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Biological Nutrient Removal (BNR) Process Optimization and Recovery of Embedded Energy Using Biodiesel By-product

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BIOLOGICAL NUTRIENT REMOVAL (BNR) PROCESS OPTIMIZATION AND RECOVERY OF EMBEDDED ENERGY USING BIODIESEL BY-PRODUCT

by

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ABSTRACT

Enhanced biological phosphorus removal (EBPR) as well as biological nitrogen removal require a carbon source to be carried out. Volatile fatty acid (VFAs) (mainly acetic and propionic acids) are the major driving force for EBPR. Many domestic wastewaters have an insufficient amount of VFAs. However, carbon sources such as acetic and propionic acids can be produced using primary solids fermentation process. Due to the cost of VFA production, an external carbon source can be added to the biological nutrient removal (BNR) system that can be fermented to provide the desired VFAs. Glycerol (biodiesel by-product) offers a solution to reduce carbon addition cost if can be fermented to acetic and propionic acid or can be used directly as an external carbon substrate for EBPR and denitrification. Using glycerol in wastewater treatment can also offset the biodiesel plant disposal cost and reduce the BNR chemical cost. The main objective of this study was to optimize the prefermentation process and optimize the BNR system using glycerol as an external carbon source. In this work, Optimization of the prefermentation process using glycerol, mixing, and hydrogen gas addition was evaluated. EBPR performance within an A_2O-BNR system was evaluated using either a direct glycerol method to the anaerobic zone or by co-fermentation with primary solids. Also, optimization of the nitrogen removal (specifically denitrification) efficiency of a 5-stage Bardenpho^{TM} BNR system using either a direct glycerol method to the second anoxic zone or by co-fermentation with primary solids was evaluated. It was found in this study that glycerol was an efficient external carbon substrate for EBPR as well as biological nitrogen removal.

The prefermentation experiment showed that glycerol co-fermentation with primary solids produced significantly higher ($p<0.05$) VFAs than primary solids fermentation alone, even more than the possible value from the added glycerol (427 mg-COD/L). The increased VFAs imply that the glycerol addition stimulated additional fermentation of primary solids. Lowering the prefermenter mixing energy (50 to 7 rpm) resulted in a significant increase in VFAs production (80%). Also, purging the headspace of the prefermenter with hydrogen gas did not lead to more VFAs, but significantly ($p<0.05$) increased the propionic acid to acetic acid ratio by 41%. In the A_2O-BNR pilot plant experiment, it was found that glycerol is a suitable renewable external substrate to drive enhanced EBPR as well as denitrification. The results from both locations of glycerol addition (direct vs. fermented) were beneficial to the BNR system. Both systems had similar effluent quality and achieved total nitrogen (TN) and total phosphorus (TP) removals up to 86% and 92% respectively. The 5-stage Bardenpho^{TM} BNR experiment investigated the location of glycerol addition (direct vs. fermented) on the performance of denitrification in the second anoxic zone and the overall performance. The results from both systems were that glycerol was beneficial to the BNR system and had virtually similar effluent quality. Both systems achieve complete denitrification and excellent removal of TN and TP up to 95% and 89% respectively. Also, the pilot that received fermented glycerol had significantly higher VFAs loading and lower observed yield. The side-stream prefermenter effluent flowing to the second anoxic reactor did not cause high effluent ammonia (NH_3) concentration.

In summary, the location at which glycerol was added did not affect effluent quality

for nitrogen and phosphorus. However, glycerol addition and mixing energy did impact prefermenter performance and effluent quality.

Dedicated to my parents (Khalid and Etimad), and my wife (Hanan).

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I would also like to express my appreciation to all who worked in this project. I would also like to show my appreciation to the staff at the Iron Bridge Regional Water Reclamation Facility (Orlando, FL), and the staff of Glendale Wastewater Treatment Plant (Lakeland, FL).

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LIST OF ABBREVIATION AND ACRONYMS

AE	Aerobic reactor
AE II	Second aerobic reactor
AN	Anaerobic reactor
AX	Anoxic reactor
AX II	Second anoxic reactor
BNR	Biological nutrient removal
BPR	Biological Phosphorus Removal
CG	Crude glycerol
Cla	Secondary clarifier
DO	Dissolved oxygen
EBPR	Enhanced biological phosphorus removal
FID	Flame ionization detector
FS	Factor of Safety
GAOs	Glycogen accumulating organism
HAB	Harmful algal blooms

HRT	Hydraulic retention time
IX	Ion Exchange
JHB	Johannesburg WWTP configuration
MCL	Maximum contaminant level
MCRT	Mean cell resident time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
NARCY	Nitrate recycle
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NOx	Nitrate+Nitrite
P	Phosphorus
PAHs	Polycyclic Aromatic Hydrocarbons

PAOs	Polyphosphate accumulating organisms
PC	Pollution Control
PF	Prefermenter reactor
PO_4^{3-}	Orthophosphate
PP	Pilot plant
RAS	Return activated sludge
rb-COD	Readily biodegradable chemical oxygen demand
SBR	Sequencing batch reactor
s-COD	Soluble chemical oxygen demand
SDR	Specific denitrification rate
SOP	Ortho-Phosphorus
SRP	Soluble Reactive Phosphorus
SRT	Solid retention time
TCOD	Total chemical oxygen demand
TDP	Total Dissolved Phosphorus
TDS	Total Dissolved Solids

TIN	Total inorganic naitrogen
TKN	Total Kjeldahl Nitrogen
TMDL	Total maximum daily load
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
U.S. EPA	United States Environmental Protection Agency
UASB	Up-flow anaerobic sludge blanket
UCF	University of Central Florida
V/H	Vertical/Horizontal
VDR	Volumetric denitrification rate
VFA	Volatile fatty acid
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

CHAPTER ONE: INTRODUCTION

Wastewater from residential and industrial areas contains a high nutrient concentration, and could cause significant environmental problems (e.g. eutrophication, algal bloom) if discharged to receiving water without proper treatment (Walsh, 2012; Wanielista et al., 2008; Xuan, Chang, Daranpob, & Wanielista, 2009). Wastewater nutrient removal can be achieved chemically through precipitation or biologically through biological nutrient removal (BNR). Biological removal usually consists of multiple zones in series (anaerobic, anoxic, and aerobic). Many well established BNR systems already exist such as A/O, A^2O, University of Cape Town (UCT), and 5-stage Bardenpho™ (Metcalf&Eddy, 2014). Typically, BNR process require a sufficient carbon source to provide high denitrification and enhanced biological phosphorus removal (EBPR) efficiencies, which cause concern since many domestic wastewaters lack sufficient carbon sources (Bernat, Kulikowska, & Godlewski, 2016; Wu, Peng, Li, & Wang, 2010). Many studies were dedicated to find the efficiency of different carbon sources on BNR systems. Different organic carbon sources such as acetic acid, propionic acid, and methanol have been studied for their potential effectiveness as a carbon substrate for nitrate removal (Aspegren, Nyberg, Andersson, Gotthardsson, & la Cour Jansen, 1998; Moser-Engeler, Udert, Wild, & Siegrist, 1998; Rahmani, Rols, Capdeville, Cornier, & Deguin, 1995). Lee and Welander (1996) studied the effectiveness of many carbon sources on denitrification in a long-term batch test. The results showed the acetate provided the highest specific denitrification rate (SDR) and lower sludge yield followed by methanol. Chen, Wang, Li,

Yang, and Zeng (2015) also tested acetate, ethanol, glucose, methanol, and propionate as a carbon substrate for BNR. VFAs (acetate and propionate) were the best suitable carbon source that provided the highest nitrogen and phosphorus removal. Glucose caused a deterioration in phosphorus removal from 99% with VFAs to 54% (Chen et al., 2015). VFAs such as acetic and propionic acids are the most favorable carbon source for EBPR (Shen & Zhou, 2016). Propionic acid was found to be more effective than acetic acid and resulted in a more stable phosphorus removal. Although, acetate is less effective than propionate, but can occasionally favor glycogen accumulating organism (GAOs) over polyphosphate accumulating organism (PAOs) over time, causing EBPR failure. GAOs compete with the PAOs for the VFAs but do not contribute to the phosphorus removal (Chen, Randall, & McCue, 2004; Shen & Zhou, 2016; Wu et al., 2010). Lopez-Vazquez et al. (2009) showed that optimal EBPR was obtained with a 50:50 or 75:25 mixture of acetic:propionic acid. However; propionic acid supplementation for full-scale BNR is to some extent cost prohibitive. The more economical and sustainable way to produce VFAs is fermentation using either wastewater or inexpensive waste-product carbon sources.

Fermentation is carried out in three phases: hydrolysis, acidogenesis, and acetogenesis, respectively. The first phase is the reduction of polymers to simple monomers (e.g. fatty acids) followed by the second phase which is conversion of fatty acids into VFAs other than acetic acid (e.g. propionic and butyric acids). The last phase is the conversion of propionic acid and the other intermediates into acetic acid, carbon dioxide, and hydrogen (H_2) (Henze, 2008; Jia, Furumai, & Fang, 1996; Metcalf&Eddy, 2014; Valcke &

Verstraete, 1983). Prefermentation of primary solids mainly results in the production of propionic and acetic acids (Henze, 2008; Metcalf&Eddy, 2014). Merzouki, Bernet, Delgenès, and Benlemlih (2005) were not able to establish biological nutrient removal before adding a prefermentation reactor to his sequencing batch reactor (SBR). The prefermenter significantly improved the performance of the system and resulted in 99% nitrogen and phosphorus removal. McCue et al. (2004) studied prefermentation's effect on a UCT process with regard to denitrification and EBPR. The results showed a significant increase in the denitrification rate after prefermentation use, but no significant effect was recorded for EBPR. Propionic acid was found to be a better suited carbon source for BNR systems than acetic acid when pH >7. The reason is that propionic acid requires less energy and less C/Prelease ratio (Shen & Zhou, 2016). Glycerol can also be fermented to provide VFAs.

With the increasing demand for biodiesel energy as an alternative sustainable energy source, the disposal cost of biodiesel by-products (mainly glycerol) increases. For wastewater treatment, glycerol could be used as a sustainable and cheap external carbon substrate for biological nutrient removal. Using glycerol as a carbon source for denitrification is very effective, and the best C-glycerol/N ratio is ≈ one, which means that glycerol has a lower denitrification requirement than methanol (2.6 C-methanol/N) (Grabińska-ńoniewska, Słomczyński, & Kańska, 1985). Methanol is used in most full-scale wastewater treatment plants. However, glycerol is proven to have a higher denitrification rate (up to three times) than methanol. Also, glycerol is more economical to use since

methanol prices are increasing and pose flammability risks. Also, using glycerol may offset the biodiesel waste disposal costs (Lu & Chandran, 2010). The addition of crude glycerol to the denitrification tank in full-scale wastewater treatment plants increased the denitrification by 2-5 mg NO_2-N/L, and the NOx (nitrite + nitrate) removal up to 65% (Bernat et al., 2016). Co-fermentation of waste activated sludge and crude glycerol for denitrification increased the denitrification rate 0.23 mg-N/mg-VSS*day in a sequencing batch reactors with synthetic wastewater (Bernat et al., 2016). An 800-day study was run using laboratory-scale sequencing batch reactors (SBRs) filled with raw wastewater to test the potential of crude glycerol (CG) as a direct carbon source addition for EBPR. The experimental data found that prefermentation of crude glycerol resulted in unstable EBPR even though the GAO fraction was less than 4.9%. Raw CG addition achieved excellent phosphorus removal and better EBPR stability than fermented products (Coats, Dobroth, & Brinkman, 2015). However; Shen and Zhou (2016) suggested that glycerol fermentation is essential for EBPR to utilize it as readily biodegradable carbon oxygen demand (rbCOD) (mainly propionic and acetic acid). Also, most of the glycerol for EBPR driven studies are short-term studies which cannot guarantee the stability of EBPR with a complex carbon source. When glycerol was co-fermented with waste activated sludge, it resulted in a significant VFAs production and superior phosphorus removal. In the same study, direct glycerol addition caused EBPR failure when substituted for acetate in lab-scale batch reactors (Yuan et al., 2010). Guerrero, Tayà, Guisasola, and Baeza (2012) found that glycerol can be directly added to the anaerobic zone if allowed enough time to ferment

inside the reactor and produce VFAs. It was found that the optimal conditions are using 4 hours anaerobic and 3.5 hours aerobic. However; the PAOs did not directly use the glycerol, but the long anaerobic conditions allowed degradation of glycerol to VFAs.

Study Objectives

Most studies in the literature regarding the use of glycerol (glycerin) as a carbon source for nutrient removal focus on EBPR or nitrogen removal and mainly are done in a lab scale setting. Both phosphorus and nitrogen compete for the same resources, and thus, a combined effect of glycerol as carbon source is needed. Direct addition of glycerol for nitrogen removal is well studied. However, EBPR studies using glycerol are not consistent. The main objectives of this study are:

- Optimize primary solids fermentation using glycerol co-fermentation, mixing intensity, and hydrogen gas.
- Optimize the performance of the A_2O-BNR system using the glycerol addition points (Prefermenter versus direct addition to the anaerobic zone).
- Study the effects of the side-stream prefermenter (PF) mixing intensity on the performance of the PF and of the A_2O-BNR systems.
- Optimize the performance of the 5-stage Bardenpho^{TM} BNR system using either a direct addition to the second anoxic zone or co-fermentation with primary solids.

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CHAPTER TWO: LITERATURE REVIEW

Biological Wastewater Treatment

Domestic biological wastewater treatment is used to produce an acceptable end-product from dissolved and particulate biodegradable pollutants through biological floc or biofilm. Additional objectives include nutrient removal (N and P). In some cases, domestic wastewater treatment should include the removal of specific constituents that cause result in detrimental effects on public health or the environment (Cornwell, 2013; Metcalf&Eddy, 2014).

Biological Nutrient Removal

Biological nutrient removal (BNR) is a considered an advanced treatment process to remove nitrogen and phosphorus from wastewater. Municipal wastewater can have high levels of nitrogen and phosphorus present, which can promote eutrophication when discharged into the ecosystem, (Henze, 2008; Metcalf&Eddy, 2014).

Eutrophication is a phenomenon where an excess amount of nutrient causes excessive growth of harmful algal blooms (HABs) that cause harmful effects on aquatic life via oxygen depletion, and reduction in transparency (Walsh, 2012). Also, eutrophication is associated with health risks such as Methemoglobinemia (a fatal blood syndrome that affects infants and is also known as a “blue-baby syndrome”), spontaneous

abortions, diabetes, osteoporosis and kidney or liver failure (Wanielista et al., 2008; Xuan et al., 2009).

Wastewater nitrogen is removed biologically by a nitrification and denitrification process. Phosphorus can be removed chemically or biologically by Enhanced biological phosphorus removal (EBPR) process (Cornwell, 2013; Henze, 2008; Metcalf&Eddy, 2014).

Biological Nitrogen Removal

Nitrification

Nitrification is a two-step biological process to convert ammonia to nitrate-nitrogen in the presence of dissolved oxygen. Both steps are carried out by chemoautotrophic bacterias known as nitrifying bacteria. In the first step, nitrifying bacteria such as *Nitrosomonas Europea* converts ammonia to nitrite. The stoichiometry of the first step of nitrification is shown in Equation 1. In the second step, an organism such as *Nitrobacter* converts nitrite to nitrate as shown in Equation 2. Equation 3 describe the summary reaction for the entire nitrification process (Metcalf&Eddy, 2014).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

$$2NO_2^- + 2O_2 \rightarrow 2NO_3^- \quad (2)$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

However, nitrification is affected by many phenomena like biochemical oxygen demand (BOD_5), alkalinity, pH, temperature, plug flow conditions, and mean cell residence time (MCRT). High BOD_5 levels reduce the nitrification efficiency and the ratio of $\frac{BOD5}{\text{Total Kjeldahl Nitrogen (TKN)}}$ must be under 3 for optimum nitrification conditions. Nitrification typically requires a minimum of 2.0 mg/l dissolved oxygen to occur. 4.57 grams of oxygen is needed to oxidize 1 gram of ammonia during the nitrification process (3.43 g O_2/g NH4-N + 1.14 g O_2/ NO_2-N) (Carroll Murphy, 2007; Metcalf&Eddy, 2014). The mixed liquor suspended solids (MLSS) should maintain a pH value of 8.4 for optimum nitrification and must not exceed a minimum pH of 7.2. Alkalinity is another limiting condition for nitrification since oxidation of each mg of ammonia requires 7.14 mg alkalinity as $CaCO_3$. The consumption of alkalinity produces carbon dioxide CO_2 which can significantly reduce pH. Nitrification can be carried out at low temperatures, but require a minimum of 10 °C to be efficient. Plug flow conditions are important to for the growth of nitrifying bacteria. Minimum range of MCRT varies with temperature, but it is 10-20 days for nitrification with the optimum condition being at 20-30 days. Nutrient removal facilities operate on the low end of this range or lower. Toxic compounds can inhibit ammonia oxidation and deactivate the nitrifying bacteria even at very low concentration compared with aerobic heterotrophic bacteria (Aboobakar et al., 2013; Carroll Murphy, 2007; Metcalf&Eddy, 2014; Ward, Arp, & Klotz, 2011).

Denitrification

Denitrification is a process to convert nitrate to nitrogen gas. Denitrification is carried out a dissimilation process by a broad range of heterotrophic groups of bacteria, including, but not limited to - *Pseudomonas, Acinetobacter, Agrobacterium, Micrococcus, Alcaligenes, Archromobacter, Spirillum, and Bacillus*. Dissimilation is a reduction process in which denitrifying bacteria uses the chemically bound oxygen in nitrate and nitrite for the respiratory process. Equation 4 describe the intermediate products in the dissimilation process (Gerardi, 2003; Metcalf&Eddy, 2014).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (4)$$

The denitrification process produces alkalinity and thus raises the pH of the mixed liquor. Denitrification recovers approximately half the alkalinity destroyed in the nitrification process. Optimum denitrification pH is 7 to 7.5. (Gerardi, 2003; Metcalf&Eddy, 2014).

Denitrification rate is affected by the BOD_5 concentration, dissolved oxygen, pH, and temperature. Dissolved oxygen $\geq$ 0.2 mg/l can inhibit denitrification. Temperatures below 5°C inhibits the denitrification process. This temperature inhibition can be compensated partially by increasing the mixed liquor volatile suspended solids (MLVSS). Also, simultaneous nitrification/denitrification can occur in the aerobic tank due to insufficient aeration or poor mixing. The denitrification process requires a constant supply of a carbon source (organic matter). Many carbon sources are studied for denitrification

like methanol and acetic acid. The most used in BNR systems are methanol and then glucose based on their cost. Recent studies introduced glycerol from biodiesel waste as an alternative cheap carbon source (Gerardi, 2003; Henze, 2008; Her & Huang, 1995; Metcalf&Eddy, 2014).

Enhanced Biological Phosphorus Removal

Enhanced biological phosphorus removal (EBPR) is a specific modification of the activated sludge systems to maximize phosphorus removal. Phosphorus can be removed chemically from wastewater, also but for the purpose of this study only biological phosphorus removal was discussed (Wentzel, Comeau, Ekama, van Loosdrecht, & Brdjanovic, 2008). In the early 1960s, biological phosphorus removal was discovered by accident when Srinath, an Indian professor, noticed an excessive biological phosphate uptake in some treatment plants when aerated (Henze, 2008; Srinath, Sastry, & Pillai, 1959).

In EBPR, Polyphosphate Accumulating Organisms (PAOs) capture phosphorus in cells. Phosphorus is then removed through sludge wasting. Phosphorus removal from wastewater takes place in two main environments: anaerobic and aerobic. In the first phase, the lack of oxygen gives the PAOs advantage over the other bacteria populations in the system since PAOs can take up VFAs. Then, PAOs are exposed to an aerobic environment where they grow rapidly and uptake phosphorus. The last step is the clarifier where the separation of water and waste sludge occur (Henze, 2008; Merzouki et al., 2005;

Metcalf&Eddy, 2014). The typical EBPR configuration is shown in Figure 1.

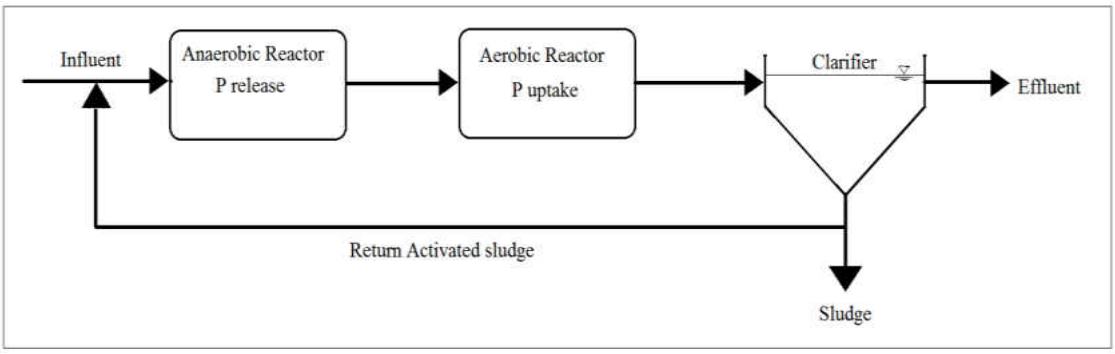

Figure 1 Typical EBPR configuration

In the anaerobic tank, PAOs uptake volatile fatty acids (VFAs) to form poly-hydroxy-alkanoates (PHAs). To provide energy for this, poly phosphate (poly-P) is broken down, releasing inorganic P outside the cell. Intercellular glycogen is also broken down to glucose.

In the aerobic tank, rapid growth of PAOs happens using PHAs and dissolved oxygen. In the process, glycogen and poly-P are replenished, and inorganic P is removed from the bulk wastewater (Güngör, Müftügil, Ogejo, Knowlton, & Love, 2009; Henze, 2008; Merzouki et al., 2005; Metcalf&Eddy, 2014). Figure 2 depicts the metabolism of the PAOs in the absence and presence of dissolved oxygen.

Glycogen accumulating organisms (GAOs) consumes glycogen and convert it to PHAs. GAOs and PAOs co-exist in EBPR and compete for the carbon source (mainly VFAs). Even though GAOs consumes the VFAs, but it does not contribute to the phosphorus removal. It is important to monitor the PAOs/GAOs ratio in the anaerobic zone because failure of EBPR is mainly caused by undesirable dominant of GAOs over PAOs (Oehmen, Saunders, Vives, Yuan, & Keller, 2006). Also, nitrite in the anaerobic zone due to inadequate monitoring or incomplete denitrification can cause instability or even complete EBPR failure. Anaerobic-nitrite will reduce the uptake of nitrifying phosphorus in the anoxic and aerobic zones. As a result, causing a favorable environment for the GAOs over the PAOs. pH values < 7.3 can also cause undesirable reduction of the PAOs/GAOs ratio (Saito, Brdjanovic, & van Loosdrecht, 2004; Shen & Zhou, 2016).

