NO_3-N (mg/L)	12	7	13	0	20
TIN rem. %	~80–85	75	78	70	35
PO_4-P (mg/L)	n/r	n/r	n/r	n/r	n/r
PO_4-P rem. %	n/r	n/r	n/r	n/r	n/r
TSS (mg/L)	n/r	166	n/r	n/r	n/r

Note: n/r = not reported.
References: A (Chen et al., 2013); B (Beun et al., 2000a); C (Chen et al., 2011); D (Wang et al., 2012); E (Shi et al., 2011).

5.4 Ammonia and Nitrite Oxidation

This section discusses research results on aerobic granular sludge systems operated for only ammonia and/or nitrite oxidation. The wastewaters fed to these systems contained none or little biodegradable COD. A major area of interest for these studies was to determine if autotrophic growth from ammonia and/or nitrite oxidation could result in a granular sludge, and how such granules compared to aerobic granular sludge with mainly heterotrophic bacteria growth. Another area of research interest was the use of nitrifying granular sludge to enhance nitrification in flocculent sludge systems by bioaugmentation, with the nitrifying granular sludge grown on high-strength ammonia feed from industrial wastewaters or centrate/filtrate return flows from dewatering anaerobic sludge digestion effluent.

5.4.1 Reactor Design and Operating Conditions

A summary of design and operating conditions for several lab-scale, ammonia-oxidizing aerobic granular sludge systems is shown in Table 5-6. These systems were selected because they include a range of nitrogen loading rates, feeding modes, granule sizes, and seed sludge sources and feed types. Applied volumetric NH_3-N loading rates ranged from 0.16 to 2.4 kg/m^3-day. Systems B and C were taken from different operational periods within a single reactor study.

Table 5-6. Operating and Design Conditions of Laboratory Aerobic Granular Sludge Systems for Ammonia Oxidation

System	A	B	C	D	E
Study duration, day	~20	60	880	410	180
Avg. granule diameter, mm	0.25	3	3	0.75	4.3
Feed organic carbon source	None	None	None	None	Anaerobic digester supernatant
Temperature, °C	25	18–24	18–24	30	30
Inoculum	Flocs	Granules	Granules	Flocs	Mixture of flocs & granules
Reactor configuration	Bubble column SBR	Bubble column SBR	Bubble column SBR	Bubble column SBR	Conventional SBR
H/D ratio	24	5.5	5.5	11	3
Cycle fill fraction	0.50	0.50	0.50	0.50	0.50
Equivalent HRT (V/Q), hr	12	6	6	6	8
Total cycle time, hr	6	3	3	3	4
Feeding step Feed type Feed time, min	 Static 4	 Static 3	 Aerated 171	 Static 3	 Aerated 150
Aeration step Sparge air Time, min Supplemental mixing	 Continuous 322 None	 Continuous 171 None	 Continuous 171 None	 Continuous 341 None	 Continuous 236 None
Settling time, min	30	2	2	10	1
Decant time, min	4	4	4	6	3
DO control	No	No	No	n/r	No
DO, mg/L	>1.8	7.5–8.5	7.5–8.5	n/r	~8
Superficial gas velocity, cm/s	3.5	0.5	0.5	n/r	1.4
SRT, day	n/r	n/r	n/r	n/r	n/r
NH_3-N load (kg/m^3-day)	0.16	0.40	0.80	1.2	2.4

Note: n/r = not reported.
References: A (Tay et al., 2002c); B (Vázquez-Padín et al., 2010b); C (Vázquez-Padín et al., 2010b); D (Liu et al., 2008); E (Lopez-Palau et al., 2011).

All systems used SBRs with a 50% fill fraction, and the H/D ratios ranged from 3 to 24. Systems A, B, and D were pulse fed, whereas feeding for Systems C and E occurred over an extended time during the aeration period to avoid having a high initial NH_3-N concentration and pH, to cause a high enough FA concentration to inhibit NH_3-N oxidation. Operating temperatures were between 18 and 30°C. The SRT in the systems was not reported. Although all systems in Table 5-6 utilized SBRs, ammonia-oxidizing aerobic granular sludge has been grown in other reactor configurations, described in Section 5.6.

With the exception of System E, all systems were fed a synthetic wastewater without biodegradable carbon. System E was fed anaerobic municipal sludge digester supernatant with reported average concentrations of 800 mg/L and 340 mg/L for NH_3-N and sCOD, respectively. Though the sCOD concentration appears low with an sCOD to NH_3-N ratio of 0.43, it does suggest that the bCOD level due to VFA was low. Moen et al. (2003) observed effluent VFA concentrations of less than 100 mg/L for a mesophilic digester with a 15-day SRT treating municipal sludge. The sCOD in that case was 1600 mg/L, and the sCOD to NH_3-N ratio was 0.85.

A high DO concentration is shown in Table 5-6 for four of the five systems, for which DO concentration was reported. In addition to the high DO concentrations shown for Systems B and E, the reference noted that the ammonia-oxidizing granules were also maintained at a DO of 2.0 to 3.5 mg/L.

Superficial gas velocity ranged from 0.5 cm/s for Systems B and C to 3.5 cm/s for System A. Systems B and C were seeded with mature nitrifying granules, which were maintained with a superficial gas velocity of 0.5 cm/s. However, it cannot be concluded that ammonia-oxidizing granules can be grown from flocculent seed sludge in the laboratory at this lower gas sparging intensity.

The average size of granules in these systems ranged from 0.25 to 4.3 mm. The smallest granules were obtained in System A, which had a 30-minute settling period, which is not a favorable selective settling pressure for obtaining large granules. The growth of granules in this case was likely favored by the high superficial gas velocity and aeration shear. In System D, 0.75 mm granules were obtained under a moderate settling time of 10 minutes. In Systems B and C, the granular sludge size did not significantly change after seeding with granules, and an average granule size of 3.0 mm was maintained. The largest granules were obtained in System E, which had the lowest settling time of one minute. Particles with a settling velocity less than 10 m/hr would be washed out of this system because of its H/D ratio of 3.0 and short settling time.

5.4.2 Treatment Performance for Ammonia Oxidation

The treatment performance for ammonia-oxidizing granular sludge systems is summarized in Table 5-7. The effluent NH_3-N concentration was near zero for Systems A and B, which had the lowest NH_3-N loading, high DO concentration, and pH ranging from 7.0 to 8.5. System C had double the NH_3-N load of System B and had an elevated effluent NH_3-N concentration in spite of sufficient alkalinity and high DO concentration. System D had 1.5 times the NH_3-N loading of System C but had a lower effluent NH_3-N concentration. The difference in effluent NH_3-N concentrations between Systems B, C, and D may have been influenced by factors other than NH_3-N loading, such as SRT, MLSS concentration, and granule size.

System E was fed anaerobic digester dewatering centrate with equimolar concentrations of bicarbonate alkalinity and NH_3-N and thus nitrification, which requires 2.0 equivalents of bicarbonate alkalinity per equivalent of NH_3-N, was alkalinity limited. The effect of the alkalinity limitation is shown by the lower pH and high effluent NH_3-N concentration with about 48% NH_3-N oxidation.

NO_2-N accumulation occurred in Systems C and E. The higher-loaded System D did not have significant NO_2-N accumulation in spite of a high FA concentration at the start of each cycle. The FA concentration

was estimated to be as high as 11.6 mg N/L based on the bulk liquid NH_3-N concentration of 150 mg/L, at 30°C and pH 8.0. The smaller granule size of 0.75 mm in System D may have contributed to achieving more complete nitrification than that for System C because smaller granules have a larger aerobic volume fraction than larger granules at equivalent bulk liquid DO concentrations. For System E, the effluent NO_3-N concentration was near zero and the NO_2-N concentration was approximately 370 mg/L. Two possibilities may be considered for the elevated NO_2-N concentration in System E and lack of NO_3-N production. The first is that the high bulk liquid NH_3-N concentration may have led to rapid NH_3-N oxidation by AOB in the outer layer of the granule, leaving little DO for NO_2-N oxidation by NOB in the interior layers of the granule. The second is that the elevated NO_2-N concentration and low pH could result in a higher concentration of free nitrous acid (FNA) to further inhibit NOB activity.

Table 5-7. Treatment Performance Summary of Laboratory Aerobic Granular Sludge Systems for Ammonia Oxidation

System	A	B	C	D	E
Influent					
sCOD (mg/L)	0	0	0	0	340
N load (kg/m^3-day)	0.16	0.40	0.80	1.2	2.4
NH_3-N (mg/L)	80	100	200	300	810
Reactor					
pH	7.5–8.2	7–8.5	7–8.5	6.5–8	5.9–6.7
MLSS (mg/L)	n/r	~1900	~3900	2600	11,000
MLVSS (mg/L)	n/r	~1700	~3500	n/r	9200
%MLVSS	n/r	~90	90	n/r	84
Effluent					
NH_3-N (mg/L)	~0	~0	20	<5	~420
NH_3-N rem %	>99	>99	90	>98	48
NO_2-N (mg/L)	~10	~0	130	<10	~370
NO_3-N (mg/L)	~70	~100	40	~280	~0
TSS (mg/L)	n/r	5–50	5–50	n/r	25
Other notes	Effluent values taken from 5.5 hr point of 8 hr batch test because effluent for normal 6 hr cycle was not explicitly reported; peak NO_2-N during batch test cycle was ~30 mg/L	MLSS concentration interpolated from gradual MLVSS increase and volatile solids fraction reported by authors	MLSS concentration interpolated from gradual MLVSS increase and volatile solids fraction reported by authors		Influent TSS = 690 mg/L No supplemental alkalinity addition

Note: n/r = not reported.
References: A (Tay et al., 2002c); B (Vázquez-Padín et al., 2010b); C (Vázquez-Padín et al., 2010b); D (Liu et al., 2008); E (Lopez-Palau et al., 2011).

5.4.3 Aerobic Granular Sludge Systems for Nitrite Oxidation

A limited number of studies have been reported for aerobic granular sludge developed from nitrite-oxidizing bacteria. Their operating conditions and performances are summarized in Table 5-8. System A was a conventional SBR seeded with nitrifying granules from a laboratory granular sludge reactor. System B was a bubble column SBR seeded with flocculent activated sludge from a municipal WRRF. Temperature in the systems was between 20 and 23°C.

System A employed a continuously aerated feed period with a high DO concentration of ~8 mg/L. System B employed a single pulse feed followed by on/off aeration to control the DO concentration between 2.75 and 3.25 mg/L. The average diameter of seed granules in System A was 0.8 mm, which decreased slightly to 0.75 mm over the course of the 220 days of operation. System B had a longer settling time, and the size of the granules was smaller than those in System A, ranging from 0.2 to 1.3 mm in diameter.

The NO_2-N oxidation in System A was 68% with an effluent concentration of 160 mg/L. The biomass concentration of 600 mg VSS/L may not have been at a sufficient level for complete NO_2-N oxidation. Due to the objectives of the study, which focused on soluble microbial product utilization, the NO_2-N removal performance under the granular sludge growth conditions was not determined for System B.

Table 5-8. Operating and Design Conditions and Treatment Performance Summary of Laboratory Aerobic Granular Sludge Systems Oxidizing Nitrite Only

System	A	B
Study duration, day	220	>120
Avg. granule diameter, mm	0.75	0.2–1.3 mm size range
Organic carbon feed	None	None
Temperature, °C	20–23	20
Inoculum	Nitrifying granules	Flocs
Reactor Configuration	Conventional SBR	Bubble column SBR
H/D ratio	2.7	14
Cycle fill fraction	0.275	0.50
Equivalent HRT (V/Q), hr	21.8	12
Total cycle time, hr	6	6
Feeding step Feed type Feed time, min	 Continuously aerated feeding 345	 Static 5
Aeration step Sparge air Time, min Supplemental mixing	 Continuous 345 None	 Continuous 340 None
Settling, min	10	5
Decant time, min	5	10
DO control	No	Yes (on/off)
DO, mg/L	7.5–8.3	2.7–3.3
Superficial gas velocity, cm/s	n/r	n/r
pH	7.7–8.3	~7.3
SRT, day	n/r	n/r
MLVSS, mg/L	600	n/r
N load, kg/m^3-day	0.55	1
Influent NO_2-N, mg/L	500	500
NO_2-N removal, %	68%	n/r
Effluent VSS, mg/L	6	n/r
Comments	Average diameter of seed granules was 0.8 mm.	Study focused on side batch kinetic tests; long-term performance data not reported; granules formed after 4 months of operation

Note: n/r = not reported.
References: A (Vázquez-Padín et al., 2009); B (Ni et al., 2011).

5.4.4 Nitrification Bioaugmentation Using Ammonia-Oxidizing Granules

A potential approach to improve the ammonia oxidation capacity in low-SRT flocculent or cold temperature sludge systems is bioaugmentation by adding nitrifying granular sludge into the flocculent sludge system. The physicochemical characteristics of aerobic granular sludge, including larger size and faster settling velocity, may enable granules to be selectively retained in the flocculent sludge system to allow a decoupling of the granular activated sludge SRT and the limited SRT of the flocculent activated sludge. Thus, the retained nitrifying granules can accomplish nitrification in a low-SRT flocculent sludge system.

Nitrification bioaugmentation using ammonia-oxidizing granules grown in a continuously fed aerobic upflow sparged reactor with a solid-liquid separator (Tsuneda et al., 2003; see Section 5.6) has been investigated in two studies. Though these studies used ammonia-oxidizing granules grown on an autotrophic medium, the broader concept of nitrification bioaugmentation could be extended to other types of granules containing nitrifiers.

In the first study (Tsuneda et al., 2006b), granules were mixed with flocculent sludge to treat a wastewater containing a complex synthetic organic carbon composition in a flow-through, completely mixed reactor that from all accounts was similar to the baffled, completely mixed reactor described in Section 5.6. The inoculated granular and flocculent sludge concentrations were 1200 and 1500 mg/L, respectively. The complex synthetic organic carbon-containing wastewater was characterized by DOC, total nitrogen (TN), and NH_3-N concentrations of 180, 100, and 60 mg/L, respectively. The reactor pH was controlled at 7.0 to 7.1 using sodium bicarbonate. A 100-200 um thick heterotrophic biofilm developed on the nitrifying granules, and an increase in the gas sparging aeration rate was necessary to provide sufficient oxygen penetration to maintain nitrifying activity (Tsuneda et al., 2006b). For the initial gas sparging condition of 0.5 L/min at 20% oxygen concentration, the product of ammonia oxidation shifted from nitrate to nitrite over the first 20 days of exposure with flocculent sludge and the organic wastewater. Full ammonia oxidation to nitrate was recovered after doubling the aeration rate while maintaining the 20% oxygen condition. The DO concentrations at these conditions were not reported. During this operation, there were no negative changes in granule morphology and integrity.

In a similar second study by the same research group (Kishida et al., 2011), nitrifying granules and flocculent sludge were again added to an aerated, continuously fed, baffled, completely mixed reactor at initial MLSS concentrations of 1200 and 1500 mg/L, respectively. Parallel reactors were operated at various COD/N ratios of 0, 2.6, and 13.2 using acetate, while the NH_3-N concentration was fixed at 50 mg/L. The pH in the reactors was controlled between 7.0 and 7.2 using sodium bicarbonate. The initial aeration rate of 1.0 L/min was increased on day 12 to maintain a DO concentration greater than 2 mg/L. NH_3-N removal efficiencies of approximately 85%, 80%, and 65% were achieved in the reactors with influent COD/N ratios of 0, 2.6, and 13.2, respectively. The decrease in NH_3-N removal efficiency with increasing influent COD/N ratio was attributed to heterotrophic growth on the seeded granules. It was noted that heterotrophic growth on the granules reduced their settling velocity.

5.5 Nitrous Oxide Emissions in Laboratory Aerobic Granular Sludge Systems

In an effort to curb greenhouse gas emissions, recent research efforts have focused on identifying and quantifying the sources of nitrous oxide (N_2O) emissions from wastewater treatment facilities. Nitrous oxide has received particular attention because it has a 100-year global warming potential, which is approximately 300 times that of carbon dioxide (IPCC, 2007). For purposes of this report, the biological and physicochemical mechanisms for N_2O production are not reviewed in detail, and the reader is referred to a comprehensive WE&RF report on the subject (Chandran et al., 2012).

Table 5-9 presents a summary of process conditions and N_2O emissions reported for aerobic granular sludge systems with NDN-PAO granules. All systems were bench-scale reactors with the exception of System D, which the authors classified as a pilot reactor. This reactor was a conventional SBR with a volume of 60 L and height and diameter of 1.0 and 0.3 m, respectively. Nitrogen loading rates ranged from 0.11 kg/m^3-day in System D to 0.27 kg/m^3-day in System A. N_2O emissions are reported as a percentage of the applied nitrogen load. Systems are presented in order of increasing effluent NO_2-N concentration. This approach is also used later in Table 5-10 for other granule types.

The NDN-PAO granular sludge Systems A and B did not contain detectable NO_2-N in the effluent, and N_2O emissions were low compared to other systems. System A was operated at a DO concentration of 1.8 mg/L, and it was reported that the off-gas was analyzed several times by gas chromatography but that N_2O was not detected. System B was operated at a DO concentration near saturation, and N_2O emissions were reported to be 2%. These systems exhibited little potential for NO_2-N accumulation during reactor cycles. In System A, the NO_x-N concentration was always less than 2 mg/L, and in System B, the maximum NO_2-N accumulation was 0.8 mg/L.

NDN-PAO granular sludge Systems C and D contained measurable effluent NO_2-N concentrations up to 14 mg/L. System C used an intermittent aeration regime of high-frequency cycling between a DO concentration of approximately 6 mg/L and unaerated conditions. Aerobic NO_2-N accumulation in System C was confirmed by batch activity tests with NH_3-N spiked in the System C mixed liquor. N_2O emissions were 5.2% in this system. Higher N_2O emissions in this system with NO_2-N present in the bulk liquid and frequent transitions from anoxic to oxic conditions is consistent with findings for flocculent sludge systems where these operating conditions resulted in increased N_2O emissions (Ahn et al., 2011; Chandran et al., 2011; Kampschreur et al., 2009). System D treated saline domestic wastewater where Cl^- concentration was between 5.8 and 7.0 g/L. Nitrification was incomplete in this system, with NH_3-N being oxidized to a mixture of NO_2-N and NO_3-N. N_2O emissions were between 2.3 and 6.8% of the applied TKN load. The observation of NO_2-N accumulation and higher N_2O emissions is consistent with the impact of elevated salt concentrations reported in other studies of aerobic granular (Pronk et al., 2013) and flocculent (Kampschreur et al., 2009) sludges.

N_2O emissions data from pilot or full-scale Nereda® reactors were not available in the literature. These reactors employ slow anaerobic upflow feeding followed by aeration at a DO concentration of approximately 2 mg/L and would be most similar to Systems A and B.

Table 5-9. Summary of Process Conditions and N_2O Emissions in Bench-Scale NDN-PAO Granular Sludge Systems

System	A	B	C	D
Avg. granule diameter, mm	1.3	0.9	n/r	2-3
Carbon feed	Acetate	Acetate	Acetate + propionate	Domestic wastewater
Temperature, °C	20	18–22	20	>25
Reactor configuration	Airlift SBR	Bubble column SBR	Bubble column SBR	Conventional SBR
H/D ratio	14	20	21	3
Cycle fill fraction	0.53	0.56	0.50	0.27
HRT, hr	5.6	5.4	6.4	11.6
Total cycle time, hr	3	3	3.2	3.2
Anaerobic step Feed type Feed time, min	 Upflow 60	 Upflow 60	 Upflow 60	 Upflow 60
Aeration step Sparge air Time, min Supplemental mixing	 Continuous 112 None	 Continuous 112 None	 Intermittent 120 None	 Continuous 120 None
Settling time, min	3	3	5	6–8
Decant time, min	5	5	5	2.5
DO control	Yes	No	No	Yes
DO, mg/L	1.8	>8	20 min air ON at 5.5-6.5 mg/L; 20 min air OFF without mixing	1–4
Superficial gas velocity, cm/s	5.3	1.5	2.8	0.12–0.35
pH	6.8–7.2	6.9–7.1	7–7.3	n/r
Infl. COD, mg/L	370	409	400	~490 avg.
Infl. NH_3-N, mg/L	46.5	60	50	34 avg. (53 avg. TKN)
N load, kg/m^3-day	0.20	0.27	0.16	~0.07 avg. NH_3-N basis ~0.11 avg. TKN basis
Effl. NH_3-N, mg/L	0.1	~0	1-3	<2
Effl. NO_2-N, mg/L	0	~0	<4	0–14
Effl. NO_3-N, mg/L	1.7	~35	<1	2–15
N_2O emission as % of influent N load	N_2O not detected	~2	5.2	3.7–11 (TIN basis) 2.3–6.8 (TKN basis)
Other notes		Data for low-salinity operation at 0.2 g Cl^-/L		N_2O emissions lowest for high DO and low NO_2 concentrations

Note: n/r = not reported.

References: A (de Kreuk et al., 2005b); B (Pronk et al., 2013); C (Lochmatter et al., 2014); D (van den Akker et al., 2015).

Process conditions and N_2O emissions reported for systems using granule types other than NDN-PAO granules are shown in Table 5-10 and include OHO, NDN-OHO, and NIT granules. All systems were bench-scale reactors with the exception of System I, which was a 150 L pilot-scale reactor with height and diameter of 2.4 and 0.3 m, respectively. Nitrogen loading rates ranged from 0.13 kg/m^3-day in System E to approximately 1.5 kg/m^3-day in System I.

The pulse-fed aerobic System E with OHO granules was operated at the lowest N loading rate, exhibited complete nitrification, and possessed the lowest N_2O emission rate, 0.6%. In this system, the N_2O emission rate was highest at the start of aeration and declined throughout the aerobic period. The NO_2-N concentration in this system was near zero at the start of aeration and accumulated to a maximum of ~3 mg/L during the aerobic period.

Pulse-fed anoxic-aerobic Systems F and G with NDN-OHO granules were seeded with granules performing full and partial nitrification, respectively, and evaluated in parallel reactors as part of the same study. Both reactors were operated at an N loading rate of 0.40 kg/m^3-day. System G, performing partial nitrification to only nitrite, had higher N_2O emissions (11.5%) than System F, which performed full nitrification (7.5%). The general observation of higher N_2O emissions from a partially nitrifying reactor compared to a completely nitrifying reactor is consistent with earlier findings using activated sludge that was not granular (Ahn et al., 2011).

Systems H and I were operated with N loading rates greater than 1.0 kg/m^3-day and exhibited a high degree of NO_2-N accumulation, with NO_2-N representing 92% and 100% of effluent NO_x-N, respectively. System H was a pulse-fed anoxic-aerobic reactor with NDN-OHO granules. The N_2O emission rate in System H was 4.7%, and N_2O emission during the anoxic period was not significant. System I was a flow-through, continuously aerated airlift reactor with NIT granules and treated municipal anaerobic sludge digester dewatering reject water, which was fed semi-continuously to maintain an NH_3-N concentration between 40 and 50 mg/L in the reactor. An N_2O emission rate of 2.2% was consistently measured for DO concentrations between 4.5 and 7.5 mg/L, while N_2O emissions gradually increased from 2.2% to 6.0% as DO concentration was decreased from 4.5 to 1.0 mg/L. The pattern of increasing N_2O emissions with decreasing DO is consistent with studies of flocculent sludge (Kampschreur et al., 2009).

In these laboratory systems with high aeration intensity as indicated by superficial gas velocity, the measured absolute value of N_2O emissions from aerobic granular sludge reactors may not be indicative of otherwise comparable full-scale reactors because the intense aeration in the bench-scale systems likely increased the relative rate of dissolved N_2O stripping compared to that expected from a full-scale system under lower aeration intensity. For comparison, N_2O emissions from full-scale partial nitrification reactors where N_2O emissions would be expected to be high have ranged from 0.24 to 1.7% of the influent N load (Ahn et al., 2010; Kampschreur et al., 2008).

Table 5-10. Summary of Process Conditions and N_2O Emissions in Other Bench- and Pilot-Scale Granular Sludge Systems

System	E	F	G	H	I
Granule type	OHO	NDN-OHO	NDN-OHO	NDN-OHO	NIT
Avg. granule diameter, mm	2-3	3	3	2	0.5
Carbon feed	Glucose + acetate	Glucose	Glucose	Glucose + acetate	Anaerobic digester dewatering reject water
Temperature, °C	15–21	25–28	25–28	20–30	30
Reactor configuration	Airlift SBR	Bubble column SBR	Bubble column SBR	Bubble column SBR	Flow-through airlift reactor
H/D ratio	10	8	8	6	8.4
Cycle fill fraction	0.50	0.50	0.50	0.50	n/a
HRT, hr	12	12	12	8	9.6–14.4
Total cycle time, hr	6	6	6	4	n/a
Feed type	Static	Static	Static	Static	Semi-continuous
Sequence and duration of feeding and reaction periods	Feed = 10 min Oxic = 312 min	Feed = 5 min Anoxic = 25 min Oxic = 300 min	Feed = 5 min Anoxic = 25 min Oxic = 300 min	Feed = 6 min Anoxic = 60 min Oxic = 168 min	Continuous aeration
Anoxic mixing method	n/a	n/r	n/r	Stirring	n/a
Settling time, min	3	2	2	2	n/a
Decant time, min	5	5	5	4	n/a
Idle time, min	30	23	23	0	n/a
DO control	No	No	No	No	Yes
DO, mg/L	n/r	~8	~8	6–8	1–7.5
Superficial gas velocity, cm/s	2.5	0.95	0.95	1.3	n/r
pH	7–7.8	8–8.8	8–8.8	7.5–8.5	7.3–7.7
Infl. COD, mg/L	1200	600	600	500	n/r
Infl. NH_3-N, mg/L	63	200	200	350	700–800
NH_3-N load, kg/m^3-day	0.13	0.40	0.40	1.05	~1.5 avg.
Effl. NH_3-N, mg/L	<0.1	1.7	2.5	<7	40–50
Effl. NO_2-N, mg/L	<0.1	0.7	98.9	225	600–720
Effl. NO_3-N, mg/L	23	156	13.8	20	<2
N_2O emission as % of influent TIN load	0.6	7.5	11.5	4.7	2.2% for DO from 4.5 to 7.5 mg/L; 6% for DO of 1 mg/L

Notes: n/r = not reported; n/a = not applicable.
References: E (Kong et al., 2013); F (Wei et al., 2014); G (Wei et al., 2014); H (Shi et al., 2011); I (Pijuan et al., 2014).

5.6 Other Laboratory Granular Sludge Reactor Configurations

Other laboratory reactor configurations that have been used to investigate aerobic granular sludge growth are summarized in Table 5-11. These include several continuous-flow reactor configurations and an SBR that was reported to contain granular sludge in addition to biofilm carriers and associated attached growth. The types of granules grown in these respective reactors are discussed below. Applications in which stable long-term granular sludge growth was not achieved are also noted.

A continuously fed, aerobic upflow sparged reactor with a solid-liquid separator (Table 5-11a) was used by Tsuneda et al. (2003) and Kishida et al. (2010) to grow NIT granules from flocculent sludge on synthetic autotrophic wastewaters containing no organic carbon. In the former study, the hydraulic retention time was gradually reduced over a two year period to select for faster-settling granules, and an external settling tank was used for periodic sludge return. Other studies have reported the formation and subsequent deterioration of OHO granules grown on acetate (Kishida et al., 2012a) and domestic wastewater (Niu et al., 2013) using this reactor configuration. The latter study listed above reported transient granule formation (0.8 to 1.0 mm diameter granules with an SVI of 35 mL/g and compact morphology) but subsequent deterioration and loss of granular sludge due to filamentous growth.

A continuously fed, baffled, complete mix reactor with a solid-liquid separator (Table 5-11b) was used by Kishida et al. (2012b) to grow NIT granules following seeding with NDN-PAO granular sludge. Reactor dimensions and upflow velocity in the solid-liquid separator zone were not reported.

Liu et al. (2014a) used a continuously fed reactor with a separate granular sludge selection screening tank (Table 5-11c). The reactor had an H/D ratio of 16, a 7.5 L volume, and a 9 hr HRT. No details were available for the sludge selection tank. The screen aperture was gradually increased from 0.1 to 1.0 mm for granule selection. Sludge retained on the screen was intermittently returned to the reactor by pumping. A favorable granule morphology and SVI (35 mL/g) were obtained. Performance data indicated that simultaneous EBPR and N removal occurred despite continuous feeding and aeration, where the DO concentration was reported to be 3 to 6 mg/L. Average effluent TN and total phosphorus (TP) were less than 1 and 2 mg/L, respectively, during a 30-day period of operation with influent COD (as glucose), TN, and TP concentrations of 400, 40, and 8 mg/L, respectively. The authors did not provide an explanation for the apparent occurrence of EBPR in this continuously aerated reactor. A potential explanation is that the sludge selection tank was serving as a return activated sludge (RAS) fermenter. RAS fermentation has been shown to select for PAO growth, particularly that of *Tetrasphaera* PAOs, which appears to have an advantage in fermentative conditions (Marques et al., 2016; Tooker et al., 2016). The microbial community in this system was not investigated.

Several studies have investigated the growth of aerobic granular sludge in membrane bioreactors (MBRs) with continuous feeding and aeration (Juang et al., 2008; Wang et al., 2008c; Yu et al., 2009; see Table 5-11d). Effluent permeation occurred semi-continuously. For example, 8 min of permeation and 2 min of relax was employed by Wang et al. (2008c). Aerobic granular sludge grown in SBRs was used as seed sludge in these studies. The study by Wang et al. (2008c) provided the most useful information on aerobic granular sludge growth in an MBR where a reactor of unspecified H/D ratio was seeded with GAO-enriched aerobic granules of 0.8 to 1.5 mm diameter obtained from an anaerobic-aerobic SBR. The MBR feed included acetate as the sole carbon source at a concentration from 117 to 312 mg COD/L and ammonium chloride at a concentration from 25 to 40 mg NH_3-N/L. The operating time was 70 days, and the SRT was given at approximately 40 days. Seed granules larger than 0.9 mm tended to disaggregate, but approximately 50% of the MLSS at the end of the experiment contained granules with a size between 0.2 and 0.9 mm. The TN removal efficiency was approximately 50%. Considering these results

and the absence of hydraulic selective pressures and feast-famine regimes in conventional MBRs, the practical application of sustaining aerobic granular sludge in MBRs appears limited.

Granular sludge has also been reported in a hybrid attached growth-granular sludge SBR (Table 5-11e), termed a sequencing batch biofilter reactor by the investigators (Di Iaconi et al., 2006, 2007). The reactor volume and H/D ratio were 27 L and 4.0, respectively. Granular sludge and the attached growth media were retained between two screens, and bulk liquid was recirculated through the reactor at an upflow velocity of 20 m/hr during the aerobic reaction period. Media consisted of 8 mm diameter Kaldnes K1 carriers (690 m^2/m^3), and the initial bed porosity was 0.75 prior to biomass growth. The authors reported that granule formation was facilitated by biofilm detachment, subsequent transition to granular morphology, and retention within the media bed by the retaining screens. The granule size and morphology were not given. The biomass concentration within the bed volume between the retaining screens was as high as 38 g TSS/L. This type of hybrid reactor was used successfully at the pilot scale to treat domestic and tannery wastewaters (Di Iaconi et al., 2008) with 90% TKN removal for domestic wastewater treatment at an HRT of 8 hr. The feed COD concentration was from 200 to 500 mg/L, and the TKN concentration was from 35 to 60 mg/L.