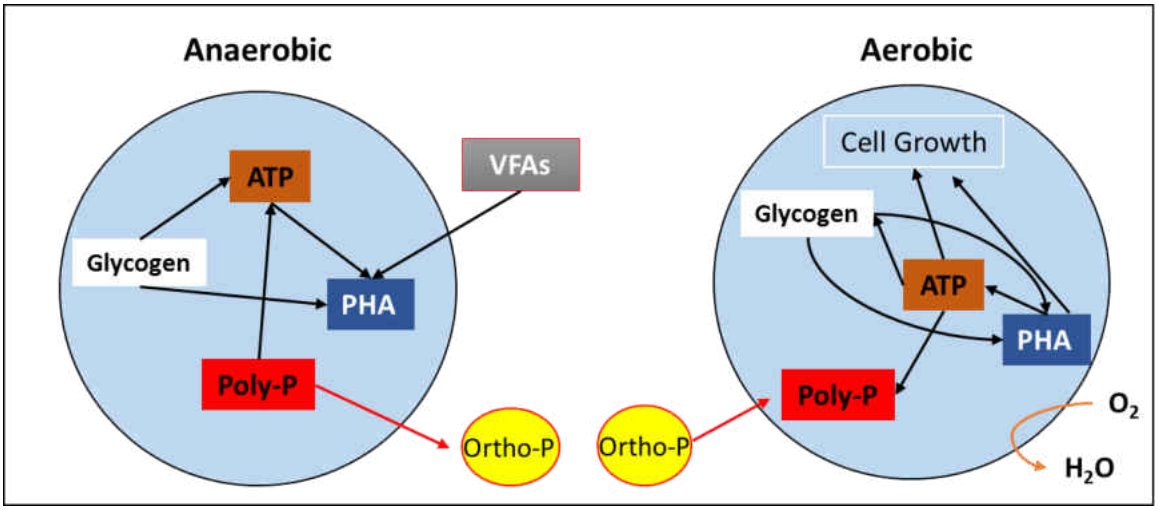

Figure 2 PAOs metabolism in anaerobic and aerobic conditions, adapted from Henze (2008).

Fermentation

The fermentation process is typically part of a methanogenic process done in four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis as described in Figure 3 (Henze, 2008; Metcalf&Eddy, 2014). Prefermentation consists of the first three processes but is not methanogenic.

In the hydrolysis phase, polymers (lipids, polysaccharide, protein and nucleic acids) are reduced to simple monomers (fatty acids, monosaccharides, amino acids, purines, pyrimidines, and simple aromatics). Acidogenic is treatment processes leading to short chain VFAs other than acetate (3-5 carbon atoms mostly). Acetogenesis is term process leading to acetic acid. H_2 and CO_2 can be produced from both types of fermentation. Methanogenesis uses the products from the fermentation processes and produces methane or methane and CO_2. There are two types of methanogens bacteria. Type one is acetoclastic methanogens responsible for converting acetic acid to methane gas and CO_2. Type two is the hydrogen utilizing methanogens responsible for converting H_2 and CO_2 to methane gas (Henze, 2008; Jia et al., 1996; Metcalf&Eddy, 2014; Valcke & Verstraete, 1983).

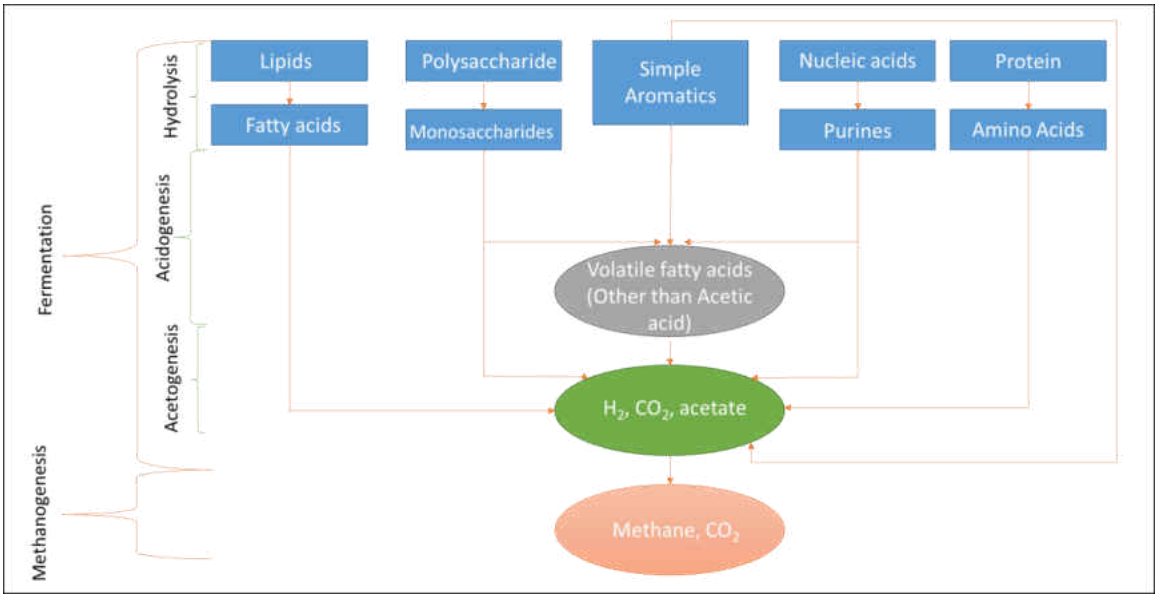

Figure 3 Fermentation and methanogenesis process schematic, adapted from (McCarty & Mosey, 1991; McCarty & Smith, 1986).

Prefermentation

Prefermentation is a fermentation process associated with BNR systems for nonseptic wastewater using primary sludge to increase EBPR. It is a common practice in Canada, Australia, and South Africa. However, it is minimally applied in full-scale systems in the United States (McCue et al., 2004) although this is changing in some states.

Two main designs are known for prefermentation applications, online and offline. Only offline prefermentation is of interest in this study. Usually, offline fermentation is a tank that receives primary solids in anaerobic conditions. The BNR system receives

fermented solids or supernatant from the prefermentation tank. Temperature increase has a positive effect on the net VFA production. However, hydraulic retention time (HRT) increase has a negative effect on the acidogensis process by reducing the acetate/ propionate ratio (Henze, 2008; McCue et al., 2004; Xu & Nakhla, 2007).

Prefermentation Effect on Denitrification and EBPR

VFAs from primary solid fermentation are mainly composed of propionic and acetic acids (Metcalf&Eddy, 2014). A study in China used a plug-flow A_2O process to study the effect of acetate and propionate as a carbon source on BNR functions. The data revealed that both acetate and propionate had no significant effect on nitrogen removal due to the carbon being the limiting factor for TN. The study found that propionate was more efficient carbon source than acetate in biological nitrogen and phosphorus removal (Wu et al., 2010). Chen et al. (2004) also found that higher propionic ratio improved the EBPR when he studied the effect of propionic to acetic acid ratio on EBPR performance in two (SBRs). The results showed superior performance at a ratio of 2.06 than 0.16 with P removal of 95% and 68%, respectively.

Similarly, Shen and Zhou (2016) discussed both acetate and propionate and concluded that propionate is more effective carbon source in BNR systems than acetate. High acetate loading will eventually favor GAOs over PAOs. Monitoring pH > 7.5 is very essential to maintain a higher fraction of the PAOs. Propionate requires less energy and lower C/P release ratio than acetate. Consumption of propionate by the GAOs is

insignificant compared to PAOs consumption. Propionate can provide excellent performance at pH > 7.

Merzouki et al. (2005) studied the effect of prefermentation on biological nitrogen and phosphorus removal in an anaerobic–anoxic sequencing batch reactor (SBR) coupled with a fixed-bed nitrification reactor from slaughterhouse wastewater. The results showed that before using the prefermenters, biological nutrient removal could not be carried out. However, BNR performance improved significantly by the addition of prefermenters due to the increase in VFA production which increased the COD/P ratio. Removal of P, COD, and N averaged at 99%, 99%, and 85%, respectively. McCue et al. (2004) found that adding prefermenter improved the denitrification rate in the study using bench-scale University of Cape Town (UCT) BNR systems. However, the data showed no significant improvement in the EBPR performance.

Biodiesel

Due to the limitation of the existing petroleum energy sources and its negative impact economically and environmentally, scientists are trying to find better renewable energy alternatives. Biodiesel is a fuel produced from vegetable oils or animal fats (in the presence of a catalyst) through a transesterification reaction. The reaction also results in a glycerol as a by-product (Figure 4) (Leoneti, Aragao-Leoneti, & De Oliveira, 2012). Biodiesel contributes to air pollution prevention, or it results in zero carbon emission, and a desirable effect on the energy self-sufficiency rate. Biodiesel is also considered a

sustainable energy source (Eguchi, Kagawa, & Okamoto, 2015). Furthermore, studies support that when biodiesel is used in diesel engines, no noticeable effect was recorded regarding fuel consumption or engine performance. Also, investigations showed that biodiesel fuel had a reduced effect on hydrocarbons, carbon monoxide, and particulate matter emissions, but increased nitrogen oxides emissions when compared with diesel fuel (Correa & Arbilla, 2008; Hoekman & Robbins, 2012; Usta et al., 2005).

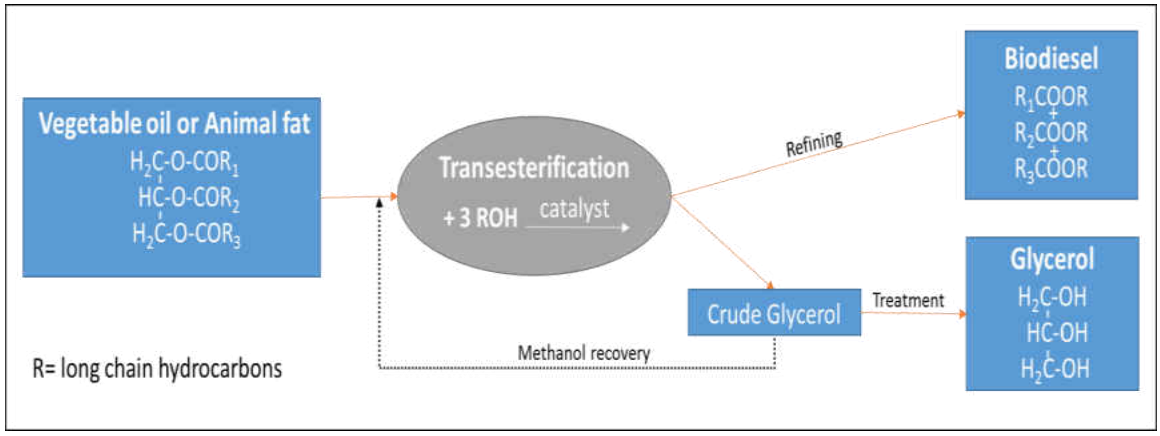

Figure 4 Transesterification reactions for Biodiesel production, adapted from Leoneti et al. (2012).

The primary limiting factor for the slow growth of biodiesel full-scale plants is the operational and disposal cost which is significantly higher than that of fossil fuel (Demirbas, 2008). Current researchers are trying to reduce the disposal cost by glycerol (glycerin) recovery and reuse. Glycerol is a biodiesel by-product. Roughly, for every million gallon biodiesel produced, 383 tonnes of a 99.9% pure glycerol will be produced (Yang, Hanna, & Sun, 2012). One pound of crude glycerol can be composed of 0.3 lb

glycerol, 0.5 lb Methanol, 0.13 lb soap, 0.02 lb moisture, 0.04-0.06 lb other impurities (Wijesekara, Nomura, Sato, & Matsumura, 2008). However, the composition of the crude glycerol can be site specific.

There are more than 2000 industrial uses for pure glycerol, Crude glycerol, however, require must be refined to be used as pure glycerol (Leoneti et al., 2012; Quispe, Coronado, & Carvalho Jr, 2013). Crude glycerol can be used without refining in chemical products, fuel additives, fuel cells, animal feed, and co-digestion and co-gasification (Leoneti et al., 2012). Pure and crude glycerol can be used in wastewater treatment as a carbon source after fermentation to VFAs (Leoneti et al., 2012).

Glycerol Effect on EBPR

When using glycerol as a carbon source for EBPR, prefermentation is required to promote readily biodegradable carbon (mainly propionic and acetic acid). Many full-scale WWTPs use side stream fermentation to produce VFAs for the BNR system. Shen and Zhou (2016) believe that most of the glycerol studies are short-term studies which cannot guarantee the stability of EBPR with a complex carbon source (Shen & Zhou, 2016). However, an 800-day sequencing batch reactors (SBRs) study testing the potential use of crude glycerol (CG) as a direct carbon source for EBPR, found that prefermentation of crude glycerol resulted in unstable EBPR even though the GAO fraction was less than 4.9%. Raw CG addition achieved excellent phosphorus removal and better EBPR stability than fermented products (Coats et al., 2015). Yuan et al. (2010) studied glycerol as a carbon

source for EBPR using co-fermentation of glycerol with waste activated sludge and using direct addition of glycerol. The co-fermentation of glycerol resulted in a significant production of VFAs and superior P removal. It was found that when acetate was replaced with glycerol, EBPR failure resulted. However, Guerrero et al. (2012) looked at the feasibility of glycerol fermentation in the anaerobic zone to produce VFAs as a carbon source for EBPR in a SBR reactors. The study found that phosphorus removal was peaked using 4 hours anaerobic and 3.5 hours aerobic cycle. A low (P mol/C mol glycerol) uptake was observed in the anaerobic phase. However, the glycerol was not directly used by the PAOs. The long anaerobic conditions allowed degradation of glycerol to VFAs (mainly propionate). Thus, sufficient hydraulic retention time will allow glycerol to be directly added to the anaerobic zone for EBPR. These findings contradict past statements by Shen and Zhou (2016) that say glycerol cannot be used for EBPR without prefermentation.

Guerrero, Guisasola, and Baeza (2015) tested the possibility of controlled CG addition to overcome EBPR failure due to nitrite presence in the anaerobic zone. The study consisted of two BNR systems A_2O and Johannesburg WWTP configuration (JHB) in addition to a computer models. It was proved that CG is considered a suitable carbon alternative for denitrification and EBPR with appropriate CG control. Also, JHB system required 18% less CG and had better phosphorus removal than A_2O, even without dose control.

Glycerol Effect on Denitrification

A research in Poland using modified Upflow Anaerobic Sludge Blanket (UASB) reactors used glycerol as a carbon source for denitrification. It was found that glycerol is a suitable carbon source. The removal of nitrogen and COD in the reactors was 97% and 94% respectively. It was found that the best C-glycerol/N ratio is ≈ 1 which means that glycerol has lower denitrification requirements than methanol (2.6 C-methanol/N) (Grabińska-ńoniewska et al., 1985). Another study compared methanol and glycerol as a carbon source and an electron donor to enhance denitrification. This resulted in three advantages for glycerol over methanol being identified. The first advantage is due to the increasing price of natural gas which is used to synthesize methanol. This increase makes glycerol more appealing as a carbon source. Also, reusing a by-product from biodiesel production offsets the disposal cost, making biodiesel more feasible to use. The third and most significant advantage is that glycerol had a higher denitrification rate (up to three times) than methanol (Lu & Chandran, 2010).

Torà, Baeza, Carrera, and Oleszkiewicz (2011) studied multiple carbon substitutions for denitrification in a lab-scale SBR. The results suggested that glycerol is a suitable carbon source and was able to achieve SDR 0.25 gN/gVSS*day. Bodík, Blšťáková, Sedláček, and Hutňan (2009b) used a full scale (25 ML/day) WWTP with insufficient nitrogen removal to test the possibility of using the addition of CG into the denitrification tank to enhance nitrogen removal. The CG dose increased the denitrification

by 2-5 mg NO_{3}-N/L. Also, the removal of COD and NO_x increase 43% and 65% after glycerol addition. Bernat et al. (2016) studied the potential effect of using co-fermentation of waste activated sludge and crude glycerol on denitrification in a SBR with synthetic wastewater. The result showed that with crude glycerol the denitrification rate increased 0.28 - 0.51 mg-N/mg-VSS*day.

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CHAPTER THREE: OPTIMIZATION OF SLUDGE FERMENTATION FOR VOLATILE FATTY ACIDS PRODUCTION

Abstract

Prefermentation of primary solids can produce volatile fatty acids (VFAs), and operational strategies may affect the propionic acid content. In this study, three prefermenter phases were used to optimize VFAs using 3 separate strategies. The phases were (i) glycerol (biodiesel by-product) co-fermentation with primary solids, (ii) the effect of mixing energy on the co-fermentation of glycerol and primary solids, (iii) the effect of hydrogen gas addition on the co-fermentation of glycerol and primary solids. The Phase 1 data showed that glycerol increased the VFAs production 1.2 times over the possible value from the added glycerol alone (427 mg-COD/L), implying that the glycerol addition stimulated additional fermentation of primary solids. In phase 2, low mixing energy in glycerol increased the VFAs production by 80% while slightly favoring propionic acid over acetic acid compared to the higher mixing energy. The addition of hydrogen gas in Phase 3 did not increase the VFAs total production, but significantly increased the concentration of propionic acid by 41%. All three optimization approaches performed well and were able to increase the VFAs production and/or increase propionic acid concentration relative to acetic acid.

Keywords fermentation; glycerol; hydrogen gas; mixing energy; volatile fatty acids; propionic acid.

Introduction

Biological nutrient removal (BNR) is considered one of the most economical processes to meet the wastewater treatment plants increasingly strict discharge requirements for nitrogen and phosphorus (Broughton, Pratt, & Shilton, 2008; Coats et al., 2015). Both enhanced biological phosphorus removal (EBPR) and nitrogen removal require a carbon source as an electron donor to complete the removal process (Wu et al., 2010). Many studies were dedicated to finding the efficiency of different carbon sources on BNR. Different organic carbon sources such as acetic acid, propionic acid, and methanol have been studied for their potential effectiveness as a carbon substrate for nitrate removal (Aspegren et al., 1998; Moser-Engeler et al., 1998; Rahmani et al., 1995). For EBPR, it was found that volatile fatty acids (VFAs) such as acetic and propionic acids are the most favorable carbon source (Shen & Zhou, 2016). Small quantities of VFAs can be found in the wastewater, but often not enough for EBPR and denitrification to reach completion (Bernat et al., 2016).

Propionic acid was found to be more effective and to result in more stable phosphorus removal, and acetate is also effective but can occasionally favor GAOs over PAOs over time, causing EBPR failure (Chen et al., 2004; Shen & Zhou, 2016; Wu et al., 2010). Also, propionic acid was found to provide better nitrogen removal by Wu et al. (2010). Lopez-Vazquez et al. (2009). showed that optimal EBPR was obtained with a 50:50 or 75:25 mixture of acetic:propionic acid. However; propionic acid supplementation for

full-scale BNR is to some extent cost prohibitive. The most economical and sustainable way to produce VFAs is fermentation using either wastewater or inexpensive waste-product carbon sources.

Prefermentation is an established process to produce VFAs (mainly acetic and propionic acids), and is a common practice in Canada, Australia, and South Africa (McCue et al., 2004). However, it is minimally applied in full-scale systems in the United States (McCue et al., 2004) although this is changing in some states. Fermentation is carried out in three phases: hydrolysis, acidogenesis, and acetogenesis, respectively. The first phase involves the reduction of polymers to simple monomers (e.g. fatty acids) followed by the second phase which is conversion of fatty acids into VFAs other than acetic acid (e.g. propionic and butyric acids). The third phase is the conversion of propionic acid and the other intermediates into acetic acid, carbon dioxide, and hydrogen (H_2) (Henze, 2008; Jia et al., 1996; Metcalf&Eddy, 2014; Valcke & Verstraete, 1983). Hydrogen is potentially a major factor that can inhibit fermentation of propionic and butyric acid to acetic acid. Hydrogen ($> 10^{-4}$ atm) in the fermentation process should, in theory, inhibit propionic acids further fermentation to acetic acid (acetogenesis) allowing the accumulation of propionic acid in the prefermenter (Fukuzaki, Nishio, Shobayashi, & Nagai, 1990; Metcalf&Eddy, 2014). In the case of prefermenters the accumulation of propionic acid is desirable to produce a mixture of acetic and propionic acids. Also, the fermentation operational conditions such as temperature, pH, and mixing could be used to further maximize VFAs production. Many studies in the past evaluated properties such as mixing

and pH to determine the optimum operational conditions that can enhance the VFAs production from the fermentation process (Banister & Pretorius, 1998; Danesh & Oleszkiewicz, 1997).

Glycerol (a biodiesel by-product) is being investigated as an economical and sustainable enhancement of the VFAs production. The addition of glycerol to the prefermentation reactor significantly improved the production of VFAs (Yuan et al., 2010). Coats et al. (2015) was able to put glycerol directly into an anaerobic zone directly and obtained low effluent phosphorus for a phosphorus limited wastewater. Addition of glycerol also was used to drive denitritation in biological nitrogen removal (Bernat et al., 2016). Thus there is great interest in using glycerol for BNR.

The aim of this study is to optimize the VFAs production, reduce the HAc/HPc, and reduce the operational cost of the prefermentation process through a study divided into three phases:

- Compare the VFAs production of primary solids and glycerol co-fermentation with primary solids fermentation.
- Study the effect of mixing intensity on VFAs yields during co-fermentation of glycerol and primary solids using 7 and 50 rpm mixers.
- Test the effects of hydrogen addition to the co-fermentation of glycerol and primary solids in terms of the HAc/HPc ratio. To the best of the author's knowledge, hydrogen use to increase the propionic acid content of prefermenter VFAs has not

been studied yet.

Materials and Methods

Source of Wastewater, Primary solids, and Glycerol

Wastewater was obtained from Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) and was screened on-site with a 1/4 inch mesh, then used to fill a 400 L tank. The tank was cleaned and filled on a daily basis. The primary solids were obtained from Glendale Wastewater Treatment Plant (Lakeland, Florida) on a weekly basis and stored in a 4C° freezer. The glycerol ($C_3H_8O_3$) was obtained from Fisher Scientific (Tampa, FL).

Process Configuration for Glycerol Effect and Mixing Intensity

Prefermentation experiments were carried out in two pilot scale 10 L prefermentation reactors. Both were operated at a 5 day SRT to prevent methanogenesis. Two liters of primary solids were manually added to the prefermenters daily. Also, prefermenter supernatant was pumped at a 2 L/day flowrate.

For the glycerol effect experiment, both prefermenters were mixed at 50 rpm. The first reactor (PF1) received a constant 0.5 L/day glycerol dose using a stock solution with a concentration of 7000 mg pure glycerol/L. This resulted in an initial concentration in the prefermenter of 350 mg-VFAs/L (427 mg COD/L). The second reactor (PF2) was operated

without glycerol addition. For the mixing intensity experiment, both prefermenters received a constant 0.5 L/day glycerol dose of stock solution with a concentration of 7000 mg pure glycerol/L. The experimental variable between the two reactors was that PF3 was mixed at 7 rpm while PF4 was mixed at 50 rpm. A summary of experimental variables can be found in Figure 5 and Table 1. Phase 1 and 2 study lasted for 160 days including a 60 days acclimation period. Phase 1 experiment contains 16 sampling events and was run for 60 days. Phase 2 experiment contains 6 sampling events and was run for 40 days.

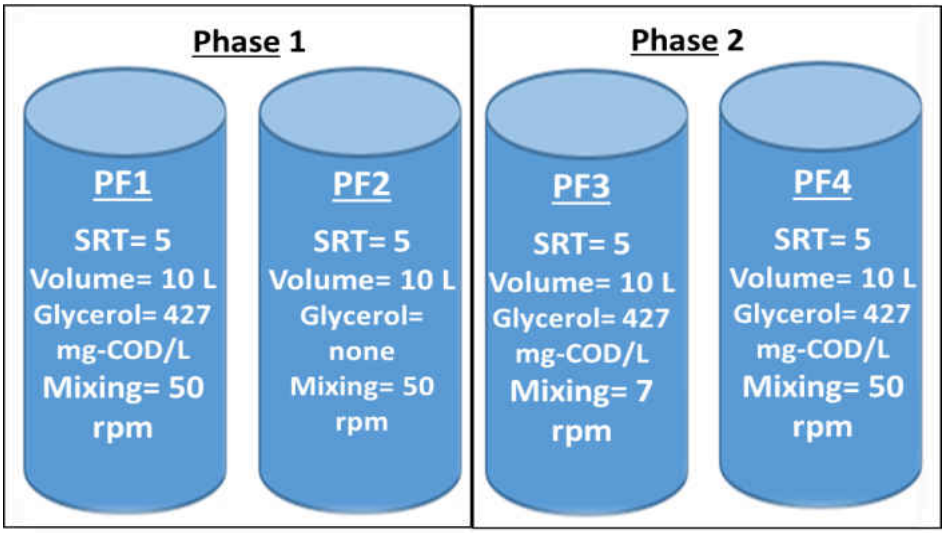

Figure 5 Phase 1 and 2 prefermenters configuration

Table 1 Summary of all phases, reactors and experimental variables.