Table 5-11. Other Aerobic Granular Sludge Reactor Configurations Investigated in Laboratory Studies

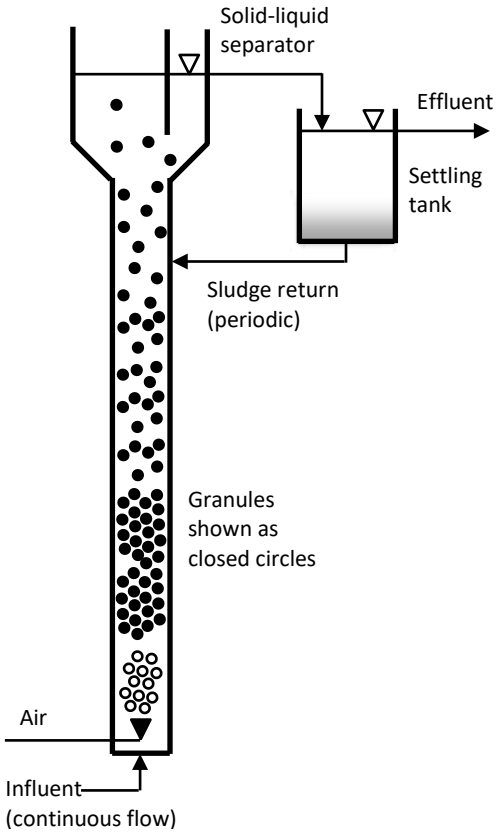
Reactor Configuration	Description
(a) Continuously fed, aerobic upflow sparged reactor with solid-liquid separator and external settler 	The granular sludge reactor configuration involves continuous upflow feeding and aeration in a tall column reactor (H/D ratio >50) that includes a solid-liquid separator at the top of the column. An optional settling tank following the solid-liquid separator has been used to allow sludge return.

Table 5-11. Other Aerobic Granular Sludge Reactor Configurations Investigated in Laboratory Studies (continued)

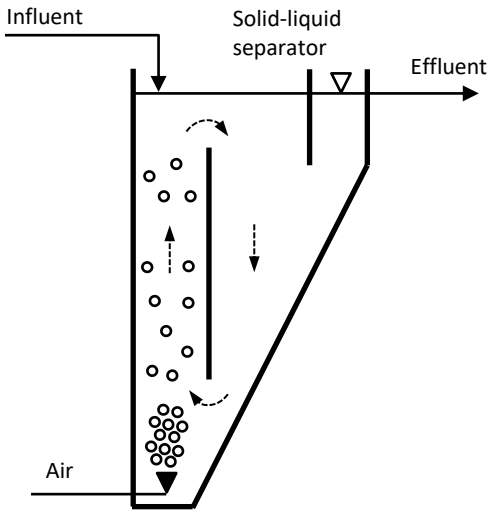
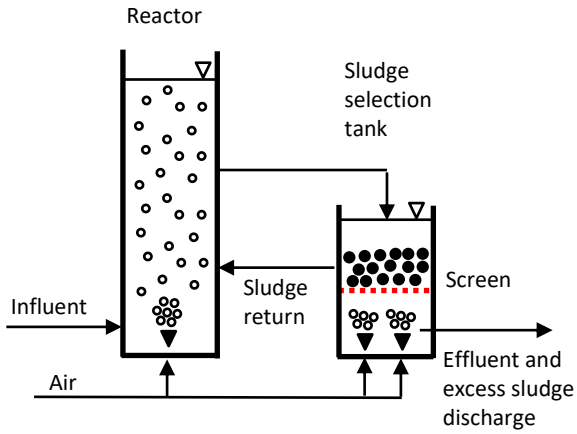
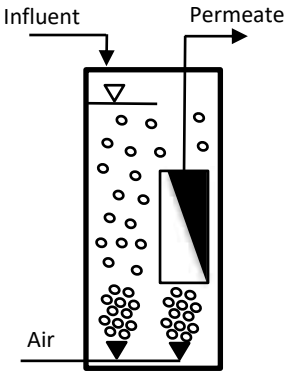
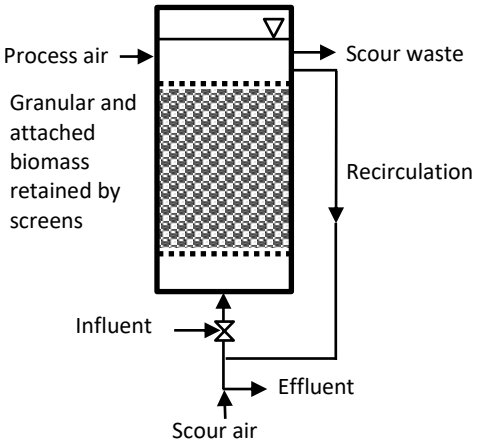
Reactor Configuration	Description
(b) Continuously fed, baffled, complete mix reactor with solid-liquid separator 	The continuously fed and aerated granular sludge reactor uses an internal baffle and aerator placement to obtain a recirculating mixing pattern as shown. An additional baffle creates an upflow solid-liquid separator zone for effluent discharge.
(c) Continuously fed reactor with a granular sludge selection screening tank 	The granular sludge reactor configuration includes a continuously fed, continuously aerated granular sludge reactor and separate granular sludge selection tank that uses a screen to select for larger granular sludge particles that are returned to the reactor by pumping. The screen aperture can be gradually increased over time for granular sludge selection. Effluent is discharged continuously from the sludge selection tank downstream of screening, and smaller particles passing the screen are washed out in the effluent stream. Scour air is provided intermittently in the screening tank to prevent blockage.

Table 5-11. Other Aerobic Granular Sludge Reactor Configurations Investigated in Laboratory Studies (continued)

Reactor Configuration	Description
(d) Membrane bioreactor seeded with granules 	In membrane bioreactors (MBRs), treated effluent is referred to as permeate and is drawn from the biological reactor across a membrane, which provides liquid-solids separation. Several studies have investigated the growth of aerobic granular sludge in MBRs with continuous feeding and aeration. Stable long-term granular sludge growth in this configuration has not been demonstrated.
(e) Hybrid attached growth-granular sludge SBR 	The hybrid attached growth-granular sludge SBR contains a bed of granular sludge and attached-growth media retained by screens. The reactor operates in SBR mode with unaerated pulse feeding followed by an aerobic reaction period during which bulk liquid is recirculated through the bed and process air is added at the top of the reactor. The bed is occasionally cleaned by air scour. No settling period occurs. Effluent is drawn from the bottom of the reactor in a brief discharge period.

CHAPTER 6

Removal of Micropollutants and Industrial Chemicals in Bench-Scale Aerobic Granular Sludge Systems

In recent years, there has been increasing concern about the presence of pharmaceuticals and personnel care products (PPCPs) in municipal WRRF effluents. These substances are typically at low ng/L to ug/L concentrations and are included in what has been referred to as trace organics or micropollutants. PPCP compounds include analgesics, lipid regulators, synthetic hormones, steroids, fragrances, sunscreens, shampoos, and cosmetics. Their presence in surface waters and groundwater and have been reported in a number of studies (Barnes et al., 2008; Benotti et al., 2009; Focazio et al., 2008; Kolpin et al., 2002). Another class of trace organics that include certain pharmaceuticals, human hormones, and industrial chemicals are endocrine-disrupting compounds (EDCs). EDCs can have adverse effects on aquatic organisms at low ng/L concentrations in streams by affecting both male and female reproductive hormones (Blazer et al., 2007; Caldwell et al., 2012; Guillette and Gunderson, 2001; Jobling et al., 2006; Milnes et al., 2006; Vajda et al., 2008). The fate of PPCPs and EDCs have been studied in flocculent activated sludge WRRFs in Water Environment & Reuse Foundation (WE&RF) projects (Ohlinger et al., 2014; Stephenson and Oppenheimer, 2007) and by many others (Andersen et al., 2003; Clara et al., 2005; Joss et al., 2004; Joss et al., 2006). Removal of many trace organic compounds has occurred to varying degrees in activated sludge systems by biodegradation and sorption to waste solids.

There has been a significant amount of laboratory research with flocculent activated sludge processes and enriched cultures to study PPCP and EDC biodegradation efficiency, microbial populations, and removal mechanisms. However, there has been limited research on the fate and biodegradation of these compounds in aerobic granular sludge systems. Initial investigative work has shown similar removal mechanisms of biodegradation and sorption as for flocculent activated sludge (Andersen et al., 2003). However, the effect of potentially different microbial population selections and of granular biofilm diffusion limitations is not known. Results from a limited amount of laboratory experiments with several endocrine-disrupting and pharmaceutical compounds are presented in this section.

The removal of the EDCs estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), bisphenol A (BPA), and 4-*tert*-octylphenol (4t-OP) from municipal primary effluent wastewater by a pilot-scale hybrid attached-growth/granular sludge reactor (SBBGR; see Table 5-11e in Section 5.6) has been investigated (Balest et al., 2008). The concentration of EDCs in the influent to the secondary process was dependent solely on the nature of the municipal wastewater after primary treatment. For all compounds, the SBBGR EDC removal efficiencies were higher than those of the full-scale activated sludge system. The removal efficiencies of E1, E2, BPA, and 4t-OP were 60, 69, 93, and 81% for the SBBGR versus 53, 41, 72, and 67% for the flocculent activated sludge plant, respectively, over the 4 month experiment. For EE2 the effluent concentrations for both systems were below the quantification limit of 0.3 ng/L using the HPLC-MS analytical method. The enhanced performance of the SBBGR was attributed to its high biomass concentration and long SRT of 40 g/L and 180 days, respectively.

Studies on the treatment of industrial chemicals (Maszenan et al., 2011) with aerobic granular sludge have focused on the removal of phenol or substituted phenolic compounds. Using a flocculent seed sludge that was acclimated to phenol, aerobic granular sludge was grown on phenol as the sole carbon source in an aerobic pulse-fed bubble column SBR at 25°C with a 50% fill fraction (Tay et al., 2004b). During the last 50 days of the 150-day operating period, the reactor contained granules with an average diameter of 1.2 mm and MLSS concentration of 8.2 g/L. During this period, 99.96% phenol degradation

efficiency was achieved for an influent phenol concentration of 833 mg/L, which corresponded to a loading rate of 2.5 g phenol/L-day.

A study by Duque et al. (2011) evaluated the bioaugmentation of a 2-fluorophenol-degrading strain into an established granular sludge system. The granular sludge bubble column SBR involved slow anaerobic bottom feeding of synthetic wastewater with an acetate COD concentration of 370 mg/L as the sole carbon source. The SRT was controlled at 30 days, and pH varied between 6.2 and 7.8. After 210 days of operation to cultivate granular activated sludge, the 2-fluorophenol-degrading strain was added to the reactor. Though not explicitly stated, the bioaugmentation was a single seeding event. Settling time was increased 20 min with seeding to retain the newly added biomass, and then was gradually decreased over a 90-day period to the previous settling time of 3 min (cumulative day 300). During days 300-444, greater than 99% removal 2-fluorophenol was achieved at influent concentrations of 0.22 and 0.44 mM, which were varied during the experiment. Mass balances indicated that nearly all of this removal was due to biodegradation.

A study on the biodegradation of *p*-nitrophenol by aerobic granular activated sludge grown on acetate was done by Yarlagadda et al. (2012). First, the aerobic granular activated sludge was cultivated for two months in an aerobic pulse-fed SBR on a synthetic wastewater containing acetate as the sole carbon source at a COD concentration of 450 mg/L. The granular sludge was then used to inoculate two SBRs: one fed *p*-nitrophenol as the sole carbon source and the other fed acetate and *p*-nitrophenol. The reactor receiving only *p*-nitrophenol demonstrated an ability to rapidly adapt to the new feed substrate. After five days of operation, greater than 99% removal of 50 mg/L influent *p*-nitrophenol was achieved and sustained until day 30 when the experiment was terminated. The reactor receiving acetate and *p*-nitrophenol adapted to *p*-nitrophenol degradation but at a lower rate. As a consequence, the influent *p*-nitrophenol concentration was halved. From day 20 to 30, this reactor achieved greater than 99% removal of *p*-nitrophenol at a 25 mg/L influent concentration.

A study utilizing a pulse-fed aerobic airlift SBR with a 70% fill fraction, 23-27°C temperature, and 6.5-6.9 pH demonstrated that granules could be grown from flocculent seed sludge using 4-chloraniline as the sole carbon source (Zhu et al., 2008). The study evaluated different 4-chloraniline loads prior to stable operation, which occurred by day 150, when 99.9% removal of 4-chloraniline was achieved at an influent concentration of 180 mg/L. The average diameter of the granules was 0.6 mm, and the SVI_{30} was approximately 40 mL/g. The reactor MLVSS concentration was approximately 10 g/L, and the SRT was only four to six days as a result of the high effluent TSS concentration, which ranged from 500 to 800 mg/L.

Aerobic granular activated sludge was also grown from flocculent seed sludge using nitrobenzene as the sole carbon source (Zhao et al., 2011a). This system used slow anaerobic bottom feeding followed by aeration in a bubble column SBR. The reactor fill fraction was 50%, and the pH was 6.8 to 7.2. During 140 days of operation, various experimental conditions were investigated. The maximum loading conditions with greater than 99% nitrobenzene removal were at a nitrobenzene loading rate of 0.33 kg/m^3-day at an influent concentration of 600 mg/L. The MLSS concentration was approximately 5.5 g/L. The average granule diameter was 1.4 mm, and the SVI_{30} was under 35 mL/g.

Using MTBE and ethanol as co-substrates, aerobic granules were grown from flocculent sludge in an aerobic pulse-fed bubble column SBR (Zhang et al., 2008) at a DO concentration greater than 4 mg/L. Over the course of the study, the influent ethanol concentration was gradually decreased from 500 to 100 mg/L while the MTBE concentration was increased from 50 to 400 mg/L. In the last 30 days of the 280-day operating period, the MTBE removal efficiency exceeded 99.9% for 400 mg/L influent concentration. It should be noted that about 20% of the MTBE removal was from volatilization. The

granular sludge had an average diameter of 0.4 mm, and the mixed liquor SVI_{30} was approximately 50 mL/g.

A series of studies investigated the biodegradability of mono-, di-, and trichlorophenol in aerobic granular sludge (Khan et al., 2011a, 2011b, 2011c). In these studies, flocculent seed sludge was acclimated to chlorinated phenols for 15 to 30 days prior to inoculation into granular activated sludge reactors. The granular reactors were aerobic pulse-fed bubble column SBRs operated with a 50% fill fraction and an SRT of about 20 days. The first study found that granular sludge with 2-chlorophenol degradation capacity could be grown at room temperature on a synthetic wastewater containing glucose and 2-chlorophenol as the carbon sources. After 64 days of operation, the MLVSS concentration reached 2.6 g/L, the granule size ranged from 1 to 2 mm in diameter, and the SVI_{30} was 35 mL/g. A 90% COD removal efficiency was achieved for 1617 mg COD/L influent consisting of 1065 mg/L as glucose and 552 mg/L as 2-chlorophenol. The corresponding influent 2-chlorophenol concentration was 140 mg/L. The second study found that the granular sludge could degrade 2,4-dichlorophenol at an operating temperature of 28-32°C and pH controlled between 7.5 and 7.7. After 70 days of operation, a 90% COD removal efficiency was achieved for an influent 2,4-dichlorophenol concentration of 70 mg/L. No co-substrate appeared to be added in this study. The system MLVSS concentration was 2.2 g/L, and the granule size ranged from 0.5 to 1.0 mm in diameter. The MLSS concentration was 5.8 g/L such that the volatile solids fraction was only 38%. This was not addressed by the authors, but likely occurred as a consequence of elevated pH and the high PO_4-P concentration of 600 mg/L in the synthetic feed. Similar mineral concentrations were used in the first study of 2-chlorophenol, but only MLVSS was reported. The third study demonstrated the capacity of the aerobic granular sludge to degrade 2,4,6-trichlorophenol. Two parallel systems were fed 2,4,6-trichlorophenol and either glucose or acetate as co-substrates. The operating temperature was 30 to 40°C, and the pH was 8 to 9. After 100 days of operation, the granular sludge MLVSS concentration reached 6.5 g/L, and the SVI_{30} was less than 35 mL/g in both systems. A COD removal efficiency of greater than 90% was achieved for a total influent COD concentration of 4000 mg/L. The influent 2,4,6-trichlorophenol COD concentration was about 2900 mg/L. The mineral concentrations for this system were similar to those of the above systems.

CHAPTER 7

Pilot and Full-Scale Aerobic Granular Sludge Process Applications

At the time of this publication, the only operating full-scale systems designed for BNR with aerobic granular activated sludge instead of the common flocculent activated sludge use the Nereda® process. The use of full-scale Nereda® systems for the treatment of municipal (Table 7-1) and industrial (Table 7-2) wastewaters has been ongoing since 2009 and 2005, respectively. A "hybrid" Nereda® process has also been employed at full scale (Table 7-3). The hybrid process involves the treatment of the influent wastewater by a parallel Nereda® process and flocculent activated sludge process with wasting of excess granular sludge from the Nereda® process to the flocculent activated sludge process.

Municipal Nereda® plants have been designed to treat a range of average daily flow rates and peak hour flow conditions. The largest Nereda® plant is located in Rio de Janiero, Brazil, with a design average daily flow rate of 86,400 m^3/day (22.8 mgd). Design hourly flow peaking factors are shown for the municipal installations in Tables 7-1 and 7-3 and range between 1.5 and 7.5. Influent feed tanks may be used to buffer peak flows, as discussed later in Section 7.1.2. For example, two plants using influent feed tanks are Garmerwolde and Vroomshoop, which have design hourly flow peaking factors of 3.5 and 6.4, respectively (Giesen, 2016; Pronk et al., 2015b).

This chapter provides 1) a description of the Nereda® process including the design and operation, configuration options, instrumentation and controls, and ancillary process considerations; 2) process performance information; 3) a desktop comparison of energy requirements between a Nereda® process and flocculent sludge anaerobic-anoxic-oxic (A2O) process for BNR; and 4) a discussion of the Nereda® process benefits and possible limitations.

Table 7-1. Nereda® Process Municipal Installations in Operation or Under Construction as of July 2016

Facility	Country	Start-Up Year	Average Daily Flow (m^3/day)	Hourly Flow Peaking Factor	Population Equivalent	Notes
In Operation						
Gansbaai	South Africa	2009	5000	1.9	63,000	A
Epe	The Netherlands	2011	8000	4.5	54,000	B
Dinxperlo	The Netherlands	2013	3100	4.4	15,700	
Garmerwolde	The Netherlands	2013	28,600	3.5	140,000	
Wemmershoek	South Africa	2014	5000	3	39,000	
Ryki	Poland	2015	5300	1.9	41,000	C
Clonakilty	Ireland	2015	4900	3.1	20,500	
Carrigtohill	Ireland	2015	6750	3	30,000	
Rio de Janeiro	Brazil	2016	86,400	1.7	480,000	
Under Construction						
Hartebeestfontein	South Africa		5000	6	44,000	
Kingaroy	Australia		2700	4	16,000	
Dublin	Ireland		21,700	7.5	94,000	D
Dublin	Ireland		117,000	1.9	400,000	E
Cork Lower Harbour	Ireland		18,280	2.4	65,000	
Simpelveld	The Netherlands		3670	6.2	11,900	

A: Receives a high fraction of septage. Contribution of septage to COD load not specified.
B: Slaughterhouse waste contributes ~25% of COD load.
C: Septage and vegetable processing waste contributes ~60% of COD load.
D: Initial retrofit of one flocculent activated sludge SBR cell at Dublin Ringsend plant with anticipated start-up in 2016.
E: Complete scope of granular sludge upgrades at Dublin Ringsend plant with anticipated start-up in 2019.

Source: Giesen, 2016.

Table 7-2. Nereda® Process Industrial Installations in Operation as of July 2016

Plant	Country	Start-Up Year	Average Daily Flow (m^3/day)	Population Equivalent	Industrial Waste Type
Ede	The Netherlands	2005	250	5000	Cheese waste
Rotterdam	The Netherlands	2006	700	30,000	Food processing waste
Oldenzaal	The Netherlands	2006	360	10,000	Food processing waste
Oosterwolde	The Netherlands	2009	500	5000	Food processing waste
IJsselstein	The Netherlands	2015	1400	43,000	Slaughterhouse waste

Source: Giesen, 2016.

Table 7-3. Hybrid Nereda®/Activated Sludge Process Municipal Installations in Operation as of July 2016

Plant	Country	Start-Up Year	Average Daily Flow (m^3/day)	Peaking Factor	PopulationEquivalent
Vroomshoop	The Netherlands	2013	1500	6.4	12,000
Frielas	Portugal	2014	12,000	---	40,000

Source: Giesen, 2016.

7.1 Process Description

The Nereda® process is based around a sequence batch reactor (SBR) operation, but in contrast to the conventional SBR process used for flocculent activated sludge systems, it has a very short settling period and does not have a separate effluent decanting step with drawdown of the reactor liquid level.

7.1.1 Reactor Design and Operating Conditions for Nutrient Removal

The sequential operating steps for the Nereda® process are illustrated in Figure 7-1 and are as follows: 1) simultaneous anaerobic feeding and effluent overflow, 2) aeration, and 3) quiescent settling. The approximate times used for these steps and process functions during each phase are summarized in Table 7-4. During feeding, the influent flows in a plug flow mode through the settled granular sludge, which at the same time displaces treated effluent at the top of the tank to an effluent overflow. Anaerobic conditions exist in the granular blanket, which provides an environmental condition for the selection of PAOs and/or GAOs and their uptake of VFA in the influent and from rbCOD fermentation. Influent particulate bCOD is sorbed to the granule surface and converted to rbCOD by hydrolysis, allowing further carbon uptake by the PAOs and/or GAOs.

During the aeration period, the DO concentration is controlled in the range of 1.0 to 2.0 mg/L, which results in nitrification in the outer oxygen-penetrated region of the granule and denitrification in the inner anoxic region of the granule. Denitrifying PAOs and/or GAOs in the inner region use stored carbon substrates for NO_x reduction to nitrogen gas, with the PAOs taking up soluble P during NO_x reduction. OHOs present in the inner anoxic zone may also contribute to NO_x reduction by using rbCOD remaining after the anaerobic contact period and produced from particulate hydrolysis and endogenous decay during the aeration period. Because anaerobic feeding favors rbCOD uptake by PAOs and/or GAOs, the contribution of ordinary heterotrophs to NO_x reduction is less significant. PAOs and/or GAOs may also be present in the oxygen-penetrated outer layer and aerobically oxidize internal COD storage products. OHOs in the outer aerobic layer may also oxidize any rbCOD remaining in the bulk liquid after the anaerobic period and produced from particulate hydrolysis and endogenous decay in the outer layer.

The short settling period after aeration, typically about 10 minutes, allows the formation of a granular sludge bed at the bottom of the reactor due the rapid settling rates of granules. The time for the process sequence steps is dependent on the wastewater characteristics and targeted effluent concentrations. The total cycle time may range from two to nine hours (van Haandel and van der Lubbe, 2012).

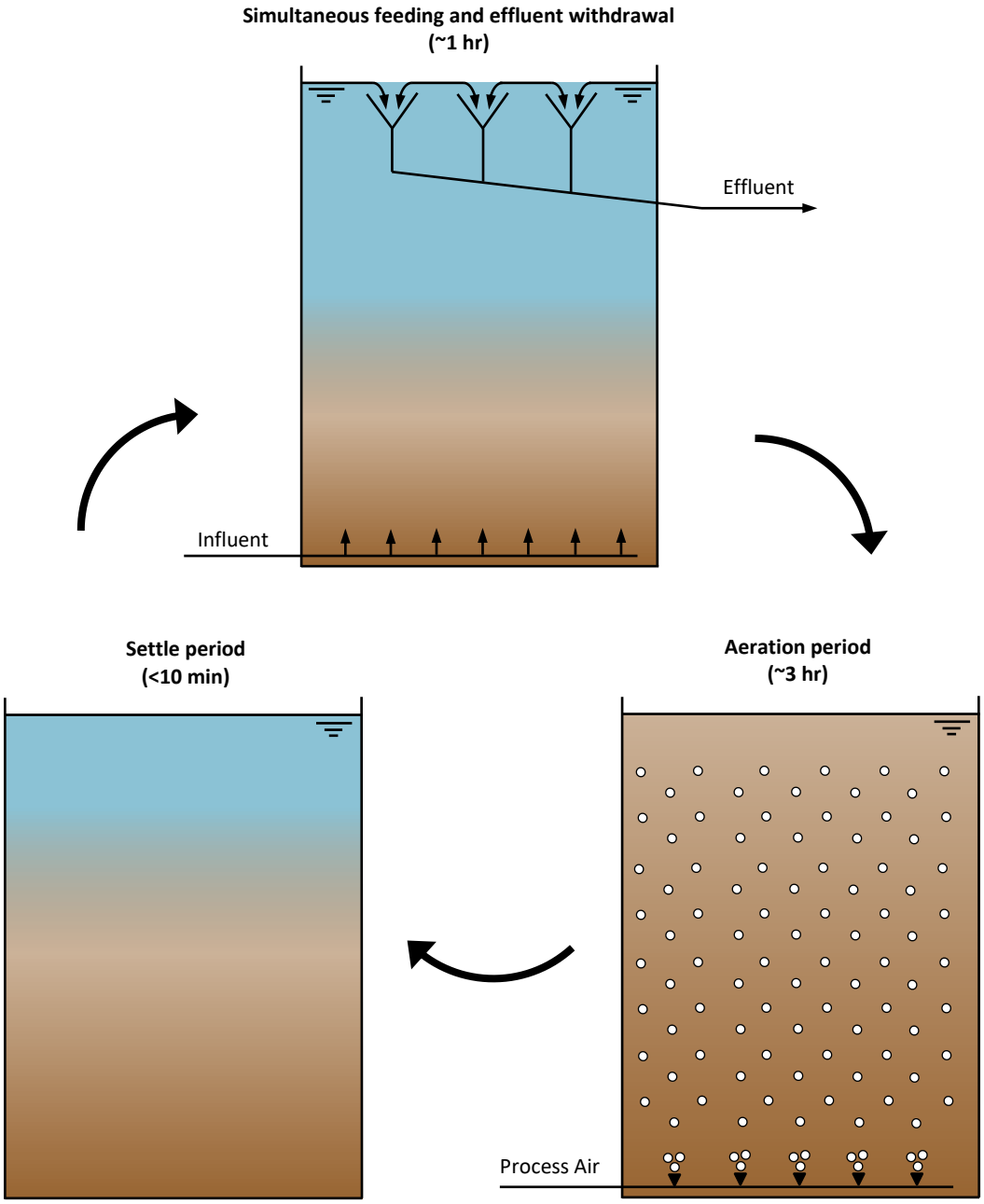


Figure 7-1. Sequential operating steps of feeding, aeration, and settling in the Nereda® granular sludge process.

Table 7-4. Function of the Operating Steps in the Nereda® Granular Sludge Process

Sequence Step	Typical Duration, hrs	Process Function
Anaerobic feed / Effluent overflow	0.5–1	Uptake of rbCOD by PAOs and/or GAOs Sorption and hydrolysis of particulate bCOD to rbCOD Upflow feed displaces treated effluent
Aeration	3–5	NH_3-N and NO_x removal by simultaneous nitrification-denitrification PAO use of stored carbon and P uptake Biological degradation of particulate bCOD remaining after anaerobic contacting
Settling	0.1–0.2	Granular sludge blanket formation at bottom of reactor before feeding

Key design features of the Nereda® reactor are shown in Figure 7-2, and an aerial image of the Epe plant with three SBRs is shown in Figure 7-3. Proper design of the feed distribution and effluent collection systems is critical to ensure an upflow plug flow feeding regime without short circuiting. A minimum side water depth of approximately 5 m is used to facilitate plug-flow feeding, limit the reactor area required, reduce the cost of the hydraulic distribution system components, and increase the oxygen transfer efficiency. At the end of the feeding period, a supplementary effluent drain valve is opened in order to provide space for the expansion of the liquid volume during aeration. Besides NO_x reduction during the aeration period, the reactor design provides provision for additional NO_x reduction by the ability to recycle treated liquid with NO_x in the upper portion of the reactor to the granular sludge bed feed distribution system. Although fine bubble membrane diffusers are typically used for aeration, an existing jet aeration system was retained in an early industrial SBR conversion to the Nereda® process without adversely impacting granulation. Waste sludge may be discharged at the end of the settling phase or during aeration. Selective wasting from the top of the settled sludge bed can enhance the selection of faster settling PAO-enriched granules (Bassin et al., 2012a; Winkler et al., 2011).

Common Nereda® design and process parameters are listed in Table 7-5. The maximum upflow feeding velocity is 5 m/hr, which allows granules with typical settling velocities between 10 and 50 m/hr to remain settled during feeding. The upflow feeding velocities applied in this feeding mode allow for the presence of well-settling flocculent biomass with the granular sludge. The settling of flocculent sludge may continue during the upflow feeding step after the short quiescent settling step. The flocculent sludge fraction is discussed in greater detail in Section 7.2.2. The volumetric OLR is determined by the practical limits on hydraulic loading rates and cycle times for the treatment of conventional domestic wastewaters of low to moderate strength. Volumetric OLRs are typically 1 to 3 kg COD/m^3-day, but due to the high biomass concentration in the reactors (8 to 12 g/L), the corresponding F/M range is approximately 0.1 to 0.3 kg COD/kg VSS-day, which is comparable to many flocculent activated sludge systems. The fill fraction per cycle may range between 5 and 70% of the reactor volume. Smaller fill fractions would typically be associated with more concentrated industrial wastes. High fill fractions are employed during peak wet weather conditions to maximize reactor throughput.

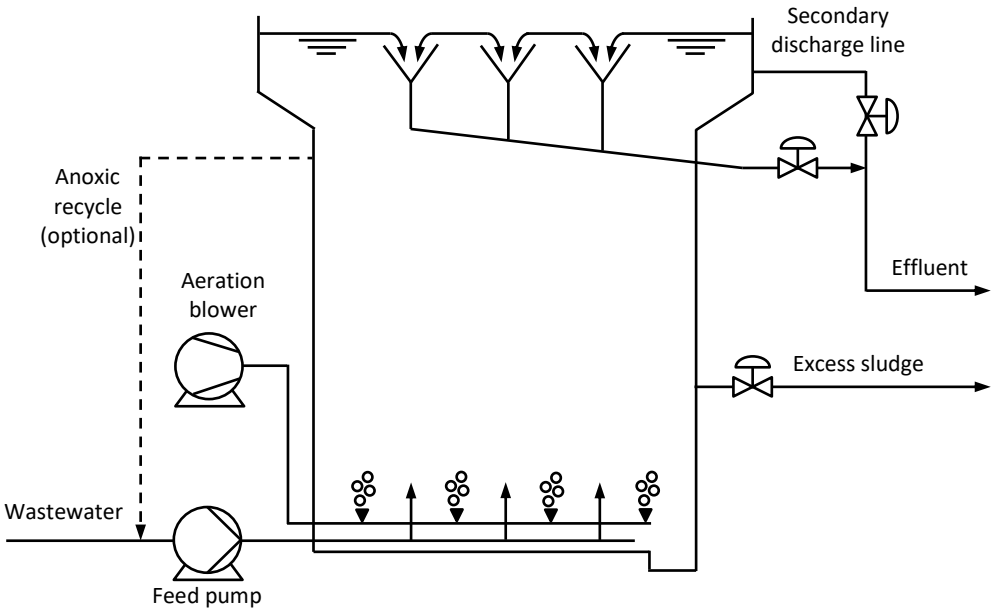


Figure 7-2. Schematic showing Nereda® reactor design features.
Adapted with permission from van Haandel and van der Lubbe (2012). Copyright 2012 IWA Publishing.

Figure 7-3. Aerial image of Nereda® Plant in Epe, The Netherlands.
Reprinted with permission from the Netherlands Water Partnership. Copyright Netherlands Water Partnership. Retrieved from http://www.dutchwatersector.com/news-events/news/2704-official-commissioning-nereda-at-wwtp-epe-wonder-granule-keeps-its-promise.html

Table 7-5. Typical Value Ranges for Several Design and Process Parameters in Nereda® Reactors

Parameter	Value	Parameter	Value
Avg. upflow feed velocity	2–3 m/hr	SVI_5 / SVI_{30}	<1.1
Max. upflow feed velocity	5 m/hr	SVI_{30}	<50
Granule settling velocity	10–50 m/hr	DO setpoint (bulk)	1–2.5 mg/L
Settling time	≤10 min	Reactor height	>5 m
Reactor MLSS	8–12 g TSS/L	Typical aeration	Fine bubble
Volumetric organic loading rate	1–3 kg COD/m^3-day	Fill fraction	5–70%

Sources: Pronk et al., 2015b; van Haandel and van der Lubbe, 2012.