	Reactor name	Glycerol Dose	Mixing rpm	Other
Phase 1	PF1	3500 mg Glycerol/day	50	none
	PF2	No glycerol addition	50	none
Phase 2	PF3	3500 mg Glycerol/day	7	none
	PF4	3500 mg Glycerol/day	50	none
Phase 3	R1	6500 mg of pure glycerol	none	H2 addition
	R2	6500 mg of pure glycerol	none	none

Process Configuration for Hydrogen Addition

Two bench-scale semi-continuous reactors with a volume of 1500 mL per reactor were used to study the effect of hydrogen gas on VFA production at an SRT of 4 days. The reactors were called R1 and R2. Both reactors initially received 1.5 liters of 50:50 mix of primary solids and raw wastewater. Each day, 375 mL (0.375 L) was removed and replaced with 375 mL of a 50:50 mix of primary solids and raw wastewater plus 6500 mg of pure glycerol. This resulted in an initial glycerol concentration of 1625 mg/L (1982 mg-COD/L) in the prefermenters. No mixing was applied to the reactors except when sampling and feeding. The procedure was done at the beginning of each cycle (i. e. every 24-hours). R1 received a daily 30-second dose of H_2 gas (purging the headspace). It was sealed airtight, so H_2 could come to equilibrium with the liquid in the reactor. R2 did not receive H_2 gas. The experimental variable was H_2 gas addition (H_2 partial pressure, although this was not measured). Figure 6 show the reactors configuration, and the experimental variables are summarized in Table 1. This phase of the experiment lasted for 70 days including a 30 day

acclimation period. Phase 3 experiment contains 6 sampling events and was run for 40 days.

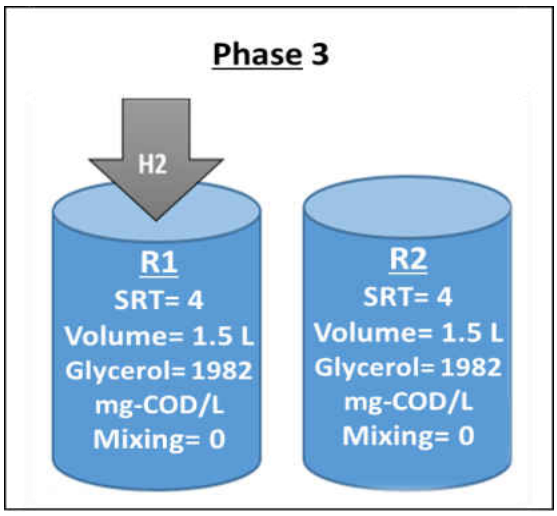

Figure 6 Phase 3 prefermenters configuration

Analytical Techniques

VFAs, COD, TSS, VSS, and pH were measured in the reactors. The samples were filtered immediately on site in Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) with a glass fiber filter (Whatman™, 1827-025, Pittsburgh, Pennsylvania). Following that they were filtered with 0.45µm membrane filters (Fisherbrand™, SA1J791H5). Short-chain volatile Fatty Acids (SCVFAs) were measured using a Shimadzu gas chromatography (GC) 14-A (Kyoto, Japan). The gas chromatograph was equipped with a flame ionization detector (FID) and Supelco Nukol column and Shimadzu

auto-sampler AOC-20I. The oven initial temperature was 110° and increased at a 5° C/min rate until reached the final temperature of 190° which was held for 10 minutes. The temperature of the injector and detector port were maintained at 220°. Standard curves were developed using 10mM volatile free acid mix (46975-U; Shimadzu, St. Louis, MO).

The total and soluble chemical oxygen demand (COD) was measured using the closed reflux titrimetric standard method C Section 5220 (Eatone, Closceri, & Greenberg, 1995) with Lovibond® Tintometer® 2420726 kit (Sarasota, FL). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured using Standard Method sections 2450 D and E (Eatone et al., 1995). pH was monitored using EcoTester^{TM} pH2 (Oakton, IL) on a daily basis. A paired-samples t-test was conducted to compare the VFAs and VFAs composition in both reactors of each phase.

Results and discussion

Glycerol Co-fermentation Effect

Glycerol is an inevitable by-product for bioethanol and biodiesel processes. The search for renewable energy sources, caused a significant increase in bioethanol and biodiesel production which caused a reduction in glycerol prices (Clomburg & Gonzalez, 2013). This part of the study is aimed to test the potential of optimizing the VFA production from primary solid fermentation using glycerol as a substrate. The experimental results show that there was a significant VFAs increase with the glycerol co-fermentation

(M=1949, SD=822) and with no glycerol (M=932, SD=471); t(15)=6.6, p = 0.000. The addition of 427 mg-COD/L glycerol to PF1 led to a total VFAs production of 1949 mg-COD/L. PF2 (no glycerol) had a total VFA production of 932 mg-COD/L which is approximately half the production from the reactor with glycerol co-fermentation. The VFA yield increased from 0.2 mg-VFAs/mg-VSS to 0.5 mg-VFAs/mg-VSS. The glycerol effect on VFA production in PF1 was 811 mg-COD/L, which is 89.9% more than the expected value (427 mg-COD/L) from glycerol conversion alone. This could imply some type of synergy between glycerol addition and primary solids fermentation. Glycerol fermentation may have resulted in a higher biomass with the glycerol fermenter microorganisms also contributing to fermentation of primary solids.

The HAc/HPc decreased from 0.89 to 0.85 with glycerol addition (Figure 7). There was a significant increase in propionic acid production in the reactor with the glycerol and primary solids co-fermentation (M=875, SD=314) and the rector with no glycerol (M=637, SD=445); t(15)=2.44, p = 0.027. This means that the addition of glycerol to the reactor favorably increased the production of propionic acid over acetic acid during the fermentation process. This may be because both glycerol and propionic acid are three carbon-chain molecules. The average VSS in PF1 and PF2 were similar with 2945 and 3388 mg/L respectively. PF1 had a considerably higher s-COD than PF2 with 1850 and 800 mg/L, consistent with the higher VFA production observed. The results indicated that adding glycerol as a carbon source to the primary solid fermentation process is favorable. Glycerol addition increased the VFAs yield and resulted in a more optimal mixture of

acetic and propionic acid.

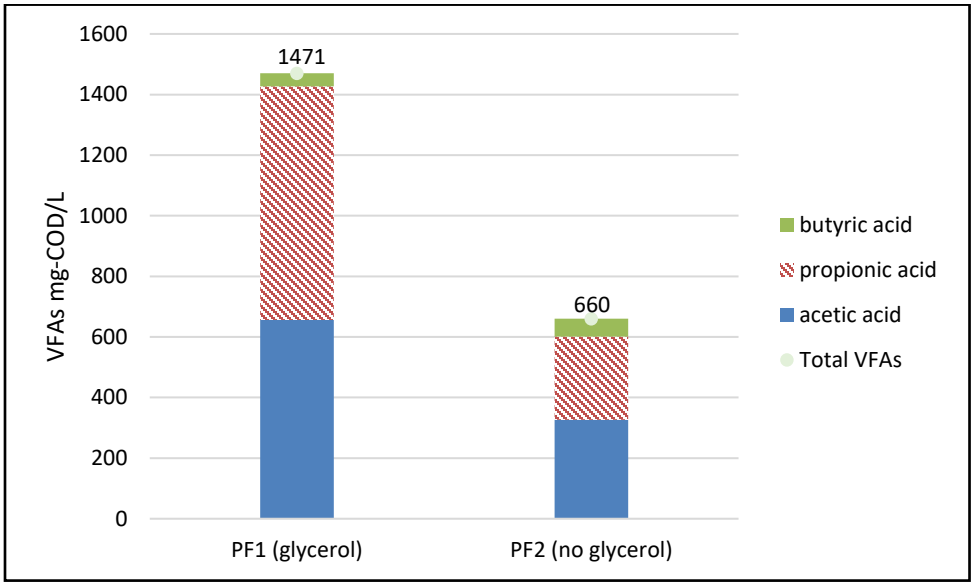

Figure 7. VFAs distribution in the effect of glycerol/primary sludge co-fermentation.

Mixing Intensity

Prefermentation mixing is applied to increase the contact between microorganisms and the substrate by causing suspension of the organic material (Yuan, Sparling, & Oleszkiewicz, 2011). Both reactors in this phase of the study were operated exactly the same except that PF3 was mixed at 7 rpm and PF4 was mixed at 50 rpm. The results (Figure 8) showed that mixing has an inverse correlation with VFA production. There was a significant VFAs increase in the lower mixed prefermenter (M=2429, SD=813) and the higher mixed prefermenter (M=845, SD=321); $t(5)=4.03$, $p = 0.010$. At 50 rpm, the total VFA production was 845 mg-COD/L while at 7 rpm, it was 2429 mg-COD/L. Lower mixing in the prefermentation reactor resulted in almost double the VFA production

compared to the highly mixed reactor. Also, PF3 (7 rpm) resulted in a significantly higher VFA yield ($p<0.05$) than PF4 (50 rpm) with 0.9 and 0.4 mg-VFAs/mg-VSS respectively. This could imply that lower mixing energy caused higher hydrolysis and solubilization rates.

The lower mixing prefermenter (M=943, SD=227) significantly increased the production of VFAs compared with the higher mixed prefermenter (M=550, SD=191); $t(5)=3.55$, $p = 0.016$. Reduction of the mixing energy resulted in a favorable higher propionic acid production and thus lower HAc/HPc ratio of 0.61. PF4 had a ratio of 0.73 (Figure 8). Biomass stratification and lower sheer force at low mixing energy probably increased the hydrogen transfer during the acidification process, favoring the production of propionic acid. This is because production of propionic acid often requires hydrogen to drive it, and stratification may facilitate the transfer of hydrogen for that purpose. Also, it could be caused by the fact that the external substrate (glycerol) is a 3-carbon molecule like propionic acid. The absence of acid consumption in both reactors means that they did not go methanogenic. Mixing energy had a direct relation with VSS since PF3 had 3222 mg/L and PF4 had 4069 mg/L. An average of 35% more s-COD was found when lower mixing was applied. PF3 and PF4 had an s-COD of 2737 and 2032 mg/L respectively. The experimental results indicate that lower mixing energy increased the VFA yield, propionic acid production, the s-COD, and the solids consumption.

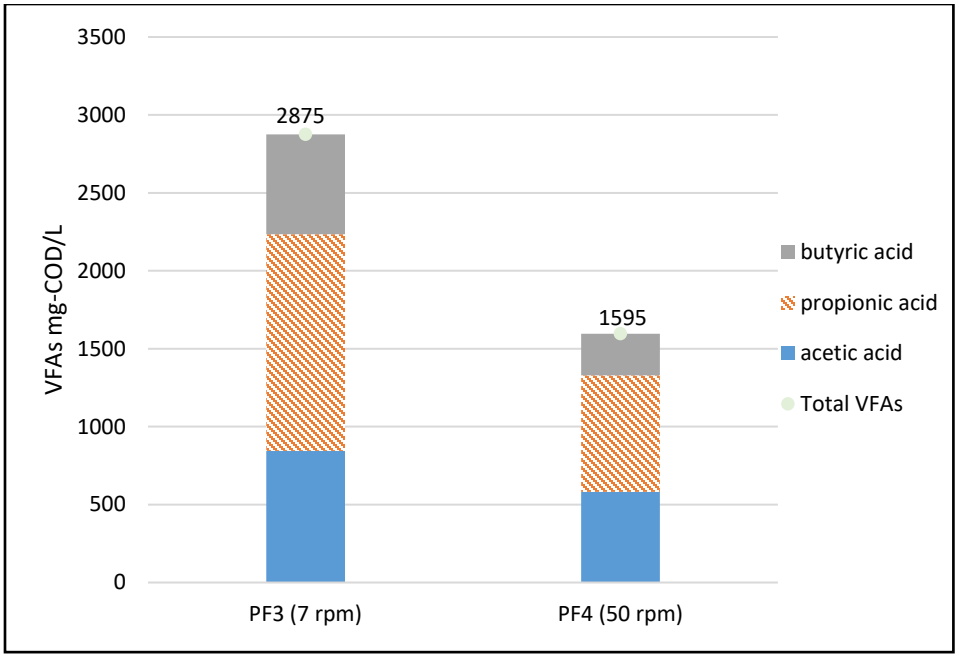

Figure 8 VFAs distribution in the study of mixing energy effects on glycerol and primary sludge

Hydrogen Effect in the Absence of Mixing

The fermentation process is very hydrogen sensitive. If hydrogen in the system exceeds 10^{-4} atm, it could inhibit acetogenesis. This sensitivity could be used to increase the propionic acid production by adding H_2 to the process. Also, hydrogen can be produced on site from the wastewater using different types of anaerobic biofilm reactors or co-fermentation of waste activated sludge with crude glycerol (Barca, Soric, Ranava, Giudici-Orticoni, & Ferrasse, 2015; Varrone et al., 2013). This phase of the study was carried out in two reactors: R1 (glycerol+hydrogen) and R2 (glycerol only).

The experimental results show that both reactors performed well regarding VFAs production. There was no significant difference in the TVFAs production in the reactor with hydrogen addition (M=4893, SD=1875) and the reactor without hydrogen addition (M=4526, SD=1431); $t(5)=0.401$, $p = 0.705$. R1 produced 4883 mg-COD/L, and R2 produced 4526 mg-COD/L. This corresponds to a VFAs yield of 1.00 for both reactors which means both reactors had similar VFAs production potential with and without the hydrogen gas addition. This is very similar to the yield found in the pilot prefermenter with glycerol addition and low mixing energy which proves again that lower mixing or no mixing, in this case, increases the VFAs production potential.

Even though the VFAs produced in both reactors are the same, the HAc/HPc ratio was positively affected by the hydrogen addition (Figure 9). The HAc/HPc ratio in the hydrogen reactor was on average 67% lower than R2. This indicates that there was a significant HAc/HPc reduction in the reactor with hydrogen addition (M=0.23, SD=0.23) and the reactor without hydrogen addition (M=0.70, SD=0.49); $t(5)=-2.757$, $p = 0.04$. The HAc/HPc ratio for R1 was 0.17 and for R2 was 0.50 (Table 2). As mentioned before, a higher propionic acid fraction is required (along with acetic acid) to fully optimize EBPR (Chen et al., 2004; Lopez-Vazquez et al., 2009; Shen & Zhou, 2016; Wu et al., 2010).

A significant amount of butyric acid was found in both reactors at approximately 12% of the total average VFAs. Soluble COD was higher in R1 (glycerol+hydrogen) than in R2 (glycerol only) with 15085 mg-COD/L and 13343 mg-COD/L. The VSS

concentration in R1 and R2 were 4888 mg/L and 4519 mg/L respectively. Both reactors had a VFAs yield of about 1.00 mg VFA/mgVSS which is the highest observed yield throughout the study. Also, the addition of hydrogen gas caused the lowest HAc/HPc ratio in the entire study. The results suggest that the hydrogen either drives the formation of propionic acid or instead that it inhibits conversion of the propionic acid to acetic acid.

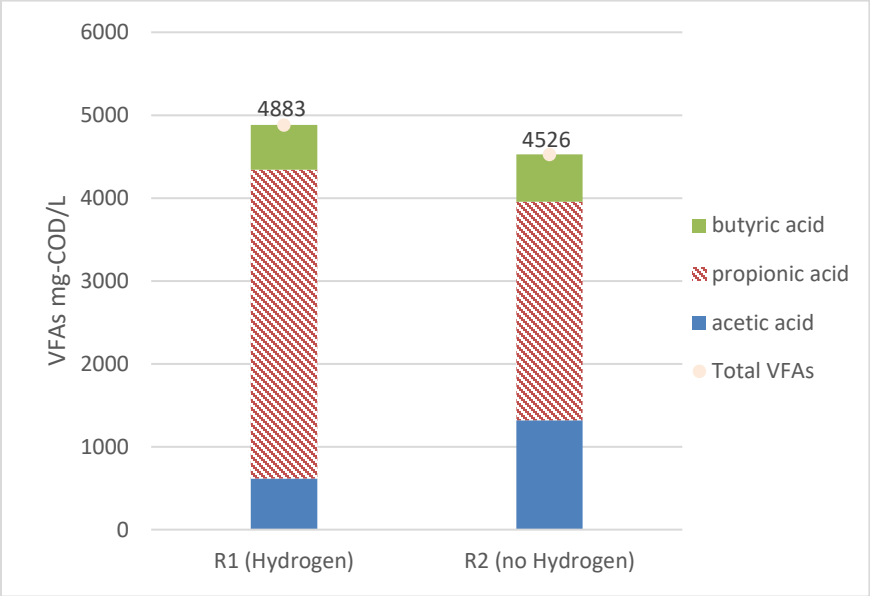

Figure 9 VFAs distribution in the hydrogen gas effect experiment.

Table 2 Supernatant concentrations for the hydrogen addition experiment.

	acetic acid	propionic acid	Total VFAs	s-COD	VSS	pH	HAc/HPc
	mg-COD/L			mg/L			
R1	617	3726	4883	15085	4888	4.1	0.17
R2	1321	2635	4526	13343	4519	4.2	0.50

Conclusion

Optimization of prefermentation performance using renewable substrate was demonstrated in both pilot and lab scale experiments to increase the VFAs production, reduce the HAc/HPc ratio, and potentially lower operational costs at full-scale BNR facilities with low COD wastewaters. Glycerol addition to the prefermenter increased the VFAs yield 1.2 times compared to the prefermenter without glycerol. Lowering the mixing energy from 50 rpm to 7 rpm in the glycerol enriched reactor enhanced the VFAs production by 80% and caused an increase in the fraction of propionic acid in the VFA mix. Hydrogen gas addition to the headspace of an unmixed, glycerol enriched prefermentation reactor had a similar VFA yield to the reactor without H_2. However it significantly ($p<0.05$) increased the production of propionic acid by 41%, probably by driving propionic acid production (which often requires reducing equivalents) or through acetogenesis inhibition. The three approaches (glycerol addition, lower mixing, and H_2 addition) were successful in optimizing the production of VFAs and increasing the propionic acid fraction of the VFA mix. This study also may result in a reduction to prefermenters operational cost (if there is a need for a supplemental carbon source) because

glycerol has become relatively affordable due to biodiesel manufacturing. In addition low or no mixing strategies could directly reduce power consumption at plants. The use of hydrogen in the prefermenter is more uncertain since any explosive hazard would need to be eliminated and that might be expensive. In addition it did not result in more VFA production like glycerol addition and low mixing did. However, it did result in more propionic acid being produced, and it may be possible that hydrogen could be produced on site from wastewater or wastewater solids if current research advances. Of all three possible strategies, low or no mixing is the most promising since it directly reduces costs and also directly increases VFA production as well as favoring a significant propionic acid fraction. Glycerol addition will probably only be desirable for plants treating COD limited wastewaters.

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CHAPTER FOUR: GLYCEROL PERFORMANCE AS AN EXTERNAL SUBSTRATE FOR BIOLOGICAL NUTRIENT REMOVAL

Abstract

Four 27.4 L pilot scale anaerobic-anoxic-aerobic (A2O) systems combined with a side-stream prefermenter were operated to study biological nutrient removal (BNR) using glycerol. The research was focused on testing the effects of glycerol addition (prefermenter versus anaerobic rector), and to test the effects of prefermenter mixing intensity on the co-fermentation of glycerol and primary sludge. It was found that glycerol is a suitable renewable external substrate to drive enhanced biological phosphorus removal (EBPR) as well as denitrification. The results from both glycerol adding points were beneficial to the BNR system, and had similar effluent quality. Total nitrogen (TN) removal ranged between 79% and 86% (48.3 - 52.7 mg-N/L), also phosphorus removal ranged between 85% and 93% (4.55 - 4.96 mg-P/L) during the whole study. Direct addition of glycerol had the lowest observed yield (Y_{obs}) in the experiments. Co-fermentation of glycerol caused a significant ($p<0.05$) increase in the volatile fatty acids (VFAs) production (especially propionic acid) even higher than the theoretical glycerol dose effect (assuming 100% conversion = 427 mg-COD/L) implying higher fermentation of the primary solids has occurred. Lower mixing intensity also caused a significant ($p<0.05$) increase in VFAs production (especially propionic acid).

Keywords biological nutrient removal; enhanced biological phosphorus removal; fermentation; glycerol; mixing energy; volatile fatty acids.

Introduction

Due to the limitation of the existing petroleum energy sources and their negative impact economically and environmentally, scientists are searching for better renewable energy alternatives. Biodiesel is a sustainable, environmentally friendly option to provide clean energy. However; the primary limiting factor for the slow growth of full-scale biodiesel plants is the operational and disposal costs which is significantly higher than that of fossil fuel (Demirbas, 2008). Biodiesel by-products typically contain about 60% crude glycerol (Eguchi et al., 2015).

In biological nutrient removal (BNR), glycerol can be used in two ways. One, glycerol can be used directly as an external carbon substrate. The other option is glycerol fermentation to produce volatile fatty acids (mainly propionic and acetic acid). Prefermentation of primary or activated sludge is a common practice in Canada, Australia, and South Africa, but starting to spread in the United States (McCue et al., 2004). Fermentation is a three-stage process (hydrolysis, acidogenesis, and acetogenesis) that results in VFAs production (Henze, 2008; Jia et al., 1996; Metcalf&Eddy, 2014; Valcke & Verstraete, 1983). Danesh and Oleszkiewicz (1997) studied the effect of the fermentation process mixing intensity on anaerobic sequencing batch reactor using raw wastewater and found that production of VFAs can be optimised by reducing the mixing intensity.

Using glycerol as a carbon source for denitrification is very effective, and the best C-glycerol/N ratio is ≈ one, which means that glycerol has a lower denitrification requirements than methanol (2.6 C-methanol/N) (Grabińska-ńoniewska et al., 1985). Methanol is used in most full-scale wastewater treatment plants. However; glycerol is proven to have a higher denitrification rate (up to three times) than methanol. Also, glycerol is more economical to use since methanol prices are increasing. Furthermore; using glycerol may offset the biodiesel waste disposal costs (Lu & Chandran, 2010). The addition of crude glycerol to the denitrification tank in full-scale wastewater treatment plants increases the denitrification by 2-5 mg NO_2-N/L, and the NOx (nitrite + nitrate) removal up to 65% (Bernat et al., 2016). Co-fermentation of waste activated sludge and crude glycerol for denitrification increased the denitrification rate 0.23 mg-N/mg-VSS*day in a sequencing batch reactors with synthetic wastewater (Bernat et al., 2016).

An 800-day study was run using laboratory-scale sequencing batch reactors (SBRs) filled with raw wastewater to test the potential of crude glycerol (CG) as a direct carbon source addition for EBPR. The experimental data found that prefermentation of crude glycerol resulted in unstable EBPR even though the glycogen accumulating organisms (GAO) fraction was less than 4.9%. Raw CG addition achieved excellent phosphorus removal and better EBPR stability than fermented products (Coats et al., 2015). However; Shen and Zhou (2016) suggested that glycerol fermentation is essential for EBPR to utilize it as readily biodegradable carbon oxygen demand (rbCOD) (mainly propionic and acetic acid). When glycerol was co-fermented with waste activated sludge, it resulted in a

significant VFAs production and superior phosphorus removal. In the same study, direct glycerol addition caused EBPR failure when substituted for acetate in lab-scale batch reactors (Yuan et al., 2010). Guerrero et al. (2012) found that glycerol can be directly added to the anaerobic zone if allowed enough time to ferment inside the reactor and produce VFAs. It was found that the optimal conditions are using 4 hours anaerobic and 3.5 hours aerobic. However; the polyphosphate accumulating organisms (PAOs) did not directly use the glycerol, but the long anaerobic conditions allowed degradation of glycerol to VFAs. The aim of this study is to optimise the performance of the A_2O-BNR system by investigating the effect of glycerol adding locations (co-fermentation with primary solids versus direct addition to the anaerobic or anoxic zone) on EBPR and denitrification. Also, the effect of mixing intensity on the glycerol co-fermentation was studied with respect to VFAs production, and the BNR system performance.