7.1.2 Nereda® Process Application Options

The Nereda® process can be applied for secondary treatment as an entirely new or replacement facility or as a retrofit depending on site-specific conditions as shown in Figure 7-4. In both cases pretreatment consists of conventional headworks and either primary clarification or fine screening. In Treatment Scheme 1, three or more reactors are used to enable continuous feeding of incoming wastewater. An optional influent feed tank (IFT) for flow equalization is considered (Treatment Scheme 2) depending on the characteristics of the influent diurnal flow patterns. For highly variable flow rates, equalization may reduce the required reactor volume and capital cost of the secondary treatment process.

For retrofit applications, the Nereda® process may be installed as a new parallel system (Treatment Scheme 3) to provide additional biological treatment capacity to upgrade the facility to BNR or to handle higher future flows and loadings. Influent flow equalization may also be considered for the parallel Nereda® operation. Another option for this treatment scheme is to direct the Nereda® process waste sludge to the existing conventional flocculent activated sludge system to increased MLSS settleability and concentration. Wasting of Nereda® process waste sludge at the Vroomshoop, the Netherlands plant during start-up and operation of the parallel Nereda® system from June 2013 to December 2014 was reported to result in a gradual decrease in the SVI_{30} of the flocculent activated sludge system from approximately 100 mL/g to 60 mL/g (Giesen et al., 2015; Robertson et al., 2015). Further work is needed with a controlled experiment to better understand changes in mixed liquor characteristics and to more reliably predict the benefits of adding waste sludge from a Nereda® process to a flocculent activated sludge system.

The effluent from a parallel Nereda® process would normally be blended with the effluent from the existing activated sludge system prior to downstream disinfection or further treatment such as an effluent filtration process. Depending on site-specific conditions, Nereda® effluent may be blended upstream of the existing secondary clarifier if it offers a more convenient point of discharge or if chemical polishing for phosphorus removal is to be done via the secondary clarifier (Nereda® effluent routing a versus b, Treatment Scheme 3).

A different retrofit application would be the conversion of the existing flocculent activated sludge or SBR tankage to the Nereda® process (Treatment Scheme 4).

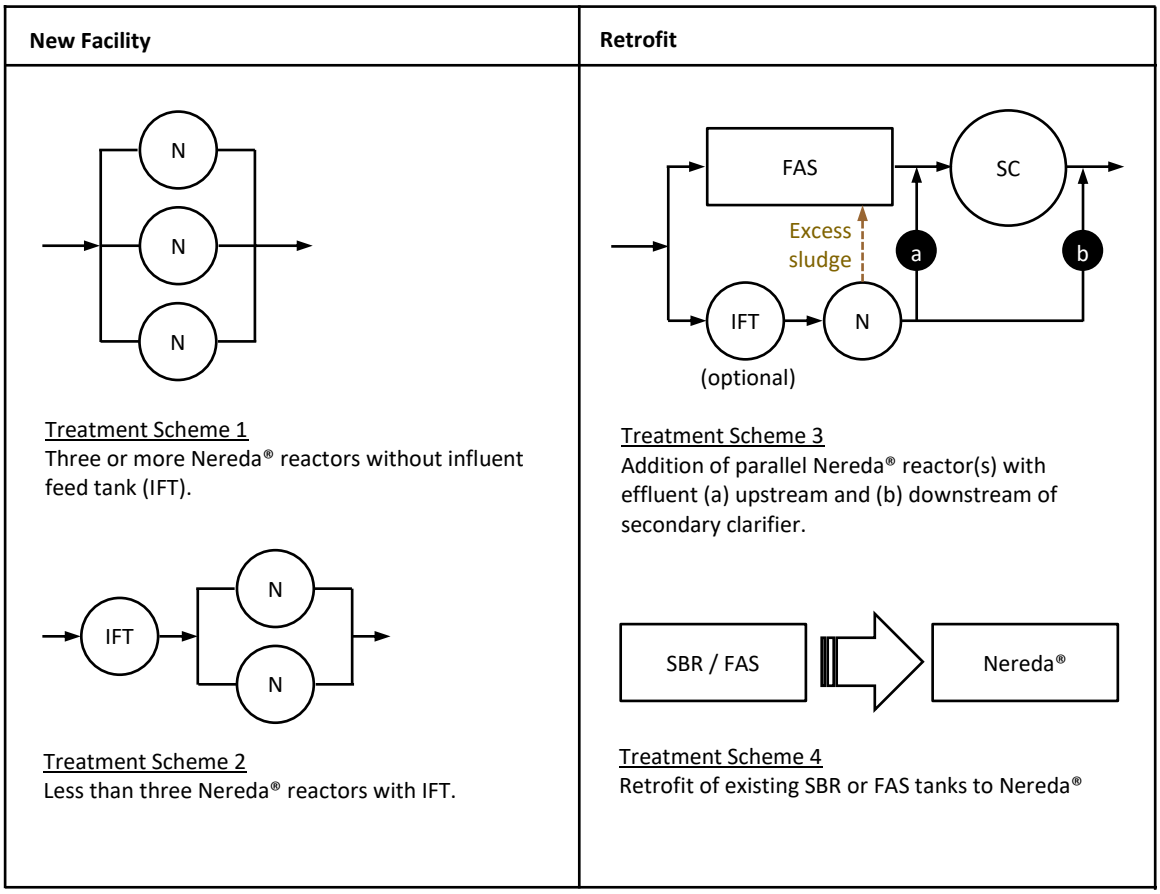


Figure 7-4. Nereda® process application configuration options.
Notes: N – Nereda® process; IFT – influent feed tank for flow equalization;
FAS – flocculent activated sludge; SC – secondary clarifier.

7.1.3 Instrumentation and Controls

On-line instrumentation is used with the control system for 1) aeration and DO control for process performance and to minimize energy costs, 2) control and optimization of nutrient removal, and 3) process effluent monitoring. The flexibility of the Nereda® control system affords the opportunity to optimize the process such that treatment requirements are met while minimizing energy demand and maximizing throughput.

The extent of on-line instrumentation depends on plant size, effluent quality targets, and optimization objectives. For larger plants, a higher level of instrumentation is used to reduce operating costs, while for smaller plants or those with less stringent effluent limits, only a basic level of instrumentation may be used. Basic on-line measurements include DO, ORP, temperature, and water level. Other instrumentation includes on-line turbidity, TSS, NH_3-N, NO_3-N, and PO_4-P measurements. If the plant is controlled remotely, a complete instrumentation package is installed. For example, at the Epe plant, an operator is present at the plant approximately twice per week, and the plant operation is monitored from a central control room for the entire water district.

A programmable computer is provided with the Nereda® process to control the reactor cycles and sequential step times and has been interfaced with on-line instruments to optimize energy costs and nutrient removal. The process controller is also integrated with the facility programmable logic controller (PLC) and supervisory control and data acquisition (SCADA) system. Cycle times may be

constant with variable batch volume or dynamic with fixed batch volume depending on flow and loading conditions.

For plants with on-line NH_3-N measurement, aeration is typically controlled by the NH_3-N signal. A minimum amount of aeration is provided to meet treatment objectives by stopping aeration when the target NH_3-N concentration is reached and varying DO concentration within and/or between cycles based on the NH_3-N signal. In the absence of on-line NH_3-N measurement, aeration is controlled based on DO setpoints. In certain cases aeration time may be governed by on-line PO_4-P measurement as discussed below. For effluent limits based on TN removal, both on-line NO_3-N and NH_3-N measurements are made. In this case, the NH_3-N concentration target may be elevated to minimize the energy demand while meeting the TN target. When aeration is terminated based on the NH_3-N measurement, the settle period may commence or an anoxic period may be introduced prior to the settle period, as discussed below.

An operating option unique to the Nereda® process reactor configuration is to use a post-anoxic recycle period to reduce the effluent NO_x concentration to meet discharge limits for NO_x-N, TIN, or TN concentrations. On-line NO_3-N measurements may initiate the need for this period depending on the final NO_3-N concentration following simultaneous nitrification-denitrification during the aeration period after the target NH_3-N concentration has been attained. Additional denitrification can be achieved by recycling liquid from the top of the reactor through the granular sludge bed with the aeration turned off (see Figure 7-2). The design internal recycle ratio is typically less than 1.0, which is much lower than that used in conventional anoxic-aerobic activated sludge processes (2.0 to 4.0). Anoxic recycle periods may also be introduced in the middle of the cycle between periods of aeration. A pre-anoxic period at the start of the cycle concurrent with influent feeding is avoided because it would decrease the sCOD uptake and storage by PAOs and/or GAOs during the anaerobic feeding.

The use of on-line PO_4-P measurement can assist in optimizing phosphorus removal. For instance, at peak loads, the time needed by PAOs to uptake PO_4-P may be greater than that required for ammonia removal. Thus, the PO_4-P measurement after the anaerobic feed period will govern the required aerobic react time. Additionally, the PO_4-P measurement may be used to call for a trim dose of metal salts as necessary to supplement EBPR to meet effluent P concentration targets.

7.2 Treatment Performance

The treatment capability of the Nereda® process for BOD, TSS, and nutrient removal has been demonstrated in a variety of full-scale applications. Process design, operating parameters and performance data are provided in this section for various locations and site conditions. An evaluation and comparison of the energy requirements for an A2O (Tchobanoglous et al., 2014) flocculent sludge system and Nereda® system are also included.

7.2.1 Range of Wastewater Strength at Nereda® Plants

The Nereda® process has been applied to high-strength industrial wastes, moderate strength municipal wastewaters, and dilute municipal wastewaters with an average influent BOD as low as 100 mg/L. The pretreatment type and average influent COD and BOD concentrations reported for a number of operating full-scale and pilot-scale Nereda® plants treating municipal wastewater are shown in Table 7-6.

Table 7-6. Average Influent Wastewater COD and BOD Concentrations at Operating Nereda® Plants Treating Municipal Wastewater

Plant	Country	Feed Pretreatment	COD, mg/L	BOD, mg/L
Gansbaai	South Africa	Screened raw wastewater	1170	---
Wemmershoek	South Africa	Screened raw wastewater	805	---
Epe	The Netherlands	Screened raw wastewater	750	330
Vroomshoop	The Netherlands	Screened raw wastewater	705	258
Ryki	Poland	Screened raw wastewater	650	---
Dinxperlo	The Netherlands	Screened raw wastewater	645	253
Clonakilty	Ireland	Screened raw wastewater	544	---
Garmerwolde	The Netherlands	Screened raw wastewater	484	212
Lisbon	Portugal	Primary effluent	450	182
Pilot	United Kingdom	Primary effluent	490	226
Pilot	Ireland	Primary effluent	436	170
Pilot	Switzerland	Primary effluent	350	---
Pilot	UK Scotland	Primary effluent	---	100

Source: Giesen, 2015.

7.2.2 Effective Start-Up Conditions and Time to Develop a Granular Sludge Mixed Liquor

The time required to fully develop a granular sludge mixed liquor at full-scale facilities may vary depending on the amount of granular biomass in the seed sludge, wastewater characteristics, and the assumed metrics for a granular sludge mixed liquor. Granular sludge was considered present in the Garmerwolde WRRF approximately six months after seeding with waste sludge from the full-scale Epe Nereda® plant to a MLSS concentration of 1 g/L (Pronk et al., 2015b). The seed waste sludge was more representative of a good-settling flocculent activated sludge; the SVI_5 was 145 mL/g and the SVI_{30} was 90 mL/g for an SVI_5/SVI_{30} ratio of 1.6, versus a typical value of 1.0 to 1.1 for mainly granular sludge. The flocculent nature of excess sludge removed from the top of the settled sludge bed in Nereda® reactors is shown in Figure 7-5 and discussed later in this section. The design volumetric loading rate was reached after three months of operation, and nutrient removal efficiency fluctuated during this period. After a total of 6 months of operation during what may be considered the "granulation phase," the SVI_{30} decreased from 70 mL/g to 50 mL/g, and the mixed liquor granule mass fraction was 30% based on retention on a 212 μm sieve size. During the next 6 months, SVI values continued to decrease and stabilize at an SVI_5 of 45 mL/g and SVI_{30} of 35mL/g, for an SVI_5/SVI_{30} ratio of 1.3. The mixed liquor granule mass fraction also increased to greater than 80%. A similar granulation development phase was reported for Vroomshoop, where it took approximately 14 months to reach an SVI_5/SVI_{30} ratio of 1.25 with an SVI_5 and SVI_{30} of 50 and 40 mL/g, respectively (Giesen et al., 2015).

At the Epe pilot plant, the time to achieve a greater than 80% mixed liquor granule mass fraction was shortened to approximately 5 months by having a granule mass fraction of approximately 10% in the seed mixed liquor at start-up (de Kreuk, 2006). Similarly, the start-up of the Clonakilty plant in Ireland was expedited by seeding with a large fraction of granular sludge (Giesen, 2015). The plant was seeded with 6 truckloads of granular sludge and 20 truckloads of flocculent sludge from a BNR plant. After seeding, the MLSS concentration reached 7,000 mg/L in about one month.

In addition to the availability of seed granules, the rate of developing granular sludge growth after start-up is also faster at a warmer temperature and at a higher influent rbCOD:bCOD concentration fraction. A higher temperature naturally results in higher bacteria growth rates, which allows more aggressive increases in feeding after start-up due to the faster assimilation and increase in biomass concentration. Faster hydrolysis rates at a warmer temperature can also result in more rbCOD available for PAOs and thus a faster growth of PAOs, which provide a good nucleus for granular sludge development. A higher influent rbCOD:bCOD ratio also favors more growth of PAOs over OHOs, thus enhancing the growth of smooth, rapid-settling granules. Granular sludge growth in the initial Nereda® installations was demonstrated with the treatment of relatively high strength wastewaters. However, successful granular sludge growth was also demonstrated in Scotland for the treatment of a more dilute, low strength wastewater at a concentration closer to what would be more common in the United States; this is discussed in Section 7.2.4.

After long-term operation of a granular activated sludge system, the mixed liquor can still contain some flocculent sludge. The flocculent sludge MLSS may be in the range of 5% to 15% of the total MLSS (Giesen, 2015; Pronk et al., 2015), and most of these slower settling particles are found in the top of the bed after the short settling period, as illustrated in Figure 7-5. The upper layer flocculent sludge fraction may help the removal of colloids and small particles to achieve a lower effluent TSS concentration by sludge blanket filtration during the settling and simultaneous fill/decant periods.

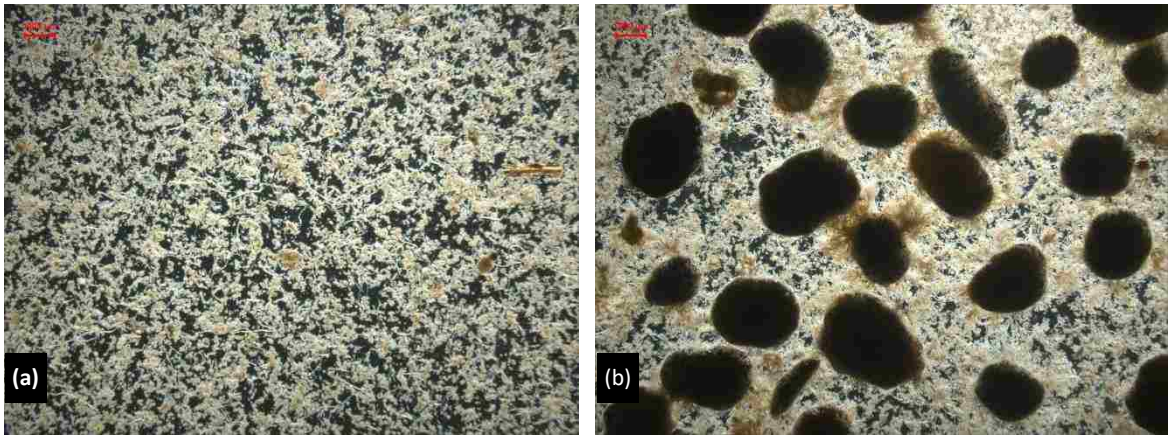


Figure 7-5. Photomicrograph of granular sludge mixed liquor sample at Nereda® Garmerwolde Plant treating domestic wastewater.

Scale bar = 1mm. (a) Top of settled sludge bed; (b) mixed sample from aeration period.
Courtesy of Mario Pronk, Delft University of Technology Department of Biotechnology. Copyright 2016 Mario Pronk.

7.2.3 Demonstrated Effluent TSS Performance

Comparison of the TSS effluent concentration in the Nereda® process to that for flocculent activated sludge processes is of interest in view of treatment needs and the differences in MLSS concentrations, sludge physical characteristics, and applied settling times. Reported Nereda® process effluent TSS concentrations have been comparable to those of flocculent activated sludge processes, with average values ranging from 5 to 20 mg/L. Elevated effluent TSS concentrations during start-up with selective settling pressure for granule selection over flocculent sludge can occur, but the effluent TSS concentration has often been limited to approximately 30 mg/L (Giesen, 2015).

Examples of effluent TSS concentrations from Nereda® systems treating domestic wastewater are summarized in the following: The effluent TSS concentration averaged 5 mg/L over a 1.5-month period in 2015 (Figure 7-6) for the 1500-m^3/day demonstration-scale Nereda® plant in Utrecht, the

Netherlands, treating screened raw wastewater. At a pilot plant in Switzerland treating municipal primary effluent, the Nereda® process effluent TSS concentration was consistently under 10 mg/L for a three month period from February 2015 to April 2015 (Giesen, 2015). The effluent TSS concentration averaged 10 mg/L for a 12-month period in 2014 at the full-scale Vroomshoop plant treating screened raw wastewater (Giesen et al., 2015). At the full-scale Garmerwolde plant, the effluent TSS concentration averaged 20 mg/L during a nine-month period from March 2014 to December 2014 after the first six months of operation (Pronk et al., 2015b).

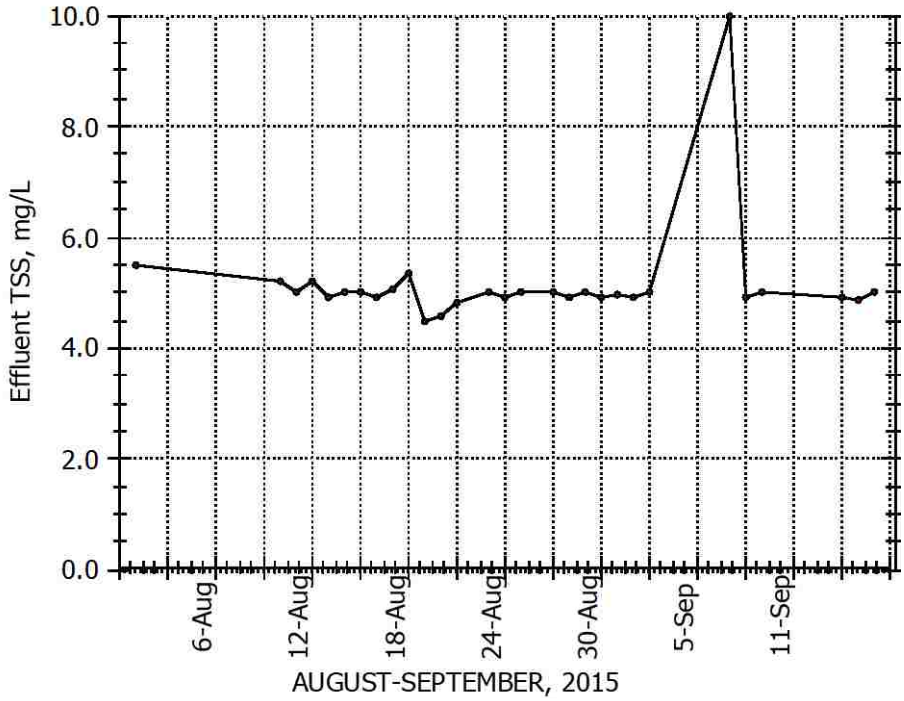


Figure 7-6. Effluent TSS concentration over a 1.5-month period at the Nereda® Utrecht Plant.
Source: Giesen, 2015.

7.2.4 Demonstrated Nutrient Removal Performance

Examples of reported nutrient removal performance for the Garmerwolde, Vroomshoop, and Epe full-scale Nereda® plants located in the Netherlands are summarized in this section. The respective permit limits are also presented, as the permit limit affects the treatment goals and operating conditions. To minimize energy demand, Nereda® plants with full on-line instrumentation packages are often operated aggressively to more closely meet but not exceed permit removal requirements.

The Nereda® Garmerwolde plant (Pronk et al., 2015b) provides an example of nutrient removal for the Nereda® process for municipal wastewater without a significant industrial waste component, which is often present in influent wastewater at WRRFs in the Netherlands. Garmerwolde operational parameters and process performance are summarized in Tables 7-7 and 7-8 for March 2014 to December 2014 after the design loading rates were reached. The average influent flow given in Table 7-8 is 97% of the design flow. The plant started operation in September 2013. In March 2014, the mixed liquor SVI_{30} and SVI_5 were 50 mL/g and 70 mL/g, respectively. By December 2014, these SVIs decreased to 35 mL/g and 45 mL/g, respectively, which suggests that some degree of granule maturation occurred with time. During the period for the average performance shown in Table 7-8, the reactor temperature

ranged from 12°C to 21°C, the MLSS concentration ranged from 6.5 g/L to 8.5 g/L with a MLSS volatile solids fraction of 0.75, and the SRT was between 20 days and 38 days. On-line NH_3-N monitoring was used to control the DO concentration during the aeration period. During the initial aeration time after the feeding period, the reactor DO concentration was between 1.8 mg/L and 2.5 mg/L, and the NH_3-N concentration decreased to the targeted effluent concentration. Then the DO concentration was lowered to approximately 0.5 mg/L for the remainder of the SBR aeration cycle to promote denitrification. The volumetric loading rates, process temperature, range of SRT and MLSS concentrations given in Table 7-7, and aeration strategy described for the Garmerwolde plant should be representative of other Nereda® plants located in the Netherlands.

The annual average Garmerwolde effluent permit limits of TN <7.0 mg/L and TP <1.0 mg/L were met during the reported performance period. Because the plant does not have a NH_3-N discharge limit, a higher residual NH_3-N concentration is maintained to minimize aeration. Notable operational parameters to improve nutrient removal included the use of anoxic recycle periods with an average recycle rate of 0.3 times the feed flow to increase denitrification and the use of a trim dose of metal salt addition for phosphorus removal during wet weather flow conditions.

Table 7-7. Average Values for Operational Parameters at the Nereda® Garmerwolde Plant (March-December 2014)

Parameter	Unit	Value
Number of reactors	–	2
Volume per reactor	m^3	9600
Reactor height	m	7.5
Temperate range	°C	12–21
SRT	day	20–38
MLSS	g/L	6.5–8.5
MLVSS/MLSS	–	0.75
Average daily flow	m^3/day	27,840
Effective HRT[(a)]	hr	17
COD F/M	kg COD/kg MLSS-day	0.10
Nitrogen loading	kg TN/m^3-day	0.08
Max anoxic recycle ratio	–	0.3
Fe(III) / P molar ratio[(b)]	–	0.18
DO concentration[(c)]	mg/L	1.8–2.5

[(a)]Total reactor volume divided by average daily influent flowrate.
[(b)] Fe dosed upstream of Nereda® only during peak wet weather flows. The reported Fe/P molar ratio is based on the influent total P concentration.
[(c)]During initial aeration period characterized by NH_3-N removal to reach targeted effluent NH_3-N concentration. DO concentration is lowered to 0.5 mg/L after targeted effluent NH_3-N concentration is met.
Source: Pronk et al., 2015b.

Table 7-8. Average Influent and Effluent Concentrations and Permit Limits for TSS, COD, BOD, and Nutrient Concentrations at the Nereda® Garmerwolde Plant (March-December 2014)

Influent (mg/L)				Effluent (mg/L)		
Parameter	**Minimum**	**Maximum**	**Average**	**Average**	**Permit Limit**	**Compliance Conditions**
TSS	101	465	223	20	30	Maximum day
COD	146	715	506	64	125	Maximum day
BOD	60	420	224	9.7	20	Maximum day
TP	1.9	9.7	6.7	0.9	1	Annual average
PO_4-P	1.5	6.8	4.4	0.4	–	–
TN	14	81	49.4	6.9	7	Annual average
NH_3-N	13.4	56.5	39	1.1	–	–

Sources: Pronk et al., 2015b; Giesen, 2016.

The Nereda® Vroomshoop plant was operating at its design average daily flow of 1500 m^3/day during the 2014 calendar year (Giesen et al., 2015). The summary of the average process performance and permit limits during this period in Table 7-9 shows that effluent nitrogen and phosphorus concentrations were well within the permitted limits and the effluent TN concentration averaged 7.2 mg/L, well below the permit limit of 10 mg/L. Less stringent NH_3-N discharge limits of 2 mg/L in the summer and 4 mg/L in the winter allowed the plant to limit nitrification, resulting in an annual average effluent NH_3-N/L concentration of 2.2 mg/L. The effluent TSS concentration averaged 10 mg/L, and the average effluent TP concentration of 0.9 mg/L is well below the permit limit of 2 mg/L.

Table 7-9. Influent, Effluent, and Permit Limit Concentrations for TSS, COD, BOD, and Nutrient Concentrations at the Nereda® Vroomshoop Plant (January-December 2014)

Influent (mg/L)		Effluent (mg/L)		
Parameter	**Average**	**Average**	**Permit Limit**	**Compliance conditions**
TSS	317	10	10	Daily limit with 3 exceedances per year up to 30 mg/L permitted
COD	720	55	125	Daily limit with 3 exceedances per year up to 250 mg/L permitted
BOD	263	4	10	Daily limit with 3 exceedances per year up to 20 mg/L permitted
TP	8.9	0.9	2	10 day moving average
PO_4-P	–	0.6	–	–
TN	–	7.2	10	Annual average
TKN	66	5.2	–	–
NH_3-N	–	2.2	Summer = 2 Winter = 4	Average, May 1 to Nov 1 Average, Nov 1 to May 1
NO_x-N	–	–	–	–

Sources: Giesen et al., 2015; Giesen, 2016.

The Nereda® Epe plant was designed to meet the most stringent nutrient effluent permit concentration limits in the Netherlands, TN < 5 and TP < 0.3 mg/L during the summer permit compliance period. About 30% of the COD load to the plant is from slaughterhouse wastewater with the balance being domestic wastewater. Process performance results for a three-month process verification period (March-May 2012) are summarized in Table 7-10 along with permit limits and compliance conditions. The process temperature was 14-16°C during the verification period, and the design average flow of 8000 m^3/day was reached prior to the verification period. Pre-existing gravity sand filters with alum addition were used to polish the effluent for TSS and TP removal. Naicker et al. (2015) reported that the alum dose was 0.05 mol Al / mol P based on the influent P concentration to the Nereda® reactor. The system had the ability to improve nitrogen removal after the aeration period by recycling liquid from the upper volume through the upflow distribution piping system to promote NO_3-N reduction in the sludge bed. The maximum possible recycle ratio relative to the average design influent flow was 1.25. Average effluent TN, NH_3-N, TP, and PO_4-P concentrations during the process verification period met the permit limits and were less than 4.0, 0.1, 0.3, and 0.1 mg/L, respectively.

Table 7-10. Average Influent and Filtration Effluent Concentrations and Permit Limit for TSS, COD, BOD, and Nutrient Concentrations at the Nereda® Epe Plant for March to May 2012

*The < indicates that all values are below the value shown

Influent (mg/L)		Filtered Effluent (mg/L)		
Parameter	**Average**	**Average**	**Permit Limit**	**Compliance Conditions**
TSS	341	<5*	30	Annual average
COD	879	27	125	Maximum day
BOD	333	<2*	7	Annual average with maximum day limit of 15
TP	9.3	0.3	Summer = 0.3 Winter = 0.5	Average, Apr 1 to Oct 1 Average, Oct 1 to Apr 1
PO_4-P	5.8	<0.1*	–	–
TN	–	<4*	Summer = 5 Winter = 12	Average, Apr 1 to Oct 1 Average, Oct 1 to Apr 1
TKN	77	1.4	–	–
NH_3-N	54	0.1	–	–

Sources: Inocêncio et al., 2013; Naicker et al., 2015; Giesen, 2016.

The biological nitrogen removal efficiency of 86 to 95% and influent BOD/TKN ratio of 4.0 to 4.5 for the three facilities reported here (Table 7-11) is comparable to the values of many biological nitrogen removal facilities in the United States. The ability to achieve effluent TN concentrations of less than 7.5 and 4.0 mg/L at the high influent TKN concentrations of 66 and 77 mg/L, respectively, by nitrification-denitrification in the aeration period is notable. For such high strength TKN wastewaters, the commonly used flocculent activated sludge modified Ludzack-Ettinger process (anoxic-aerobic) would normally not be able to produce a comparable effluent. It most likely would have to be followed by a post-anoxic reactor such as that used in the five-stage Bardenpho process.

The effluent NH_3-N concentrations for the facilities were a function of the treatment goals and selected aeration strategy. The Epe facility demonstrated the ability to achieve a very low effluent NH_3-N concentration of 0.1 mg/L.

EBPR was demonstrated for the full-scale Nereda® granular activated sludge process with very good phosphorus removal efficiencies, ranging from 87 to 97%. The influent BOD/P ratio was very favorable for EBPR, and comparable effluent performance would be expected for a flocculent activated sludge EBPR system. Similar polishing steps as those used for flocculent activated sludge systems can be applied to accomplish additional soluble P removal by metal salt addition and additional TSS and particulate P removal. Sand filtration has been used at Epe to reduce the effluent TSS concentration. Other methods for effluent polishing of TSS from granular sludge treatment were investigated in bench-scale testing as part of a STOWA study (Berkhof et al., 2010). The study showed that rotary screening (10 um) and compressible media filtration could produce effluent with a turbidity of less than two nephelometric turbidity units (NTU). Associated effluent TSS concentrations were not reported.

Table 7-11. Comparison of Nutrient Removal Performance Reported for Full-Scale Nereda® Facilities

	Facility		
Average Influent Concentration, mg/L	**Garmerwolde**	**Vroomshoop**	**Epe**
BOD	224	263	333
TKN	49.4	66	77
TP	6.7	8.9	9.3
BOD/TKN	4.5	4	4.3
BOD/P	33	30	36
Average Effluent Concentration, mg/L			
TN	6.9	7.2	<4
NH_3-N	1.1	2.2	0.1
TP	0.9	0.9	0.3
PO_4-P	0.4	0.6	<0.1
%N removal	86	89	95
%P removal	87	90	97

Results obtained at two pilot plant operations in Daldowie and Dalmarnock, Scotland, United Kingdom, in 2015 showed the capability of the Nereda® process to obtain granular sludge on a low-strength municipal wastewater and to maintain a high degree of NH_3-N removal and EBPR at low temperatures (Giesen, 2015; Giesen, 2016). The pilot reactor influent BOD after primary treatment averaged approximately 100 mg/L at both facilities. One pilot reactor was seeded with mature granular sludge, and the other was seeded with flocculent activated sludge. Granules were successfully grown from flocculent seed sludge on this low-strength wastewater feed, and the reported performance after granule development was similar for both systems. Treatment goals were based on discharge limits of 1 mg/L for both NH_3-N and TP. A process temperature as low as 3°C is possible during winter wet weather periods at these facilities, so cold weather nitrification and EBPR performance was of interest in this study. At one of the U.K. pilot plants, the influent wastewater temperature (7 to 10°C) was lowered with a chiller to between 3.0 and 4.5°C during a two-week period from February 17 to March 3, 2015. Though the in-situ process temperature was not monitored, the effluent temperature was checked in grab samples and was less than 5.0°C during the low-temperature operating period. During this operating period, the effluent NH_3-N concentration was between 0.1 and 0.6 mg/L and the effluent PO_4-P concentration was between 0.1 and 0.4 mg/L without supplemental VFA or metal salts addition.

7.2.5 Energy Demand Evaluation and Comparison

The energy required for the Nereda® process has been compared to that of flocculent activated sludge systems in desktop evaluations and full-scale side-by-side comparisons. In these evaluations, energy savings of 25-50% for the Nereda® system have been suggested (Giesen et al., 2015; Pronk et al., 2015b; van Haandel and van der Lubbe, 2012). However, these evaluations did not provide specific details on the assumptions or operating conditions impacting the energy demand associated with the processes, particularly those of the flocculent activated sludge process. These include but are not necessarily limited to tank depth, aerator type, aerator age and extent of fouling, blower or compressor efficiency, aeration alpha value, SRT, and MLSS and DO concentrations. Some energy savings naturally occur due to the nature of the Nereda® process, which reduces or eliminates internal recycle pumping and eliminates mechanical mixers, return sludge pumps, and secondary clarifier drives. Compared to common designs used for SBRs with flocculent sludge, the energy savings for the Nereda® SBR process are related to the elimination of mechanical mixing and to having aeration under a constant full tank depth versus the aeration with influent feeding that is done for some flocculent sludge SBR applications.

An energy demand analysis is provided here between a Nereda® process and a flocculent sludge A2O process as an example to illustrate the major design components and design conditions that result in the different operating energy needs. This flocculent sludge process is commonly referred to as the A2O process. This illustration provides a ballpark estimate of possible energy differences, but actual differences will vary for different applications depending on the site conditions, wastewater characteristics, treatment needs, tank configurations, and design conditions. The analysis shows differences in the energy demands associated with aeration, pumping, mixing, and ancillary needs for the biological treatment processes. The differences in energy demands for the biological processes associated with pre-treatment, post-treatment, and solids handling were assumed to be negligible. The influent and effluent design values; process design assumptions; aeration, pumping, and mixing energy design assumptions; and design summaries are given in Table 7-12 through Table 7-17. The estimated energy demands for the Nereda® process and A2O process are compared in Table 7-18.

The influent and effluent design values used in this scenario to illustrate energy demand differences between the Nereda® and A2O processes are summarized in Table 7-12. An influent flowrate of 38,000 m^3/day (10 mgd) following primary treatment and a biological reactor temperature of 15°C were assumed. The influent wastewater concentrations for BOD, bCOD, TKN, and TP concentrations were assumed at 138, 220, 37, and 5 mg/L, respectively. This evaluation assumes a permit limit of less than 10 mg/L and 1 mg/L for TN and TP, respectively. A design goal of a TN of 8.0 mg/L was used in this evaluation to address the 10 mg/L limit. Thus, the nutrient removal treatment performance was assumed to result in effluent NH_3-N, NO_3-N, TN, and TP concentrations of less than 0.5, 6.0, 8.0, and 1.0 mg/L, respectively. The NO_3-N production in both systems was about 32 mg/L after accounting for the influent nitrogen used in biomass synthesis and the effluent NH_3-N concentration of 0.50 mg/L. The necessary phosphorus removal is assumed to be met by EBPR in this evaluation. Other process addition options are possible for the granular and flocculent sludge systems developed here if lower effluent TN and TP concentrations are needed. These include effluent filtration with metal salts, external carbon addition to the biological processes, and denitrification filters.

Table 7-12. Influent, Effluent, and Reactor Design Basis Assumptions for an Energy Demand Analysis for the Nereda® and A2O Processes

Parameter	Units	Value
Influent		
Flow rate	m^3/day (mgd)	38,000 (10)
BOD	mg/L	138
bCOD	mg/L	220
TKN	mg/L	37
TP	mg/L	5
Reactor		
Temperature	°C	15
NO_3-N produced	mg/L	32
Effluent		
bCOD	mg/L	<5
NH_3-N	mg/L	<0.5
NO_3-N	mg/L	<6
TN	mg/L	<8
TP	mg/L	<1

Key assumptions used in the biological process design for the two systems are summarized in Table 7-13. The A2O process design SRT of 20 days was based on the desktop design procedure for biological nitrogen removal given by Tchobanoglous et al. (2014). First, based on nitrification kinetics, an aerobic SRT of 9.4 days was determined to produce the design effluent NH_3-N concentration of 0.50 mg/L at a DO concentration of 2 mg/L. Assuming a safety factor of 1.5 to account for domestic diurnal load variations, the aerobic SRT was 14 days. The anaerobic zone and pre-anoxic volume required to remove 26 mg/L NO_3-N increased the SRT to 20 days. The internal recycle flow to feed NO_3-N produced in the aerobic zone was at a recycle ratio of 4.0 based on the influent flow rate, and the return activated sludge recycle ratio was set at 0.50. Table 7-13 shows higher yield values with oxygen as the electron acceptor. These differences in yields had a slight effect on the total oxygen demand between the two systems in addition to the effect of the SRT.

Table 7-13. Process Design Assumptions for the Nereda® and A2O Processes

Parameter	Units	Nereda®	A2O
Temperature	°C	15	15
SRT	day	32	20
Nitrification safety factor	-	-	1.5
rbCOD fraction of influent bCOD	g/g	-	0.20
MLSS concentration	mg/L	-	3500
Net sludge yield	g TSS /g BODr	0.61	0.67
Internal recycle flow ratio	-	-	4
Return activated sludge flow ratio	-	-	0.50
Anaerobic feed time	hr	2	-
Aeration time at 2 mg/L DO	hr	2	-
Aeration time at 0.5 mg/L DO	hr	2	-
Effluent displacement depth fraction	-	0.5	
Biomass synthesis yield with O_2	g VSS/g COD	0.45	0.45
Biomass synthesis yield with NO_3-N	g VSS /g COD	0.37	0.37
Specific endogenous decay rate	g VSS /g VSS-day	0.12	0.12
g O_2 used/g NO_3-N produced	g O_2/g NO_3-N	4.57	4.57
O_2 equiv. of NO_3-N reduced	g O_2/g NO_3-N	2.86	2.86

Three SBR reactors were assumed for the Nereda® process so that one reactor was always being fed with an anaerobic feed time of 2.0 hr and total cycle time of 6.0 hr. After feeding, the total aeration time was 4.0 hr minus a short settling period of 5-10 min within the 4.0 hr period just before the feeding step. Thus, the total cycle time for each reactor was 6.0 hr. Assuming an SVI of below 50 mL/g for the granular activated sludge, the settled sludge blanket was low enough to allow for displacing half of the total liquid depth by feeding an equal influent volume at each cycle. Based on information on the range of SRTs used in the full-scale Nereda® processes in the Netherlands, an SRT of 32 days was assumed. This resulted in an aerobic SRT of 21.3 days, which compares to an aerobic SRT of 14 days for the A2O process. The longer SRT is needed to compensate for a lower specific nitrification for the Nereda® process in view of the diffusion limitations of DO and NH_3-N diffusing into the granular sludge particles. The Nereda® process cycle time and displacement fraction determined the required tank volume. Each

of the three SBR tanks handles four cycles per day with a cycle time of six hours. This equates to 12 tank feedings per day or an average feed volume of 3154 m^3/cycle. With a feed displacement volume fraction of 0.50, the volume of each SBR tank is 6308 m^3. For three SBR tanks the total Nereda® process volume for this analysis is 19,000 m^3 (Table 7-14). The MLSS concentration then depends on the daily sludge production rate and SRT.

The total volume of the A2O process was determined from the assumed MLSS concentration of 3500 mg/L and the total TSS mass in the system, which was equal to the daily TSS production rate times the system SRT of 20 days. The daily TSS production rate was determined by multiplying the net solids yield (g TSS/ g BOD removed) by the BOD removed in kg/day. The net solids yield was obtained from Figure 8-7 in Tchobanoglous et al. (2014), which gives a net solids yield in g VSS/g BODr as a function of SRT. Assuming a VSS/TSS ratio of 0.75, the net solids yield was 0.66 g TSS/g BODr, which resulted in a total volume of 20,500 m^3 as shown in Table 7-14. The total system HRT and the HRT of the three zones is also given in the table. The volumes associated with the anoxic zone HRTs are used in the mixing energy determination. The net solid yield for the Nereda® process was 0.61 g TSS/g BODr, which resulted in calculating a MLSS concentration of 5400 mg/L for the 32-day SRT and 19,000 m^3 volume.

Table 7-14. Biological Process Design Summary for the Nereda® and A2O Processes in the Comparative Energy Demand Evaluation

Parameter	Units	Nereda®	A2O
Total tank volume	m^3 (Mgal)	19,000 (5)	20,300 (5.4)
MLSS concentration		5400	3500
Bioreactor total equivalent HRT	hr	12	12.9
Anaerobic zone HRT	hr	–	1
Anoxic zone HRT	hr	–	3.4
Aerobic zone HRT	hr	–	8.5

As shown in Table 7-15, the daily oxygen requirements for the Nereda® and A2O processes are 9850 and 9440 kg/day, respectively. The table also shows key components for the oxygen mass balances to arrive at the actual oxygen requirement (AOR) used for the aeration design in this energy demand analysis. The kg/day of bCOD removed from the influent is accounted for by the oxygen equivalent of the biomass produced and the oxidation of the influent bCOD during cell synthesis and endogenous decay. The net effect is carbonaceous oxygen demands of 7130 kg O_2/day and 6720 kg O_2/day for the Nereda® and A2O processes, respectively. There is a lower biomass production rate for the Nereda® process due to the associated lower biomass yield coefficient value at the longer SRT. The oxygen required for nitrification and the oxygen equivalent provided by denitrification are the same for both systems (5540 and 2820 kg O_2/day) due to an equal amount of NH_3-N oxidation (32 mg/L) and NO_3-N reduction (26 mg/L). For nitrification a ratio of 4.57 g O_2/g NO_3-N produced and for denitrification a ratio of 2.86 g O_2 equivalent/g NO_3-N reduced were used.

Table 7-15. Net Daily Oxygen Requirement and Oxygen Calculation Components for the Nereda® and A2O Processes in the Comparative Energy Demand Evaluation

Parameter	Units	Nereda®	A2O
Influent bCOD load	kg/day	8330	8330
Net biomass VSS production	kg/day	840	1140
O_2 equivalent of biomass production	kg/day	(1190)	(1610)
O_2 required for bCOD removal	kg/day	7130	6720
O_2 equivalent of NO_3-N removed	kg/day	(2820)	(2820)
O_2 required for nitrification	kg/day	5540	5540
Net actual O_2 requirement (AOR)	kg/day	9850	9440

The aeration energy is a major portion of the energy demand for both systems. Fine-pore membrane diffused aeration was assumed for both systems. The aeration design procedure given in Chapter 8 in Tchobanoglous et al. (2014) was used to determine the respective air application rates from the AOR values shown in Table 7-16. Because the diffuser oxygen transfer efficiency is defined at standard conditions (SOR) of clean water at sea level, zero DO, and 20°C, the AOR values were adjusted to determine the necessary clean water SOR by accounting for the effect of site DO concentration, diffuser submergence depth, mixed liquor negative effects on oxygen transfer (α and β), temperature, elevation, and diffuser fouling. The assumptions that accounted for these effects and were used to calculate the air application rates are summarized in Table 7-16. The air application rates were then used to determine the respective energy requirements for a centrifugal blower using the procedure given in Chapter 5 in Tchobanoglous et al. (2014)

A side water depth of 7.6 m (25 ft) as shown in Table 7-16 was used as a typical liquid depth for the Nereda® process, whereas a typical liquid depth for the A2O process was assumed to be 4.9 m (16 ft). In view of the fact that the reactor liquid depth impacts the aeration efficiency and that the A2O process is not limited at a 4.9 m depth, an energy evaluation was also done for the A2O process at the same side water depth as the Nereda® process. The air release depth of the fine-pore membrane diffusers was assumed to be at 0.3 m (1.0 ft) above the tank floor.

Table 7-16. Assumed Values and Air Application Rate Calculation Results for the Nereda® and A2O Processes in the Comparative Energy Demand Evaluation

Parameter	Units	Nereda®	A2O (Typical Depth)	A2O (Deep Tank)
Assumptions				
Side water depth	m (ft)	7.6 (25)	4.9 (16)	7.6 (25)
Diffuser submergence	m (ft)	7.3 (24)	4.6 (15)	7.6 (25)
Site elevation pressure	m	10.33	10.33	10.33
AOR, DO = 2 mg/L	kg/day	8340	9440	9440
AOR, DO = 0.5 mg/L	kg/day	1510		---
Process temperature	°C	15	15	15
Alpha (α) factor	---	0.55	0.55	0.55
Beta (β) factor	---	0.95	0.95	0.95
Diffuser fouling factor	---	0.90	0.90	0.90
Standard O_2 transfer eff.	%	48	30	48
Results				
DO = 2 mg/L				
Aeration time per day	hr	8	24	24
SOR	kg O_2/hr	2450	940	920
Air rate at std. cond.	m^3/min (scfm)	304 (10,750)	187 (6610)	115 (4050)
DO = 0.5 mg/L				
Aeration time per day	hr	8		
SOR	kg O_2/hr	260		
Air rate at std. cond.	m^3/min (scfm)	32 (1130)		

Typical aeration operating conditions for the Nereda® and A2O processes were used for the assumptions of the DO concentration corresponding to the actual oxygen consumption. For the A2O process a typical DO concentration of 2.0 mg/L was used with the continuous aeration condition in meeting the process AOR. For the Nereda® process the aeration set point and oxygen consumption amount vary with time in the batch-fed cycle. Evaluation of changes in oxygen consumption rates and NH_3-N concentration with time in a cycle for the Garmerwolde facility (Pronk et al., 2015b) suggested that the nitrification oxygen demand was satisfied in the initial part of the aerobic period at a DO concentration between 1.8 and 2.3 mg/L. The DO setpoint was reduced from approximately 2 to 0.5 mg/L once the target NH_3-N concentration was met. For this energy demand analysis, the DO concentration was assumed to be 2.0 mg/L for the first two hours of the cycle and 0.50 mg/L for the

second two-hour period to encourage further denitrification at the lower oxygen demand rate. The assumed portions of the total AOR satisfied during these periods are shown in Table 7-16 for each DO setting. During the 2 mg/L DO concentration period all of the nitrification oxygen demand was assumed to be satisfied and 65% of the carbonaceous oxygen demand was assumed satisfied. During the last two hours of aeration at a 0.50 mg/L DO concentration the remaining 35% of the carbonaceous oxygen demand was assumed satisfied.

The aeration alpha (α) value was assumed to be equal to 0.55 in both systems. The 0.55 value is a reasonable expectation for the A2O process with an MLSS concentration of 3500 mg/L and an SRT of 20 days (Tchobanoglous et al., 2014). Information on which to base the Nereda® process α value is limited. Alpha values determined by off-gas testing in Nereda® systems have not been reported. The Nereda® process design in this evaluation has a much higher MLSS concentration than the A2O process, and lower α values have been associated with higher MLSS concentrations (Cornel et al., 2003; Krampe and Krauth, 2003). The decreased α values have been attributed to an increased viscosity at higher MLSS concentrations. It is likely that the more compact granular sludge matrix results in different rheological characteristics than those of flocculent activated sludge, and thus there was no basis to assume a lower α value for the Nereda® process in this aeration energy analysis. The same values were also assumed for the beta (β) and diffuser fouling factors (0.95 and 0.90, respectively).

For both systems, the fine bubble membrane diffuser standard oxygen transfer efficiency (SOTE) was assumed to be 6.6% per meter (2% per foot) of submergence. It is noted that the fine bubble membrane diffuser SOTE per foot of submergence is not linear but tends to decrease with increasing submergence and diffuser flux. However, within the assumed range of diffuser submergence depths, the quantity of diffusers can be selected such that the combination of membrane flux and submergence depth allows the assumed SOTE of 2% per foot of submergence to be a reasonable assumption.

The results for the air application rate determination summarized in Table 7-16 show that the air application rate and required blower energy demand varied for each of the 2 hr aeration periods, whereas the aeration energy demand for the A2O process was constant over 24 hours. For the ease of the energy comparison, the total kWh used for aeration for the Nereda® process was divided by 24 hours to provide a direct comparison to the energy demand of the A2O process.

Assumptions used for the blower energy demand, based on the blower adiabatic compression power calculation, were blower and motor efficiencies of 75% and 93%, respectively, and an ambient air temperature of 20°C.

The design basis for the energy demands associated with pumping, mixing, and secondary clarifier drives is summarized in Table 7-17. Both tanks were assumed to have the same influent elevation and excavation depth. Thus, the differential influent pumping head for the Nereda® process was due to the difference in the Nereda® and A2O tank depths of 2.7 m (9.0 ft). The influent pumping head for the A2O system was based on an assumed headloss of 1.4 m (4.6 ft) between the primary and secondary effluent channels. Internal recycle pumping energy in the A2O system was based on a recycle ratio of 4.0 and 0.5 m (1.6 ft) of friction and minor losses. Static head in the internal recycle pumping system was assumed to be negligible. The return activated sludge (RAS) pumping energy demand was based on an RAS ratio of 0.50 and 1.7 m (5.6 ft) of total dynamic head (TDH) composed of 1.2 m (4.0 ft) of static head between the secondary clarifier and biological process influent channel and 0.5 m (1.6 ft) of friction and minor losses. It was assumed that pipe velocities would be limited to a maximum of approximately 1.2 m/s (4.0 ft/s). For pumping energy calculations, the pump and motor efficiencies were assumed to be 75% and 93%, respectively. The specific mixing energy demand for the anaerobic, pre-anoxic, and post-anoxic tanks in the A2O process was assumed to be 1.5 W/m^3 based on using high-efficiency slow-speed top-entry mixers. The energy demand of the mechanical drives in the secondary clarifiers for the

A2O process was based on having four clarifiers with a 24 m (80 ft) diameter and power draw of 3 kW (4 hp) for each drive.

Table 7-17. Summary of Assumptions Used to Determine Energy Requirements for Pumping, Mixing, and Secondary Clarifier Drive Mechanisms for the Nereda® and A2O Processes in the Comparative Energy Demand Evaluation

Category / Parameter	Units	Nereda®	A2O (Typical Depth)
Influent pumping			
Flow rate	m^3/day (mgd)	38,000 (10)	38,000 (10)
Total dynamic head	m (ft)	2.7 (9)	1.4 (4.6)
Comments/Basis	–	Higher head due to taller tank and same bottom excavation level	Headloss between primary effluent and secondary effluent channels
Internal recycle pumping			
Ratio relative to influent flow	–	–	4
Total dynamic head	m (ft)	–	0.5 (1.6)
RAS pumping			
Ratio relative to influent flow	–	–	0.50
Total dynamic head	m (ft)	–	1.7 (5.6)
Mixing			
Specific energy input	W/m^3	–	1.5
Anaerobic zone mixing volume	m^3	–	1580
Anoxic zone mixing volume	m^3	–	5360
Comments/Basis	–	---	Slow-speed top-entry mixing
Secondary clarifier drives			
Energy demand	kW	–	3
Comments/Basis	–	–	4 clarifiers with 0.75 kW drives

A comparison of the energy demand for the Nereda® and A2O processes in Table 7-18 shows that the Nereda® process results in a 16 to 21% energy reduction over the A2O process; the total continuous energy demand for the Nereda® process is 182 kW compared to 230 and 216 kW for the A2O process with tank depths of 4.9 m and 7.6 m, respectively. In comparing the 4.9 m-deep A2O process to the Nereda® process, about 52% of the energy savings is due to the increased aeration efficiency of the Nereda® process and 48% due to the savings from reactor mixing and recycle pumping. The aeration energy reduction was achieved by efficiencies gained from increased tank depth and the inclusion of low-DO operation periods. When the A2O process was designed with the same liquid depth as the Nereda® process, the aeration energy was close, and about 88% of the energy savings for the Nereda® process was due to the elimination of the recycle pumping and mixing energy necessary for the A2O process. This energy analysis and the final numbers are a function of the many assumptions presented for the process design, aeration design, and pumping and mixing design. These results are not intended to be interpreted as an energy difference that applies in all cases. Such an analysis for other design and site conditions will produce different numerical results, but this analysis does illustrate the potential for a significant energy savings when using the Nereda® process for a biological nitrogen and phosphorus removal application.

Table 7-18. Comparison of the Estimated Energy Demands for the Nereda® and A2O Processes

	Equivalent Continuous Energy Demand (kW)		
Process	**Nereda® (7.6 m depth)**	**A2O (4.9 m depth)**	**A2O (7.6 m depth)**
Aeration	165	190	169
Influent pumping	17	9	17
Internal recycle pumping	-	12	12
RAS pumping	-	5	5
Mixing—anaerobic zone	-	2	2
Mixing—anoxic zone	-	8	8
Secondary clarifier drives	-	3	3
Total non-aeration	17	40	47
Total energy demand	182	230	216
% energy savings with Nereda®		21%	16%
% of energy savings due to non-aeration elements		48%	88%

7.3 Waste Sludge Handling

Information from the initial Nereda® process facilities suggests the same solids handling characteristics as at flocculent activated sludge facilities. Because EBPR is a basic element in the process, mechanical thickening is used at plants with P limits to avoid phosphorus release due to longer holding times and anaerobic conditions in gravity thickening.

There is limited data on thickening and dewatering performance. Experience with a pilot plant waste sludge in the range of 1.4 to 2.2 g TSS/L is summarized in Table 7-19. The polymer dose and percent solids achieved are within the range reported for flocculent WAS (Tchobanoglous et al., 2014).

Table 7-19. Thickening and Dewatering Results for Nereda® Process Waste Sludge

Method	Key Conditions	Polymer Dose, g/kg TSS	Product %TS	Comments
Lab gravity thickening test	24 hr holding	2	2.5	P release
Pilot belt thickening test	–	3	5–6%	Belt loading not specified
Lab mini-press for dewatering	6.9 atm	10	16–18.5%	–

Source: Berkhof et al., 2010.

A recent lab study with a PAO granular sludge from a bubble column SBR lab reactor at the University of Washington showed very rapid gravity thickening in a few minutes with only small increases in thickening at extensive holding times (Wei, 2016). In the study, 500 mL of granular sludge with an MLSS concentration of 8000 mg/L was placed in a 1000 mL graduated cylinder, and the sludge blanket level was recorded with time. The settled sludge volume after three minutes was such that the SVI_3 was 33 mL/g. At 30 and 180 minutes, the SVI_{30} remained at 33 mL/g. Three minutes after gently stirring the granules, the SVI dropped to 29 mL/g and remained there for the next five hours. The thickened solids concentration at SVIs of 33 and 29 mL/g were 3% and 3.4%, respectively.

Gravity belt thickening is used for the full-scale Nereda® Epe installation and produces 4.1% TS at an active polymer dose of 1.7 g/kg dry solids. The thickened solids content of 4% was selected to ensure that the thickened sludge easily pumped to an off-site anaerobic digestion facility where the solids are combined and digested with other solids streams (Monita et al., 2015).

No data has been made available or problems reported on dewatering after the anaerobic digestion of waste granular sludge (Section 9.1).

7.4 Process Benefits

Advantages for the Nereda® process are related to 1) nutrient removal, 2) space requirements, 3) energy demand, and 4) equipment needs. Another benefit is the potential for resource recovery, which is discussed in Chapter 9.

7.4.1 Nutrient Removal

The Nereda® process design allows for optimal conditions for BNR under variable loadings and with process control simplicity (Giesen et al., 2015). Nitrification-denitrification occurs during the aeration period due to the biofilm thickness of the granule, which allows for nitrification near the surface of the granule and NO_x-N reduction in the inner volume. With current on-line NH_3-N and NO_x-N analyzers, the aeration can be controlled to meet target effluent NH_3-N and NO_x-N concentrations (Pronk et al., 2015b). The low NO_x-N concentration that can be achieved at the end of the react phase, combined with upflow feeding through the settled sludge bed, provides conditions to allow most if not all of the influent rbCOD and rbCOD produced form anaerobic hydrolysis and fermentation to be available for PAOs. In addition, the rbCOD needed for nitrogen and phosphorus removal is minimized due to the denitrification by the PAOs using carbon stored during the anaerobic feeding period.

7.4.2 Space Requirements

As an SBR process, Nereda® systems inherently reduce plant footprint space requirements by eliminating secondary clarifiers and associated return sludge pumping facilities. Space requirements are further reduced by 1) the higher MLSS concentration afforded by granular sludge growth, which is closer to that used by flocculent sludge membrane bioreactors than to that used by conventional flocculent activated sludge SBRs; 2) the taller reactor configuration; and 3) the shorter settling times employed. A desktop analysis comparing an oxidation ditch process to the Nereda® process estimated that the Nereda® system would require only ~30% of the footprint required for the oxidation ditch system (van Haandel and van der Lubbe, 2012). The flowrate-normalized volume for the Garmerwolde Nereda® plant was reported to be 33% lower than that of the parallel A-B activated sludge process (Pronk et al., 2015b). Although a similar comparison was not made with respect to space requirements, the reduced volume requirement for the Nereda® system would be expected to yield space savings, which can be confirmed by aerial images of the plant (Giesen et al., 2015). The ability to intensify treatment capacity within the available plant space and possibly to reserve space for expansion if necessitated by population growth is a significant benefit.

7.4.3 Energy Demand

As discussed in Section 7.2.5, energy demand for the Nereda® process was estimated to be 21% less than that needed for an A2O activated sludge process with the same treatment objectives and aeration equipment. These energy savings were attributed to the elimination of the energy needed for mixers, recycle pumping, and clarifier mechanisms as well as efficiencies gained in oxygen transfer as a consequence of deep tanks and inclusion of periods of low DO concentration.

7.4.4 Mechanical Equipment and Piping Needs

The SBR process reduces the number of mechanical equipment items by eliminating the need for mixers, secondary clarifiers, and return sludge pumping systems. Internal recycle pumping systems can potentially be eliminated, but if internal recycle pumping capability is included, SBR feed pumps are used in lieu of separate internal recycle pumps. It appears that no special foam control equipment such as surface wasting of mixed liquor and chlorine spray systems for tank water surfaces is necessary for the Nereda® process. Elimination of this equipment reduces infrastructure and operating and maintenance costs.

7.5 Process Limitations and Special Considerations

Process limitations or special considerations are related to: 1) the ease of retrofitting existing facilities, 2) process start-up, 3) waste sludge management, and 4) instrumentation maintenance and process control. These are discussed as follows.

7.5.1 Plant Retrofits

The most obvious limitation of the Nereda® process is that it would not be an easy retrofit for many existing activated sludge facilities. Unique aspects of the process that could make retrofits difficult are: 1) special influent distribution feed and effluent collection systems are needed at the tank bottom and top, respectively; 2) it may not be compatible with the existing plant hydraulic profile; 3) the existing tankage may not be compatible with the preferred Nereda® reactor dimensions for height and diameter or width; and 4) the quantity of SBRs that may be required for plants with high flowrates. For some flocculent activated sludge plants, capital investment to convert to the Nereda® granular sludge process may not be competitive if only minor upgrades are necessary to achieve necessary nutrient removal requirements or increases in capacity.

7.5.2 Plant Start-Up

In the absence of a large enough amount of seed sludge, more time will be needed to develop the granular activated sludge mixed liquor than that needed by flocculent activated sludge systems. An allowance for granulation time must be built in to the project schedule. Experience at Nereda® plants in the Netherlands indicates that the granular sludge maturation and performance optimization period can be up to 12 months (Section 7.2.2). In addition, the granulation and granular growth period must be managed to avoid excessive biomass losses and permit violations. It is also possible to have elevated TSS concentrations during part of the start-up period.

7.5.3 Waste Sludge Management

Special consideration must be given to the sludge wasting strategy. Excess sludge wasting may be done during the aeration period to waste a homogeneous mixture of PAOs and other heterotrophs and granular and flocculent solids. In this way, the reactor SRT is easily managed for target values and PAOs are wasted for phosphorus removal. Another approach is to preferentially waste the lighter sludge at the top of the settled granular bed during or before feeding to favor a greater proportion of faster-settling granules, which would tend to be larger and have a higher density due to PAOs (Section 3.4.8). This results in different SRTs for different fractions of the mixed liquor, and it must be managed to avoid excessive accumulation of precipitate-laden granules and/or having too long of an SRT for the PAOs. The ultimate phosphorus removal pathway is via the PAOs contained in waste sludge. At longer SRTs, more endogenous respiration occurs, and the amount of PAO biomass wasted per day would decrease, which could then lead to less phosphorus removal than desired.

7.5.4 Instrumentation, Maintenance, and Process Control

On-line instrumentation and effective process control is critical for proper operation of the Nereda® process and to assure that effluent nutrient concentration goals are consistently met. Instrumentation and process control also provide a means for the optimization of nitrogen and phosphorus removal. The facility must have skilled and properly trained personnel to maintain and use the instrumentation and process control systems.

CHAPTER 8

Modeling Aerobic Granular Sludge

Mechanistic simulation models are commonly used in full-scale plant analysis and design and in research for flocculent activated sludge processes. Similarly, mechanistic simulation models have been developed to analyze aerobic granular activated sludge reactors in research studies. This chapter provides an overview of the key elements considered in mechanistic models for granular activated sludge processes and the types of models demonstrated. The final section discusses model applications for activated sludge processes containing both flocculent and granular sludge.

8.1 Key Elements in Mechanistic Models for Aerobic Granular Sludge

Mechanistic models for aerobic granular sludge are based on well-established models used previously for attached growth biofilms on a fixed media that account for mass transport processes, mass balance, and key microbial populations, as well as bioconversions and biokinetics for substrate and electron acceptors. Mass transport equations describe the flux of substrates by diffusion and advection across a mass transfer boundary layer between the biofilm and bulk liquid and within the biofilm where bioconversion processes occur. In one-dimensional biofilm models, illustrated by Figure 8-1, the biofilm is represented by a series of discrete planar elements perpendicular to the direction of substrate flux, and the substrate concentration profiles are solved in one dimension based on the distance from a reference point such as the interface of the support media and biofilm. The granular activated sludge does not have a support media, but similar one-dimensional models can be used by assuming spherical shaped biofilms with an infinitely small spherical carrier.

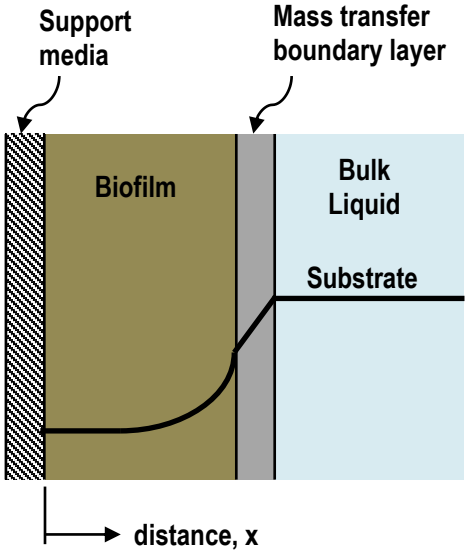


Figure 8-1. Schematic representation of an idealized attached-growth biofilm system showing substrate concentration across the biofilm, biofilm boundary layer, and bulk liquid.

The granular activated sludge mechanistic models for nutrient removal require incorporating a number of important bacteria species and their kinetics, including PAOs, GAOs, OHOs, AOB, and NOB. Their growth yields, and endogenous decay, substrate, and electron acceptor utilization rates are defined using appropriate equations. The biokinetics may be related to the Monod model growth kinetics, or the Michaelis-Menten substrate utilization rate model may be used. These bioreactions and kinetics and their coefficients have also been defined in flocculent activated sludge models, such as ASM2d (Henze et al., 2000), and similar equations and values may be used in the granular sludge models. Inclusion of storage-growth phenomena is necessary for an accurate mechanistic description of aerobic granular sludge with PAOs for EBPR. In addition to these basic modeling elements, simulation models may also consider including chemical equilibria expressions to account for pH and alkalinity within the biofilm.