Materials and Methods

Pilot plant Configuration and Operation

Two activated sludge pilot plants were constructed at the Iron Bridge Wastewater Reclamation Facility (IBWRF) (Oviedo, Florida). The process schematic are shown in Figure 10. Design, and operational parameters are listed in Table 3. The mainstream consisted of an A_2O BNR process (anaerobic/ anoxic/ aerobic) followed by a secondary clarifier. The working volume of the reactors were: anaerobic (3.6 L), anoxic (5.9 L), and aerobic (18 L). A 10 L side-stream prefermenter reactor was added to each pilot system.

The raw wastewater was collected daily from Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) and transported after screening with a 1/4 in steel mesh to a 400 L influent tank. The prefermentation reactor was filled daily with 2 L of primary sludge obtained weekly from Glendale Wastewater Treatment Plant (Lakeland, Florida) and stored in a 4°C refrigerator.

The prefermenter effluent in both pilots was fed into the anaerobic reactor at a flowrate of 2 L/day. To facilitate nitrogen removal, a nitrate recycle (NARCY) was established from the aerobic to the anoxic reactor at a target rate of 200% of the influent flow rate. The return activated sludge (RAS) was set at half the influent flow rate. Influent, RAS, and NARCY flow rates were obtained using flexible tubes and adjustable peristaltic pumps. 10% of the total reactor volume (added together) was wasted each day to maintain a 10 day solid retention time (SRT). To keep the mixed liquor suspended solids suspended, anaerobic, anoxic, and prefermenter reactors were equipped with 50 rpm mixers except in the prefermenter of pilot plant 4 (PP4) that was equipped with a 7 rpm mixer. The aerobic reactor was equipped with an adjustable air pump fitted with 4-inch diameter air stone disks to maintain a sufficient oxygen supply and provide optimal wastewater to microorganisms contact/mixing. The solid-liquid separation was done using a secondary clarifier between the aerobic reactor and the effluent tank. The clarifier was equipped with a 1.1 rpm skimmer. Two phases were investigated in this study.

Phase one consisted of two Pilots named PP1 and PP2 (Figure 10). The experimental variable in phase one was that PP1 received a constant flow of 0.5 L/day of a 7000 mg-glycerol/L stock solution that was pumped into the prefermenter. This corresponded to 3500 mg-glycerol/day (4270 mg-COD/day). In PP2, the same glycerol dose was added directly to the anaerobic zone (Table 4).

In phase two, the same pilot configuration (Figure 10) was used for two pilots named PP3 and PP4. The same 3500 mg-glycerol/day was pumped to the prefermenters of both pilots. The experimental variable in phase two was that PP3 prefermenter was mixed at 7 rpm while PP4 prefermenter was mixed at 50 rpm. Glycerol dose and experimental variable are listed in Table 5.

The experiment lasted for 185 days including a two month acclimation period for the biomass and the prefermenters, two month for phase one and the same for phase two. Phase one consisted of eight comprehensive sampling events and six comprehensive sampling events for phase two.

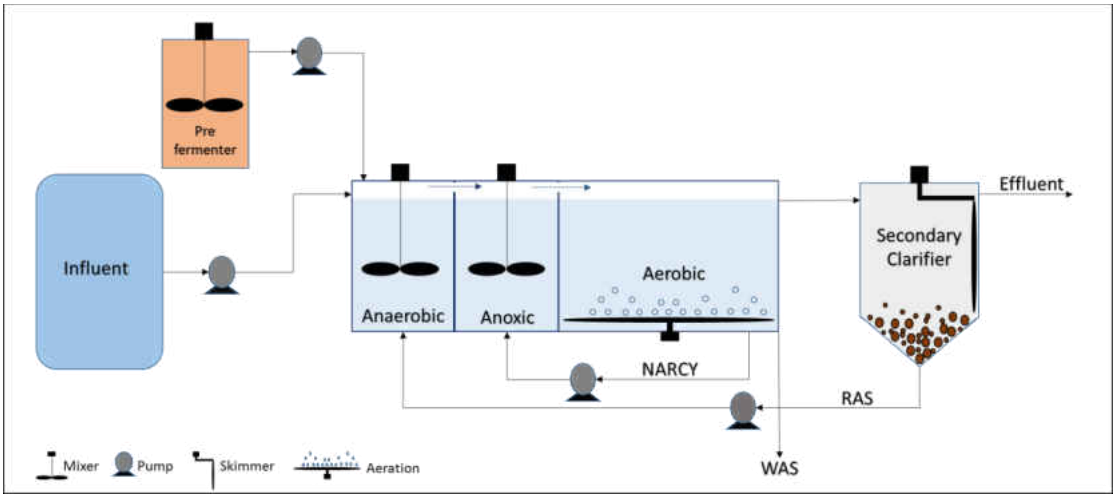

Figure 10 Pilot Plant schematic

Table 3 Pilot plant design, and operational parameters

		Volume					Flow rate				
		AN	AX	AE	Cla	PF	Influent	NARCY	RAS	WAS	PF
		L					L/day	% Influent		L/day	L/day
Phase 1	PP1	3.6	5.9	18	3.1	10	59.8	215%	63%	2.7	2.0
	PP2							223%	59%		
Phase 2	PP3	3.6	5.9	18	3.1	10	51.7	219%	68%	2.7	2.0
	PP4							200%	80%		

		HRT					SRT	pH	MLSS
		Total	AN	AX	AE	Cla			
		hour					day		mg/L
Phase 1	PP1	11	1.4	2.3	7.1	1.3	9	7.5	2952 ± 343
	PP2						11	7.5	2111 ± 746
Phase 2	PP3	12	1.6	2.7	8.1	1.5	10	7.7	2660 ± 760
	PP4						10	7.7	4480± 943

+/- = 1 standard deviation
AN= anaerobic; AX=anoxic; AE= aerobic; Cla= clarifier; PF= prefermenter

Table 4 Phase one, glycerol adding location and experimental variable

		Glycerol dose		Location of glycerol dose	Experimental variable
		mg-COD/day	mg-COD/L		
Phase 1	PP1	4270	*68.5	Prefermenter	Location of glycerol
	PP2			Anaerobic	Location of glycerol

*normalized to the combined influent flow

Table 5 Phase two glycerol dose and experimental variable

		Glycerol dose		Location of glycerol dose	Experimental variable
		mg-COD/day	mg-COD/L		
Phase 2	PP3	4270	*78.8	Prefermenter	7 rpm PF mixer
	PP4			Prefermenter	50 rpm PF mixer

*normalized to the combined influent flow

Influent Wastewater Characteristics

The pilot plant in each phase of this study received raw wastewater from the same influent tank without any chemical addition. The influent characteristics are listed in Table 6 and Table 7. It should be noted that the differences in the influent numbers between pilots in the same phase are caused by the side-stream prefermenter supernatant entering the BNR system (combined influent).

Table 6 Wastewater influent and side-stream prefermenters effluent characteristics

		Raw Influent		Prefermenters			
		Phase one	**Phase two**	**Phase one**		**Phase two**	
				PF1	**PF2**	**PF3**	**PF4**
TN	mg-N/L	42.7±4.5	52.3±18	207±117	304±170	234±92	285±104
NO_x		0.28±0.1	*0.00	0.72±0.1	0.64±0.4	0.66±0.4	0.98±0.2
NH3		30.3±7.0	33.9±6.1	41.8±4.4	51.3±11	81.3±12	81.4±14
TP	mg-P/L	5.23±1.4	4.42±1.5	52.2±14	65.1±1.8	-	-
SOP		3.70±1.2	3.40±0.9	18.30±2.9	22.9±4.6	29.1±6.2	28.8±6.0
TSS	mg/L	73.3±23	52.8±27	3465±1130	3985±4.6	3790±1898	5427±626
s-COD		155±35	121±23	1850±423	801±237	2737±88	1899±627
TCOD		252±58	209±71	6517±1310	5814±637	7515±2325	8776±1055
VFA	mg-COD/L	51.5±37	*0.00	1471±481	660±455	2875±1658	931±358

- Phase one values are the average of 8 sampling events, and phase two is the average of 6 sampling events
*below detection limit
+/- = 1 standard deviation
- PF= prefermenter

Table 7 Combined influent characteristics

		Phase one		Phase two	
		PP1	PP2	PP3	PP4
TN	mg-N/L	43.9	45.2	59.2	61.1
NO_x		0.28	0.30	0.07	0.03
NH3		30.7	31.1	37.0	38.2
TP	mg-P/L	5.66	5.85	5.35	5.33
SOP		4.18	4.44	4.36	4.34
TSS	mg/L	168	197	195	256
s-COD		205	*225	262	234
TCOD		447	*476	346	302
VFA	mg-COD/L	77.0	74.3	111	31.5
DO	mg /L	0.08		0.07	
pH		7.5		7.7	

*The number includes 68.5 mg-COD/L from direct glycerol addition.
- Phase one values are the average of 8 sampling events, and phase two is the average of 6 sampling events

Analytical Techniques

Samples were collected from the anaerobic, anoxic, aerobic, and secondary clarifier as well as influent and effluent reservoirs in two sample containers. One of the sample containers was filtered immediately on site with a glass fiber filter (Whatman™, 1827-025, Pittsburgh, Pennsylvania) before transporting to the lab. The measurements of chemical oxygen demand (COD), e.g. TCOD and s-COD, ammonia (NH_3), nitrate (NO_3), nitrite (NO_2), total nitrogen (TN), total phosphorus (TP), soluble ortho-phosphate (SOP), total suspended solids (TSS), and volatile suspended solids (VSS) were performed according to the procedures published in Standard Methods (APHA, 2005).

VFAs were measured using a Shimadzu (Columbia, Maryland) gas chromatograph equipped with a Supelco (St Louis, Missouri) Nukol column, and flame ionization detector (FID). The injection port and the detector were maintained at 220°C. Column initial temperature was 110°C and then ramped up at 5°C/min to reach a final temperature of 190°C which was held for 10 minutes. The carrier gas was helium at a flow rate of 20 cm/min, and a 10 mM volatile free acid mix was used to develop the standard curve. In addition, pH and dissolved oxygen (DO) were monitored for all reactors on a daily basis. A paired-samples t-test was conducted to compare the results in both Pilot of each phase.

Results and Discussion

Phase One: Glycerol Dose Location

Many studies agree that fermented glycerol is considered a suitable external carbon source for BNR functions (Bodík, Bisťáková, Sedláček, & Hutňan, 2009a; Coats et al., 2015; Guerrero et al., 2015; Guerrero et al., 2012; Lu & Chandran, 2010; Shen & Zhou, 2016). However, some studies consider direct addition of glycerol to the BNR system to be a leading cause for unstable BNR performance and even EBPR inhibition (Coats et al., 2015; Yuan et al., 2010). This part of the study looks at the effects of side-stream prefermentation in addition to direct (PP2) and fermented (PP1) glycerol addition to an A_2O BNR system.

The prefermenter in PP1 (M=1427, SD=537) had a significantly higher VFAs production than PP2 (M=660, SD=266); $t(7)=3.798$, $p = 0.007$, due to the co-fermentation of primary solids and glycerol. PP1 prefermenter produced 1427 mg-COD/L while the PP2 prefermenter produced 660 mg-COD/L. In fact, the additional VFAs production in PP1 was even higher than the theoretical maximum effect that should result from the addition of glycerol assuming 100% conversion (427 mg-COD/L). This could imply that the addition of glycerol to the prefermenter caused greater fermentation of the primary sludge. The type of VFAs produced was also affected by the addition of glycerol. PP1 prefermenter (M=0.82, SD=0.25) had significantly lower acetic to propionic acid (HAc/HPc) ratio than the PP2 prefermenter (M=1.48, SD=0.70); $t(7)=-2.639$, $p = 0.033$. Glycerol co-

fermentation caused a favourable increase in propionic acid over acetic acid

For the A_2O system, the NOx and SOP profiles are listed in Table 8 a and b. Also, other effluent parameters are presented in Table 9. Both pilots had an excellent SOP effluent quality. Combined influent TP in PP1 and PP2 was 5.7 mg-P/l and 5.9 mg-P/L, respectively. PP1 and PP2 had the same average effluent of 0.6 mg-N/L SOP. It can be seen in Table 10 that PP2 had a significantly higher total SOP release (29.9 mg/L) than PP1 (19.1 mg/L) but a slightly lower SOP uptake/release ratio of 1.13 versus 1.20 in PP1. PP1 had a consistent anoxic P uptake except for one date out of seven, where there was a P release. However, the PP2 anoxic zone had P release in three out of seven dates. The P uptake was dominant in both the PP1 and PP2 anoxic reactor with 3.92 and 5.31 mg/L average, respectively. The percent TP removal in PP1 was 90% and in PP2 was 89%. PP1 had a MLVSS P content of 4.46% while PP2 had 6.29%. Direct glycerol addition in PP2 caused a 41% increase in the phosphorus content, but this was offset by a lower MLVSS concentration of 1795 mg/L in PP2 versus 2509 mg/L in PP1. Nitrate concentration was not significant in the return activated sludge (RAS) and thus, has no effect on the VFAs available for the polyphosphate accumulating organisms (PAOs). It is possible that the glycerol addition in PP2 drove secondary P release (P release without the formation of PHAs) and this was the reason the SOP uptake/release ratio was lower than it was for PP1.

Table 8 Phase 1 NOx (a) and SOP (b) profiles

	(a) NOx (mg-N/L)			
	AN	AX	AE	Cla
PP1	0.2	1.0	9.6	6.3
PP2	0.2	1.9	10.6	8.1
	(b) SOP (mg-P/L)			
PP1	15.3	5.8	0.6	0.6
PP2	19.2	7.4	1.1	0.6

Table 9 Effluent parameter for phase 1

	SOP	TN	NH_3	NOx	T-COD	s-COD	TSS	pH
	mg-P/L	mg-N/L			mg/L			
PP1	0.60±0.2	10.8±2.3	*0.00	6.30±2.3	33.0±6.9	28.9±2.5	7.70±3.5	7.8
PP2	0.60±0.2	11.3±1.9	*0.00	8.10±2.6	31.6±3.1	31.1±4.2	7.00±4.2	7.6

*below detection limit
+/- = 1 standard deviation

Table 10 Phase 1 SOP release, uptake, ratios, and P content

	SOP release	SOP uptake	SOP release/ VFA	SOP uptake /Release	TP removal	TCOD/TP	P content
	mg/L	mg/L	(mg/L-p)/(mg-COD/L)			Ratio	
PP1	*19.1	*22.9	0.25	1.20	90%	**78.9-46.2	4.46%
PP2	*29.9	*33.7	0.39	1.13	89%	**81.4-46.2	6.29%

*are the total SOP release (anaerobic+anoxic), and uptake (anoxic + aerobic); anaerobic release relative to influent SOP.
**First ratio calculated from the combined influent, and the second from raw wastewater influent

The observed yield (Y_{obs}) in PP2 (0.19 mg-VSS/mg-COD) was the lowest in the whole study. PP1 had a Y_{obs} of 0.28 mg-VSS/mg-COD. This could imply that although glycerol was ultimately processed such that it could drive EBPR, perhaps this fermentation of a 3 carbon substrate in the anaerobic zone consumed energy and resulted in a low yield. As stated previously this low yield resulted in a lower P removal than might be expected since PP2 biomass had a higher P content. Another possible explanation is that glycerol might have favoured some organisms higher in the food chain that feed on the microorganisms (e.g. *Protozoa*, *Rotifera*, *Oligochaeta* and *nematodes*) and that it was microorganism predation that resulted in the yield reduction (Mayhew & Stephenson, 1997). However this is speculative since no microscopy data was obtained during the study.

The average combined influent TN was 43.9 mg-N/L and 45.2 mg-N/L for PP1 and PP2 respectively. The effluent TN in PP1 and PP2 were 10.8 and 11.3 respectively. Combined ammonia (NH_3) influent in PP1 was 30.7 mg-N/L and 31.1 mg-N/L in PP2. Effluent NH_3 was below detection limit in both pilots. PP1 removed 80% TN while PP2 removed 75%. Overall, the fermentation of glycerol (PP1) seems to have resulted in greater denitrification than direct glycerol addition to the anaerobic zone (PP2) but not significantly. The data implies that sufficient nitrifying bacteria existed in the aerobic reactors of both pilots to cause complete nitrification. However, effluent NOx suggest incomplete denitrification occurred in the anoxic zone. The specific denitrification rate (SDR) in PP2 was slightly higher (0.073 gNOx/g VSS-day) than PP1 (0.055 gNOx/g VSS-day) even though PP2 had a higher NOx concentration. The reason is believed to be the

SRT difference between PP1 and PP2, 9 and 11 day respectively. The volumetric denitrification rate (VDR) for PP1 and PP2 was 123 and 151 mg-N/L*day respectively.

Both pilots provided excellent COD removal. Influent total CODs (TCODs) in PP1 and PP2 were 447 and 476 mg/L, respectively. A stable low s-COD effluent below 32 mg/L was achieved in PP1 and PP2. No significant effect of fermented versus direct glycerol addition on COD was noticed. The COD removal across the pilot reactors was uniform, with significant removal occurring in the anoxic reactor due to denitrification as well as aerobic removal in the aerobic reactor. PP1 and PP2 removed 93% of the COD. The results from this phase showed that the location of glycerol addition had no effect on phosphorus or COD removal. However, the fermented glycerol in PP1 prefermenter resulted in a significant increase in VFAs production ($p<0.05$), and also increased the propionic acid production relative to acetic acid. This improved prefermenter performance in terms of VFA production might benefit weak wastewaters (i.e. wastewaters with TCOD/TP<40) (Randall, Barnard, & Stensel, 1998). Direct glycerol addition in PP2 resulted in slightly worse denitrification (anoxic and effluent NOx were higher than PP1) and significantly lower Y_{obs}.

Phase Two: Prefermentation Mixing Effect

Prefermentation reactors are mixed to suspend the organic matter and maximize the substrate/microorganisms contact time (Yuan et al., 2011). This phase of the study evaluated the effects of mixing intensity on the prefermenters and the subsequent impact

on the BNR system. PP3 and PP4 received the same raw wastewater influent, and the experimental variable was that the PP3 prefermenter was equipped with a 7 rpm mixer and PP4 with a 50 rpm mixer. Lower mixing energy in the PF3 prefermenter (M=2620, SD=743) increased the VFAs production significantly compared to the higher mixing PF4 (M=789, SD=324); $t(5)=4.033$, $p = 0.008$. There was also a significant increase in propionic acid production in PF3 (M=946, SD=253) compared with PF4 (M=493, SD=147); $t(5)=3.546$, $p = 0.017$. PP3 produced 2620 mg-COD/L while PP4 produced 789 mg-COD/L. PP3 produced 906 mg-COD/L acetic acid, 946 mg-COD/L propionic acid, and 768 mg/L butyric acid. PP4 produced 269 mg-COD/L, 493 mg-COD/L, and 28 mg-COD/L acetic, propionic, and butyric acids respectively. The lower mixing energy caused a significant amount of butyric acid and higher propionic acid production. This could be a result of increased hydrogen transfer in the acidification process facilitated by the biomass stratification and lower sheer force.

The PP3 and PP4 BNR effluent parameters are listed in Table 11. Also, the NOx and P profiles are listed in Table 12. Effluent NOx in PP3 (5.7 mg-N/L) was lower than PP4 (7.1 mg-N/L). Despite receiving lower combined influent TN of 59.2 mg-N/L, the effluent TN in PP3 (7 rpm) was actually higher than PP4 with 10.9 mg-N/L. However, the effluent TIN (total inorganic nitrogen) was lower in PP3 (6.0 mg-N/L) then PP4 (7.2 mg-N/L). The average TN removal of PP3 was 82% while PP4 had 86%. Effluent NH_3 in PP3 was 0.3 mg-N/L and 0.1 mg-N/L in PP4. PP3 NH_3 removal was 99%, and PP4 100% The SDR of PP3 was higher than PP4 with 0.055 gNOx/g VSS-day and 0.035 gNOx/g VSS-

day respectively, but it was caused by the significant MLVSS deference between the two pilots. The VDR for PP3 and PP4 was 125 and 147 mg-N/L*day, respectively.

Phosphorus is removed from wastewater either by assimilation or EBPR (assimilation + stored polyphosphate, i.e. enhanced assimilation). The sequence of anaerobic and aerobic zones causes the EBPR as long as VFAs are available under anaerobic conditions. The combined SOP influent for PP3 and PP4 is 4.4 mg-P/L and 4.3 mg-P/L respectively, while total phosphorus values were 5.4 and 5.3 mg-P/L respectively. PP3 had an effluent SOP of 0.8 mg-P/L while it was 0.4 mg-P/L in PP4. Despite having similar influent and effluent in both pilots, PP3 (7 rpm) had a higher total phosphorus release (34.12 mg/L; see Table 10) than PP4 (25.25 mg/L). PP3 and PP4 had a consistent anoxic P uptake throughout the study with 6.35 and 11.38 mg/L respectively. However, both had virtually similar total SOP uptake/release ratio of 1.1 and 1.2 for PP3 and PP4 respectively, and this is similar to the PP2 in phase one where higher phosphorus release did not correlate with higher phosphorus removal. The phosphorus removal for PP3 was 85%, and it was 93% for PP4. The P content in PP3 was 4.09% and in PP4 was 2.59% (Table 13). PP3 (lower mixing) received higher VFAs from the prefermenter, but that did not cause a significant improvement in phosphorus removal. This may be because the wastewaters had sufficient COD even before prefermenters effluent was mixed with them (e.g. see Table 13 TCOD/TP ratios 47.2 and 43.1 where COD limited systems have values < 40) (Randall et al., 1998).

PP3 had a 0.27 mg-VSS/mg-COD Y_{obs}, and PP4 had 0.45 mg-VSS/mg-COD. The Y_{obs} from PP3 is virtually the same as PP1 in phase one. One factor in the low yield may be the fact that the VFAs received in PP3 were high molecular weight (propionic and butyric acid) rather than more readily degradable acetic acid. The BNR organic removal performance is judged by the COD removal. PP3 had a combined influent TCOD of 346 mg/L, and achieved an effluent of 25.2 mg/L. On the other hand, PP4 had lower combined influent TCOD of 302 mg/L and higher effluent of 31.6 mg/L than PP3, and this corresponds to a removal of 90% and 93% COD for PP3 and PP4 respectively. The results from this phase indicate that there is no significant ($p<0.05$) effect of prefermentation mixing energy on phosphorus, nitrogen, or COD removal. However, lower prefermenter mixing resulted in a significant increase in VFA production ($p<0.05$) and higher propionic acid content which could be significant for VFA limited wastewater. Also, lower mixing had higher P content and lower Y_{obs}. For full scale wastewater treatment plants this means that prefermenters can be operated with lower mixing energy while producing more and better VFAs for EBPR and nitrogen removal. However the impacts of lower prefermenter mixing energy need to be evaluated for a COD/VFA limited wastewater to determine how significant improvements really are. In this study we had systems that already had ample organics in the raw influent, and the VFAs from the prefermenter and from glycerol were probably far in excess of what was needed for EBPR and downstream denitrification. In phase 1 we had P releases driven by direct addition of glycerol, and this P release may have been due to glycerol fermentation rather than formation of PHAs, making it a type of

secondary P release, and resulting in a lower P uptake/P release ratio. In phase 2 we had the system receiving higher VFAs also with a lower P uptake/P release ratio. In this cases the excess VFAs may have driven some form of secondary P release if PHA formation kinetics were already at a maximum. Obviously this hypothesis cannot be answered by our data since we were unable to measure PHAs in this study. If PHA data could be obtained in future studies the effect of excess VFAs might be better understood.