The computer program AQUASIM (Reichert, 1994, 1998) is a widely used software to model biofilm systems, and has been applied to aerobic granular sludge systems. A suite of mass transport phenomena that can be activated or inactivated by the user are built into AQUASIM, thereby allowing different model formulations. These processes are illustrated in Figure 8-2 and include 1) attachment and detachment of particulate species, 2) diffusion of dissolved species within the biofilm pore phase, 3) advection of particulate and dissolved species due to water flow, 4) advection of particulate and dissolved species due to void spaces in the biofilm created by biological growth and decay, and 5) effective diffusion of particulate species (Wanner and Reichert, 1996). The effective diffusion of particulate species is included to allow for the empirical description of phenomena arising from the mechanical deformation of the biofilm resulting in the temporary dislocation and subsequent reattachment of particulate species at another location within the biofilm matrix. This transport process can be canceled, resulting in the assumption of what is termed a “rigid” biofilm. The reader is directed to the above references and an IWA publication (Wanner et al., 2006) for detailed discussions of mechanistic descriptions of mass transport processes in biofilm systems. By building on the suite of mass transport phenomena in AQUASIM, one can define the full reaction-mass transport model by defining dissolved and particulate species of interest, as well as the associated bioconversion process kinetics and stoichiometry of all involved bacteria, as discussed above.

A spherical granular geometry is assumed in the AQUASIM one-dimensional biofilm model, and the granular sludge inventory is based on the granule size and number of granules. The biofilm area at a given radius is calculated as a function of distance (z) from the granule center. The biofilm model compartment in AQUASIM allows for a total biofilm area (A) to be calculated based on a number of spherical biomass granules (n_{sp}) of defined radius ($r_{sp} + z$) according to the equation below, where r_{sp} represents an infinitely small spherical support media.

$$A = 4\pi\, n_{sp}\, (r_{sp} + z)^2 \qquad \text{(Equation 8-1)}$$

By manipulating the detachment rate, the targeted granule size can be obtained for a given steady-state operation (Beun et al., 2001; Isanta et al., 2013; Vázquez-Padín et al., 2010a).

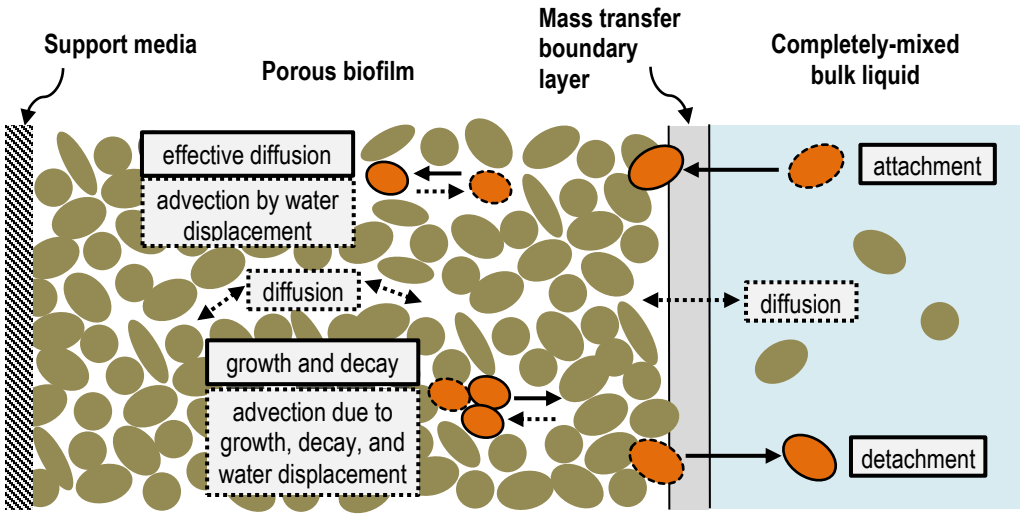


Figure 8-2. Schematic of transport processes included in the AQUASIM biofilm model.
Solid text boxes and arrows refer to particulate components.
Dashed text boxes and arrows refer to dissolved components.

Though researchers have used other computational software such as MATLAB® to construct similar biofilm model architectures, the AQUASIM software offers the convenience of a built-in model framework, flexibility, and options to conduct sensitivity analyses. The simulation of aerobic granular sludge using biofilm models in commercially available wastewater process simulation packages such as BioWin™ and GPS-X™ has yet to be reported but should be feasible using a similar model framework. Features of biofilm models in commonly used wastewater treatment simulators have been summarized in the literature (Boltz et al., 2010). A more recent modeling platform is COMSOL Multiphysics®, which has been used by Ofiteru et al. (2014) to simultaneously simulate micro-scale activated sludge floc development in two dimensions and macro-scale bioreactor performance. Conventional diffusion-reaction equations were used for bioconversions and mass balances of soluble species. This modeling platform has yet to be extended to granular activated sludge but could be utilized to simulate the development of individual granules as well as overall reactor treatment performance.

In a few cases, aerobic granular sludge models have included the settling phase of an SBR. In these cases, the effect of the settling time as a selective pressure to determine granule size is accounted for. In one study (Kagawa et al., 2015), particles were modeled as having discrete Stokian settling behavior, and in another (Su et al., 2013), a hindered settling model was used, with the zone settling parameter being a function of particle size. Due to the computation requirements driven by the inclusion of dynamic changes in granule size, C++ and MATLAB®, respectively, were used in these studies in lieu of AQUASIM. However, most aerobic granular sludge modeling studies have simulated mature granular sludge suspensions without considering the effect of transient operating conditions on the granule size.

8.2 One-Dimensional Biofilm Models for Aerobic Granular Sludge Processes

A summary of the conditions for a number of one-dimensional model simulations of bench-scale aerobic granular sludge SBRs is shown in Table 8-1. The study conditions include the type of nutrient removal process, the feed wastewater type and carbon source, the bacteria species in the model, the model software, the use of a fixed or variable granule size, the use of a variable or uniform granule biomass density, and whether the settling selective pressure for granular size selection was used in the model. All

of these studies included simultaneous storage and growth phenomena for ordinary heterotrophs when reactor operation included the feeding of organic carbon. The majority of the simulated systems were pulse-fed aerobic (PF-aerobic) nitrogen removal processes without EBPR. Studies C, I, and J were EBPR processes with anaerobic bottom feeding (BF-anaerobic) and simultaneous nitrogen removal and phosphorus uptake in the subsequent aerobic period. Though most studies did not include microbial groups that compete for the same substrate, Study I included both PAOs and GAOs, and Study J included two types of NOB representing *Nitrospira* and *Nitrobacter*.

As shown in Table 8-1, most models assumed a single uniform granule size for the reactor. The uniform granule size was estimated or equal to the weighted average granule diameter in the experimental reactor. Studies C and J successfully demonstrated good model fits to the experimental data with this approach for BF-anaerobic laboratory SBRs that had simultaneous nitrification-denitrification and EBPR. The simulation results closely matched experimental data for acetate, NH_3-N, NO_2-N, and NO_3-N concentrations during the SBR cycles. Studies using this uniform granule size framework have also included sensitivity analyses evaluating treatment performance for different granule sizes (Studies C, H, and J). In some studies (B, E, and I), the model framework allowed for a distribution of granule sizes in the model. Study B reported that for an otherwise equivalent model scenario, results were significantly different when a uniform granule size was used versus when a normal distribution of granule sizes with up to 200 incremental size groups was used. However, this type of modeling comparison was not done against experimental data to determine which more closely fit the reactor performance. Similarly, no experimental data was available in Studies E and I with simulation models that also used a distribution of granule sizes.

The mass transfer processes in the mechanistic AQUASIM biofilm model are impacted by biofilm porosity, which is calculated from the relative amounts of particulate species and their associated densities. As shown in Table 8-1, in most cases the densities of the microbial groups have been specified as being uniform. However, a range of microbial densities has been reported (Vázquez-Padín et al., 2010a). In some studies (G and H), different densities have been assigned to microbial groups, with autotrophs being assigned higher densities than heterotrophs. Because studies using uniform microbial group densities within ranges reported in the literature have accurately simulated reactor performance, the sensitivity of results to this parameter does not appear to be great.

Table 8-1. Summary of Conditions for One-Dimensional Biofilm Model Applications for Aerobic Granular Sludge SBR Processes

Description of Process	Wastewater Type and Organic Carbon Source	Microbial Species	Software	Granule Size	Density of Bacterial Groups	Settling Included	Study
N removal in PF-aerobic reactor	Synthetic; acetate	OHOs Nitrifiers	AQUASIM	Uniform	Uniform	No	A
N removal in PF-aerobic reactor	Synthetic; acetate	OHOs Nitrifiers	MATLAB	Variable with normal distribution	Uniform	No	B
EBPR and N removal in BF-anaerobic reactor	Synthetic; acetate	PAOs AOB NOB	AQUASIM	Uniform	Uniform	No	C
N removal in PF-aerobic reactor	Effluent from lab acidogenic reactor fed synthetic waste	OHOs Nitrifiers	AQUASIM	Uniform	Not specified	No	D
Ammonia oxidation in PF-aerobic reactor	Synthetic; inorganic autotrophic medium	OHOs AOB NOB	AQUASIM	Variable with unspecified distribution	Uniform	No	E
N removal in PF-aerobic reactor	Domestic wastewater	OHOs Nitrifiers	AQUASIM	Uniform	Uniform	No	F
N removal in PF-aerobic reactor	Synthetic; acetate	OHOs AOB NOB	AQUASIM	Uniform	Variable	No	G
N removal in PF-aerobic reactor	Swine slurry with high rbCOD	OHO AOB NOB	AQUASIM	Uniform	Variable	No	H
EBPR and N removal in BF-anaerobic reactor	Synthetic; acetate	OHOs PAOs GAOs AOB NOB	C++	Variable size per selective settling pressure	Uniform	Yes	I
EBPR and N removal in BF-anaerobic reactor	Synthetic; acetate	PAOs AOB Nitrospira Nitrobacter	AQUASIM	Uniform	Uniform	No	J

Notes: PF-aerobic = pulse-fed aerobic SBR. BF-anaerobic = anaerobic bottom feeding through settled sludge bed.

Sources: A (Beun et al., 2001); B (Su and Yu, 2006a, 2006b); C (de Kreuk et al., 2007); D (Ni et al., 2008); E (Fang et al., 2009); F (Ni et al., 2009); G (Vázquez-Padín et al., 2010a); H (Isanta et al., 2013); I (Kagawa et al., 2015); J (Winkler et al., 2015).

A common process model schematic for aerobic granular sludge SBRs is shown in Figure 8-3. Two completely mixed compartments are used. A fixed-volume biofilm compartment contains the granular sludge biomass inventory that is represented by a specified number of spherical particles as discussed above. A variable-volume compartment serves to modulate the total liquid volume during feeding and effluent withdrawal. A high recirculation ratio between the compartments is applied so that the bulk liquid concentrations are essentially equal in both compartments. Because effluent is discharged from a completely mixed compartment, sedimentation does not occur in this configuration. Simulation results for effluent TSS concentrations were not reported in the studies summarized in Table 8-1. However, the purpose of the models was to simulate biologically mediated substrate conversions and not to simulate liquid-solids separation performance. It should be noted that Study I in Table 8-1 simulated discrete Stokian sedimentation in a one-dimensional model for an initial period of 10 days, after which a representative average granule was constructed and used in subsequent, two-dimensional modeling.

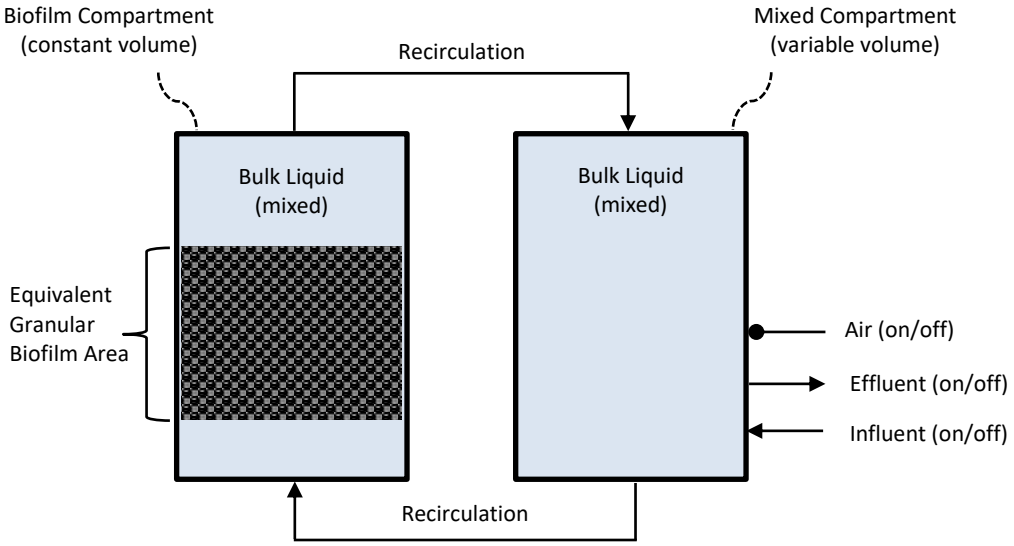


Figure 8-3. Schematic of commonly used process configuration in modeling of aerobic granular sludge SBRs.
Lines with arrows represent advective links. Lines with circles represent diffusive links.

Because the full-scale aerobic granular sludge processes employed to date for simultaneous EBPR and nitrogen removal have involved slow BF-anaerobic through the granular sludge bed, some additional aspects of the model setup for Study C (de Kreuk et al., 2007b), which simulates a bench-scale system of this nature, are summarized here. It should be noted that the completely mixed nature of both compartments used in the model (see Figure 8-3) does not represent the more plug-flow regime and higher influent concentrations to which the granule sludge bed is exposed during slow BF-anaerobic. However, this setup was used because a more realistic representation of the feeding regime would have presented excessive complexity. To compensate for the lower influent substrate concentrations to which the granular biomass was exposed in the model, a higher diffusion coefficient for acetate was used in order to represent the expected deeper substrate penetration of the real system exposed to higher influent concentrations. Additionally, phosphorus precipitation was not included in this study (or others). In order to compensate for this, the maximum fractions of stored poly-phosphate and PHB were increased for PAOs.

No aerobic granular sludge models with simultaneous EBPR and nitrogen removal processes have been applied to the treatment of domestic wastewaters. However, Study F included the simulation of a pilot-scale aerobic pulse-fed nitrogen removal process treating low strength domestic primary effluent where

the total COD concentration was less than 200 mg/L. In this model, hydrolysis of particulate COD was simulated by OHO-mediated surface reaction kinetics as in ASM3 with a maximum hydrolysis rate of 3 day^{-1}.

In some studies, two-dimensional models have been used to simulate the spatial arrangement of individual cells or other constituents such as particulate inert material from decay across a granule section (Kagawa et al., 2015; Matsumoto et al., 2010; Xavier et al., 2007). These models are more computationally intensive than one-dimensional models and currently of limited practical use to the design of engineered wastewater treatment systems (Boltz et al., 2010).

8.3 Modeling a Mixed Suspension of Flocs and Granules

In the typical setup for modeling the performance of laboratory-scale aerobic granular sludge SBRs (Figure 8-3), the liquid-solids separation process is not simulated. Consequently, smaller particles that arise due to biofilm detachment are rapidly washed out of the system, which is representative of typical conditions in laboratory-scale SBRs with short settling times followed by rapid decanting. However, full-scale aerobic granular sludge reactors performing simultaneous EBPR and nitrogen removal have been shown to contain a significant flocculent MLSS fraction (approximately 10-20% by mass, as discussed in Section 7.2.2). The presence of this non-negligible flocculent biomass fraction is a consequence of the less stringent selective settling pressure resulting from the employment of the simultaneous upflow feeding and a more moderate effluent overflow rate in contrast to a rapid decant period following the settling period in many granular SBR systems. Thus, a more representative model setup for existing full-scale aerobic granular sludge processes would account for the presence of both granular and flocculent biomass fractions.

A mixture of flocculent and granular biomass has yet to be simulated in aerobic granular sludge processes performing EBPR and nitrogen removal, but has been simulated in a model-based study of a nitritation-anammox process (Hubaux et al., 2015) aimed at investigating the impact of a small mass fraction of flocs (5%) compared to the assumption of a completely granular suspension. In the study, the continuously fed system was modeled in AQUASIM using a biofilm compartment with recycle from the effluent stream to the biofilm reactor compartment in order to achieve the targeted flocculent solids retention time. The approach used in this continuously fed system is not feasible for intermittently fed aerobic granular sludge reactors, but a similar strategy of including flocculent biomass could be accommodated by introducing a liquid-solids separation step into the aerobic granular sludge model setup.

CHAPTER 9

Resource Recovery with Aerobic Granular Sludge

Methane production in anaerobic digestion and nutrient recovery are common resource recovery options available to granular activated sludge WRRFs just as they are to flocculent activated sludge WRRFs. In addition, granular activated sludge offers a unique resource recovery product in the form of an alginate-like biomaterial. Resource recovery experience with the processing of waste granular activated sludge (WGAS) is discussed in this chapter.

9.1 Methane Production from Anaerobic Digestion of Waste Granular Activated Sludge

The amount of methane produced from solids destruction in anaerobic digestion is a critical parameter for gas handling and energy recovery designs in WRRFs. Methane production in anaerobic digestion is directly proportional to the volatile solids (VS) destruction efficiency. The VS destruction efficiency is a function of the digester temperature, SRT, and feed sludge characteristics. For the mesophilic anaerobic digestion of combined primary and secondary waste sludge, VS destruction efficiencies of 50 to 60% are considered typical at an SRT of about 20 days and 35°C temperature (Tchobanoglous et al., 2014; Water Environment Federation, 1998).

However, waste activated sludge (WAS) is not as biodegradable as primary sludge due to the fact that most of the biodegradable material is contained inside the bacteria cell wall or within an extracellular polymeric matrix (Neyens and Baeyens, 2003). The VS destruction efficiency for WAS has been reported to range from 35 to 45% (Bhattacharya et al., 1996; Bolzonella et al., 2005). In the anaerobic digestion of WAS from a conventional 5-day SRT activated sludge process, the VS destruction efficiency was reported as 37.4% at a 20-day anaerobic digester SRT at 35°C. The VS destruction efficiency was about 40% in a two-stage, 14-day SRT (2-day SRT plus 12-day SRT) mesophilic digester fed WAS from a BNR process operated with a 10-day SRT (Ge et al., 2011).

There have been limited studies on the VS destruction efficiency in the anaerobic digestion of WGAS, but the results show a similar range of destruction efficiency as that observed for WAS from flocculent activated sludge processes. The VS destruction efficiency of WAS from the Epe Nereda® process fed to a lab-scale mesophilic digester at a 20-day SRT was reported at 42% (Hogendoorn, 2013). A photomicrograph of the Epe waste sludge (Hogendoorn, 2013) showed a high proportion of flocculent activated sludge due to the operational practice of normally wasting from the top of the sludge blanket. The WGAS from a laboratory 30-day SRT aerobic granular sludge reactor with nitrogen removal and EPBR treating a synthetic municipal wastewater was fed to a 26-day SRT mesophilic digester, and the VS destruction efficiency was 49%, based on total COD removal (Val del Río et al., 2011). The VS destruction efficiency was 32% for lab-scale mesophilic digestion of WGAS from an aerobic pulse-fed 100 L pilot bubble column SBR treating swine manure at a 5-day SRT (Morales et al., 2013; Val Del Río et al., 2014). Based on these studies, there is no basis to expect a different anaerobic digestion VS destruction efficiency for the digestion of WGAS versus WAS from a flocculent activated sludge system at the same digester operating conditions, but a side by side study of the digestion of waste sludge from flocculent and granular sludge systems treating the same wastewater at the same SRT would provide a more conclusive comparison.

Thermal pretreatment of WGAS in the above studies was shown to improve digestibility for operation at the same digester SRTs. For the Epe Nereda® waste sludge, thermal pressure hydrolysis pretreatment at 160°C for two hours and 6-8 bar pressure increased the VS destruction from 42 to 48% (Hogendoorn, 2013). For the synthetic municipal wastewater WGAS, thermal pretreatment at 190°C and 210°C for 20 minutes increased the VS destruction efficiency from 49% to 56% and 58%, respectively (Val del Río et al., 2011). For the swine manure WGAS, thermal pretreatment at 133°C for 20 minutes improved the VS destruction efficiency from 32% to 47% (Val Del Río et al., 2014).

9.2 Nitrogen and Phosphorus Recovery from Waste Granular Activated Sludge

At present, there are no reported studies on nitrogen or phosphorus recovery from WGAS processing. WGAS at the Nereda® Epe plant is thickened to 4% total solids and transported off-site to a centralized anaerobic digestion facility where it is co-digested with other solids streams (Monita et al., 2015). Based on WGAS biology and observed digestibility, the sludge processing, resource recovery, and ultimate biosolids application methods demonstrated with flocculent activated sludge should be applicable to WGAS. For systems without anaerobic digestion, this would include the land application of biosolids after aerobic digestion or composting to reuse the nitrogen and phosphorus in the biosolids. Similar methods may apply to anaerobic digestion biosolids. In addition, similar processes such as ammonia stripping and recovery and struvite ($MgNH_4PO_4 \cdot 6H_2O$) recovery via crystallization processes (Tchobanoglous et al., 2014) due to the ammonia- and phosphorus-rich digester liquid should be applicable. An emerging technology to recover phosphorus from municipal WAS that also applies to WGAS is the production of phosphorus-rich biochar fertilizer via pyrolysis processes. An example is the PYREG® process, which recently had its first municipal WRRF installation in Linz-Unkel, Germany in 2015.

9.3 Alginate-Like Biomaterial Harvesting from Waste Granular Activated Sludge

Alginate is a valuable commercial product that is extracted from seaweed, ultimately purified and concentrated as sodium alginate, and used as a gelling, thickening, or emulsifying agent in various industrial applications including human and animal food production, cosmetics and pharmaceuticals, and textile printing (McHugh, 1987). The production and role of alginate-like exopolysaccharides (ALE) in aerobic granular sludge was discussed earlier (Section 2.7). ALE can be recovered from aerobic granular sludge and potentially introduced to the marketplace as a lower-cost alternative to seaweed-derived alginate depending on the end-use application and on processing and purification methods that are currently under development. This resource recovery option has the potential to produce a revenue stream for the aerobic granular activated sludge facility (Giesen et al., 2015).

It is presently expected that ALE from aerobic granular activated sludge can be used for similar purposes as commercial alginate, excluding food and personal care product applications. Though ALE extracts derived from granular activated sludge have not been evaluated for pathogens, the use of these ALE extracts in the production of agricultural or industrial goods for human consumption is not anticipated. ALE extracts from the Epe Nereda® plant have been characterized as amphiphilic, having both polar water-soluble and nonpolar water-insoluble groups. This type of ALE formed thin films on hydrophilic surfaces, creating a water-resistant barrier that could potentially be used in a similar manner as alkenyl succinic anhydride in surface coating applications for paper, paper products, and gypsum board (Lin et al., 2015a) or as a coating to extend the life of steel and concrete (Lin et al, 2015b).

Using existing laboratory-scale processes (Figure 9-1) modeled after those that are well-established processes for commercial alginate extraction from seaweed (Figure 9-2), the initial investigation of ALE recovery from Nereda® excess sludge from the Epe facility showed that chemical doses and centrifugation times were acceptable, but that the ALE may contain a signification amount of protein (Hogendoorn, 2013). At a dosage of 3 mmol Na^+/g VS an ALE yield of 23% (as g of ALE VS per g of WGAS VS) was obtained, which is in the range of 15 to 25% claimed for the ALE yield for Nereda® excess sludge (Giesen et al., 2015). The ALE yield was independent of centrifugation times between five and 50 minutes in the final separation step, suggesting that only a brief final centrifugation step would be required. However, the ALE contained a significant amount of protein (20-40%). The impact of this protein content on commercial uses and/or downstream purification requirements is not yet known.

ALE recovery after lab-scale mesophilic anaerobic digestion at a 20-day SRT using Nereda® WGAS from the Epe facility was not statistically different than that from the WGAS before digestion (approximately 25% as g of ALE VS per g of WGAS VS at a dose of 7.5 mmol Na^+/g VS, when corrected for initial VS prior to digestion; Hogendoorn, 2013). These results indicate that ALE are only slowly degraded in anaerobic digestion such that most ALE remain available for extraction after anaerobic digestion. However, the preferred location of ALE extraction is before anaerobic digestion because the digestibility and dewaterability of the solids after digestion are improved (Giesen et al., 2015).

Recognizing the potential opportunity for wastewater-based alginate recovery, the public-private Dutch National Alginate Research Program, estimated at €14 million, was launched in 2013 to further develop ALE extraction, purification, and potential market applications. As part of this program, two demonstration-scale alginate extraction facilities will be installed – one treating Nereda® excess sludge from Epe, Dinxperlo, and Vroomshoop and another planned as part of a future Nereda® industrial wastewater treatment facility (Giesen et al., 2015).

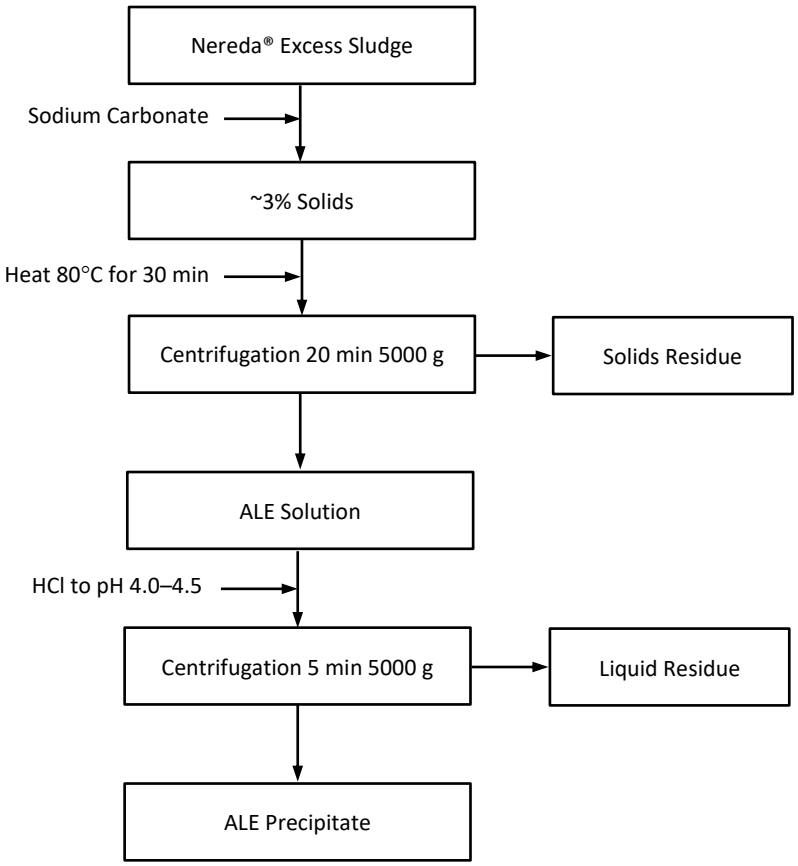


Figure 9-1. Flow chart of laboratory method for harvesting alginate-like extracts from Nereda® excess sludge.
Based on protocol of Hogendoorn (2013).

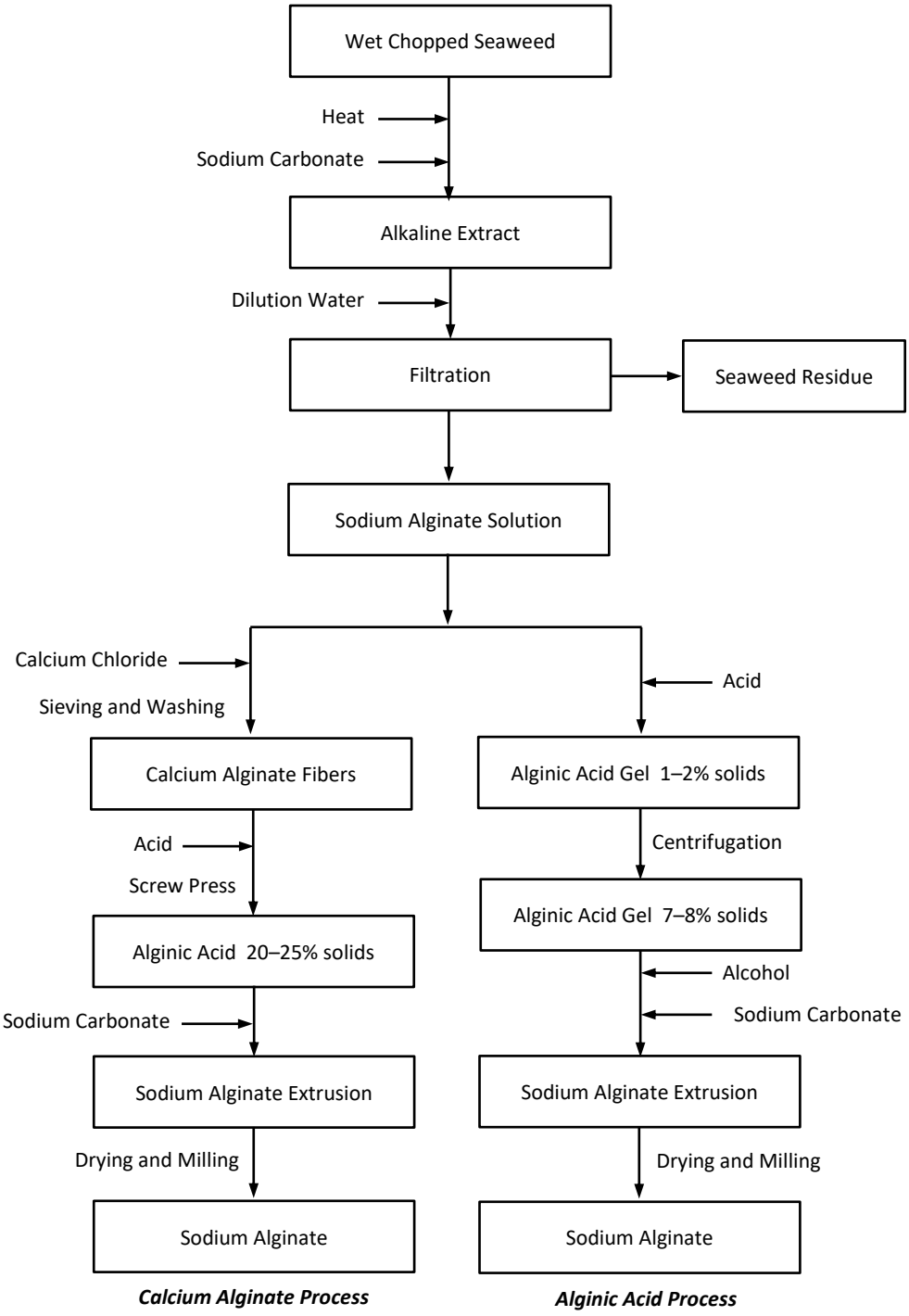


Figure 9-2. Flow chart of key steps for the production of alginate from seaweed.
Based on methods described by McHugh (2003).