Table 11 Phase two effluent parameters

	SOP	TN	NH3	NOx	T-COD	s-COD	TSS	pH
	mg-P/L	mg-N/L			mg/L			
PP3	0.80±0.4	10.9±3.0	0.30±0.1	5.71±2.7	30.7±3.7	25.2±12	9.26±6.4	7.8
PP4	0.38±0.2	9.00±0.7	0.1±0.3	7.12±2.0	36.3±4.2	31.6±2.2	10.0±6.3	7.8

+/- = 1 standard deviation

Table 12 NOx and SOP profiles

	NOx (mg-N/L)			
	AN	AX	AE	Cla
PP3	0.0	1.8	8.3	5.7
PP4	0.0	2.2	9.8	7.1
	SOP (mg-P/L)			
PP3	21	7.4	0.8	0.8
PP4	19	5.3	0.3	0.4

Table 13 Phase two SOP release, uptake, ratios, and P content

	SOP release	SOP uptake	SOP release/ VFA	SOP uptake /Release	TP removal	TCOD/TP	P content
	mg/L	mg/L	(mg/L-p)/(mg-COD/L)			Ratio	
PP3	*34.12	*37.64	0.3	1.1	85%	**64.72-43.09	4.09%
PP4	*25.25	*29.64	0.8	1.2	93%	**56.55-43.09	2.59%

*The total SOP release (anaerobic + anoxic) and uptake (anoxic + aerobic)
**First ratio calculated from the combined influent, and the second from raw wastewater influent

Conclusions

This work investigated the effects of direct glycerol addition to the anaerobic zone and glycerol co-fermentation in the side-stream prefermenter on an A_2O-BNR system. The prefermenters mixing intensity effects on the BNR systems were also studied. The experimental data showed that:

- In phase one, glycerol directly addition to the anaerobic zone had beneficial effects on the A_2O system similar to prefermentation of the glycerol and made no significant difference ($p>0.05$) in the effluent quality with respect to both P and N.
- Direct addition of glycerol to the anaerobic zone in PP2, resulted in the lowest Y_{obs} in the whole study. In addition a low Y_{obs} was also observed in the system (PP3, in comparison to PP4 observed yields) that received high prefermenter VFAs resulting from low mixing energy in the prefermenter. However, the VFAs had a large propionic and butyric acid content. It may be that the metabolism of 3 and 4 carbon molecules resulted in the low observed yields. These 3 and 4 carbon compounds also resulted in the highest anaerobic P releases, but the lowest P uptake/release ratios. Although the effluent SOPs were similar, these systems reached that result exhibiting very different behavior than the other systems which received VFAs in a more even distribution of acetic and propionic acid (PP1 and PP4). The low observed yields also coincided with high MLVSS P content but total removals didn't exceed that of the systems in parallel with them since there was less sludge

to waste. Another theory is that the low yields may coincide with microorganism predation that resulted in yield reduction, but this conclusion requires further study to confirm it.

- The co-fermentation of glycerol and primary sludge in the prefermenter of PP1 resulted in a significant VFAs increase ($p<0.05$) even beyond the theoretical estimated additional VFAs from the glycerol addition (assuming 100% conversion) suggesting that glycerol caused a higher fermentation of the primary sludge. This synergistic effect could be important in rbCOD or VFAs limited BNR systems.
- Lower prefermenter mixing in PP3 increased the VFAs production significantly ($p<0.05$) (especially propionic acid) but did not correlate with superior EBPR effluent quality. This was possibly because the VFAs were being received in excess of what was required, and it is possible that some benefit from the increased VFAs would be observed in COD limited wastewaters. However, this needs to be evaluated in a future study.

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CHAPTER FIVE: GLYCEROL AS AN EXTERNAL CARBON SUBSTRATE FOR ENHANCING HETEROTROPHIC DENITRIFICATION

Abstract

Two pilot-scale 5-stage Bardenpho^{TM} biological nutrient removal (BNR) systems were coupled with side-stream prefermenters used to improve the BNR systems performance. Direct glycerol addition and fermented glycerol were used to test the suitability of glycerol as a sustainable carbon source for denitrification as well as enhanced biological phosphorus removal (EBPR). The results from both systems were beneficial to the BNR system and resulted in similar effluent quality. Both systems achieved complete denitrification and excellent removal of total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD). Removal of TN in the system with direct glycerol and the fermented glycerol were 92% and 95%, respectively. Similarly, TP removal were 82% in the pilot with direct glycerol addition and 89% in the system with fermented glycerol. Co-fermentation of glycerol and primary solids resulted in a significant increase in VFA production. The pilot that received fermented glycerol had a significantly higher VFA loading ($p<0.05$) and lower observed yield. Also, the side-stream prefermenter supernatant flowing to the second anoxic reactor did not cause high effluent ammonia (NH_3) concentration.

Keywords biological nutrient removal; denitrification; fermentation; glycerol; enhanced biological phosphorus removal; volatile fatty acids.

Introduction

The wastewater from residential and industrial areas cause significant environmental problems if discharged to receiving waters without proper treatment. Wastewater nutrient removal can be achieved chemically through precipitation or biologically through BNR. Biological removal usually consists of multiple zones in series (anaerobic, anoxic, and aerobic). Many well established BNR systems already exist such as A/O, A^2O, University of Cape Town (UCT), and 5-stage Bardenpho^{TM} (Metcalf&Eddy, 2014). BNR process requires a sufficient carbon source to provide high denitrification and enhanced biological phosphorus removal (EBPR) efficiencies, which causes concern since some domestic wastewaters lack sufficient carbon source (Bernat et al., 2016; Wu et al., 2010). Many studies suggested that methanol, propionate, and acetate can be used to meet the carbon requirement for the system (Ahmed et al., 2008; Shen & Zhou, 2016). However, volatile fatty acids (VFAs) (mainly acetic and propionic acids) was found to be the driving force for EBPR. The effect of propionic and acetic acids is well studied (Chen et al., 2004; Lopez-Vazquez et al., 2009; Oehmen, Zeng, Yuan, & Keller, 2005). One way to produce VFAs is the prefermentation process.

The fermentation process is a three step process (hydrolysis, acidogenesis, and acetogenesis) that results in the production of VFAs. Prefermentation of primary solids

mainly results in the production of propionic and acetic acids (Henze, 2008; Metcalf&Eddy, 2014). Merzouki et al. (2005) was not able to establish biological nutrient removal before adding a prefermentation reactor to his sequencing batch reactor (SBR). The prefermenter significantly improved the performance of the system and resulted in 99% nitrogen and phosphorus removal. McCue et al. (2004) studied the prefermentation effect on a UCT process with regard to denitrification and EBPR. The results showed a significant increase in denitrification rate after prefermentation use, but no significant effect was recorded for EBPR. Propionic acid was found to be a better-suited carbon source for BNR systems than acetic acid when pH >7. The reason was thought to be that propionic acid required less energy and less C/Prelease. Also, acetic acid accumulation will favor the glycogen accumulating organisms (GAOs) over time. GAOs consume the VFAs, but they do not contribute to the phosphorus removal (Shen & Zhou, 2016).

Glycerol is a biodiesel by-product which can also be fermented to VFAs. With the increasing demand for biodiesel energy as an alternative sustainable energy source, the disposal cost of biodiesel by-products (mainly glycerol) increases. For wastewater treatment, glycerol could be used as a sustainable and cheap external carbon substrate for biological nutrient removal. Glycerol could be added directly to the BNR process or after fermentation to VFAs. Glycerol has a lower denitrification requirement than methanol with a 1 C-glycerol/N requirement and a 2.6 C-methanol/N requirement for methanol (Grabińska-ńoniewska et al., 1985). Torà et al. (2011) achieved 0.25 g-N/g-VSS*day specific denitrification rate (SDR) using direct glycerol addition in a lab-scale SBR. Other

studies also found that glycerol caused SDR increase (Bernat et al., 2016; Bodík et al., 2009b). Coats et al. (2015) found that fermented crude glycerol caused unstable EBPR performance, and direct addition of crude glycerol to the system resulted in a much better phosphorus removal. However; Yuan et al. (2010) found that substituting acetate with glycerol resulted in EBPR frailer. One way for the direct addition of glycerol to provide excellent phosphorus removal is to allow sufficient anaerobic and aerobic zone hydraulic retention times (HRT) at 4 and 3.5 hours respectively. The anaerobic conditions will cause glycerol degradation to VFAs which will then be used by the Polyphosphate accumulating organisms (PAOs) (Guerrero et al., 2012).

In this study, two identical 5-stage Bardenpho^{TM} pilot plants, Pilot A and Pilot B, were used. Pilot A received glycerol directly into the second anoxic zone, where the VFAs from the side-stream prefermenter were also added. In Pilot B, glycerol was added to the side-stream prefermenter, and then the increased VFAs flowed to the second anoxic tank. Both pilots received raw wastewater in the anaerobic zone. Experiments were conducted on these pilot systems to determine if fermented glycerol or direct glycerol were suitable external substrates for heterotrophic denitrification and EBPR.

Materials and Methods

Source of Wastewater, Primary Solids, and Glycerol

Raw wastewater was obtained from Iron Bridge Wastewater Reclamation Facility

(Oviedo, Florida). Before the wastewater was transported to the 400 L influent tank, it was screened with a 1/4 inch steel mesh. A weekly supply of primary solids was received from Glendale Wastewater Treatment Plant (Lakeland, Florida) which was refrigerated at 4°C. A 99.5% pure glycerol ($HOCH_2CH(OH)CH_2OH$) was obtained from Fisher Scientific (Tampa, FL).

Pilot Plant Configuration and Operation

Two identical BNR pilots were built at Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida), named Pilot A and Pilot B. The pilot consisted of a five-stage Bardenpho^{TM} BNR system as the mainstream (anaerobic, anoxic I, aerobic I, anoxic II, and aerobic II), and a side-stream 10 L prefermenter. A 400 L influent tank was cleaned and filled daily with raw wastewater. Then, the wastewater was pumped using flexible tubes and peristaltic pumps to the anaerobic zone at a target flow rate of 50 L/day. A 3.1 L secondary clarifier fitted with a 1.1 rpm skimmer received the pilot effluent (second aerobic effluent) to facilitate the liquid-solid phase separation. The anaerobic, anoxic I, anoxic II, and the side-stream prefermenter were equipped with 50 rpm mixers to keep the solids suspended. Adjustable air pumps with 4 and 2 inches stone disks were installed in aerobic I and aerobic II respectively to oxygenate and suspend the mixed liquor, and optimize the microorganisms contact with the wastewater. Two recycle lines were established using flexible tubes and peristaltic pumps. The first was the nitrate recycle (NARCY) line which was pumped from the aerobic I to the anoxic I at a target flow rate of 200% of the influent

flow. The second was the return activated sludge (RAS) which was pumped from the secondary clarifier to the anaerobic zone at a target flow rate of 50% of the influent flow. 10% of the total reactors volume was manually wasted daily from the aerobic I zone through the waste activated sludge (WAS) line to maintain a 10 day system solid retention time (SRT). The side-stream prefermenter was filled daily with 2 L primary solids. Also, 2 L/day of the side-stream prefermenter effluent was pumped to the anoxic II zone. Pilot A received a glycerol dose of 3500 mg-glycerol/day (4270 mg-COD/day, which equals 76.3 mg-COD/L per liter combined influent flow; i.e. raw influential + prefermenter effluent) dose that was pumped to the anoxic II zone. In Pilot B, the same glycerol dose was pumped to the side-stream prefermenter reactor resulting in 76.3 mg-COD/L per liter combined influential flow. The pilot schematics and additional design and operational information can be found in Figure 11, Table 14, and Table 15. The experiment lasted for 120 days including a 60 day biomass acclimation period for the biomass and the prefermenters. The study consisted of eight comprehensive sampling events.

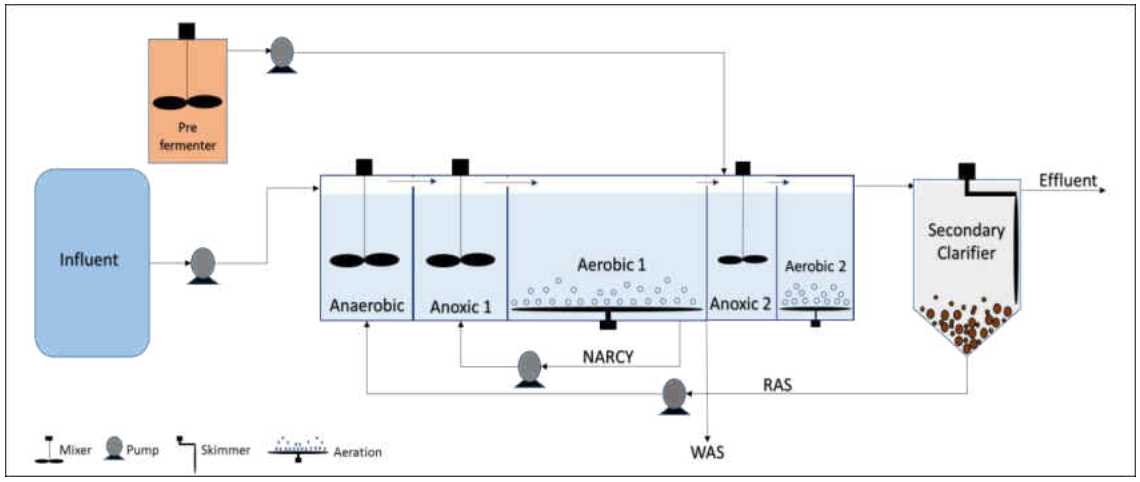

Figure 11 Pilot schematic

Table 14 Pilot design and operational information

Volume (L)	AN	3.6	**Pilot plant**			**A**	**B**
	AX I	5.9					
	AE I	18	**SRT**		day	10	10
	AX II	3.3					
	AE II	0.8	**pH**			7.5	
	total	31.6					
	Cla	3.1	**MLSS**		mg/L	4452 ±969	3238 ±595
	PF	10					
HRT (hour)	AN	1.6					
	AX I	2.6	**Flow rate**	**Influent**	L/day	53.5	
	AE I	7.8					
	AX II	1.4		**WAS**		3.2	
	AE II	0.3		**PF**		2	
	total	13.7		**NARCY**	% Influent	343%	316%
	Cla	1.4		**RAS**		50%	59%

+/- = 1 standard deviation
AN= anaerobic; AX=anoxic; AE= aerobic; Cla= clarifier; PF= prefermenter

Table 15 Experimental variable and glycerol dose

		Glycerol dose		Location of glycerol dose	Experimental variable
		mg-COD/day	mg-COD/L $Influent_{comb}$		
Pilot	A	4270	*76.3	Anoxic II	Location of glycerol
	B			Prefermenter	Location of glycerol

*is normalized to the combined influent
+/- means 1 standard deviation

Influent Wastewater Characteristics

The raw wastewater was pumped from the same influent tank to both pilots at the same time. Also, each side-stream prefermenters effluent was pumped to the anoxic II in Pilot A and Pilot B. The combined influent consisted of both raw influent and prefermenter effluent normalized to their combined flow rate. The raw influent, prefermenter effluent, and the combined influent characteristics are listed in Table 16.

Table 16 Influent characteristics

		Raw Influent		Prefermenters		Combined Influent	
		Pilot A	Pilot B	PF A	PF B	Pilot A	Pilot B
TN		43.3±4.9		323±134	316±135	53.5±8.1	53.3±7.1
NO_x	mg-N/L	*0		1.16±0.2	0.88±0.1	*0	*0
NH3		34.6±2.9		154±62	89.5±12	39.0±3.2	36.6±3.0
TP	mg-P/L	5.01±0.9		-	-	6.0±1.0	5.8±1.1
SOP		3.50±1.3		33.8±4.7	30.3±13	4.7±1.3	4.3±1.4
TSS		74.1±12		5955±1075	5468±1588	287±41	270±65
s-COD	mg/L	146±42		1597±533	2345±437	265±61	226±52
TCOD		235±40		9088±1514	9223±2258	393±59	400±34
VFA	mg-COD/L	*0		1219±278	2469±737	44.0±10.3	88.3±25
DO	mg /L	0.08					
PH		7.5±0.3					

*below detection limit
+/- means 1 standard deviation

Analytical Techniques

Two 50 mL amber bottles were collected from the influent, prefermenter effluent, secondary clarifier, and all reactors (anaerobic, anoxic I, aerobic I, anoxic II, and aerobic II). One bottle was filtered on site immediately with a glass fiber filter (Whatman 934-AH, Pennsylvania) to allow more accurate results for soluble species. The other bottle was not filtered. TN, NH_3, nitrate (NO_3), nitrite (NO_2), TP, soluble ortho-phosphate (SOP), COD, and total suspended solids (TSS) were analyzed in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 1995). Dissolved oxygen (DO) and pH were measured daily using a YSI Field Dissolved Oxygen probe (Yellow Springs,

Wyoming) and Oakton EcoTestr pH 2 (Vernon Hills, IL) respectively.

VFAs samples were analyzed using a Shimadzu gas chromatograph fitted with Supelco Nukol column (Supelco, Missouri) and flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 20 cm/min. The Nukol column initial temperature was 110°C. Then, the temperature was raised at 5°C/min to reach a final temperature of 190°C which was kept for 10 mins. The injector port and the FID detector temperature were 220°C. All samples were filtered with a 45µm membrane filter. The 1.5 mL GC vial was filled with 1 mL of the sample and 0.5 mL 5% formic acid to adjust the pH. A paired-samples t-test was conducted to compare the results in both Pilot systems.

Results and Discussion

Prefermenter (PF) VFAs Analysis

Pilot A and B were operated the same, each with a side-stream prefermenter discharging 2 L/day into the corresponding second anoxic reactor. The only difference between the pilots was the location of the glycerol dose. The glycerol was added directly to the second anoxic reactor in Pilot A and was added to the prefermenter in Pilot B to be fermented to VFAs before entering the system with the prefermenter effluent. The experimental results showed that the Pilot prefermenter A (PFA, i.e. with no glycerol to the prefermenter) (M=2469, SD=737) produced significantly lower total VFAs than Pilot prefermenter B (PFB) (M=1219, SD=278); $t(7)=5.92$, $p = 0.001$, Figure 12. Propionic acid

was the dominant species in both prefermenters PFA and PFB, followed by acetic acid. There was no significant different in propionic acid production with glycerol co-fermentation (M=980, SD=326) and with no glycerol (M=989, SD=307); t(7)=-0.078, p = 0.940. Butyric acid accounted for 27% of the total VFAs in PFB, while PFA had no butyric acid. PFA and PFB had an acetic to propionic acid ratio of 0.26 and 0.83 respectively. Co-fermentation of glycerol and primary solids in PFB produced double the total VFAs and increased the propionic acid portion significantly.

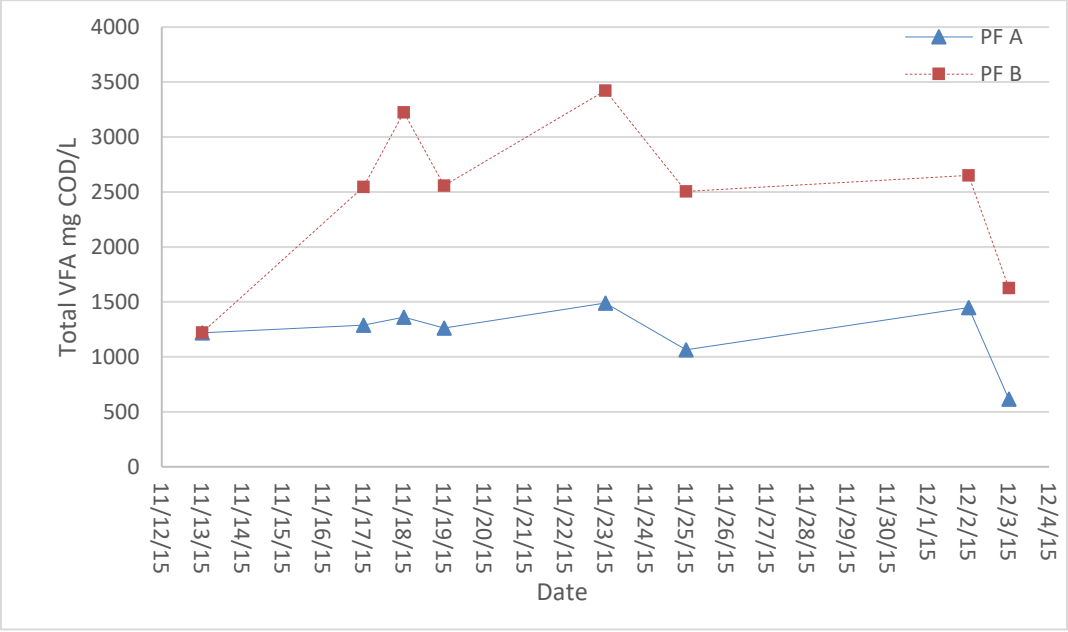

Figure 12 Total VFAs over time for PFA and PFB

Nitrogen Removal - Glycerol Fermentation vs. Pure Glycerol

Both pilots performed in a similar manner regarding ammonia. NH_3 concentration showed a reduction in value from anaerobic to first anoxic zone (Figure 13) and reached 0.6 mg-N/L in Pilot A (direct glycerol) and 0.1 mg-N/L in B (fermented glycerol) in the second aerobic tank. NH_3 reduction from the influent to the anaerobic reactor was caused by the return activated sludge (RAS) dilution. Then NH_3 slightly increased in the second anoxic zone due to the prefermenter effluent entering the reactor. The second aerobic zone oxidized the additional NH_3 entering the second anoxic from the prefermenter effluent (no ammonia breakthrough). Both systems showed a slight increase in first anoxic, NOx concentration compared to the anaerobic tank that was caused by the flow of the internal nitrate recycle (NARCY). Pilot B first anoxic reactor had a complete denitrification (0.7 mg-N/L < 1), and Pilot A did not (1.5 mg-N/L). However, both pilots achieved complete denitrification in the second anoxic (0.8 mg-N/L). Pilot A had slightly higher second aerobic NOx concentration than B, but significant denitrification accrued in the clarifier that caused the overall system (A and B) to have a complete denitrification (Figure 13 and Table 17.). The SDR in Pilot A was lower than B with 0.046 and 0.054 gNOx-N/g VSS-d respectively. However, Pilot A had a higher volumetric denitrification rate (VDR) of 182 mg-N/L*day compared with 159 mg-N/L*day for Pilot B. Even though Pilot A had a higher effluent TN than Pilot B, The effluent TIN ($NOx+NH_3$) in pilot A was actually lower. TN removal for Pilot A and B was 92% and 95% respectively, and both had 98% TIN removal. Both pilots had an excellent performance. Thus, there is no significant

difference in fermented glycerol versus direct glycerol addition with respect to denitrification. However, since almost all the nitrogen is removed in both system, the actual capacity of the pilots remains unknown.