CHAPTER 10

Research Needs

Research on aerobic granular activated sludge has been ongoing for almost 20 years, and full-scale biological treatment facilities with aerobic granular sludge have been in operation for the last 7 years. During this time much has been learned about selective pressures for granular sludge, types of granular sludge and their microbial ecology, and the design and operating conditions to achieve nutrient removal, but the range of experience and applications are very small compared to that for flocculent activated sludge, which has benefited from many more decades of investigations. Work so far has shown the potential for aerobic granular processes to reduce reactor volumes, capital cost, and energy cost for BNR. Research is needed to expand the range of applications of granular sludge and to increase the ability to predict the behavior and performance of granular sludge processes. This section discusses the research needed to: 1) increase understanding of the ability to develop aerobic granular sludge for different wastewater characteristics; 2) determine the long term performance of nutrient removal for different wastewater characteristics and temperature and the ability to meet the very low effluent nitrogen and phosphorus concentrations demonstrated with flocculent activated sludge; and 3) better understand other specific design and performance issues, including oxygen transfer characteristics in granular sludge reactors, effluent suspended solids removal, micropollutant removal, and the effect of a granular sludge operation on disinfection process designs. Research needs and opportunities to convert existing continuous flow systems to granular sludge or granular sludge/flocculent sludge hybrid systems, as well as ways to use granular sludge in bioaugmentation processes, are discussed. The need for improved design methodologies, including the application of mechanistic simulation models, and the need to better understand the capability and performance of granular sludge systems in handling variable loads and the types of process controls, are addressed. This chapter also addresses research needs in waste granular sludge processing and resource recovery opportunities.

10.1 Effect of Wastewater Characteristics on Granular Sludge Growth

The NDN-PAO type of granular sludge is needed for EBPR and is also preferred for nitrogen removal as it is a more reliable type of granular growth with lower potential for disadvantageous OHO growth on the outer layer of the granule. Growth of PAOs for flocculent sludge systems has been directly related to the availability of VFA and indirectly related to higher concentrations of soluble biodegradable COD (sbCOD) and/or higher bCOD:P ratios. The experience with flocculent sludge PAO growth thus suggests that the amount and relative fractions of rbCOD, sCOD, and particulate biodegradable COD (pbCOD) are important for the growth of NDN-PAO granular sludge. Most of the research on NDN-PAO granular sludge has used wastewaters with rbCOD as the sole or dominant portion of the influent COD concentration. There was no information provided on the influent bCOD fractions for the full-scale granular sludge facilities with good EBPR, but there was good success with apparent NDN-PAO granular sludge growth for a range of influent wastewaters, which is encouraging. There were no focused research reports on the effects of different fractions of rbCOD, sbCOD, and pbCOD and of wastewater strength on the development of the ability to grow and maintain granular sludge and to establish an effective NDN-PAO granular sludge system.

As described in Section 3.4.1, pbCOD is sorbed to the granule surface where hydrolysis occurs. If the pbCOD is completely hydrolyzed to rbCOD and taken up by PAOs and/or GAOs during the anaerobic feeding period, the maintenance of smooth, compact, and dense granules is expected. If pbCOD is not completely hydrolyzed during the anaerobic feeding period, it will be available for degradation during

aeration and may cause filamentous outgrowths that adversely impact granule settleability and increase effluent suspended solids. The impact of influent wastewater pbCOD and sbCOD fractions on granular sludge morphology, settling velocity, nutrient removal, and effluent suspended solids should be systematically evaluated.

Because the potential adverse impacts of higher influent pbCOD fractions can be avoided by sufficient anaerobic hydrolysis of pbCOD, the hydrolytic activity of granular sludge suspensions should be characterized in greater detail to inform the appropriate design and operation of granular sludge systems. Because most of the granule biomass is not available to mediate the hydrolysis of pbCOD at the granule surface, different mechanistic models to describe hydrolysis in granular sludge systems should be considered.

There is also limited information on the effect of influent wastewater strength and whether a minimum sbCOD concentration is needed to maintain a reliable granular sludge system. The ability to select for granular sludge growth on a low-strength municipal primary effluent where BOD was reported to be less than 100 mg/L has been demonstrated in a pilot-scale reactor with slow anaerobic bottom feeding through the settled sludge bed (Section 7.2.4). However, information on the sbCOD fraction was not available. In the event that granule growth on low-strength wastewaters is difficult to achieve, strategies to increase the effective instantaneous F/M ratio during feeding should be explored. In the case of upflow-fed reactors, for example, deeper tanks would result in a higher effective F/M in the bottom layers and may be more favorable for granule formation.

Although there have not been reports of detrimental grit accumulation in existing full-scale aerobic granular activated sludge reactors, it is possible that inadequate removal of very fine grit or “sugar sand” may result in grit accumulation in granular sludge processes located in geographic regions where this wastewater constitutent is known to be present. In this instance, periodic wasting of the faster-settling bottom layer of the sludge bed may be necessary.

10.2 Long-Term Performance and Reliability of Aerobic Granular Sludge Systems for Nutrient Removal

Though there are a number of full-scale granular sludge facilities in operation outside of the United States that report good nitrogen and phosphorus removal, there is very little detailed long-term performance data available for these systems. On the other hand, a large amount of long-term performance data can be found for flocculent activated sludge systems under a number of different design configurations. The database for granular sludge systems for nutrient removal needs to be increased so that designers can better understand the process capabilities, reliability, and limitations for different wastewaters and temperatures. The approach to increasing and evaluating the performance database for the granular sludge nutrient removal facilities should follow the technology performance statistics methods used by Bott and Parker (2011) in a WE&RF report on a comprehensive study of 22 BNR flocculent activated sludge facilities. The technology performance statistics recognize the reality of variable performance in full-scale facilities by defining, for example, effluent nitrogen and phosphorus species concentrations less than or equal to values that occur 95, 75, and 50% of the time. The closer the 95 and 50% values, the less variable the process performance.

10.3 Achieving Low Effluent Nitrogen and Phosphorus Concentrations

In the United States, typical low effluent TN discharge limits ≤3.0 mg/L have been met with BNR flocculent activated sludge processes with membrane liquid-solids separation or followed by filtration and with carbon addition. The effluent TN is composed mainly of soluble inorganic nitrogen and soluble organic nitrogen (SON), as the effluent suspended solids removal process results in particulate TN concentrations below 0.10 mg/L. Typical effluent concentrations of the soluble nitrogen species are <0.3 mg /L, <1.5 mg /L, and <1.0 mg/L for NH_3-N, NO_x-N, and SON, respectively. Low effluent TP discharge limits ≤0.1 mg/L have also been met in BNR flocculent activated sludge processes with membrane liquid-solids separation or followed by filtration. Phosphorus removal to low levels has been accomplished by EBPR in the BNR flocculent activated sludge systems with chemical polishing or mainly by chemical addition. Both low effluent TN and TP discharge limits of ≤3.0 mg/L and ≤0.1 mg/L, respectively, have been met by BNR flocculent activated sludge systems with highly efficient effluent suspended solids removal and chemical polishing. External carbon addition has been necessary to meet the effluent TN concentrations in many U.S. BNR facilities, and primary sludge fermentation to add VFAs to improve EBPR performance has been done in some cases.

As shown in Chapter 7 the existing full-scale SBR granular sludge facilities have shown good nitrogen and phosphorus removal ability but have not been required to meet TN and TP discharge limits of ≤3.0 mg/L and ≤0.1 mg/L, respectively. The lowest effluent TN and TP concentrations reported for the Epe facility were effluent TN and TP concentrations of less than 4.0 and 0.3 mg/L, respectively, without supplemental carbon. The reported feed concentrations showed favorable ratios of BOD/N of 4.3 and BOD/P of 36 for nutrient removal. Thus, research is needed to show the design and operating conditions necessary to achieve effluent TN and TP concentrations of ≤3.0 mg/L and ≤0.1 mg/L for a range of influent wastewater characteristics using the SBR granular sludge process in Chapter 7 and other types of granular sludge processes that may be developed. For systems with low influent BOD/N and BOD/P ratios, it would be important to know if adding supplemental carbon to the granular sludge process is preferred versus chemical addition in effluent polishing steps such as tertiary filtration. It would also be important to determine if the supplemental carbon should be added during the anaerobic feeding or within a post anoxic phase after the aerobic NDN period. Alternatively, the use of screening instead of primary clarification could be considered in systems with low influent BOD/N and BOD/P ratios. Additionally, the ability to out-select NOB from growing in aerobic granules would be advantageous to lower the COD required for nitrogen removal. Strategies to out-select NOB may include cycling between aerobic and anoxic conditions, which has been effective in flocculent activated sludge, as well as operation at a low DO concentration to promote only AOB growth in the granular sluge outer aerobic layer. The efficacy of these strategies for NOB out-selection in aerobic granular sludge processes and impacts on treatment capacity and performance should be investigated. For nitrogen removal, the background information presented in this document suggests that supplemental carbon addition during anaerobic feeding would maintain a more ideal granular sludge morphology compared to post-anoxic feeding of external carbon. However, feeding supplemental carbon in the anaerobic phase for later denitrification by PAOs and GAOs raises issues on process control methods to optimize the required dose and costs. Research in this area would have to address wastewater characteristics, the aerobic granular sludge process design, supplemental carbon feeding strategies, process control methods, and operating costs.

For phosphorus removal, research is needed to determine what low effluent phosphorus concentration is possible and how to best achieve it. Design and operating conditions will also be affected by wastewater characteristics such as influent BOD/P and BOD/N ratios and rbCOD fraction. The effect of process operating conditions for phosphorus uptake after carbon removal by PAOs during anaerobic

feeding needs to be understood. The aeration DO control strategy used to accomplish both nitrification and denitrification may impact the PAO phosphorus removal efficiency and/or aeration time required. For example, if there is insufficient NO_x-N produced during the aeration period to accomplish enough PAO carbon storage oxidation by denitrifying PAOs for sufficient phosphorus uptake, a longer aeration period may be necessary.

10.4 Effect of Granule Size in Biological Nutrient Removal Aerobic Granular Sludge Systems

The effect of granule size is another factor that may affect the design and operating conditions needed to achieve low effluent TN and TP concentrations. The ability to control the granule size and its effects needs further research. The information presented in Section 3.2.1 suggests that the settling time or liquid overflow rate affects the granular sludge sizes in a granular sludge system mixed liquor. Longer settling times and lower overflow rates should select for smaller granules and a higher fraction of flocculent sludge in a mixed liquor, and vice versa for shorter settling times. The total biofilm area for nitrification in a given granular sludge MLSS concentration and the total biofilm anoxic volume is affected by the system's average granule diameter. Using appropriate DO control, simultaneous nitrification and denitrification is achieved in the outer aerobic zone and inner anoxic zone, respectively. For a given bulk liquid DO concentration and fixed oxygen penetration depth, smaller granules will have a higher aerobic biomass fraction and lower anoxic biomass fraction than larger granules. The feasibility of this operational strategy and its impacts on NH_3-N, N, and P removals should be evaluated. For wastewaters with low BOD/N ratios, it may be more favorable to select for larger granules to maximize anoxic carbon utilization. To maximize nitrification kinetics, smaller granules with a higher aerobic total biofilm surface area may be preferred. The granule size may also affect the time needed for efficient substrate uptake by PAOs and the EBPR efficiency.

10.5 Effect of Variable Loads on Performance, Design, and Process Control

The reported performance of full-scale aerobic granular sludge systems lacks information on the effect of variable loading conditions on effluent TN, TP, and TSS concentrations. In flocculent activated sludge systems, longer SRTs and larger tank volumes, with a greater biomass inventory, are used to meet effluent treatment goals under higher variable loading conditions. An increase in biomass inventory would also be needed to handle higher variable loads to a granular activated sludge system, but it is not known if the performance of this more diffusion-limited process is more sensitive to variable loading conditions, which would thus require greater changes in the design SRT. Research is needed to develop guidelines for design and process control methods as a function of wastewater characteristics, nutrient removal performance required, and loading variability. An essential part of this research would be the use of mechanistic simulation dynamic models with full-scale plant data collection. Calibrated simulation models have proven very useful for predicting the performance of BNR flocculent activated sludge processes under dynamic loading conditions.

This work should also consider including measurements for nitrous oxide emissions, an important greenhouse gas. Other work with flocculent activated sludge systems has shown a greater propensity for nitrous oxide emissions under transient operation conditions. As noted in Section 5.5 conflicting results on nitrous oxide emissions were found in laboratory studies, and there have been no reports of measurements for full-scale granular activated sludge systems.

10.6 Microbial Diversity in Aerobic Granular Sludge

Although significant progress in characterizing aerobic granular activated sludge microbial communities has been made, addressing some new issues will provide a better understanding of this important aspect of the technology. Changes in the granule microbial community in response to different influent wastewater characteristics and operational parameters such as SRT, temperature, and DO concentration have not been studied in detail. The importance of PAO in aerobic granules performing simultaneous nitrogen removal and EBPR has been well established, but foundational studies did not investigate the abundance or spatial distribution of *Tetrasphaera* PAOs, which are now receiving greater attention in EBPR processes. Future work should investigate the abundance and spatial distribution of *Tetrasphaera* PAOs in addition to *Accumulibacter* PAOs, which have traditionally received greater attention. The impact of granule size on the abundance of different PAOs should be considered. Specifically, *Tetrasphaera* PAOs grow under deeper anaerobic conditions, and their growth niche may be in the core of large granules, while *Accumulibacter* PAOs may dominate in smaller granules.

10.7 Use of Mechanistic Models for Aerobic Granular Sludge Design and Analysis

Initial successes with mechanistic simulation models for predicting the performance of granular activated sludge systems has been demonstrated with lab-scale reactors as discussed in Chapter 8. Research is needed to expand the capability of the current models, including additional important process parameters, and to develop and demonstrate workable models that can reasonably describe the behavior and performance of full-scale granular activated sludge systems. Important process parameters to consider for further model development are chemical equilibria functions to predict pH and physical factors to predict granule sizes. Other refinements in granular sludge systems modeling could include chemistry and pH elements that can predict phosphorus precipitation within granules and functions that may account for NH_4^+-N ion exchange sorption and desorption phenomena.

The granule size is an important factor affecting the accuracy of a model's predicted performance for granular sludge systems. As discussed above in Section 10.4, smaller granules may result in higher specific nitrification rates but lower specific denitrification rates, and vise versa for larger granules. Factors that can affect the granule size are the liquid-solids separation process design and granule losses by abrasion and fragmentation.

The ability to predict granule size is a major challenge for developing reasonable model predictions of process performance for full-scale facilities. An additional challenge is the need to model full-scale aerobic granular sludge systems that contain both granular and flocculent activated sludge. Granular sludge process model development and the demonstration of the model application is needed to advance an industry-accepted process model. Further software advances may also be expected.

10.8 Oxygen Transfer Characteristics in Aerobic Granular Sludge Systems

Fine bubble diffused aeration is the most common method of oxygen supply to aerobic granular activated sludge processes, but process information is needed to apply standard aeration design protocols to size the equipment in these systems. In standard design protocols described in the references, including the EPA Fine Pore Aeration Manual (U.S. EPA, 1989) and other wastewater engineering textbooks (Tchobanoglous et al., 2014), the effect of the mixed liquor characteristics is

accounted for in a factor termed “alpha” (α), which is the ratio of the oxygen transfer efficiency in a mixed liquor to that in clean water for the same operating conditions.

Alpha values are typically less than 1.0 due to factors in the mixed liquor that decrease the oxygen transfer rates, which include viscosity, the presence of surfactants and other organic substances, and the mixed liquor concentration. Full-scale plant testing for flocculent activated sludge systems has resulted in a useful database from which alpha values are selected for site-specific design and operating conditions. A decline in alpha values has been observed in flocculent activated sludge reactors with higher MLSS concentrations; for example, a 30% decline in alpha has been reported as the MLSS concentration increased from 4000 mg/L to 8000 mg/L (Tchobanoglous et al., 2014). Higher MLSS concentrations are expected for aerobic granular sludge systems, but the effect of higher MLSS concentration on alpha may not be the same as that experienced for flocculent activated sludge systems because of the different sludge characteristics.

Full-scale or pilot-plant oxygen transfer testing is needed for different loadings, MLSS concentrations, and wastewaters to determine appropriate alpha values to use in fine bubble aeration design in aerobic granular sludge processes. For batch feeding operations, the alpha value may increase with aeration time as more impurities are biologically removed from the bulk liquid, so studies should also consider this possible effect.

10.9 Impact of Granular Activated Sludge on Downstream Unit Processes

Effluent filtration and disinfection are common unit processes following flocculent activated sludge BNR processes. Effluent filtration is needed to reach low TN and TP concentrations, and the effluent disinfection process is related to characteristics of its feed stream. There is little information on the design and performance of these unit processes following a granular activated sludge process or how the upstream granular sludge process may affect the design and operation of these important final treatment steps. Research on the microbial, colloidal, and suspended solids characteristics in effluents from full-scale granular sludge systems would be useful for determining the process design and operating conditions needed to meet necessary removal efficiencies in these downstream processes. Information on the effluent solids characteristics with regard to large particles versus more colloidal particles would be useful in relating UV performance with effluent particulates.

Based on limited data, it appears that effluent TSS concentrations from aerobic granular sludge systems are comparable to those of flocculent activated sludge systems (Section 7.2.3). Full-scale use of granular media filtration has proven to be effective for effluent TSS polishing at the Epe facility, and compressible media filtration and disc filtration has been demonstrated at smaller scales (Section 7.2.4). However, a better understanding of the nature of the different effluents can be gained by the particle size distributions in granular sludge system effluents, as has been done in the past for flocculent activated sludge systems. This type of data would provide a better basis for the design of downstream solids polishing systems with suitable hydraulic application rates and polymer doses (where applicable). Similar information is needed for membrane filtration, which has yet to be tested with aerobic granular sludge effluents. It is not known if membrane fouling rates are similar for flocculent and granular activated sludge effluents.

Data on effluent coliform levels and the fate of pathogens and viruses in aerobic granular sludge systems is not available. Compared to flocculent activated sludge systems, differences in specific surface area and hydrophobicity may result in different coliform and pathogen removal outcomes for granular sludge systems. Disinfection design needs following granular sludge treatment should be confirmed.

Granular sludge effluents should be characterized in terms of UV transmissivity, colloidal content, particle size distribution, and shielding potential to verify that the same doses as those used in flocculent activated sludge systems provide adequate disinfection.

10.10 Removal of Micropollutants in Aerobic Granular Sludge Systems

The removal of micropollutants in BNR processes should be of future concern in view of the impact that micropollutants can have on the aquatic environment at ng/L to ug/L concentrations. Of special concern are the endocrine disruptor compounds such as estrogen compounds, bisphenol A, 4-nonylphenol, and 4-tert-octylphenol. Previous work summarized in Chapter 6 showing limited research thus far on the removal of micropollutants in granular activated sludge systems points out that the microbial populations developed in granular sludge systems, the SRTs typically used, and the diffusion limited biofilm characteristics may affect the micropollutant removal performance of granular activated sludge compared to flocculent activated sludge. Attention should also be placed on the sorption characteristics of granules and effluent solids from aerobic granular activated sludge processes. Sorption characteristics may be different from those of flocculent activated sludge due to the unique EPS composition in granular activated sludge. There is a wide research need in this area.

10.11 New Process Applications and Designs for Aerobic Granular Sludge

There is increasing interest in using aerobic granular sludge for nutrient removal design configurations that are different than the currently established full-scale SBR process. Benefits of aerobic granular sludge include granular sludge growth in retrofits of continuous flow flocculent activated sludge systems to provide nutrient removal and increase capacity, granular sludge bioaugmentation to increase nutrient removal capability and/or the treatment capacity of existing flocculent activated sludge systems, and granular sludge use in separate wet weather treatment biosorption processes to improve water quality.

Retrofitting existing continuous flow flocculent activated sludge systems to the current full-scale SBR process design may not always be feasible or desirable. Thus, there is a research interest in developing other methods to incorporate granular sludge into existing continuous-flow flocculent activated sludge systems. Some efforts are underway in which larger granular particles are separated from the lighter flocculent activated sludge from the WAS flow and returned to the activated sludge basin. Separation methods include using hydrocyclones or screens. Other approaches could possibly involve variants of surface wasting configurations. The resultant hybrid flocculent/granular activated sludge system would have an increased MLSS concentration, treatment capacity, and BNR capability. Research is needed to find preferred design and operating methods that reliably develop and maintain granular activated sludge growth in existing continuous flow flocculent activated sludge systems. Attention should be paid to the extent of accumulation of fine grit or fibrous material such as toilet paper that may not be captured in upstream processes when evaluating granule separation processes.

Another approach for increasing the BNR ability and/or treatment capacity is granular sludge bioaugmentation in which nitrifying, nitrifying/denitrifying, or nitrifying/denitrifying/EBPR granules are grown on another feed stream and then added to a mainstream flocculent activated sludge treatment process to increase the rates of nitrification or BNR. This concept of bioaugmentation has been previously applied at full-scale facilities in two ways: 1) feeding the mainstream activated sludge treatment system with flocculent nitrifying activated sludge grown in a sidestream reactor fed anaerobic digestion dewatering centrate and 2) feeding anammox granular sludge also grown in a sidestream reactor fed centrate return. In the first approach, the bioaugmented biomass added is subject to the

same limiting SRT of the flocculent activated sludge, and thus only modest improvements in nitrification capacity can be realized. However, the addition of granular sludge for bioaugmentation allows decoupling of the flocculent and granular sludge SRTs because of the ability to separate and return the aerobic granular sludge, to potentially provide a much greater improvement in treatment performance and capacity. Research is needed to evaluate the long-term capability of bioaugmentation with sidestream grown granules with regard to the separation efficiency of granules from the mixed liquor, the mass of granules and SRT that can be maintained, the ability of the granules to maintain their structural integrity, and the improved substrate removal rates. The possibility of the growth of other organisms on granule sludge surfaces and these organisms' effects also needs to be investigated.

As was shown in Section 7.1.2, another scenario for granular sludge bioaugmentation would be the addition of waste sludge from a parallel mainstream granular sludge treatment system to an existing flocculent activated sludge system. In this case, the facility nutrient removal and/or treatment capacity is increased by adding a new parallel granular activated sludge process with the improvement of the nutrient removal capacity of the existing system by bioaugmentation. The same research issues described above apply to this type of bioaugmentation scenario.

The Nereda® granular sludge SBR process has shown a capability of handling high wet-weather peak flows due to its excellent granular sludge settling properties and the biosorption ability of the granules. This observation encourages consideration and investigation of its use in the treatment of peak wet-weather flows external to the main aerobic granular sludge reactor in a contact-stabilization mode of operation in a manner that is similar to that used in other ballasted biological sludge processes. Peak wet-weather flow mitigation and treatment in this manner may lead to a more optimized mainstream aerobic granular sludge process design. Research is needed to evaluate the feasibility of this type of process modification.

10.12 Granular Sludge Processing and Resource Recovery

Besides water reuse, resource recovery opportunities for granular sludge systems involve solids stream processes. These include the production of methane, phosphorus-rich fertilizer, and alginate-like biomaterial for industrial uses. Research needs pertaining to these processes are discussed here.

Thickening and dewatering are key steps in sludge processing. Initial laboratory results showed that the granular sludge thickening rate was extremely rapid compared to that for flocculent activated sludge. Research and data collection is needed to better understand the thickening and dewatering properties of granular sludge and how they impact the design loadings for mechanical sludge handling equipment and polymer dose requirements. One of the issues in studying the thickening and dewatering properties for a granular activated sludge system is that the system may not contain only granular sludge but some mixture of granular and flocculent sludge, as was shown for some of the full-scale Nereda facilities in Chapter 7.

The impact of a higher EPS content in granular sludge compared to flocculent sludge on anaerobic digestion performance is not known. Based on limited data on anaerobic digestion testing of aerobic granular sludge from the Epe facility (Section 9.1), the VS destruction efficiency appears to be similar to what would be expected with waste flocculent sludge. More research on the anaerobic digestion of aerobic granular sludge is needed to better understand the hydrolysis rates and how they compare to that in flocculent sludge. In addition, information is needed on the COD/VS ratio of the aerobic granular sludge compared to that of flocculent activated sludge. The higher EPS content of aerobic granular sludge raises the possibility of it having a higher COD content, which would result in a greater amount of methane production per kg of VS destroyed in anaerobic digestion if the EPS is digested at the same rate

of biomass. In addition, it would be useful to know if pretreatment methods such as sonication and thermal hydrolysis produce similar improvements in digestion rates for granular activated sludge as has been observed for flocculent sludge. In addition to the fate of granular activated sludge EPS in anaerobic digestion, a better understanding of the impact of undigested EPS on dewatering processes is needed.

Aerobic granular sludge for nitrogen and phosphorus removal relies on PAOs for developing desirable granular sludge characteristics in addition to achieving EBPR. Release of phosphorus from EBPR system waste sludge during thickening and anaerobic digestion is an important element of phosphorus recycle via struvite recovery systems. Research on the amount and rate of phosphorus release for waste granular sludge is needed to provide information for the design of phosphorus recovery processes and to predict their performance.

Granular sludge in facilities treating domestic wastewater and performing simultaneous EBPR and nitrogen removal contain a high content of alginate-like EPS. Extracts of this alginate-like biomaterial share many properties with the commercial alginate currently produced from seaweed. This alginate-like biomaterial holds potential to be a newly realized marketable resource recovery product for WRRFs that is not otherwise found with conventional flocculent sludge. Initial investigations of the recovery of alginate-like biomaterial from Nereda® excess sludge were promising (Section 9.3), but showed that extracts contained as much as 40% protein. The impact of this protein content on commercial uses and/or downstream purification requirements is not yet known. The impact of key extraction conditions including pH, temperature, and duration has not been studied in detail, and optimal conditions are not known. Furthermore, a market and economic feasibility analysis has yet to be conducted to better understand the cost/benefit of alginate-like biomaterial production and potential viable outlets for the material. It is not clear whether the variability in composition of real municipal and industrial wastewaters significantly impacts the composition of alginate-like extracts.

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APPENDIX B

Supplemental Information
for Chapter 4:

Comparison of different aerobic granular sludge types for activated sludge nitrification bioaugmentation potential

16 pages

2 Tables

3 Figures

B.1. Granular sludge reactor operation

In addition to the feed wastewater constituents identified in the manuscript, a trace element solution consisting of 0.40 g/L $ZnSO_4 \cdot 7H_2O$, 0.24 g/L $CoCl_2 \cdot 6H_2O$, 1.0 g/L $MnCl_2 \cdot 4H_2O$, 0.24 g/L $CuSO_4 \cdot 5H_2O$, 0.24 g/L $Na_2Mo_4 \cdot 2H_2O$, 0.20 g/L $NiCl_2 \cdot 6H_2O$, 0.16 g/L $Na_2SeO_3 \cdot 5H_2O$, and 4.0 g/L $Na_2EDTA \cdot 2H_2O$ was added at 1 mL per L of net influent. An iron solution consisting of 8.0 g/L $FeSO_4 \cdot 7H_2O$ and 5.0 g/L $Na_2EDTA \cdot 2H_2O$ was added at 0.4 mL per L of net influent. The dilution stream contained PO_4-P in equimolar parts K_2HPO_4 and $NaH_2PO_4 \cdot H_2O$ such that the PO_4-P concentration in the net influent was 20 mg/L in NIT and NDN-OHO reactors and 30 mg/L in the NDN-PAO reactor. Influent Ca^{2+} concentration in all reactors was 18 mg/L in the form of $CaCl_2$. $MgSO_4$ was included in all reactors at a net influent Mg^{2+} concentration of 10 mg/L. The NIT reactor included additional $MgCl_2$ to achieve a monovalent-to-divalent cation ratio of 0.3 in the blended feed stream based Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations to represent the use of $Mg(OH)_2$ for supplemental alkalinity in a full-scale process.

Feed streams were introduced from the top of NIT and NDN-OHO reactors and from a side port at the 100 mL level in the NDN-PAO reactor. The mixed slug feed period in the NDN-PAO was used because short-circuiting occurred when slow feeding from the side port into the settled sludge bed was attempted.

The following descriptions and details of reaction periods in the granular sludge SBRs are provided to supplement those in the manuscript. The NIT reactor N+COD feed stream was added semi-continuously during the 345 min aerobic reaction period in small feed pulses of approximately 6 mL every 10 min. The NDN-OHO reaction period consisted of four step feed intervals that were 85 minutes each with 5 minutes of deoxygenation, 20 minutes of anoxic

feeding, and 65 minutes of aeration. The N+COD stream was added in 5 pulses at 4 minute intervals during anoxic feeding. An additional 5 minutes of aeration occurred after fourth step interval prior to settling. The total NDN-PAO reactor cycle was as follows: 3 min anaerobic slug feed, 37 min additional anaerobic react, 260 min aerobic period, 1 min settling, 2 min effluent discharge, and 22 min idle. After the idle period, a 35 min period with mixing of the remaining bulk liquid and settled sludge was included for additional denitrification capacity to promote anaerobic feeding conditions in the subsequent cycle. Magnetic stirring at 200 rpm occurred during all NDN-PAO reaction periods. Intermittent N_2 sparging for 3 second pulses on 20 second intervals was applied during anaerobic and post-denitrification periods for additional mixing. Air was sparged in an on-off regime for DO control at a concentration of 1.5 mg/L during aerobic period. Gas sparging and mixing conditions in the granular sludge SBR systems are summarized in Table B-1.

Table B-1. Summary of gas sparging and mixing conditions in granular sludge SBR systems

Parameter	Units	NIT	NDN-OHO	NDN-PAO
Gas sparging condition	-	Continuous	Continuous	On-Off
Gas sparging during anoxic/anaerobic periods	cm^3/min	n/a	$N_2 = 450$ $CO_2 < 5$	$N_2 = 600$
Gas sparging during aerobic periods	cm^3/min	$N_2 = 450$ Air = 250	$N_2 = 450$ Air = 2500	Air = 1400
Aerobic period maximum superficial gas velocity	cm/s	1.0	1.0	0.6
Magnetic stirring	rpm	n/a	n/a	200

Different activated sludge seed sources were used. Seed sludges were screened with a 212 um sieve, and only particles passing the sieve were used to seed the reactors. The NDN-OHO reactor was seeded from a full-scale anoxic-aerobic flocculent activated sludge process. The NDN-PAO reactor was seeded from a mixture of sludges from full-scale anaerobic-aerobic and anoxic-aerobic flocculent activated sludge processes. The NIT reactor was seeded with a mixture of manually-crushed granules from the NDN-OHO reactor and a full-scale deammonification reactor.

B.2. Granular sludge morphology

The size, shape, and structure of granules are compared in Figure B-1. The NDN-PAO and NDN-OHO granules are much larger than the NIT granules. NDN-PAO granules have ideal morphology with a smooth outer surface and more compact biomass growth. NIT granules have an abundance of stalked ciliates on the surface. NDN–OHO granules have irregular finger-like outgrowth at the granule surface showing similarities to fingered outgrowth of *Zoogloea*-like organisms.

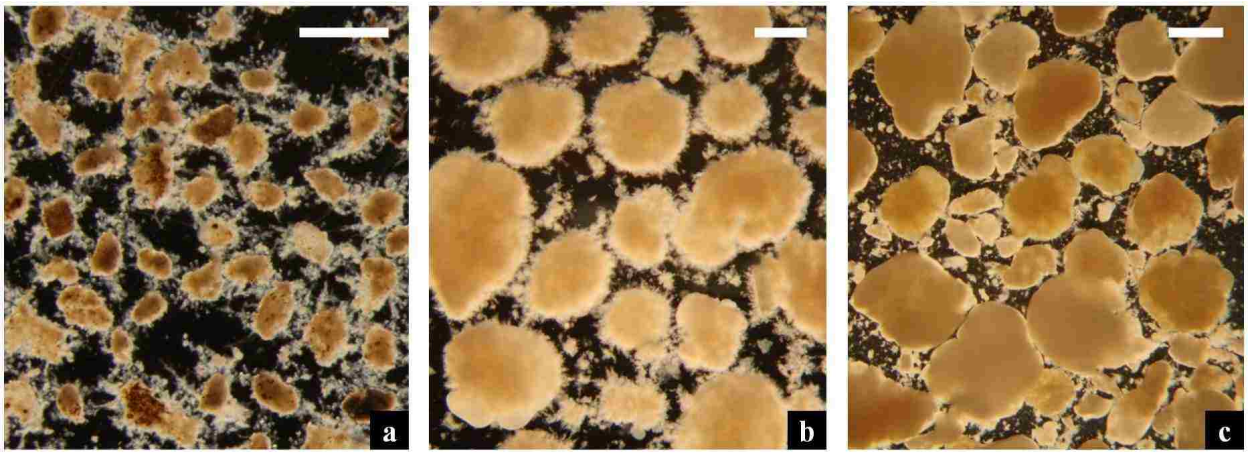

Figure B-1. Comparison of size, shape and structure of the (a) nitrifying (NIT), (b) nitrifying-denitrifying (NDN-OHO), and (c) nitrifying-denitrifying/phosphorus-accumulating (NDN-PAO granules. Scale bars = 1 mm.