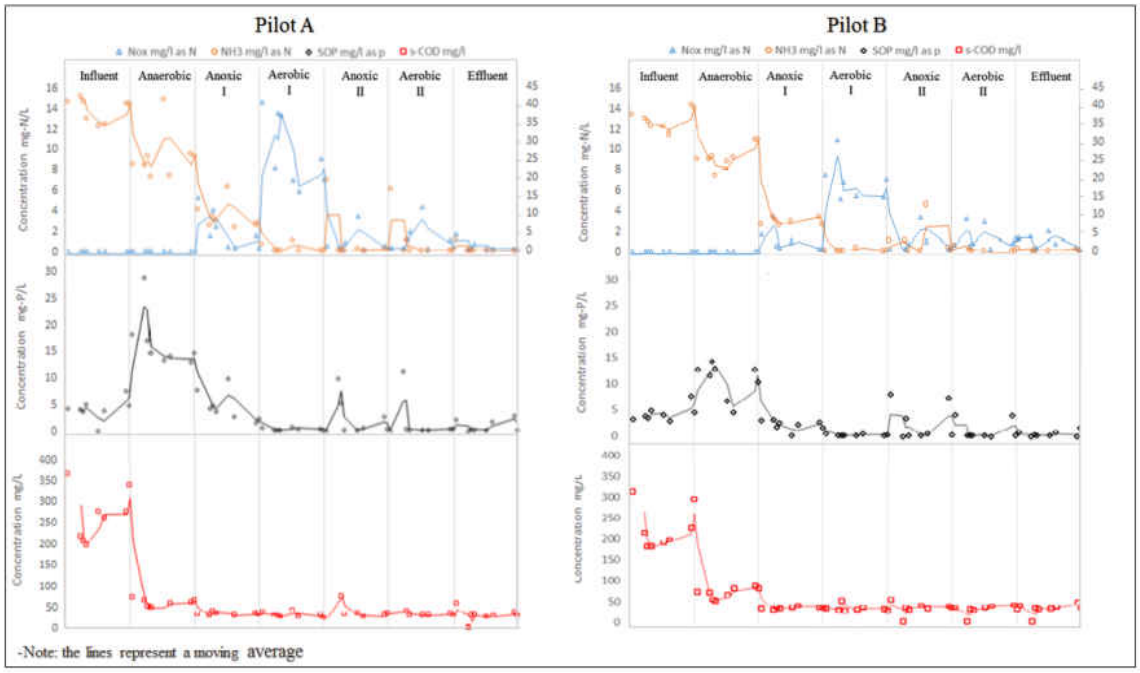

Figure 13 Concentration change in each reactor for NOx, NH3, SOP, and COD

Table 17 Effluent concentration

	SOP	TN	NH3	NOx	T-COD	s-COD	TSS	pH
	mg-P/L	mg-N/L			mg/L			
Pilot A	1.1±1.1	4.4±3.0	0.5±1.2	0.5±0.3	44.3±24	32.7±11	7.7±3.7	7.7±0.3
Pilot B	0.7±0.6	2.8±1.2	0.3±0.4	0.8±0.6	42.0±9.9	35.3±6.1	8.2±4.6	7.8±0.3

+/- means 1 standard deviation

EBPR Performance

Both pilots had anaerobic P release caused by the PAOs. However, Pilot A had a higher anaerobic release (18.2 mg-P/L) than Pilot B (15.5 mg-P/L). P uptake was dominant in the first anoxic reactor of A and B, and both performed similarly. The first aerobic reactor in Pilot A had a higher P uptake (13.7 mg/L) than Pilot B (9.3 mg/L). Even though the anaerobic release in Pilot A was greater than B, the first aerobic reactor in both systems had the same SOP concentration (0.3 mg-P/L) because of the higher uptake in the first aerobic reactor of Pilot A. A secondary P release accrued in the second anoxic reactor of the two pilots. Both Pilot A and Pilot B had a P uptake in the second aerobic tank. This means that EBPR was functional in the second anoxic/aerobic reactors of the two pilots. Clarifier-A had a slight P release, while B had a small P uptake (Figure 13). In the end, both systems performed similarly with Pilot B having a slightly lower effluent SOP (Table 17). The SOP increase in the second anoxic tank was caused partially by the prefermenter effluent entering the reactor. Pilot A had a P uptake/release ratio of 1.19, while Pilot B has a similar ratio of 1.18. TP removal achieved by Pilot A was 82%, but Pilot B had a higher TP removal of 89%. Pilot A (direct addition) had a lower MLVSS P content (3.2%) than Pilot B (fermented glycerol) (4.3%) as can be seen in Table 18. The extra (almost double) VFAs concentration in Pilot B caused a slight improvement in SOP removal.

Table 18 SOP uptake, release, ratios, and P content

	SOP release mg/L	SOP uptake mg/L	SOP release/ VFA (mg/L-P)/(mg-COD/L)	SOP uptake /Release	SOP removal	TCOD/TP Ratio	P content
Pilot A	*21.2	*25.2	0.48	1.19	82%	**65.3-46.9	3.20%
Pilot B	*19.5	*23.0	0.27	1.18	89%	**69.2-46.9	4.23%

* The total SOP release (AN + Ax I + AE I + AX II + AE II) and uptake (AN + Ax I + AE I + AX II + AE II); anaerobic release relative to influent SOP.

**First ratio calculated from the combined influent, and the second from raw wastewater influent

COD Removal and Observed Yield

As can be seen in Figure 13, the COD removal in Pilot A was 92%, and it was 91% in Pilot B. The concentration of COD in the anaerobic zone dropped an average of 89% of the COD in pilot A, and 81% in pilot B. This could have resulted from the RAS dilution. Also, the COD removal rate was higher in the first anoxic reactor than in the first aerobic. This could be caused by the nitrate being used as an electron acceptor by the heterotrophic bacteria in the first anoxic zone. An increase in COD concentration in the second anoxic zone was caused by the prefermenter effluent entering the reactor. The second aerobic zone removed most of the additional COD in the second anoxic zone. The effluent s-COD concentration in Pilot A and Pilot B were 32.7 and 35.3 mg/L respectively. From Table 19, the observed yield in Pilot B was about 25% lower than the observed yield in Pilot A, even though SRT was maintained at 10 days for both systems. It was observed that Pilot B had a significantly higher propionic and butyric acid content ($p<0.05$) and the metabolism of these 3 and 4 carbon molecules could have resulted in the lower observed yield. Also, lower observed yield coincided with higher MLVSS P content.

Table 19 Observed yield

	mg VSS-COD/mg COD	mg VSS/mg COD
Pilot A	0.60±0.15	0.41±0.10
Pilot B	0.45±0.10	0.31±0.07

+/- means 1 standard deviation

Conclusion

This study investigated glycerol as an external carbon source substrate for heterotrophic denitrification. It consisted of two 5-stage Bardenpho^{TM} BNR systems coupled with side-stream prefermenters. The experimental variable was that one of the pilots received a direct dose of glycerol in the second anoxic zone. In the second pilot, the same glycerol dose was fermented to VFAs in the side-stream prefermenter before entering the second anoxic tank. The results showed that:

- Glycerol is a suitable carbon source for EBPR and denitrification as a direct addition or after fermentation to VFAs.
- Both systems achieved complete denitrification.
- The system with direct glycerol addition achieved removals of 92% TN, 98% TIN, 99% NH_3, 82% TP, and 92% COD. The system where the glycerol was fermented to VFAs achieved removals of 95% TN, 98% TIN, 99% NH_3, 89% TP and 91% COD.

- Observed yield was lower in the pilot with fermented glycerol, but that could be a result of having significantly higher propionic and butyric acids or higher MLVSS P content.
- Also, glycerol enhanced the VFA production in the prefermenter significantly ($p<0.05$).
- The side-stream prefermenter effluent entering the second anoxic zone did not elevate the effluent NH_3 concentration.

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CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

BNR systems require a carbon source. VFAs are the most suitable for EBPR and denitrification, but full-scale supplementation is cost prohibitive, and most domestic wastewaters have low VFA concentration. One way to produce VFAs is prefermentation. The other way is to have a long enough anaerobic detention time that fermentation of rbCOD to VFAs occurs. If rbCOD is insufficient, then glycerol fermentation to VFAs or direct glycerol use as a carbon source can be used to drive nitrogen and phosphorus removals. This study consisted of three parts.

The first section of the study was to increase the prefermentation VFA production, reduce the HAc/HPc ratio, and potentially lower operational costs at full-scale BNR facilities with low COD wastewaters. The prefermentation optimization was tested using glycerol co-fermentation with primary solids, mixing intensity, and hydrogen gas (aiming to inhibit acetogenesis) as variables. The result showed that:

- Glycerol increased the prefermenters VFA production even beyond the expected value of glycerol addition alone (assuming 100% conversion). This implies that glycerol have may caused higher fermentation for the primary solids.
- Lowering the mixing intensity of the prefermenter with glycerol and primary solids from 50 rpm to 7 rpm resulted in an additional 80% increase in the VFA production, in addition to a small reduction in the HAc/HPc ratio.

- The additional VFAs production from the glycerol addition and the lower mixing energy (50 to 7 rpm) is potentially important in VFAs or rbCOD limited wastewater for biological nutrient removal. However, the wastewater in this study was not VFA limited most of the time.
- Hydrogen purging to the head space of the prefermenter reactor did not result in an increase in VFA production, but did significantly reduced the HAc/HPc ratio ($p<0.05$). However, hydrogen use poses an explosive hazard that might be expensive to control.

The second part of the study investigated the potential of using glycerol as an external carbon source for EBPR in two ways. Direct glycerol addition to the anaerobic zone, and glycerol co-fermentation in the side-stream prefermenter. This part of the study was performed using two A_2O-BNR systems coupled with side-stream prefermenters. The prefermenters mixing intensity effects on the BNR systems were also investigated. The experimental data showed that:

- Direct glycerol addition and fermented glycerol both had similar beneficial effects on the A_2O system and made no significant difference for EBPR. Thus, there is no need to ferment the glycerol to drive EBPR.
- Fermented glycerol and lower mixing fermented glycerol (50 to 7 rpm) resulted in higher VFAs loading to the system but did not correlate with superior EBPR. However, this may be significant for wastewaters with limited COD

concentrations.

The third and final part of this study investigated glycerol as an external carbon substrate for heterotrophic denitrification. It consisted of two 5-stage Bardenpho™ BNR systems coupled with side-stream prefermenters to test the effect the location where glycerol was added (direct vs. fermented) on denitrification. The data revealed that:

- Both glycerol adding locations resulted in an excellent BNR performance. This means that glycerol does not have to be fermented before being used as a carbon source.
- Complete denitrification was achieved in both systems.
- The system where glycerol was added to the second anoxic zone directly achieved an average removal of 92% TN, 99% NH_3, 82% TP, and 92% COD.
- The system where glycerol was added to the prefermenter before entering the second anoxic zone achieved an average removal of 95% TN, 99% NH_3, 89% TP and 91% COD which was not significantly higher than direct addition of glycerol.
- Fermented glycerol almost doubled the system VFAs loading but did not correlate with superior denitrification since denitrification was almost complete in both second anoxic zones. However, it may be that there would be a difference if the zones were overloaded with nitrate.
- Prefermenter effluent entering the second anoxic zone did not result in high

ammonia concentration in the effluent.

APPENDIX A:
NITROGEN MASS BALANCE AND CALCULATIONS

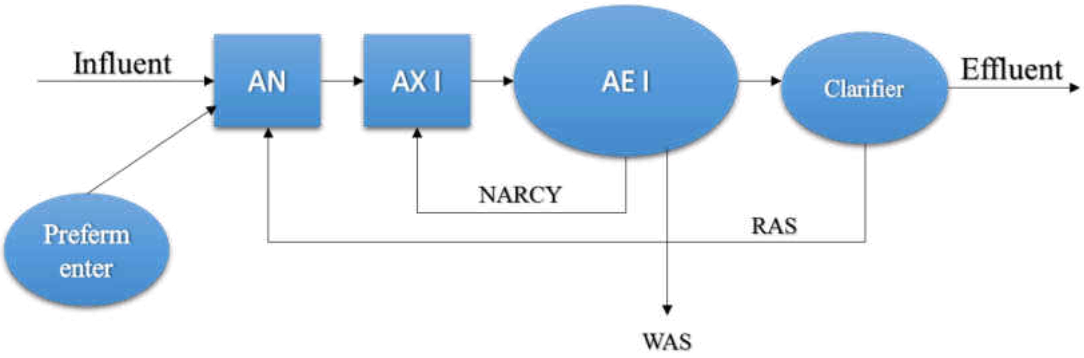


Figure 14 Pilot schematics for nitrogen sample calculations

Table 20 Source data for PP1on 8/12/2015 for nitrogen sample calculations

	Influent	Prefermenter (PF)	Anaerobic	Anoxic I	Aerobic	Cla	Effluent
	(INF)		(AN)	(AX I)	(AE)	(EFF)	(EFF)
TSS	57.0	3767	2847	3173	3053	-	10.0
VSS/TSS	0.85						
TCOD	163	6683	-	-	3878	-	28.0
sCOD	147	1944	80.0	41.0	37.0	29.0	-
TP	3.70	-	-	-	-	-	0.21
SOP	3.00	18.3	18.1	7.40	0.40	0.3	-
TN	43.45	-	-	-	-	6.66	-
NH3	33.6	41.8	22.1	8.99	0.00	0.00	-
NO3	0.31	0.00	0.25	0.28	8.44	5.10	-

***influent numbers are combined ((Cinf*Qinf+CPF*QPF)/(Qinf+QPF))** Table 21.
*** Sample calculation are from PP1 on 8/12/2015**

Table 21 Flowrate and OUR for nitrogen sample calculations

	INF	AN	AX	AE	NARCY	RAS	WAS	EFF	OUR
	L/day								mg/L/day
PAS	51.3	85.0	186	82.2	101	31.7	2.74	53.3	47.3

$Q_{An} = Q_{inf} + Q_{PF} + Q_{RAS}$
$Q_{Ax} = Q_{An} + Q_{NARCY}$
$Q_{Ae} = Q_{Ax} - Q_{RAS} - Q_{NARCY}$

Table 22 Reactor volume for nitrogen sample calculations

	AN	AX I	AE	Total A2O	PF	Cla
	L					
PAS	3.59	5.9	17.95	27.44	10	3.14

1- Calculate the total nitrogen entering the system (TN-in):

- $TN\text{-}in = Q_{inf} * TN_{inf}$
- = 53.3 L/day * 41.8 mg-N/L = 2143 mg/day

2- Calculate the nitrate change in each of the reactors (ΔNOx): negative value represent nitrification and positive value represent denitrification. (md-N/day)

- *ΔNOx Anaerobic* =
  - $(Q_{inf} * NOx_{inf} + Q_{PF} * NOx_{PF} + Q_{RAS} * NOx_{cla} – Q_{An} * NOx_{An})$

    = (51.3 L/day * 0.31 mg-N/L + 2 L/day * 0.00 mg-N/L + 31.7 L/day * 5.10 mg-N/L – 85.0 L/day* 0.25 mg-N/L) = 156 mg-N/day

- *ΔNOx Anoxic* =
    - (Q_{An} * NOx_{An} + Q_{NARCY} * NOx_{Ae} – Q_{Ax} * NOx_{Ax})

      = 85.0 L/day* 0.25 mg-N/L + 101 L/day * 8.44 mg-N/L – 186 * 0.28 mg-N/L) = 820 mg-N/day

- *ΔNOx Aerobic* =
    - (Q_{Ax} * NOx_{Ax} - Q_{NARCY} * NOx_{Ae} – Q_{Ae} * NOx_{Ae} – Q_{WAS} * NOx_{Ae})

      (85.0 L/day * 0.28 mg-N/L – 101 L/day * 0.28 mg-N/L – 82.2 L/day * 8.44 mg-N/L – 2.74 L/day * 0.28 mg-N/L) = - 1516 mg-N/day

- *ΔNOx 2nd clarifier* =
    - (Q_{Ae}* NOx_{Ae} – Q_{RAS} * NOx_{cla}– Q_{Eff}* NOx_{cla})

      (82.2 L/day* 8.44 mg-N/L – 31.7 L/day * 5.10 mg-N/L – 53.3 L/day

      * 5.10 mg-N/L) = 260 mg-N/day

3- Calculate the sum of all denitrifying reactors (ΔNOx .denitrified):

- ΔNOx .denitrified = ΔNOx Anaerobic + ΔNOx Anoxic + ΔNOx 2nd clarifier
    - ΔNOx .denitrified =156 mg-N/day +820 mg-N/day + 260 mg-N/day= 1237 mg-N/day

4- Calculate the effluent Nitrogen (N_{te}):

- $N_{te} = Q_{Eff} *(TN_{Eff} - NO_{x\ cla})$

  N_{te} = 53.3 L/day *(6.66 mg-N/L – 5.10 mg-N/L) = 83.1 mg-N/day

5- Calculate the NO_x in the effluent (N_e):

- $N_e = Q_{eff} *NOx_{cla}$

  N_e = 53.3 L/day * 5.10 mg-N/L= 272 mg-N/day

6- Calculate the nitrogen in the waste sludge (N_{waste}):

- (F_n = 0.1 mg-N/mg-VSS)
- $N_{waste}= Q_{was} * SN_{Ae} + Q_{was} * VSS_{Ae} * F_n$ (mg-N/day)

  N_{waste}= 2.74 L/day * 8.44 mg-N/day + 2.74 L/day * 3053 mg/L*0.85 * 0.1 (mg-N/day) = 689 mg-N/day

7- Calculate the NOx in the waste sludge (NOx_{waste}):

- $NOx_{waste} = Q_{was} * NOx_{Ae}$

  NOx_{waste} = 2.74 L/day * 8.44= 23.2 mg-N/day

8- Calculate the total nitrogen exiting the system (TN_{out}):

- $TN_{out} = \Delta NOx_{-denitrified} + N_{te} + N_e + N_{waste} + NOx_{waste}$

  TN_{out} = 1237 mg-N/day+ 83.1 mg-N/day+ 272 mg-N/day+689 mg-N/day+ 23.2 mg-N/day = 2304 mg-N/day

9- Calculate the percent nitrogen recovery for the system:

- % N recovery = $(TN_{in}/TN_{out})*100$

  % N recovery = (2143 mg/day /2304 mg-N/day)*100

  = **107%**

Other Nitrogen calculations

1. **Calculate assimilated nitrogen (mg/day):**
   - = Q_{was}*$MLSS_{inf\ (comb.)}$*Vss/Tss*(0.1 mg N/mg $VSS_{(Melcer,\ 2004)}$)+ Q_{Eff}*TSS_{Eff}*Vss/Tss*(0.1 mg N/mg $VSS_{(Melcer,\ 2004)}$)

     = 2.74L/day*3053mg/L*0.85* (0.1 mg N/mg VSS) + 53.3 L/day*10.0*0.85* (0.1 mg N/mg VSS) = 757 mg/day

2. **Calculate the available nitrogen for nitrification (mg-N/day)**
   - = Total Nitrogen in - assimilated N
     = 2143 mg/day - 757 mg/day= 1386 mg-N/day

3. **Calculate the percent nitrification for the total nitrogen load.**
   - = ($\Delta NO3_{AE}$/ Total Nitrogen in)
     = (1516 mg-N/day)/ (2143 mg/day) = 71%

4. **Calculate the percent nitrification for the available nitrogen for nitrification.**
   - = ($\Delta NO3_{AE}$/ Quantity of N remaining for nitrification)
     = (1516 mg-N/day) / (1386 mg-N/day) = 109%

5. **Calculate the denitrification rate (DR) (mgNOx-N/day), specific denitrification rate (SDR) (mgNOx-N/mg VSS-d), and Volumetric denitrification rate (VDR) (mgNOx-N/L-d):**

   - DR1= (Q_{inf} + Q_{RAS} + Q_{PF})*$NO3_{an}$ +Q_{narcy} *$NO3_{ae}$ -Q_{ax} *$NO3_{ax}$
   - SDR1= (DR)/ (V_{ax})*(TSS_{ax} *VSS/TSS)
   - VDR1=(DR)/(V_{ax})

     - DR1= (51.3 L/day+ 31.7 L/day+ 2 L/day)*0.25 mg-N/L+101 L/day*0.25 mg-N/L -186 L/day * 0.28 mg-N/L= 820 mgNOx-N/day

     - SDR1= (820 mgNOx-N/day)/ (5.9 L)*(3173 mg/L*0.85)= 0.052 mgNOx-N/mg VSS-d

     - VDR1=(820 mgNOx-N/day)/5.9L= 139 mgNOx-N/L-d

- *For The 5-Stage Bardenpho^{TM}:*
  - $DR2=Q_{ae1}*NO3_{ae1}+Q_{PF*}NO3_{PF}-Q_{ax2}*NO3_{ax2}$
  - $SDR2= (DR)/ (V_{ax2})*(TSS_{ax}*VSS/TSS)$
  - $VDR2=DR/V_{ax}$

APPENDIX B:
PHOSPHORUS MASS BALANCE AND CALCULATIONS

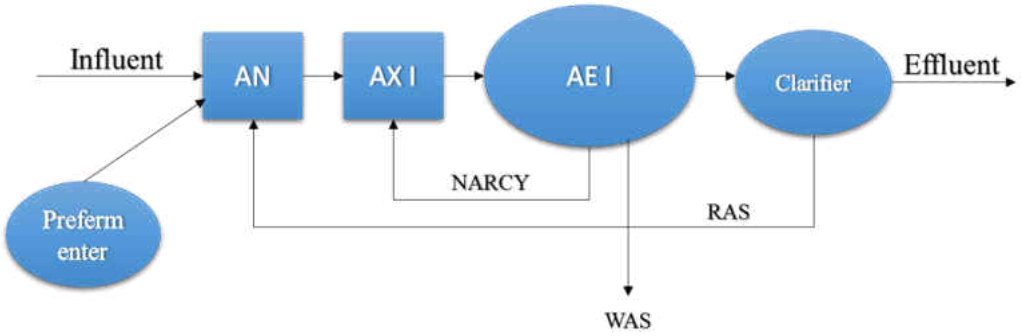


Figure 15 Pilot schematics for Phosphorus sample calculations

Table 23 Source data for phosphorus sample calculations

	Influent	Prefermenter (PF)	Anaerobic	Anoxic I	Aerobic	Cla	Effluent
	(INF)		(AN)	(AX I)	(AE)	(EFF)	(EFF)
TSS	57.0	3767	2847	3173	3053	-	10.0
VSS/TSS	0.85						
TCOD	163	6683	-	-	3878	-	28.0
sCOD	147	1944	80.0	41.0	37.0	29.0	-
TP	3.70	-	-	-	-	-	0.21
SOP	3.00	18.3	18.1	7.40	0.40	0.3	-
TN	43.45	-	-	-	-	6.66	-
NH3	33.6	41.8	22.1	8.99	0.00	0.00	-
NO3	0.31	0.00	0.25	0.28	8.44	5.10	-

***influent numbers are combined ((Cinf*Qinf+CPF*QPF)/(Qinf+QPF))**Table 24.
*** Sample calculation are from PP1 on 8/12/2015**

Table 24 Flowrate and OUR for phosphorus sample calculations

	INF	AN	AX	AE	NARCY	RAS	WAS	EFF	OUR
	L/day								mg/L/day
PAS	51.3	85.0	186	82.2	101	31.7	2.74	53.3	47.3

$Q_{An} = Q_{inf} + Q_{PF} + Q_{RAS}$

$Q_{Ax} = Q_{An} + Q_{NARCY}$

$Q_{Ae} = Q_{Ax} - Q_{RAS} - Q_{NARCY}$

Table 25 Reactor volume for Phosphorus sample calculations

	AN	AX I	AE	Total A2O	PF	Cla
	L					
PAS	3.59	5.9	17.95	27.44	10	3.14

1- Calculate the P change in each of the reactors (ΔP): negative value represents P-release and positive value represent P-uptake (mg-P/L influent).

- *ΔP Anaerobic* =

$$\frac{(Q_{inf} * SOP_{inf} + Q_{PF} * SOP_{PF} + Q_{RAS} * SOP_{cla} - Q_{An} * SOP_{An})}{Q_{inf}}$$

(51.3 L/day* 3.0 mg-P/L+ 2 L/day * 18.3 mg-P/L + 31.7 L/day * 0.3 mg-P/L – 85.0 L/day * 18.1 mg-P/L)/51.3 L/day = -26 mg-P/L influent

- *ΔP Anoxic* =

$$\frac{(Q_{An} * SOP_{An} + Q_{NARCY} * SOP_{Ae} - Q_{Ax} * SOP_{Ax})}{Q_{inf}}$$

(85.0 L/day * 18.1 mg-P/L + 101 L/day * 0.40 mg-P/L – 186 L/day * 7.40 mg-P/L)/51.3 L/day = 3.97 mg-P/L influent

- *ΔP Aerobic* =

$$\frac{(Q_{Ax} * SOP_{Ax} - Q_{NARCY} * SOP_{Ae} - Q_{Ae} * SOP_{Ae} - Q_{WAS} * SOP_{Ae})}{Q_{inf}}$$

(186 L/day * 7.4 mg-P/L - 101 L/day * 0.40 mg-P/L – 82.2 L/day * 0.40 mg-P/L – 2.74 L/day* 0.40 mg-P/L)/51.3 L/day = 25.4 mg-P/L influent

- *ΔP 2nd clarifier* =

$$\frac{(Q_{Ae} * SOP_{Ae} - Q_{RAS} * SOP_{cla} - Q_{Eff} * SOP_{cla})}{Q_{inf}}$$

(82.2 L/day * 0.40 mg-P/L – 31.7 L/day * 0.3 mg-P/L – 53.3 L/day * 0.3 mg-P/L)/51.3 L/day = 0.14 mg-P/L influent

2- Calculate the total p-release:

- Total p-release= the sum of all negative ΔP.