B.3. Representative NDN-PAO reactor cycle profiles

NDN-PAO reactor cycle profiles were periodically measured. Figure B-2 shows a profile of total inorganic nitrogen (TIN) species in the anaerobic and aerobic periods and TIN removal in the aerobic period of an NDN-PAO reactor cycle profile on day 164. Data show excellent simultaneous nitrification and denitrification performance and NO_2-N accumulation less than 1 mg/L during the aerobic period.

Soluble COD (sCOD) was measured for effluent discharged prior to the cycle and during the anaerobic period of the cycle corresponding to Figure B-2. The data showed that acetate and propionate uptake during the anaerobic period was complete and that approximately one-third of the influent biodegradable sCOD was taken up during the 3-min slug feed period.

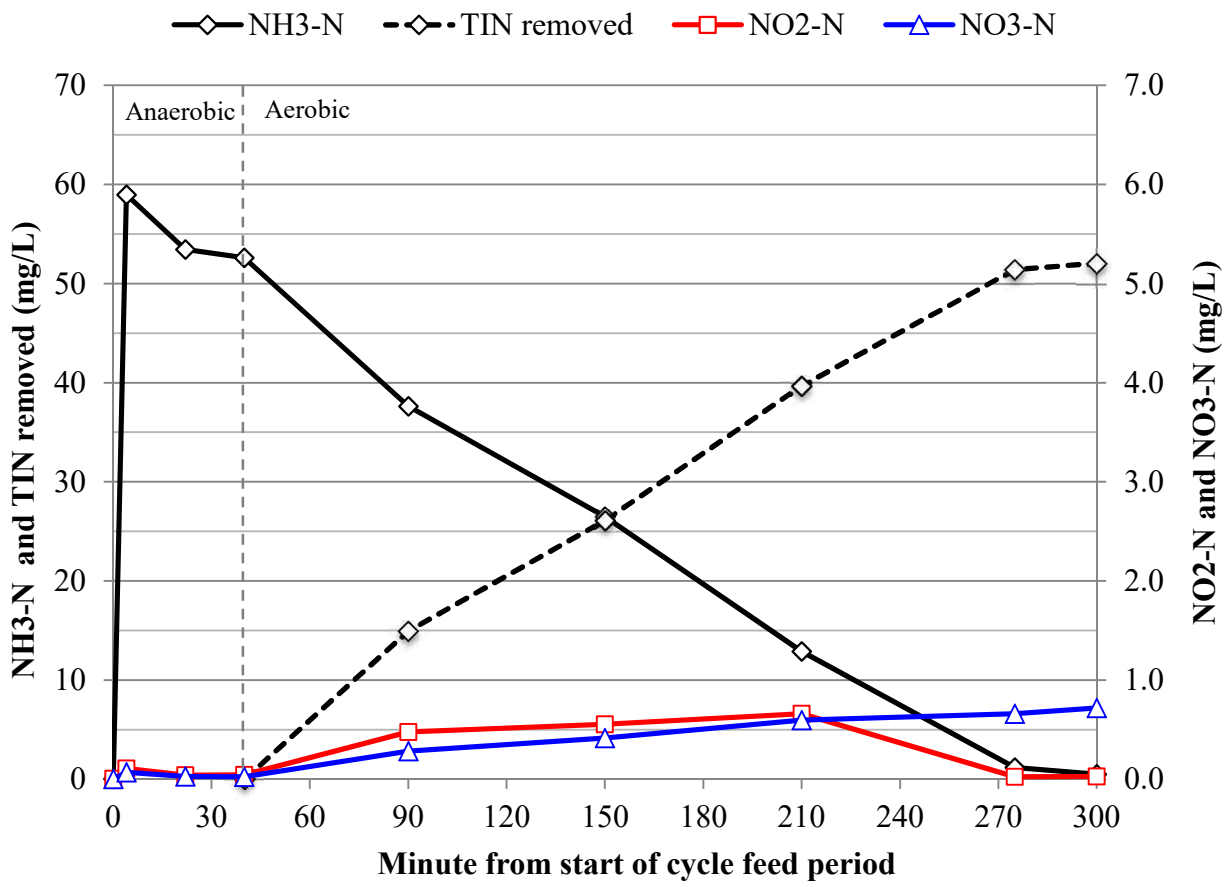

Figure B-2. Profile of total inorganic nitrogen (TIN) species in the anaerobic and aerobic periods and TIN removal in the aerobic period of a representative NDN-PAO reactor cycle on day 164. (MLSS and DO concentrations = 8800 and 1.5 mg/L, respectively).

B.4. Results of high-throughput sequencing of 16S rRNA gene amplicons

Results of microbial community diversity analysis by high-throughput sequencing of 16s rRNA gene amplicons are summarized in Table B-2 where taxonomy assignments for OTUs present at greater than 2% relative abundance are shown for each nitrifying granule type. Triplicate samples for each granule type were analyzed. Comparison of the relative abundance fraction of AOB and NOB for the three reactors show distinct differences. The NOB to AOB relative abundance ratio for the NIT, NDN-OHO, and NDN-PAO reactors was 3.2, 1.3, and 0.10, respectively.

Table B-2. Summary of microbial population taxonomy assignments and relative abundances in nitrifying granular sludge reactors

Taxonomy Assignment	Relative Abundance (%)
NIT Reactor	
Nitrospira	26.3 ±1.9
Candidatus Nitrotoga	12.2 ±0.5
Nitrosomonas	12.0 ±0.4
Cytophaga[(a)]	8.9 ±0.4
Prosthecobacter	3.7 ±0.0
Acidobacterium sp.	3.6 ±1.8
Sphaerotilus	2.9 ±0.1
Rhodobacter[(a)]	2.8 ±0.1
Dechloromonas[(a)]	2.4 ±0.1
Comamonadaceae sp.	2.1 ±0.0
All others <2% relative abundance	23.2 ±0.6
NDN-OHO Reactor	
Zoogloea	23.4 ±1.7
Thauera	17.4 ±1.2
Cytophaga[(a)]	16.0 ±1.5
Candidatus Nitrotoga	9.9 ±0.5
Sphaerotilus	7.8 ±0.3
Nitrosomonas	7.3 ±0.4
Prosthecobacter	2.2 ±0.3
Opitutus	2.1 ±0.4
All others <2% relative abundance	16.0 ±1.3
NDN-PAO Reactor	
Dechloromonas	28.2 ±1.1
Haliangium	9.9 ±0.4
Cytophaga[(a)]	9.9 ±0.0
Pedobacter	9.0 ±0.4
Candidatus Accumulibacter	6.7 ±0.2
Cystobacter[(a)]	4.4 ±0.4
Nitrosomonas	3.9 ±0.1
Haliscomenobacter[(a)]	3.4 ±0.8
Candidatus Competibacter	3.2 ±0.2
Pedobacter[(a)]	2.6 ±0.0
Flavobacterium	2.4 ±0.1
All others <2% relative abundance	19.1 ±0.2 [(b)]

[(a)] Indicates taxonomy assignment made from NCBI database

[(b)] Candidatus Nitrotoga relative abundance 0.4 ±0.0% in NDN-PAO reactor

B.5. Denitrification in NDN-OHO reactor

This section provides supplementary data on denitrification performance in the NDN-OHO reactor. Two sets of data are presented: one from an anoxic feed step of routine reactor operation and the second from an in-situ denitrification test.

Denitrification during an anoxic feed step for NDN-OHO reactor

Profiles of NO_x-N removal during single anoxic steps of NDN-OHO reactor cycles were periodically measured and showed NO_2-N accumulation. An example is reported here for intra-cycle sampling during the second anoxic period of an NDN-OHO reactor cycle on day 104. During this period, NO_3-N concentration decreased from 21.4 to 15.0 mg/L, corresponding to 6.4 mg/L NO_3-N removal. In the same period, NO_2-N concentration increased from 4.5 to 7.2 mg/L, corresponding to 2.7 mg/L NO_2-N accumulation. Thus, 3.7 mg/L NO_x-N was removed in this period. 50 mg COD was added in the feed step (50 mL feed stream at 1000 mg COD/L), and 7.0 mg NO_x-N was removed (3.7 mg NO_x-N/L removed at 1.90 L reactor volume). Therefore, the ratio of COD fed to NO_x-N removed was 7.1 g/g in this feed step.

For sampling associated with these data and other periodic measurements during anoxic feed steps, sCOD was measured at the end of the deoxygenation step preceding feeding and at the end of the anoxic period. The sCOD concentrations at these time points were within ±5 mg/L of each other for each sampling occasion. These concentrations were deemed to be equal within variability of the sCOD measurement method. Therefore, significant residual biodegradable sCOD added during the feed period was not present during subsequent aeration periods, and complete biodegradable sCOD removal occurred during anoxic feed steps.

In-situ denitrification test in NDN-OHO Reactor

A denitrification test was conducted on Day 120 to evaluate NO_x-N reduction and COD utilization with NO_2-N and NO_3-N present as available electron acceptors. The test was conducted in the NDN-OHO reactor at the start of a normal operational cycle during the anoxic period following dilution water addition and deoxygenation. N_2 and CO_2 were sparged as described for routine reaction operation. During the test, 1 mL of 1% v/v acetic acid solution (11.1 mg COD/mL) was added to the reactor every 5 minutes, corresponding to an average COD addition rate of 2.2 mg COD/min. This COD addition rate was slightly less than the COD addition rate of 2.5 mg COD/min during normal reactor operation. Bulk liquid sCOD was not measured during the test because complete influent COD uptake occurred during normal operation, as described above, at a higher COD addition rate than that applied in this test.

Results in Figure B-3 show that NO_2-N accumulated as NO_3-N was reduced and that NO_2-N concentration decreased only after NO_3-N concentration was less than 2 mg/L.

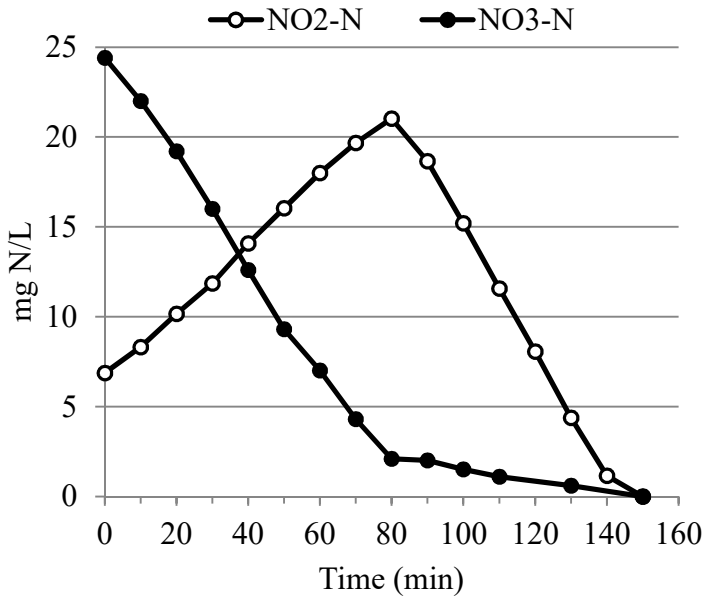

Figure B-3. Results of in-situ denitrification test in NDN-OHO reactor showing nitrite accumulation during denitrification. (Day 120 of operation).

Calculations for changes in NO_x-N species concentrations and COD utilization for NO_x-N removal are provided in the following subsections for the first 80 minutes of the test with NO_2-N accumulation (Phase A) and minutes 80 to 150 with NO_2-N reduction (Phase B). Calculations were performed to evaluate the efficiency of COD utilization for denitrification during the distinct phases of denitrification. Observed COD utilization was calculated for each phase in terms of g COD added per g NO_x-N removed. Theoretical COD requirements for NOx-N removal were also calculated for comparison. Theoretical COD/N requirements for NO_3-N reduction to N_2 and NO_2-N reduction to N_2 were assumed to be 5.0 and 3.0 g COD/g N, respectively (Tchobanoglous et al., 2014). The COD/NO_x-N removal ratio in Phase A was 12.0 g COD/g NO_x-N and 42% greater than the theoretical ratio of 8.4 g COD/g NO_x-N. The theoretical COD/NO_x-N ratio was inherently high because NO_2-N accumulation lowers net NO_x-N removal and thus the denominator in the COD/NO_x-N removal ratio. However, this mathematical artefact is accounted for in the theoretical COD/NO_x-N removal ratio calculation, and the higher observed COD/NO_x-N removal ratio suggests that COD was being utilized for processes other than denitrification and cell synthesis. Conversely, COD/NO_x-N removal in Phase B was 3.2 g COD/g NO_x-N and equal to the estimated theoretical ratio.

Phase A. NO_2-N accumulation period (Minute 0 to 80):

NO_x-N conversions:

NO_3-N removed = 24.4 - 2.1 = 22.3 mg NO_3-N/L removed

NO_2-N accumulated = 21.0 - 6.9 = 14.1 mg NO_2-N/L accumulated

Net NO_x-N removed = 22.3 mg NO_3-N/L - 14.1 mg NO_2-N/L = 8.2 mg NO_x-N/L removed

COD added per g NO_x-N removed:

NO_x-N removed = (1.8L)(22.3 mg NO_3-N/L – 14.1 mgNO_2-N/L) = 14.8 mg NO_x-N

COD added/NO_x-N removed = 177.6 mg COD / 14.8 mg NO_x-N =

= *12.0 mg COD / mg NO_x-N removed*

Estimated theoretical COD required for NO_x-N reduction observed:

The calculation is based on NO_3-N removal with adjustment for NO_2-N accumulation.

= [(5.0 mg COD/mg NO_3-N)(22.3 mg NO_3-N/L) – (3.0 mg COD/g NO_2-N)(14.1 mg NO_2-N/L)]

/ (8.2 mg NO_x-N/L net removal) = *8.4 mg COD / mg NOx-N removed*

Phase B. NO_2-N reduction period (Minute 80 to 140):

NO_x-N conversions:

NO_3-N removed = 2.1 mg NO_3-N/L removed

NO_2-N removed = 21.0 mg NO_2-N/L removed

Net NO_x-N removed = 2.1 mg NO_3-N/L + 21.0 mg NO_2-N/L = 23.1 mg NO_x-N/L removed

COD added per g NO_x-N removed:

NO_x-N removed = (1.8L)(2.1 mg NO_3-N/L + 21.0 mg NO_2-N/L) = 41.6 mg NO_x-N

COD added / NO_x-N removed = 133.2 mg COD / 41.6 mg NO_x-N =

= *3.20 mg COD / mg NO_x-N removed*

Estimated theoretical COD required for NO_x-N reduction observed:

The calculation is based on complete removal of NO_x-N species.

= [(5.0 mg COD/mg NO_3-N)(2.1 mg NO_3-N/L) + (3.0 mg COD/g NO_2-N)(21.0 mg NO_2-N/L)]

/ (23.1 mg NO_x-N/L net removal) = *3.2 mg COD / mg NOx-N removed*

B.6. Extracellular polymeric substance content in NDN-OHO granules

Granular sludge extracellular polymeric substance (EPS) content was extracted using alkaline treatment with heat after Tay et al. (2001). 10 mL samples were adjusted to pH 11 using 1M NaOH and heated at 80°C for 30 min. Samples not receiving alkaline-heat treatment was used as a control. After EPS extraction, samples were centrifuged at 10,000 g at 4°C for 20 minutes and filtered through 0.20 um filters. Filtrate was mixed with two volumes of cold ethanol and stored for a minimum of 18 hours at 4°C to precipitate crude EPS (Ramchandran and Shah, 2009). Crude EPS was weighed after drying at 110°C for 24 hr. Results were expressed as mg of crude EPS per g of MLVSS. EPS measurements were conducted in triplicate. NDN-OHO granule EPS content was 586 ±29 and 202 ±29 mg/g VSS as measured by alkaline-heat treatment and control methods, respectively.

NDN-OHO granule EPS was dominated by polysaccharides as determined by India ink staining (Jenkins et al., 2004). Alginate-like exopolysaccharide extraction and identification tests identification tests conducted based on the protocol of Lin et al. (2010) were negative.

B.7. SI References

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APPENDIX C

Supplemental Information
for Chapter 6:

Bioaugmentation of sidestream nitrifying-denitrifying phosphorus-accumulating granules in a low-SRT activated sludge system at low temperature

Contents
20 pages
1 table
11 figures

S1. SUPPLEMENTAL MATERIAL AND METHODS

S1.1. Sidestream granular sludge reactor

The centrate was measured for its phosphate concentration and supplemented with phosphate if the COD/PO_4-P ratio was greater than 20. Equal masses of $NaH_2PO_4 \cdot H_2O$ and K_2HPO_4 were added to allow for PAO enrichment. $MgSO_4$ and $CaCl_2$ were included in the influent wastewater at 10 mg/L Mg^{2+} and 18 mg/L Ca^{2+}. Iron and trace element solutions were added at 0.4 and 1.0 mL/L. Iron solution consisted of 8.0 g/L $FeSO_4 \cdot 7H_2O$ and 5.0 g/L $Na_2EDTA \cdot 2H_2O$. Trace element solution consisted of consisted of 0.40 g/L $ZnSO_4 \cdot 7H_2O$, 0.24 g/L $CoCl_2 \cdot 6H_2O$, 1.0 g/L $MnCl_2 \cdot 4H_2O$, 0.24 g/L $CuSO_4 \cdot 5H_2O$, 0.24 g/L $Na_2Mo_4 \cdot 2H_2O$, 0.20 g/L $NiCl_2 \cdot 6H_2O$, 0.16 g/L $Na_2SeO_3 \cdot 5H_2O$, and 4.0 g/L $Na_2EDTA \cdot 2H_2O$. A small dose of silicone antifoaming agent estimated at 0.3 mg/L was added to suppress foaming during centrate treatment.

Flocculent activated sludge from full-scale anoxic-aerobic and anaerobic-aerobic municipal wastewater treatment plants was used as seed sludge. Prior to centrate feeding, granules were initially grown on synthetic wastewater simulating centrate. Synthetic wastewater streams were as described in the manuscript and above but using NH_4HCO_3 to simulate centrate nitrogen and alkalinity. Centrate feeding began after 70 days of synthetic wastewater treatment. At that time, small granules were present and synthetic wastewater influent NH_3-N, PO_4-P, and COD concentrations were 125, 36, and 720 mg/L, respectively.

The centrate was obtained for secondary treatment processes at the WRRFs with following process configuration: 1) high-purity oxygen activated sludge at an SRT near 3 days, 2) anaerobic-aerobic activated sludge at an SRT near 5 days and 3) an anoxic-aerobic membrane bioreactor at an SRT near 12 days.

Although slow upflow feeding through the settled sludge bed has been widely used for growth of NDN-PAO granules (Figdore et al., 2017), the sidestream reactor inlet connections and geometry did not accommodate upflow feeding. Therefore, slow feeding through a side port at the 100 mL was attempted. However, short-circuiting of the influent through the sludge bed occurred when this feeding mode was attempted. Therefore, the stirred sludge feeding mode was used to overcome these issues and allow for complete uptake of external COD prior to aeration.

S1.2. Mainstream reactor

Mainstream influent wastewater composition was modified (Table C1) from earlier work using a complex synthetic influent (Xin et al., 2008) with adjustments to arrive at an influent wastewater with total COD, readily-biodegradable COD, NH_3-N, TN, and PO_4-P concentrations of 210, 78, 26.5, 32 and 32 mg/L, respectively. Trace elements were also added as described in prior work (Xin et al., 2008). Influent PO_4-P concentration was higher than that of typical domestic wastewaters in order to promote the growth of PAOs and provide pH buffering capacity.

Toward the end of the slug bioaugmentation phase, approximately 10% of the seeded granule mass was removed on day 14 and sacrificed for various analyses. An equal mass of sidestream granules was added after effluent measurements on day 15 to replace the removed granules.

After the slug bioaugmentation phase, a small dose of granules was added daily during the continuous bioaugmentation phase. Granule addition was in proportion to observed sidestream granule yield and typical sidestream nitrogen loads at full-scale WRRFs with anaerobic digestion. Two adjustment factors were used to accomplish this. One adjustment factor was the ratio of mainstream to sidestream TN load in the laboratory reactors. The mainstream reactor TN load was 0.19 g/d, while the sidestream reactor TN load averaged 1.09 g/d during

bioaugmentation testing. The ratio of mainstream to sidestream load for laboratory systems equals 0.17. The second adjustment factor was 0.25, which is representative of a typical sidestream TN load fraction at full-scale WWRFs with anaerobic digestion. Multiplying these two factors gives a total adjustment factor of 0.044. Consequently, only 4.4% of the daily sidestream reactor waste MLSS was added to the mainstream reactor. Specifically, an average of 75 mL of MLSS was removed daily from the sidestream reactor for SRT control, but only 3.3 mL was added to the mainstream reactor. Thus, the daily bioaugmented sidestream biomass was proportionate to this typical sidestream and mainstream nitrogen loads at full-scale WRRFs and the waste solids produced in sidestream treatment.

S1.3. Ammonia oxidation kinetics

Maximum specific NH_3-N oxidation activity

The maximum specific ammonium oxidation activities of flocculent or granular sludge fractions were measured for solids passing or retained on a 425 um sieve, respectively. Tests were conducted at room temperature (20-22°C) in reactors mixed by aeration with MLSS concentrations below 1800 mg/L. NH_4Cl and $NaHCO_3$ stock solutions were added to the test reactor to provide an initial NH_3-N concentration of 30 mg/L and alkalinity of 300 mg/L as $CaCO_3$. DO concentration was greater than 7 mg/L and pH was approximately 7.8 during activity tests. Measured activities were normalized to 20°C using a temperature-activity (θ) coefficient of 1.072 (Tchobanoglous et al., 2014).

Apparent NH_3-N half saturation concentration

Data to determine the apparent half saturation concentration for sidestream nitrifying granular sludge were obtained under conditions similar to NH_3-N oxidation activity testing, but starting the activity test at a lower initial NH_3-N concentration of 15 mg/L and extending the test

until NH_3-N oxidation was complete. Data were fitted to a fourth-degree polynomial regression with $R^2 > 0.9999$. The apparent half saturation concentration was calculated based on the NH_3-N concentration where the first derivative of the regression was half of that at the start of the test under non-limiting NH_3-N concentration.

S1.4. Nitrite oxidation activity

Granular sludge maximum specific NO_2-N oxidation activity was measured in a similar manner as NH_3-N oxidation activity but with $NaNO_2$ and $NaHCO_3$ added to the test reactor to provide an initial NO_2-N concentration of approximately 20 mg/L and 1 g alkalinity as $CaCO_3$/g NO_2-N. During NO_2-N oxidation activity tests, pH was between 7.1 and 7.3. Sidestream granules were obtained at the end of an aerobic period and subjected to an additional 60 minutes of aeration to ensure that internal carbon storage compounds were depleted prior to activity testing.

S1.5. High-throughput sequencing of 16S rRNA gene amplicons

PCR was performed prior to sequencing using HotStarTaq Plus Master Mix Kit (Qiagen, Inc.) under the following conditions: 3 min at 94°C, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 60 s, followed by the final elongation step at 72°C for 5 min. Amplicon products were mixed in equimolar concentrations and purified using AMPure XP beads (Agencourt Bioscience Corp.). Sequencing was performed on a MiSeq system (Illumina, Inc.) according to the manufacturer's protocol. Raw paired-end Illumina data were joined and reverse complimented to read in the 5'-3' orientation.

S1.6. qPCR protocols and PCR conditions

For AOB quantification with *amoA*-1F/*amoA*-2R primers (Rotthauwe et al., 1997) targeting the ammonia monooxygenase (*amoA*) gene, the PCR conditions were: a) 2 min denaturation at 95°C; b) 40 cycles of 30 sec denaturation at 95°C, 40 sec annealing at 57°C, and

40 sec extension at 72°C; c) 5 min extension at 72°C; d) holding temperature of 12°C. For *Nitrotoga* quantification with NTG200F/NTG840R primers (Alawi et al., 2007) targeting the Nitrotoga 16S ribosomal RNA gene, the PCR conditions were a) 2 min denaturation at 95°C; b) 40 cycles of 30 sec denaturation at 95°C, 50 sec annealing at 58°C, and 60 sec extension at 72°C; c) 5 min extension at 72°C; d) holding temperature of 12°C. Double-stranded DNA fragments (Integrated DNA Technologies, Inc.) consisting of the 491 and 640 bp region bound by the primers for AOB and *Nitrotoga,* respectively, in addition to the adjacent 20 bp were used as standards. qPCR was performed in an Eppendorf Mastercycler® realplex using EvaGreen® intercalating dye and associated master mix (Biotium, Inc.).

S2. SUPPLEMENTAL RESULTS

S2.1. Mainstream reactor EBPR performance

Data and calculations related to EBPR performance in the mainstream reactor are documented in this section. Anaerobic PO_4-P release is used as a metric to evaluate PAO activity and enrichment.

Mainstream influent PO_4-P concentration was 32.2 mg/L, and average PO_4-P concentrations and standard deviations at the end of the anoxic/anaerobic period and in the effluent were 43.1 ±2.6 mg/L and 27.5 ±1.1 mg/L, respectively, during bioaugmentation. PO_4-P measurements are shown in Figure S6. PO_4-P removal averaged 4.7 mg/L, which is greater than estimated P removal of 1.5 mg/L for assimilatory P removal in absence of EBPR based on the influent COD concentration of 210 mg/L and assuming a yield of 0.35 g VSS/g COD and 0.02 g P/g VSS. Therefore, EBPR was occurring in the mainstream reactor.

PO_4-P release was calculated from the average values above. Based on 1L feed volume per cycle, PO_4-P mass in the bulk liquid at the start of a new cycle and influent wastewater was 27.5 and 32.2 mg, respectively, for a total of 59.7 mg PO_4-P. PO_4-P mass in the 2L reactor at the end of the anoxic/anaerobic period was 86.2 mg, corresponding to 26.5 mg or 0.85 mmol of PO_4-P released.

In order to evaluate PAO activity based on the molar ratio of P released to C fed, the acetate equivalent of the influent complex wastewater was determined on a COD basis. Based on 1.085 g COD per g CH_3COO^-, the acetate carbon equivalent of the influent COD equals 6.6 mmol C. Therefore, the apparent PO_4-P release was 0.13 mol P per mol acetate C equivalent.

However, the effluent NO_x-N concentration was about 14 mg/L during bioaugmentation, and the presence of residual NO_x-N at the start of the subsequent feed period disturbed true anaerobic conditions preferred for EBPR. The residual NO_x-N exerts a COD demand for denitrification and therefore consumes COD from potential utilization by PAOs. Based on an effluent NO_3-N concentration of 14 mg/L as observed near the end of bioaugmentation and COD/N ratio of 5.0 g/g for denitrification, 70 mg of COD would be consumed for denitrification. Therefore, the effective influent COD mass for potential PAO utilization was approximately 140 mg/L or 4.4 mmol of acetate C equivalents. The corresponding observed PO_4-P release was 0.19 mol P per mol acetate C equivalent when accounting for this adjustment in COD.

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SUPPLEMENTAL TABLES

Table C1. Mainstream treatment reactor influent wastewater composition.

Constituent	Units	Value
Sodium acetate	mg/L	50.0
Glucose	mg/L	19.8
Lactose	mg/L	9.0
Maltose	mg/L	0.4
Sodium succinate	mg/L	1.0
Trisodium citrate dihydrate	mg/L	3.1
Lactic acid	mg/L	2.3
Ethanol	mg/L	0.8
Butanol	mg/L	0.4
Dextrin	mg/L	55.9
Yeast extract	mg/L	39.6
Peptone	mg/L	15.7
Starch	mg/L	11.2
Casein	mg/L	5.4
Gelatin	mg/L	7.7
Methionine	mg/L	0.4
Magnesium chloride	mg/L	39.0
Calcium chloride	mg/L	50.0
Sodium thiosulfate	mg/L	10.0
Ammonium chloride	mg/L	101.2
Sodium bicarbonate	mg/L	672
Sodium phosphate monobasic monohydrate	mg/L	80.0
Potassium phosphate dibasic	mg/L	80.0
Trace element solution	mL/L	2.0

SUPPLEMENTAL FIGURES

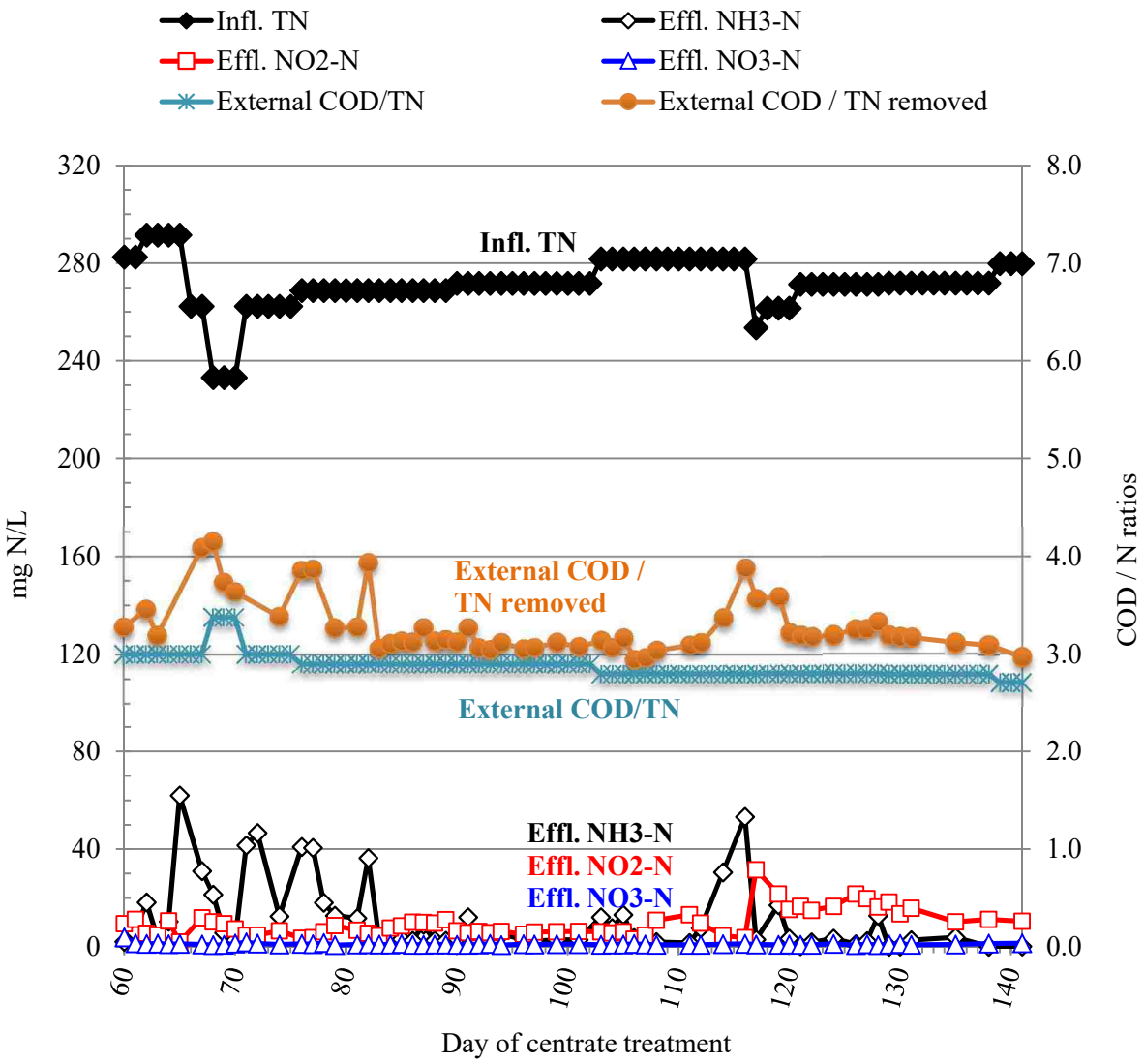

Figure C1. Sidestream nitrifying-denitrifying granular sludge reactor influent total nitrogen concentration, effluent inorganic nitrogen concentrations, and external COD/TN ratios for centrate treatment days 60 to 140. Bioaugmentation occurred during days 86 to 126.