= -26.1 mg-P/L influent

3- Calculate the total p-uptake:

- Total p-uptake= the sum of all positive ΔP

= 29.5 mg-P/L influent

4- Calculate P-removal :

- P-removal = P-uptake - |P-release|

  P-removal = 29.5 mg-P/L influent - |-26.1 mg-P/L influent | = 3.40 mg-P/L influent

5- Calculate influent P – effluent P: (mg-P/L influent):

- $P_{inf} - P_{eff} = \frac{SOP_{inf} - (Q_{eff} * SOP_{eff})}{Q_{inf}}$

  $P_{inf} - P_{eff} = \frac{3.00 \text{ mg-P/L} - (53.3 \text{ L/day} * 0.3 \text{ mg-P/L})}{51.3 \text{ L/day}}$

  = 3.26 mg-P/L influent

6- Calculate the percent phosphorus recovery for the system:

- % P recovery = (P-removal/($P_{inf} - P_{eff}$))*100

  % P recovery = (3.40 mg-P/L influent)/ (3.26 mg-P/L influent))*100

  = 104%

Phosphorus content

1. **Calculate the solid flux in the WAS.**

   - WAS Solids Flux= Q_{was}*TSS_{ae}
     WAS Solids Flux= 2.74 L/day*3053 mg/L= 8377 mg/day

2. **Calculate the solid flux in the effluent.**

   - EFF Solids Flux= Q_{Eff}*TSS_{Eff}
     EFF Solids Flux= 53.3 L/day*10.0 mg/L = 453 mg/L

3. **Calculate the mg-P/day leaving the system in the solid phase due to normal assimilation.**

   - =0.023 mg P/mg $VSS_{(van\ Haandel\ \&\ van\ der\ Lubbe,\ 2007)}$[EFF Solids

     Flux*(VSS/TSS_{eff}) +WAS Solids Flux*(VSS/TSS_{ae})]

     =0.023 mg P/mg VSS [453 mg/L*0.85 + 8377 mg/day *0.85] = 173 mg-P/day

4. **Calculate the mg-P/day leaving the system from the liquid phase to the solid phase.**

   - P Removed (mg P/d) = [$Q_{inf\ (comb.)}$*$TP_{inf\ (comb.)}$ − Q_{was}*SOP_{Ae} – Q_{eff}*SOP_{eff}]
     P Removed (mg P/d) = [(51.3+2) L/day*5.66 mg-P/L – 2.74 L/day* 0.4 mg-P/L – 53.3 L/day*0.3 mg-P/L]= 209 mg-P/day

5. **Calculate the P removal due to EBPR.**

   - = P Removed (mg P/d) - P in EFF and WAS Solids
     = 173 mg-P/day - 209 mg-P/day = 36.5 mg-P/d

6. Calculate the mg-VSS/day leaving the system.

- VSS Leaving= $VSS_{ae}*Q_{was}+VSS_{eff}*Q_{eff}$
  VSS Leaving= 3053 mg/L*0.85*2.74 L/day+10.0 mg/L*0.85*53.3 L/day
  = 7574 mg-VSS/day

7. Calculate the VSS P %.

- % P Content= [P Removed (mg P/d)/ VSS Leaving]/100
  % P Content= [209 mg-P/day / 7574 mg-VSS/day]/100
  = 2.8%

APPENDIX C:
COD MASS BALANCE AND CALCULATIONS

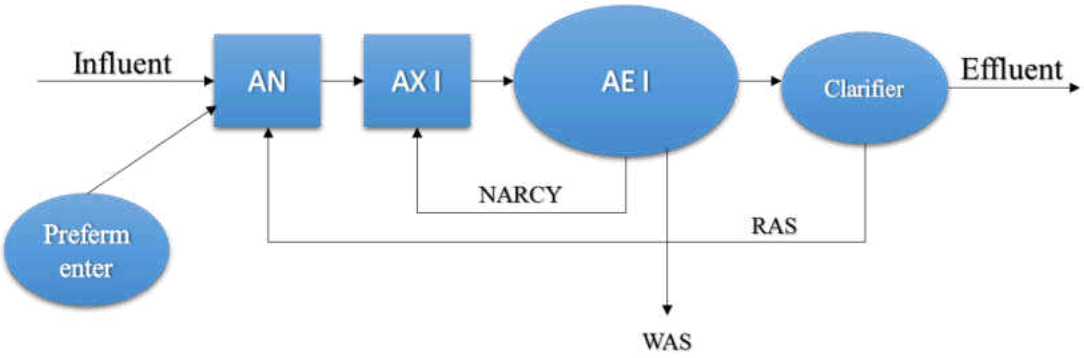


Figure 16 Pilot schematics for COD sample calculations

Table 26 Source data for PP1on 8/12/2015 for COD sample calculations

	Influent	Prefermenter	Anaerobic	Anoxic I	Aerobic	Cla	Effluent
	(INF)	(PF)	(AN)	(AX I)	(AE)	(EFF)	(EFF)
TSS	57.0	3767	2847	3173	3053	-	10.0
VSS/TSS	0.85						
TCOD	163	6683	-	-	3878	-	28.0
sCOD	147	1944	80.0	41.0	37.0	29.0	-
TP	3.70	-	-	-	-	-	0.21
SOP	3.00	18.3	18.1	7.40	0.40	0.3	-
TN	43.45	-	-	-	-	6.66	-
NH3	33.6	41.8	22.1	8.99	0.00	0.00	-
NO3	0.31	0.00	0.25	0.28	8.44	5.10	-

***influent numbers are combined ((Cinf*Qinf+CPF*QPF)/(Qinf+QPF))**Table 27.
*** Sample calculation are from PP1 on 8/12/2015**

Table 27 Flowrate and OUR for COD sample calculations

	INF	AN	AX	AE	NARCY	RAS	WAS	EFF	OUR
	L/day								mg/L/day
PAS	51.3	85.0	186	82.2	101	31.7	2.74	53.3	47.3

$Q_{An} = Q_{inf} + Q_{PF} + Q_{RAS}$
$Q_{Ax} = Q_{An} + Q_{NARCY}$
$Q_{Ae} = Q_{Ax} - Q_{RAS} - Q_{NARCY}$

Table 28 Reactor volume for COD sample calculations

	AN	AX I	AE	Total A2O	PF	Cla
	L					
PAS	3.59	5.9	17.95	27.44	10	3.14

- The COD mass balance in BNR system is defined as:

Mass of COD **entering** the system = Mass of COD exiting the system (effluent + WAS) + Mass of COD oxidized

1- Calculate the total COD entering the system ($TCOD_{in}$):

- $TCOD_{in}$ (mg-COD/day) = Q_{inf} * $TCOD_{inf}$ + Q_{PF} * $TCOD_{PF}$

  $TCOD_{in}$ (mg-COD/day) = 51.3 L/day* 163 mg/L + 2 L//day * 6683 mg/L

  = 21720 mg-COD/L

  - *In the systems with direct glycerol addition the equation becomes:*

- $TCOD_{in}$= Q_{inf} * $TCOD_{inf}$ + Q_{PF} * $TCOD_{PF}$ + $\underline{TCOD_{glycerol} * Q_{glycerol}}$ *(8540 mg-COD/L *0.5 L/day*)

2- Calculate the mass of COD exiting the system (effluent + WAS) ($MCOD_{exiting}$):

- $MCOD_{exiting}$ (mg-COD/day) = Q_{eff} * $TCOD_{eff}$ + Q_{WAS} * $TCOD_{Ae}$

  $MCOD_{exiting}$ (mg-COD/day) = 53.3 L/day* 28.0 mg/L+ 2.74 L/day* 3878 mg/L = 12132 mg-COD/day

3- Calculate the mass of COD oxidized the aerobic reactor ($MCOD_{Ae}$):

- $MCOD_{Ae}$ (mg-COD/day)= OUR * V_{An} - ΔNOx Anoxic * 4.57
  - *OUR= oxygen uptake rate (mg/L/d)*
  - *V_{An} = anaerobic tank volume*
  - *4.57 = mg-O_2/mg-NO3-produced*

  *$MCOD_{Ae}$ = 47.3 mg/L/d *3.59 L - 820 mg-N/day * 4.57 mg-O_2/mg-NO3-produced = 7775 mg-COD/day*

4- **Calculate the mass of COD oxidized in denitrification ($MCOD_{DN}$)**:

- $MCOD_{DN}$ (mg-COD/day)= ΔNOx _denitrified * 2.86
  - *2.86 = mg-O_2/mg-NO3-denitrified*
- $MCOD_{DN}$ = 1237 mg-N/day * 2.86 mg-O_2/mg-NO3-denitrified

  = 3537 mg-COD/day

5- Calculate the total COD leaving the system ($TCOD_L$)

- $MCOD_{TL}$ (mg-COD/day)$_=$ $MCOD_{exiting}$ + $MCOD_{Ae}$ + $MCOD_{DN}$

  $MCOD_{TL}$ = 12132 mg-COD/day + 7775 mg-COD/day + 3537 mg-COD/day = 23445 mg-COD/day

6- Calculate the COD percent recovery ($COD_{R\%}$)

- $COD_{R\%}$ = (23445 mg-COD/day / 21720 mg-COD/L)*100

  = 108%

Solid Retention Time *(SRT) and* Observed Yield *(Y_{obs})*

1. **Calculate the system solid retention time (SRT) (days).**

- $SRT= (VSS_{An}*V_{an}+ VSS_{ax}*V_{ax}+ VSS_{ae}*V_{ae}+ VSS_{ax2}*V_{ax2}+ VSS_{ae2}*V_{ae2}) / (VSS_{ae}*Q_{WAS}+VSS_{eff}*Q_{eff})$

  SRT= (2847 mg/L*0.85*5.9 L+ 3173 mg/L*0.85*5.9 L+ 3053 mg/L*0.85*17.95 L) / (3053 mg/L*0.85*2.74 L/day +10.0 mg/L*0.85*53.3 L/day) = 9 days

  - *In the systems with direct glycerol addition the equation becomes:*
- $SRT= (VSS_{An} *V_{an}+ VSS_{ax}*V_{ax}+ VSS_{ae}*V_{ae}+ VSS_{ax2}*V_{ax2}+ VSS_{ae2}*V_{ae2}) / (VSS_{ae}*Q_{RAS}+VSS_{eff}*Q_{eff})$

2. **Calculate the observed yield (Y_{obs}).**

- $Y_{obs\ (}$mg VSS/mg COD) = $(VSS_{ae}*Q_{RAS}+VSS_{eff}*Q_{eff}) / (TCOD_{inf(comb.)}*Q_{inf(comb.)} - sCOD_{ae}*Q_{was} - sCOD_{eff}*Q_{eff})$

  Y_{obs} = (3053 mg/L*0.85*2.74 L/day +10.0 mg/L*0.85*53.3 L/day) / (408 mg-COD/L*(51.3L/day +2 L/day) – 37.0 mg-COD/L*2.74 L/day – 29.0 mg-COD/L*53.3 L/day)= 0.38 mg VSS/mg COD

- $Y_{obs\ (}$mg VSS-COD/mg COD)= $Y_{obs\ (}$mg VSS/mg COD)* 1.48 mg COD/mg VSS (Mara & Horan, 2003)

  Y_{obs} = 0.38 mg VSS/mg COD * 1.48 mg COD/mg VSS
  = 0.56 mg VSS-COD/mg COD

APPENDIX D:
QUALITY ASSURANCE AND QUALITY CONTROL (QA&QC)

Development of quality control charts (QC):

1- Find the normalized range (I)

- I= Abs(X1-X2)/X1+X2

2- Find the upper warning limit (UWL).

- UWL= Iavrage * 2.512

3- Find the upper control limit (UCL).

- UCL = Iavrage * 3.267

4- Plot the other I values in the graph and track the quality.

Development of accuracy control charts (QA):

1- Find the percent recovery (%R):

- % R= (Final mass – Initial mass / Mass added) *100

2- Find the upper warning limit (UWL).

- UWL= $\%R_{average}$ + 2 * %R standard deviation (SD)

3- Find the upper control limit (UCL).

- UCL = $\%R_{average}$ + 3 * $\%R_{SD}$

4- Find the lower control limit (LWL).

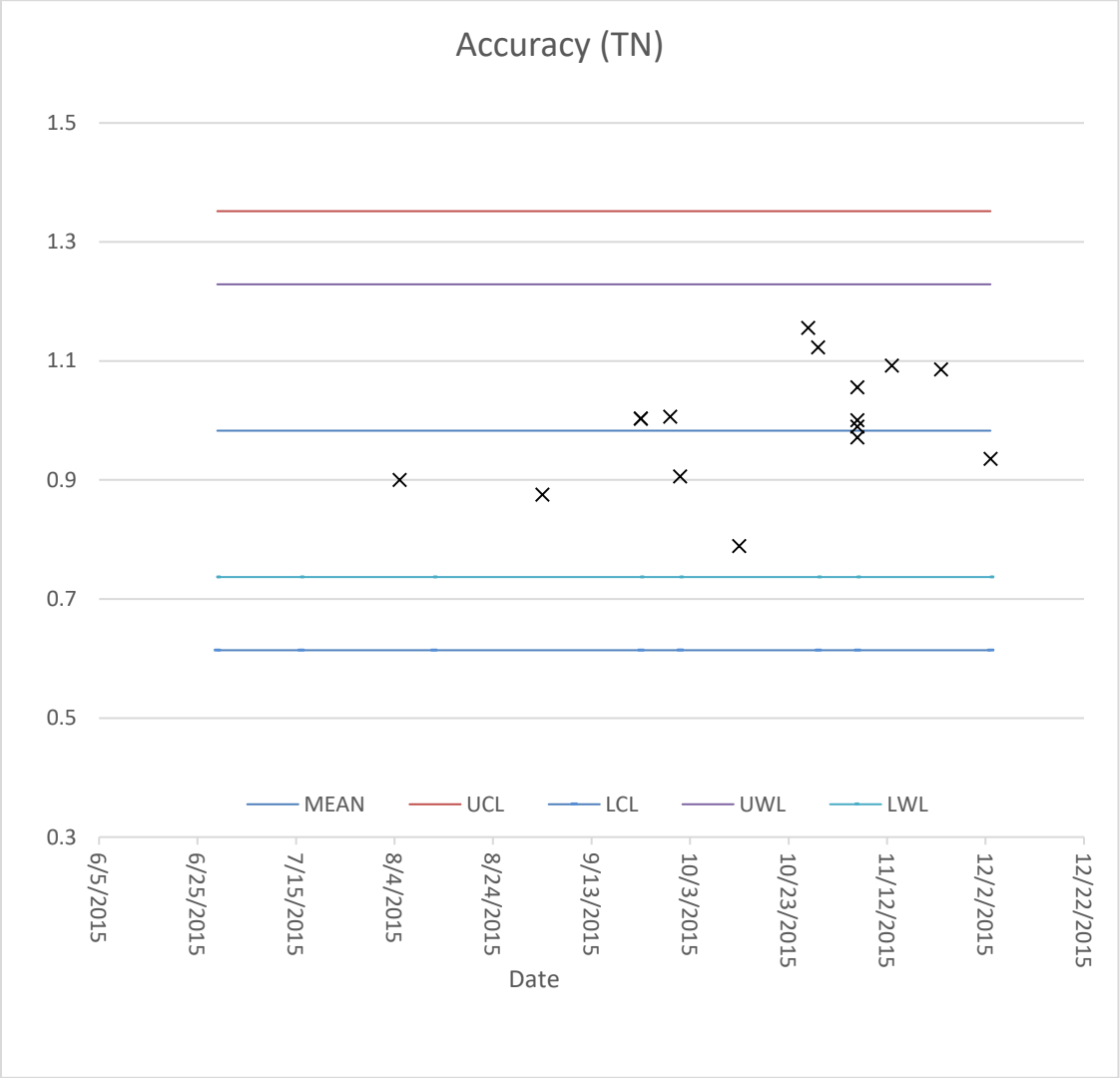
- LWL = $\%R_{average}$ - 2 * $\%R_{SD}$

5- Find the lower control limit (LCL).

- LCL = $\%R_{average} - 3 * \%R_{SD}$

6- Plot the other %R values in the graph and track the accuracy.