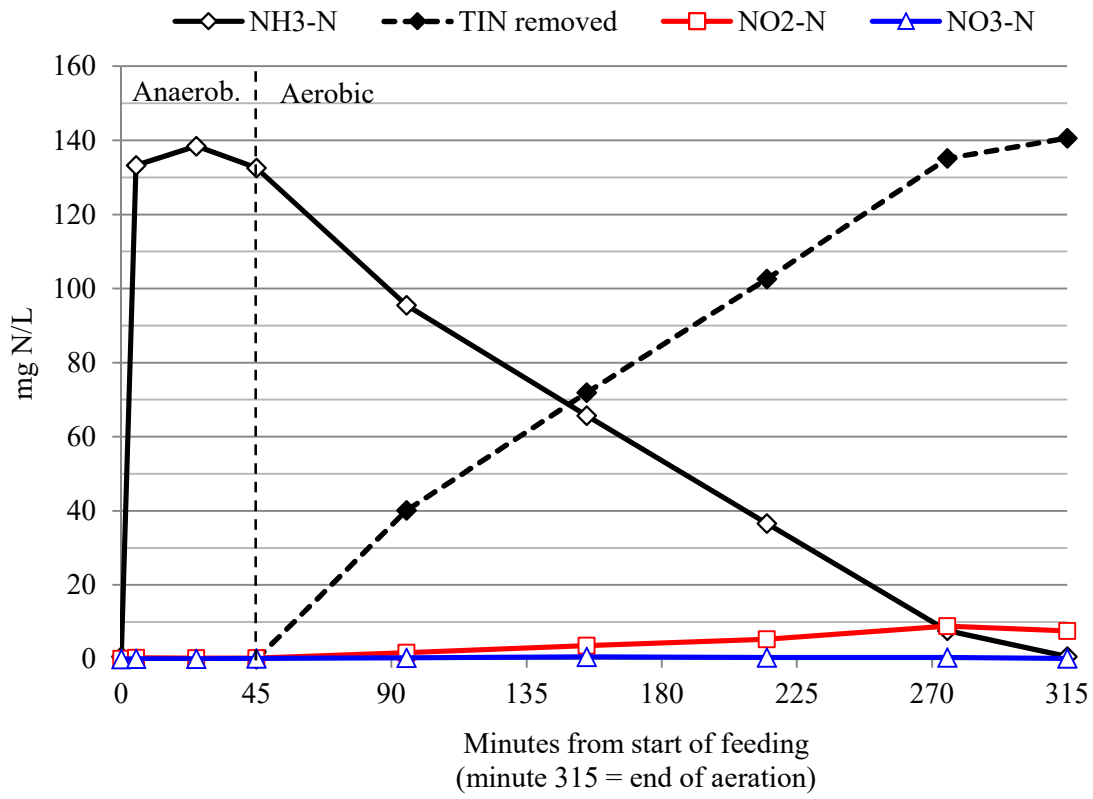

Figure C2. Profile of total inorganic nitrogen (TIN) species in the anaerobic and aerobic periods and TIN removal in the aerobic period of a representative sidestream NDN-PAO granular sludge reactor cycle on centrate treatment day 84 (bioaugmentation day 1) at 18°C. Error bars for triplicate measurements are shown but hidden by markers for average values. Dissolved oxygen concentrations were between 1.8 and 2.2 mg/L during the cycle. MLSS and MLVSS concentrations were 10,850 and 7,760 mg/L, respectively.

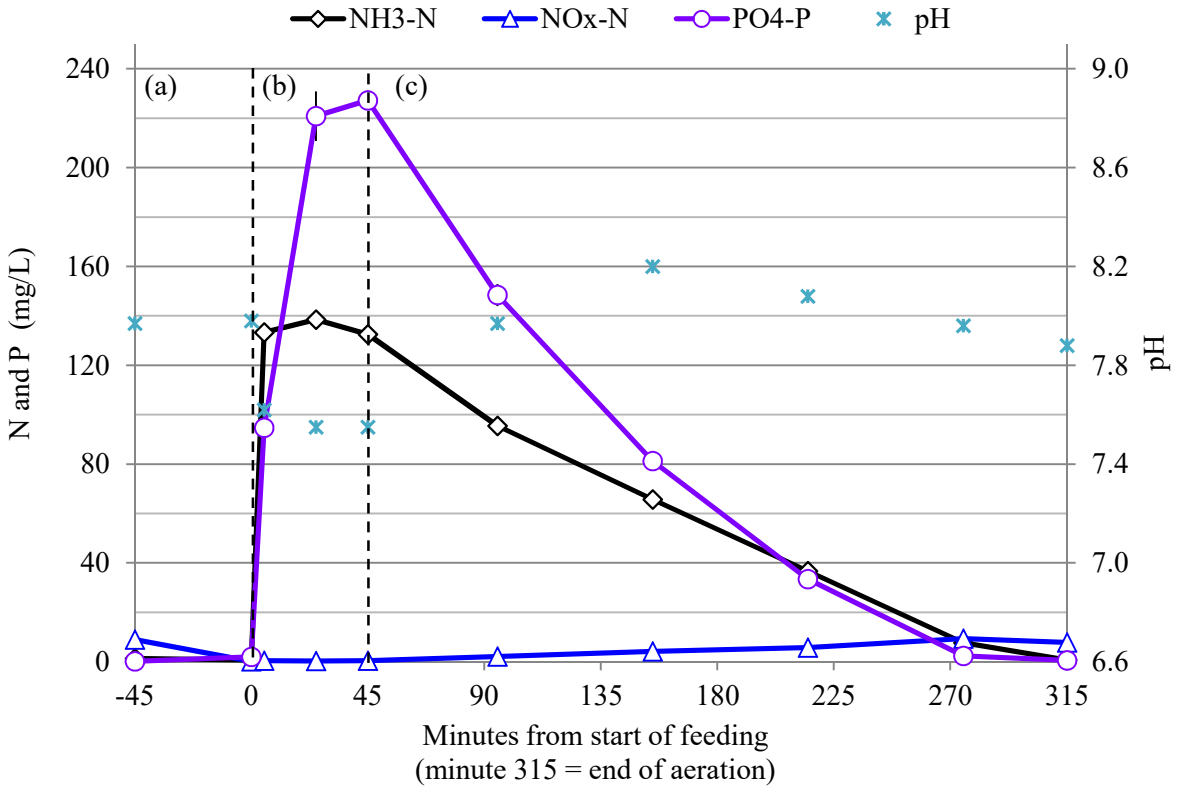

Figure C3. Cycle profile of NH_3-N, NO_x-N, PO_4-P and pH in the sidestream nitrifying-denitrifying granular sludge reactor on centrate treatment day 84 (bioaugmentation day 1) at 18°C. Error bars for triplicate measurements are shown but may be hidden by markers for average values. Idle and anoxic mixing (a), anaerobic (b), and aerobic phases (c) of the cycle are indicated. Dissolved oxygen concentrations were between 1.8 and 2.2 mg/L during the cycle. MLSS and MLVSS concentrations were 10,850 and 7,760 mg/L, respectively.

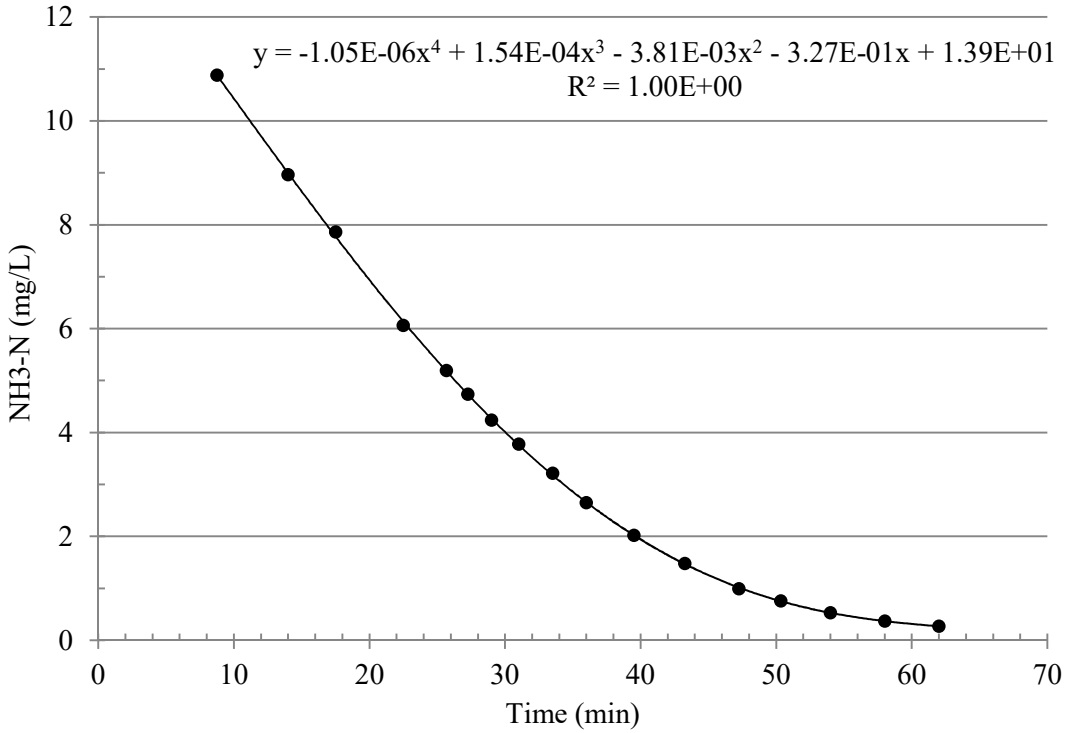

Figure C4. Experimental data for determination of apparent NH_3-N half-saturation coefficient for sidestream nitrifying-denitrifying granules. Error bars for triplicate measurements are shown but hidden by markers for average values. The value for the derivative of the regression equation at minute 38 is half that at minute 10 and corresponds to an NH_3-N concentration of 2.3 mg/L.

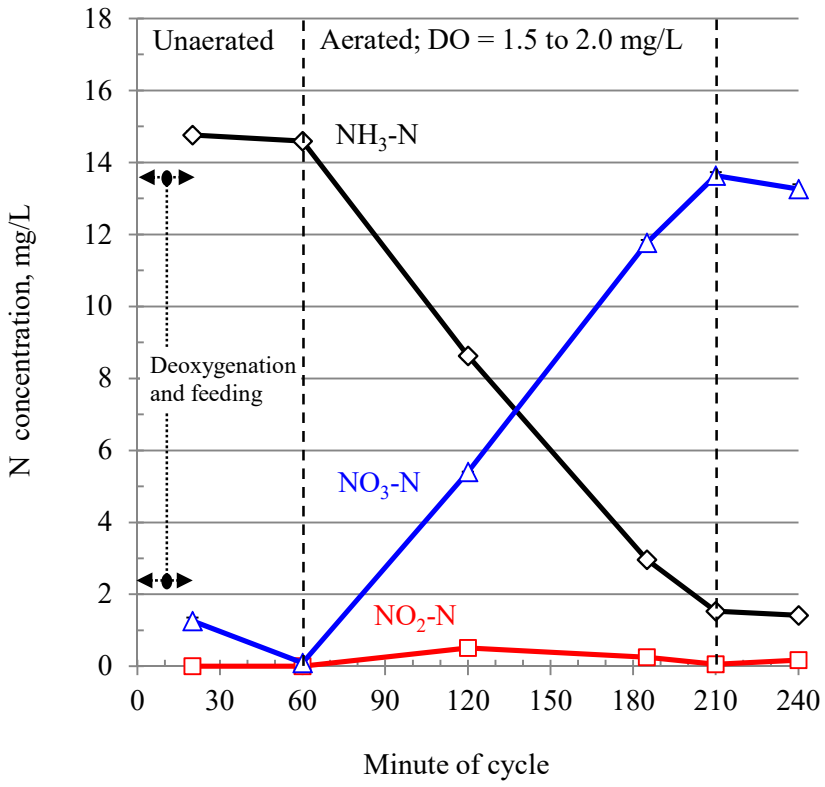

Figure C5. Changes in inorganic nitrogen species during the mainstream SBR cycle at 12°C on day 40 of bioaugmentation. Error bars for triplicate measurements are shown but hidden by markers for average values.

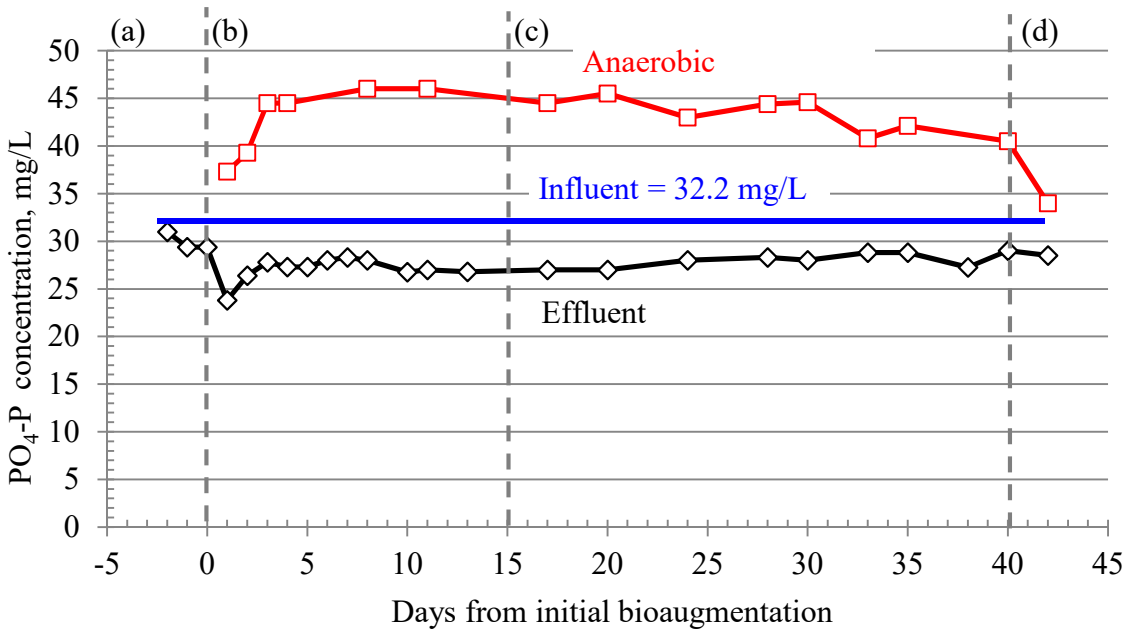

Figure C6. PO_4-P concentrations in the mainstream reactor influent, effluent, and at the end of the anaerobic period during during pre-bioaugmentation (a), slug granule bioaugmentation (b), continuous granule bioaugmentation (c) and post-bioaugmentation after granule removal (d).

Figure C7. Mainstream reactor mixed liquor suspended solids on the third day of the bioaugmentation period (6x magnification and 1mm scale bar).

Figure C8. Mainstream reactor mixed granular (left) and flocculent (right) solids fractions after sieving protocol.

Figure C9. Stratification of mainstream settled sludge bed during bioaugmentation period.

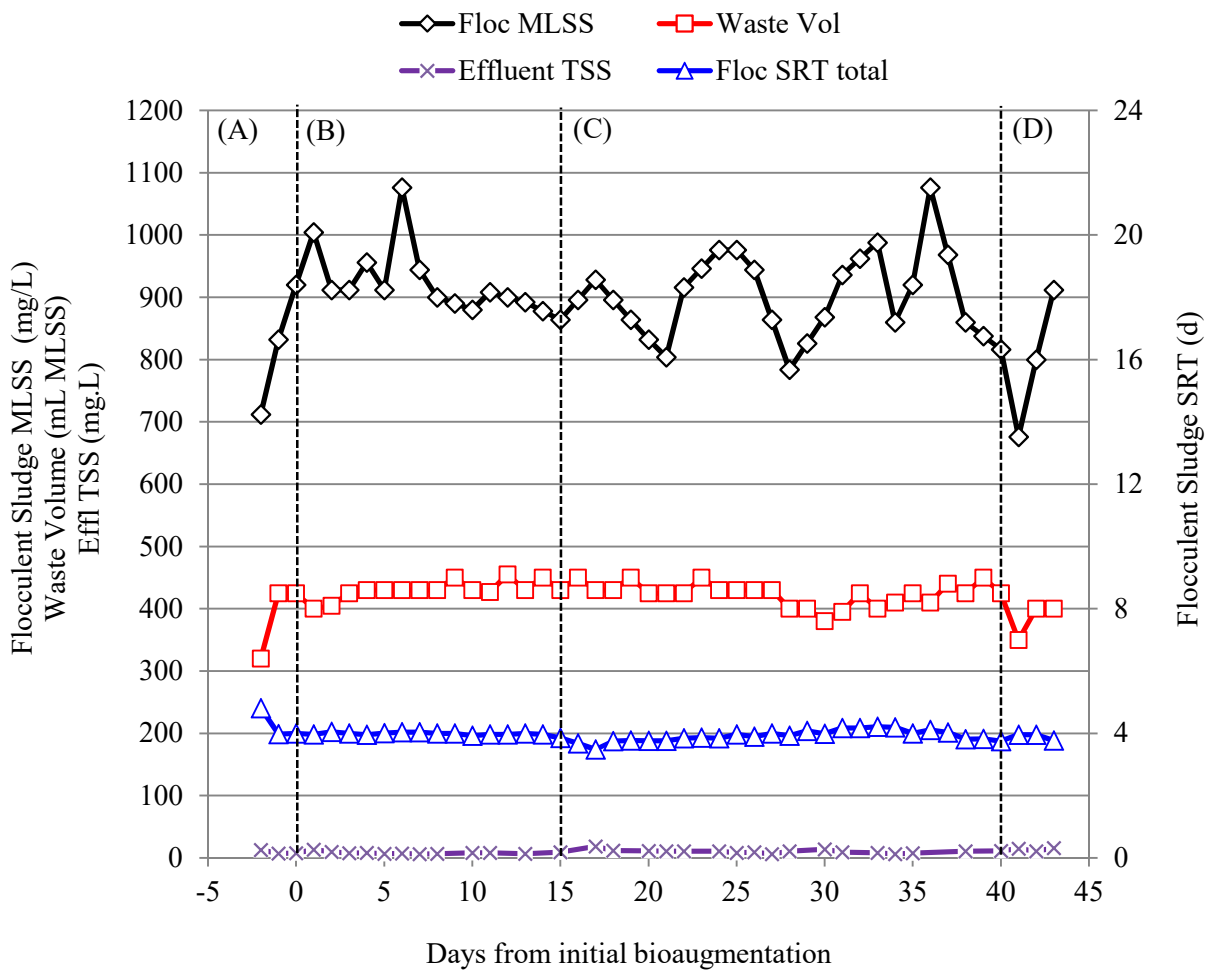

Figure C10. Mainstream reactor flocculent mixed liquor suspended solids concentration, waste mixed liquor volume, flocculent sludge solids retention time, and effluent total suspended solids during bioaugmentation testing.

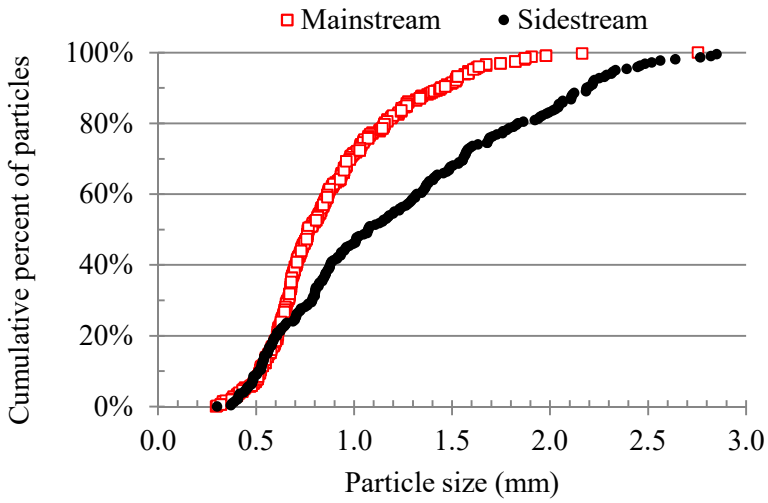

Figure C11. Cumulative particle size distribution for sidestream granules and mainstream granules at the end of bioaugmentation. Particles greater than 200 um with granular morphology were included in the analysis. Minimum sample size was 250 particles.

Bryce A. Figdore

Curriculum Vitae

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CURRICULUM VITAE IN SHORT (FOR MORE DETAILS, PLEASE SEE FULL CV)

Bryce Figdore's academic and professional interests have been motivated by a passion for responsible stewardship of water resources. With time and experience, this passion has led to a focus and expertise in wastewater treatment. Bryce graduated with distinction from The Pennsylvania State University in 2004 with a Bachelor's degree in Agricultural and Biological Engineering. As a young engineer at Mock, Roos and Associates in West Palm Beach, Florida, Bryce worked on a variety of water and wastewater infrastructure projects for municipal utilities. Motivated by exposure to the core process technologies "inside the plant fenceline" in these projects, Bryce sought to work as a process engineer. After accepting a new position at AECOM in Philadelphia, Pennsylvania, Bryce developed into a key junior wastewater process engineer supporting senior technical experts. He worked on variety of high-profile nutrient removal projects and studies in the Mid-Atlantic region. Concurrently, Bryce attained professional licensure and completed a Master's degree in 2011 from Villanova University with a thesis on anaerobic digestion modeling including competing aceticlastic methanogen populations. Drawing on opportunities and inspiration from working with wastewater treatment process experts, Bryce sought to make a contribution to the industry through research at the University of Washington (UW) Department of Civil and Environmental Engineering by joining the group of Dr. David Stensel in 2012. Bryce's research has been supported by Fellowships from the UW and King County Department of Natural Resources and Parks / Wastewater Treatment Division and centered around development of a new nitrification bioaugmentation process using sidestream aerobic granular sludge from anaerobic digester dewatering centrate treatment. The process has demonstrated ability to provide nitrification and enable nitrogen removal in non-nitrifying flocculent activated sludge at low temperature and solids retention time. The bioaugmentation process is currently being scaled-up for further study and demonstration in pilot reactor systems at King County and Los Angeles Sanitation District. Bryce expects to receive his Ph.D. degree in Civil and Environmental Engineering from the UW in the fall of 2017.

Bryce A. Figdore

Curriculum Vitae

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Educational History

Ph.D. Candidate in Civil and Environmental Engineering

University of Washington (2012 – Current), Seattle, WA

Advisor: H. David Stensel

Thesis: Nitrification bioaugmentation in mainstream flocculent activated sludge systems using sidestream aerobic granular sludge.

M.S. in Water Resources and Environmental Engineering

Villanova University (2011), Villanova, PA

Advisor: Metin Duran

Thesis: Simulation of mesophilic anaerobic digestion of municipal wastewater solids with competitive aceticlastic methanogen model structure.

B.S. in Agricultural and Biological Engineering

The Pennsylvania State University (2004), University Park, PA

With distinction and Environmental Engineering minor

Professional History

Graduate Research Assistant

2012 - Current *University of Washington, Seattle, WA*

Focus: Biological wastewater treatment with aerobic granular activated sludge

- Studied and developed a new wastewater treatment process using aerobic granules grown in a sidestream anaerobic digester dewatering centrate treatment reactor to bioaugment nitrification and enable nitrogen removal in non-nitrifying flocculent activated sludge.
- Demonstrated viability of granular sludge bioaugmentation process at low temperature and solids retention time in flocculent activated sludge.
- Designed and operated multiple laboratory-scale aerobic granular sludge reactors.

- Applied molecular biology methods to assess microbial communities in sidestream and mainstream bacterial communities by quantitative PCR, high-throughput DNA sequencing, and bioinformatics.
- Designed pilot-scale reactor systems for future research and demonstration of the nitrifying granular sludge bioaugmentation process.

Wastewater Process Engineer

2007 - 2012 AECOM, Philadelphia, PA

- Process engineer for a variety of wastewater treatment projects and studies.
- Developed expertise in process modeling for plant design and re-rating.
- Engineering design for the Nitrogen Removal Program at the 370-mgd DC Water Blue Plains facility:
    - Biological and physical-chemical process alternatives analysis for the treatment of sidestream generated from mesophilic anaerobic digestion process with thermal hydrolysis solids pretreatment. Biological processes evaluated included conventional nitrification-denitrification, nitritation-denitritation, deammonification, mainstream bioaugmentation, and aerobic-anoxic post-digestion of the anaerobically digested sludge. Developed concept design for deammonification-based treatment facility.
    - Conceptual design and planning for an enhanced clarification facility to reduce combined sewer overflows and provide treatment in wet weather events.
- Process engineer for two high-profile financial impact studies:
    - Study commissioned by the state of Pennsylvania to estimate the cost to municipal wastewater dischargers to comply with nutrient removal requirements of the Chesapeake Bay Tributary Strategy.
    - Nutrients reduction cost estimation study for the New Jersey Harbor Dischargers Group, a consortium of ten authorities in northern New Jersey that collectively discharge approximately 750 mgd of treated wastewater to the New York-New Jersey Harbor.
- Other key projects in the Mid-Atlantic region:
    - Operations support services, alternatives analysis, and facility master plan for the 5 mgd City of Millville, NJ wastewater treatment plant.
    - Process design and modeling for 0.5-mgd membrane bioreactor plant for Skillman Village in Princeton, NJ.

- Evaluation of nutrient removal upgrades at the 16-mgd wastewater treatment plant for Parsippany-Troy Hills, NJ.
- Denitrification filter and re-use system design for City of Cape May, NJ.
- Capacity assessment and re-rating of 3-mgd Logan Township, NJ plant.
- Grit removal and aeration system improvements for 12-mgd Stony Brook, NJ Sewerage Authority.

Staff Engineer / Engineer-in-Training

2004 - 2007 Mock, Roos and Assoc., West Palm Beach, FL

- Staff engineer for projects associated with a future 4.0-mgd reverse osmosis water treatment plant for the City of Lake Worth, Florida.
  - Raw water wellhead design and transmission pipeline hydraulic modeling
  - Physical and chemical pretreatment systems design
  - Reverse osmosis pilot plant monitoring
  - Reverse osmosis concentrate pump station design
  - Concentrate disposal permitting
- Construction phase engineering services for a 19-mgd wastewater pump station for the City of Lake Worth, Florida.
- Civil site design for a variety of land development projects.

Peer-Refereed Publications

Figdore, B.A., Stensel, H.D., Winkler, M.K.H., Neethling, J.B. (2017) *Aerobic Granular Sludge for Biological Nutrient Removal*. Project No. NUTR5R14h. Water Environment and Reuse Foundation: Alexandria, VA.

Figdore, B.A., Stensel, H.D., Winkler, M.K.H. Comparison of different aerobic granular sludge types for activated sludge nitrification bioaugmentation potential. *Accepted and pending publication in Bioresource Technology* as of October 2017.

Figdore, B.A., Stensel, H.D., Winkler, M.K.H. Bioaugmentation with sidestream nitrifying granules in low-SRT flocculent activated sludge at low temperature. *Accepted and pending publication in Water Environment Research* as of October 2017.

Figdore, B.A., Stensel, H.D., Winkler, M.K.H. Bioaugmentation of sidestream nitrifying-denitrifying phosphorus-accumulating granules in a low-SRT activated sludge system at low temperature. *Submitted to Water Research* in September 2017.

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Conference Proceedings

Figdore, B.A., Stensel, H.D., Winkler, M.K.H. (2017) *Achieving nitrification-denitrification in a low-SRT activated sludge system through bioaugmentation of sidestream nitrifying granules*. Proceedings of the Water Environment Federation / Internationl Water Association Nutrients Conference. Chicago, Illinios, October, 2017. (Podium presentation).

Figdore, B.A., Stensel, H.D., Winkler, M.K.H., Neethling, J.B. (2016) *Aerobic Granular Sludge for Biological Nutrient Removal*. Water Environment Federation / International Water Association Nutrients Conference. Denver, Colorado, July, 2016. (Poster presentation).

Figdore, B.A., Stensel, H.D., Winkler, M.K.H. (2015) *Sidestream Growth of Nitrifying and Nitrifying-Denitrifying Granular Sludge for Use in Mainstream Nitrification Bioaugmentation*. Proceedings of the Water Environment Federation. Chicago, Illinois. September 2015. (Podium presentation).

Figdore, B.A. (2014) *Aerobic Granular Sludge for Wastewater Treatment*. Pacific Northwest Clean Water Association annual conference. Boise, Idaho, October, 2014. (Podium presentation).

Figdore, B.A. (2014) *Aerobic Granular Sludge for Wastewater Treatment*. Water Environment Student Talks. University of British Columbia, June, 2014. (Podium presentation).

Figdore, B.A. (2012) *Deammonification – Today and Tomorrow*. Pennsylvania Water Environment Association annual conference. State College, Pennsylvania, June, 2012. (Podium presentation).

Figdore, B., Bowden, G., Stinson, B., Wett, B., Hell, M., Bailey, W., Carr, J., Derminassian, R., Murthy, S. (2011) *Treatment of Dewatering Sidestream from a Thermal Hydrolysis-Mesophilic Anaerobic Digestion Process with a Single-sludge Deammonification Process.* Proceedings of the Water Environment Federation. Los Angeles, California, October, 2011. (Podium presentation).

Figdore, B.A., Wett, B., Hell, M., Murthy, S. (2011) *Deammonification of Dewatering Sidestream from Thermal Hydrolysis-Mesophilic Anaerobic Digestion Process.* Proceedings of the Water Environment Federation Nutrient Recovery and Management Specialty Conference. Miami, Florida, January, 2011. (Podium presentation).

Figdore, B.A., Bowden, G., Bodniewicz, B., Bailey, W., Derminassian, R., Kharkar, S., Murthy, S. (2010) *Impact of Thermal Hydrolysis Solids Pretreatment on Sidestream Treatment Process Selection at the DC Water Blue Plains AWTP.* Proceedings of the Water Environment Federation. New Orleans, Louisiana, October, 2010. (Podium presentation).

Figdore, B.A. (2009) *Legislative Budget and Finance Committee Survey and Cost Study – The Impact of the Chesapeake Bay Tributary Strategy on Significant Municipal Dischargers*. Pennsylvania Water Environment Association annual conference. Lancaster, Pennsylvania, June, 2009. (Podium presentation).

Figdore, B.A. (2009) *Nitrogen and Carbon Removal Cost Study for the New Jersey Harbor Dischargers Group*. New Jersey Water Environment Association annual conference. Atlantic City, New Jersey, May, 2009. (Podium presentation).

Professional and Academic Societies

Water Environment Federation (WEF), Member

American Waterworks Association (AWWA), Member

University of Washington AWWA-WEF Student Chapter, President, 2013-2017

Professional Certifications

Professional Engineer (PE), Pennsylvania No. PE077497