QA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7/15/2015
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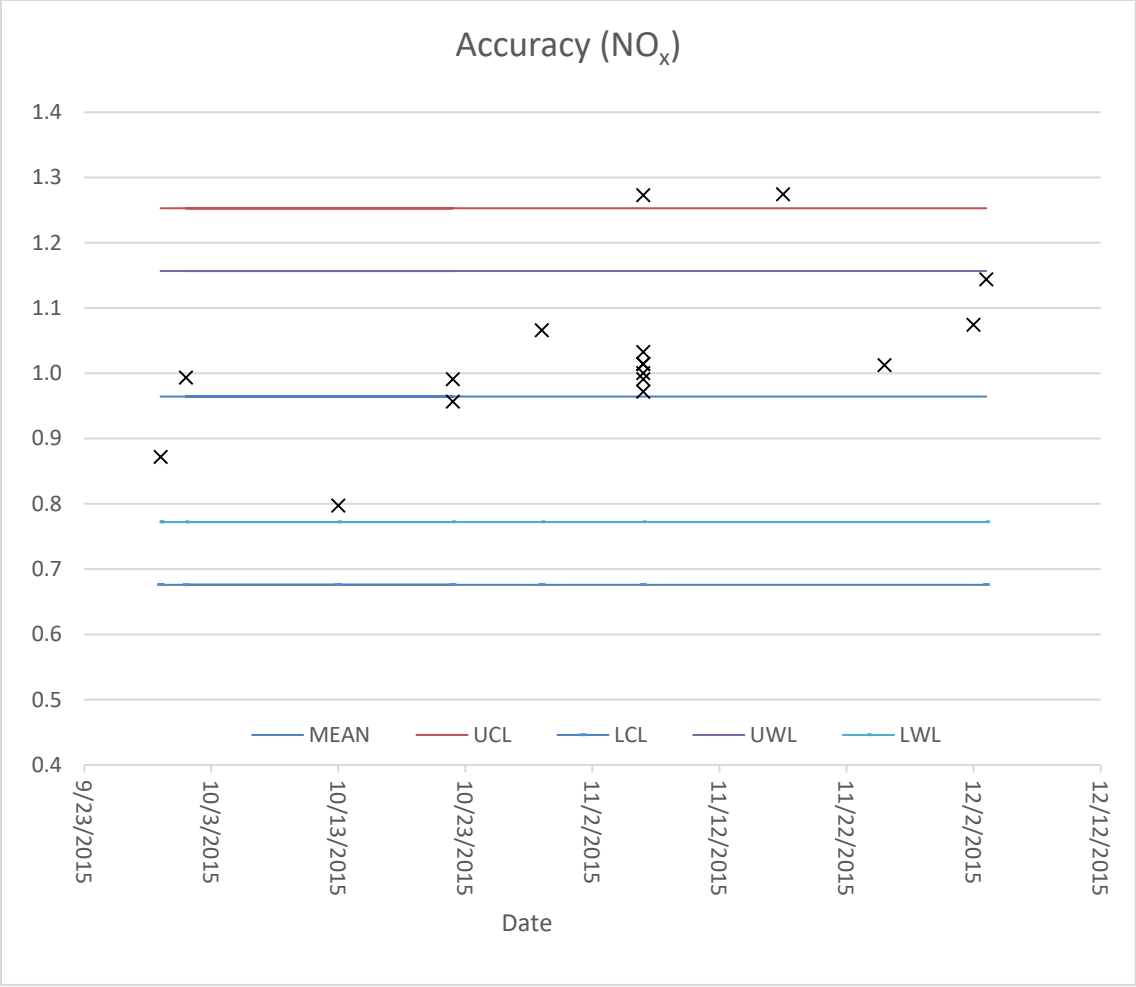
Date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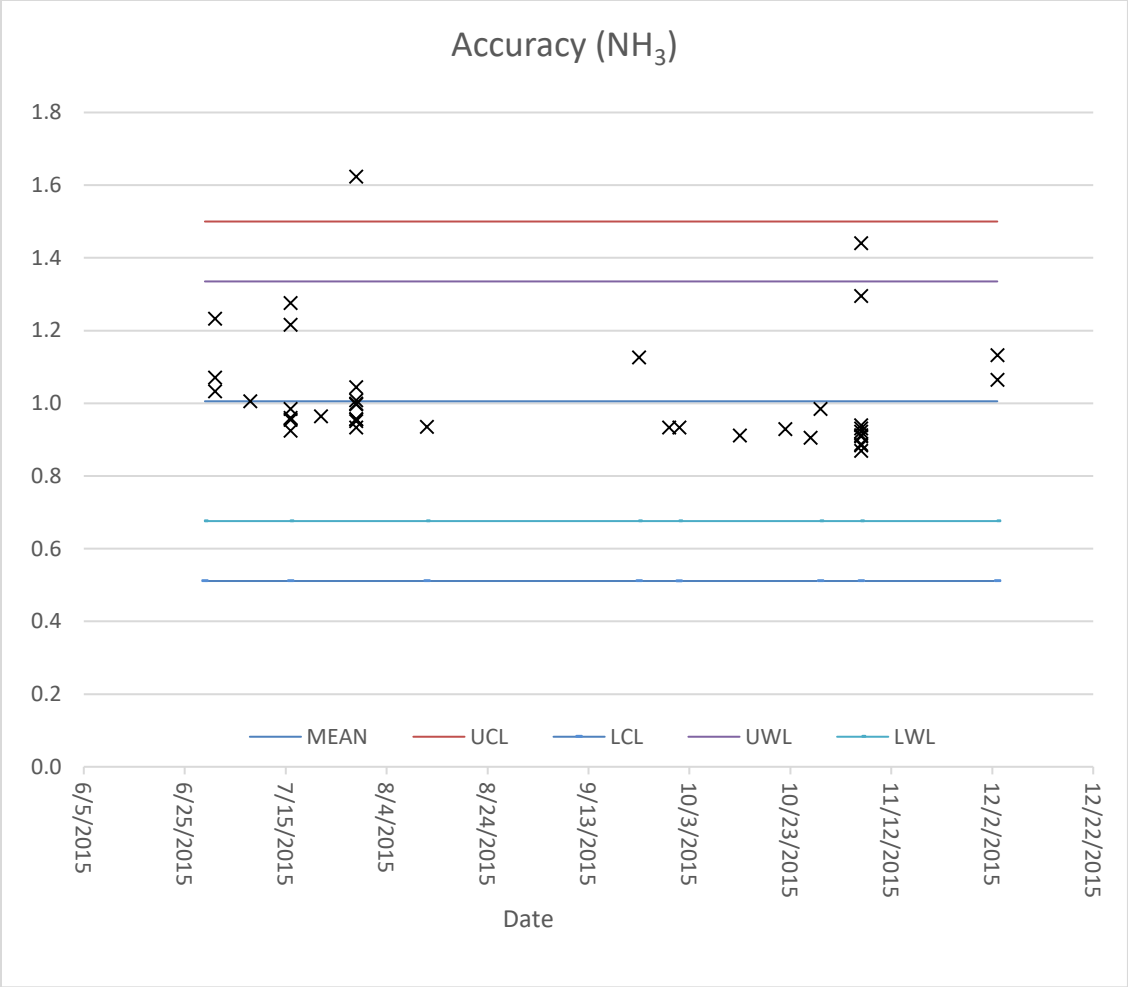
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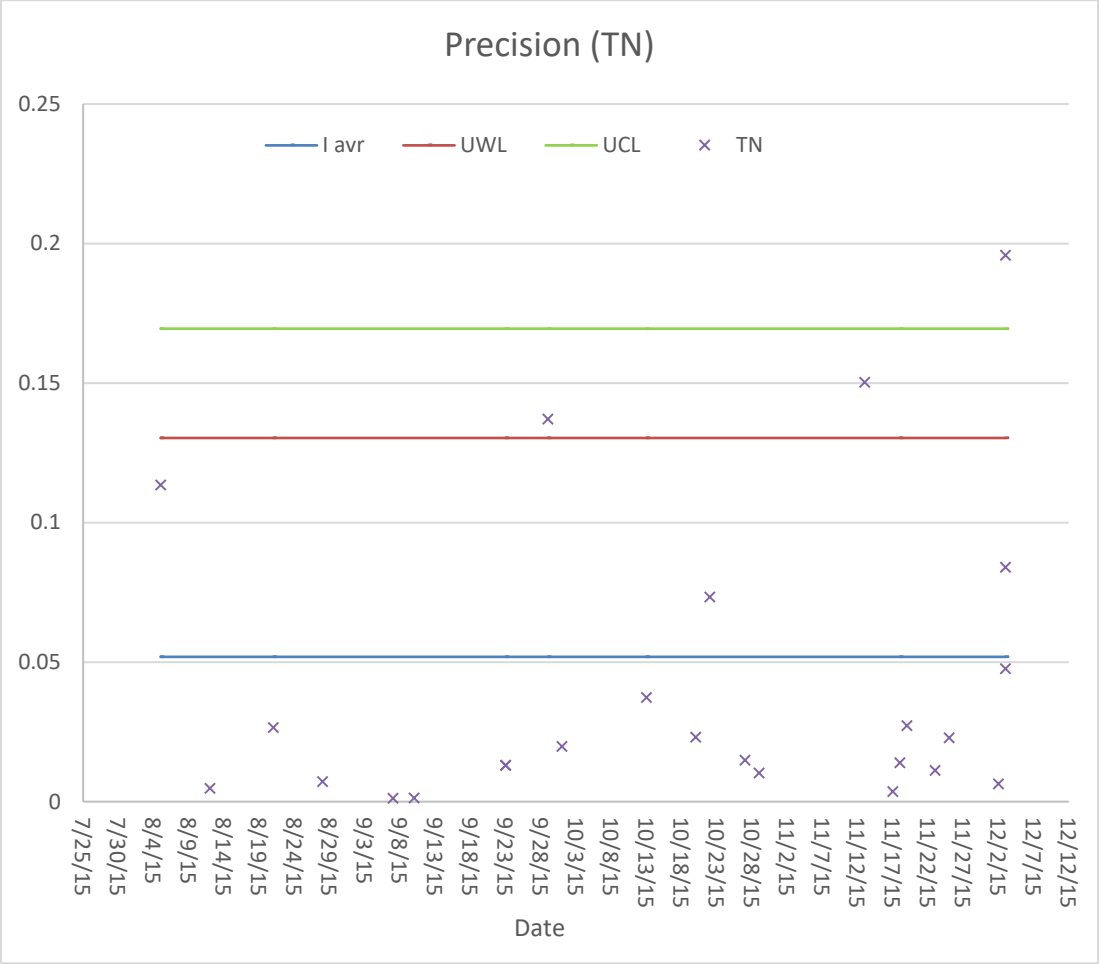
QC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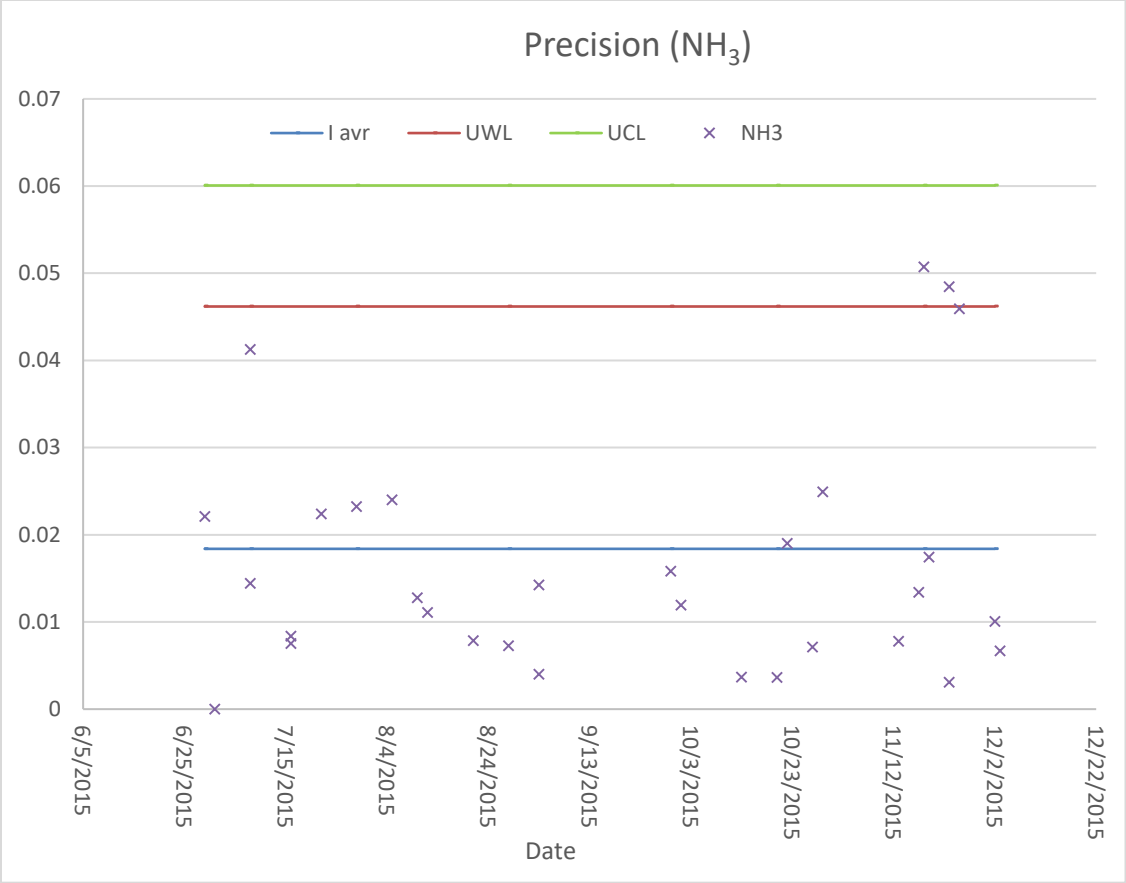
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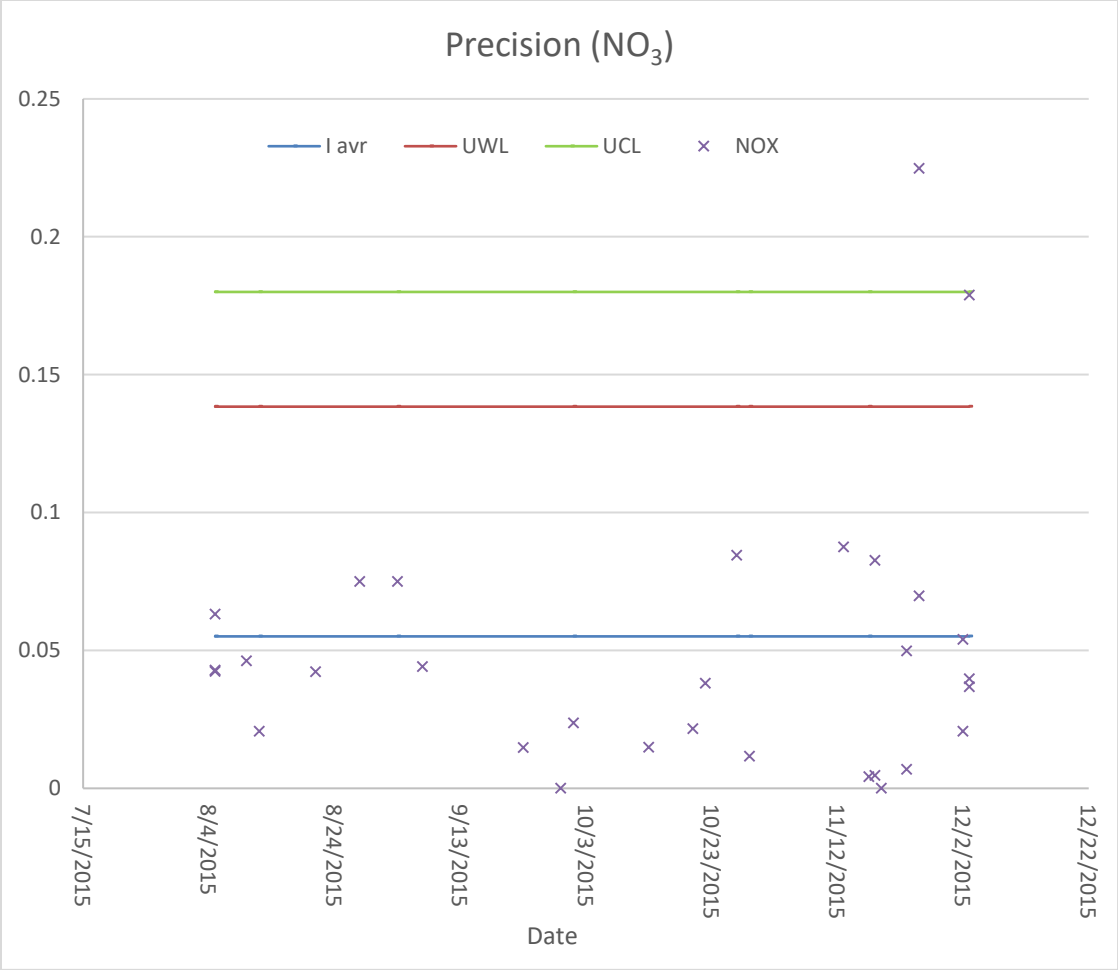
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8/24/2015
9/13/2015
10/3/2015
10/23/2015
11/12/2015
12/2/2015
12/22/2015
Date

APPENDIX E:
LOW SOLID FERMENTATION

(Low Solids Bench-Scale Prefermenters)

Methods and Materials

Three bench-scale semi-continuous reactors with a liquid volume of 1500 ml per reactor were used in this study. The reactors were named PR1, PR2, and PR3. Initially, all reactors received 1.5 L of 50:50 mix of raw wastewater and primary solids obtained from the Glendale Wastewater Treatment Facility (Lakeland, Florida). Reactors PR1 and PR2 were equipped with a 50rpm, and 7rpm Grainger mixers (Orlando, Florida) respectively to keep the solids suspended. PR3 was left with no mixing. All mixers are connected to a U shape plastic blades with 0.31-inch * 13.8-inch shaft dimensions (Cole-Parmer, Vernon Hills, Illinois. Each cycle (i. e. every 24-hour) 375 mL was wasted from each reactor and replaced with 375 mL DI water plus 6500 mg pure glycerol.

Results

PR1, PR2, and PR3 received 6500 mg glycerol daily in a 375 ml DI water and the same amount was wasted to have a 4-day SRT. Propionic acid was the only VFA produced in the reactors. However, VFA production was minimal in all reactors PR1, PR2, and PR3 with 172 ± 331 mg COD/L, 390 ± 299 mg COD/L, and 352 ± 336 mg COD/L respectively. Table 29 summarizes the VFA concentration and composition in this phase. The MLSS and the MLVSS in the reactors are summurized in Table 30.

Table 29 VFA concentration and composition for R3, R4, and R5

	HAc (mg COD/L)	HPr (mg COD/L)	HBu (mg COD/L)	VFA (mg COD/L)
PR1	0	172 ± 331	0	172 ± 331
PR2	0	390 ± 299	0	390 ± 299
PR3	0	352 ± 336	0	352 ± 336

Table 30 R3, R4, and R5 MLSS and MLVSS

	TSS mg/L	VSS mg/L
PR1	22.50	19.12
PR2	237.33	201.73
PR3	118.33	100.58

The results probably implied that a greater SRT was required to build up biomass or to acclimate the population to high glycerol concentrations without washing out the biomass. Since the study only lasted for one month, it was not possible to invistigate this hypothesis.

APPENDIX F:
ADDITIONAL METHODOLOGY INFORMATION

Prefermentation Optimization – Chapter: 3 – R1, R2

In this study, two bench-scale semi-continuous reactors with a volume of 1500 mL per reactor were used to study the effect of hydrogen gas on VFA production at an SRT of 4 days. The reactors were called R1 and R2. Both reactors initially received 1.5 liters of 50:50 mix of primary solids and raw wastewater. Each day, 375 mL (0.375 L) was removed and replaced with 375 mL a 50:50 mix of primary solids and raw wastewater plus 6500 mg of pure glycerol. This was done at the beginning of each cycle (i. e. every 24-hour). R1 received a daily 30-second dose of H_2 gas (purging at the headspace). It was sealed airtight so H_2 could come to equilibrium with the liquid in the reactor. R2 did not receive H_2 gas. The experimental variable was H_2 gas addition (H_2 partial pressure).

Process Configuration for Glycerol Effect and Mixing Intensity – Chapter: 3- PF1, PF2, PF3, and PF4

Prefermentation experiments were carried out in two pilot scale 10 L prefermentation reactors. Both were operated at a 5 day SRT to prevent methanogenesis. Two liters of primary solids were manually added to the prefermenters daily. For the glycerol effect experiment, both prefermenters were mixed at 50 rpm. The first reactor (PF1) received a constant 0.5 L/day glycerol dose using a stock solution with a concentration of 7000 mg pure glycerol/L. This resulted in an initial concentration in the prefermenter of 350 mg-VFAs/L (427 mg COD/L). The second reactor (PF2) was operated without glycerol addition.

For the mixing intensity experiment, both prefermenters received a constant 0.5 L/day glycerol dose of stock solution with a concentration of 7000 mg pure glycerol/L. The experimental variable between the two reactors was that PF3 was mixed at 7 rpm while PF4 was mixed at 50 rpm.

Activated Sludge Pilot Plant

In this experiment, two identical activated sludge pilot plants (named train A and train B) were built at Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida). The base of the plant is a wooden box painted with water resistant paint to minimize spills and protect the reactors. The containment box is also equipped with caster wheels for easy movement.

The reactors were built using 3, 4, 6 and 8-inch diameter (schedule 40) PVC pipes in a vertical orientation to have a low surface area to volume ratio minimizing the oxygen intrusion, and facilitating realistic full-scale representation. The aerobic reactors were equipped with adjustable 4-port 170-gallon Top Fin® Aquarium Air Pumps fitted with 4-inch diameter Top Fin® Aquarium Air Stone Disks in the first aerobic reactors (1), and 1-inch diameter Top Fin® Aquarium Air Stone Disks in second aerobic reactors (2) when used. All other reactors were equipped with 50 rpm Grainger mixers (Orlando, Florida) for suspension of the mixed liquor. Connection pipes were 1-inch diameter (schedule 40) PVC pipes with valves on both ends to allow for maintenance and change in plant configuration. The recycle lines were made of 3/8 inch MasterFlex® flexible tubes going through peristaltic pumps (Model CO 7553-70; Cole-Parmer, Vernon Hills) with variable speed controllers. A 400 L influent tank was fully emptied, cleaned and filled daily with raw wastewater. The two pilot plants were operated in different configurations explained below. All raw influent wastewater was collected from Iron Bridge Wastewater

Reclamation Facility (Oviedo, Florida). The volume and the height of the mixed liquor are listed in Table 31, and at least 2 inches were added to the height in each reactor for overflow protection.

Table 31 Reactor volumes and detailed design for the BNR pilot plant

Reactor	**V(L)**	**Diameter (inches)**	**Diameter (m)**	**Liquid Height (inches)**	**Liquid Height (m)**
Anaerobic	3.59	4	0.10	17.43	0.44
Anoxic 1	5.9	4	0.10	28.65	0.73
Aerobic1	17.95	8	0.20	21.79	0.55
Anoxic 2	3.33	4	0.10	16.17	0.41
Aerobic 2	0.77	3	0.08	6.65	0.17

Preliminary Phase (Acclimation Period)

For the preliminary phase, the two systems (A and B) were configured as A^2/O processes. The configuration of an A^2/O process is three reactors: anaerobic, anoxic, and aerobic, followed by a secondary clarifier. As can be seen in Figure 17, the anaerobic zone receives the return activated sludge (RAS) coming from the secondary clarifier. Also, the anoxic zone was receiving the nitrate recycle (NARCY) from the aerobic zone. The duration of this phase was two months to allow for biomass growth and steady-state conditions. The data from this phase is used to investigate the differences and similarities with other phases once the prefermenters and glycerol dosage were applied.

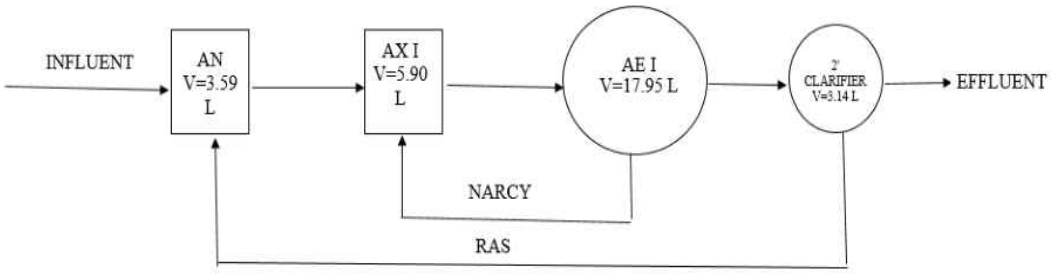

Figure 17 A2/O system setup for both A and B

Phase One (Destination of the Glycerol Dose) - Chapter: 4 - PP1 and PP2

In this phase, plants were operated as an A^2/O systems with a 10-liter pre-fermentation reactor flowing into each anaerobic reactor at a flowrate of 2 L/day. Each day, 2 liters of primary solids from Glendale Wastewater Treatment Plant (Lakeland, Florida) was transferred manually to each prefermenter to maintain a 5 days SRT. The prefermentation reactors were mixed at 50 rpm. The prefermenter A was receiving a constant 0.5 L/day glycerol dose at 7000 mg pure glycerol/L. However, in Train B, the same glycerol dose went to the anaerobic zone instead of the prefermenter making the point where glycerol that was received the experimental variable. Figure 18 illustrates the process configurations for Phase One.

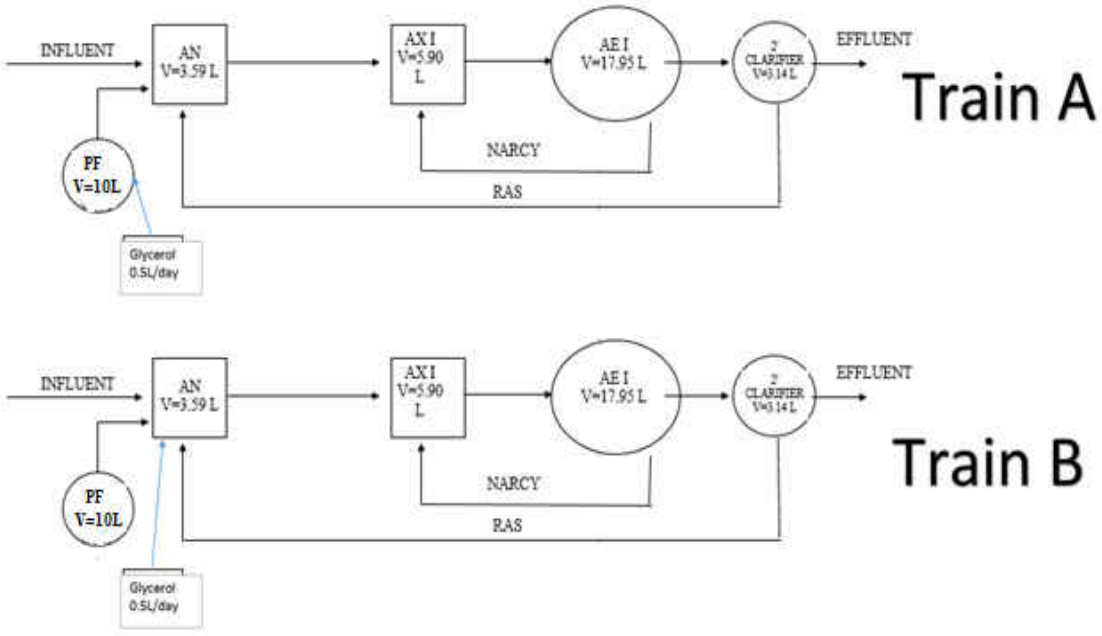

Figure 18 Phase One system configuration

Transition Phase (Effect of Glycerol Dose)

The transition phase was a quick test to study the behavior of the system when glycerol dose was eliminated. In this phase, both Pilots A and B followed exactly the A^2/O configuration in phase one. The destination of the prefermentaion reactors in both pilots (A and B) is the anaerobic reactor. The key different between the two phases is that the glycerol dose in Pilot B is terminated while pilot A still received the same 0.5 L/day pure glycerol dose at 7000 mg glycerol/L flowing to the prefermenter. Figure 19 illustrate the schematics for both trains A and B.

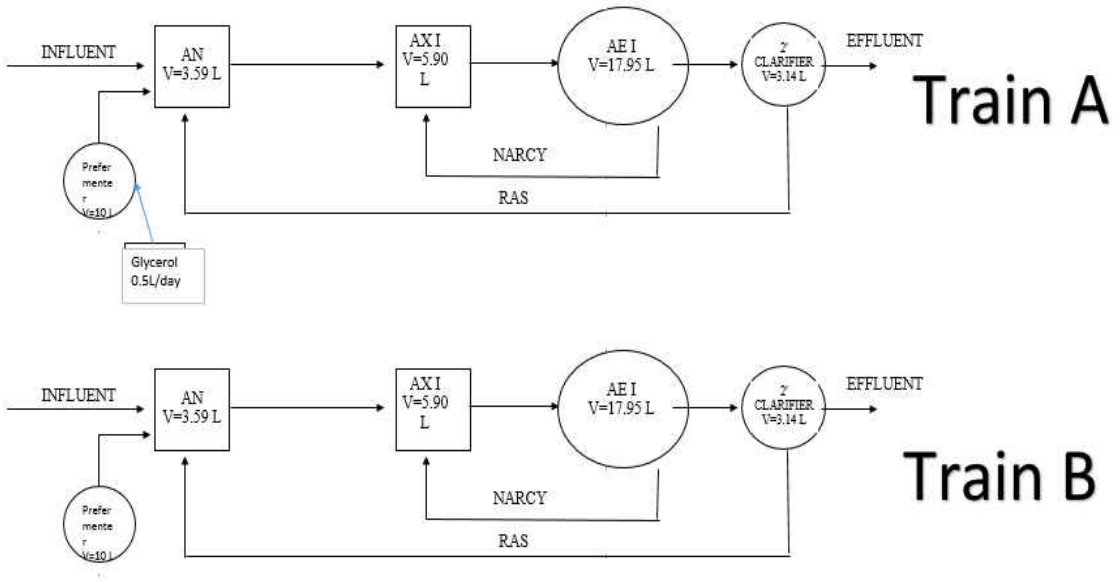

Figure 19 Transition Phase configuration for Pilot A and B

Phase Two (Mixing Intensity in the Prefermenters) - Chapter: 4- PP3 and PP4

In phase two, both systems were following the same A^2/O setup. Prefermenter A and B were dosed with the same pure glycerol (3500 mg/day). Prefermenter A was equipped with a 7rpm mixer while prefermenter B was fitted with a 50 rpm mixer. The purpose of this phase was to study the effect of mixing intensity on the Volatile Fatty Acids (VFA) production and the overall system performance. Thus, the experimental variable in this phase was the mixing intensity. Figure 20 illustrate the schematics for Phase two.

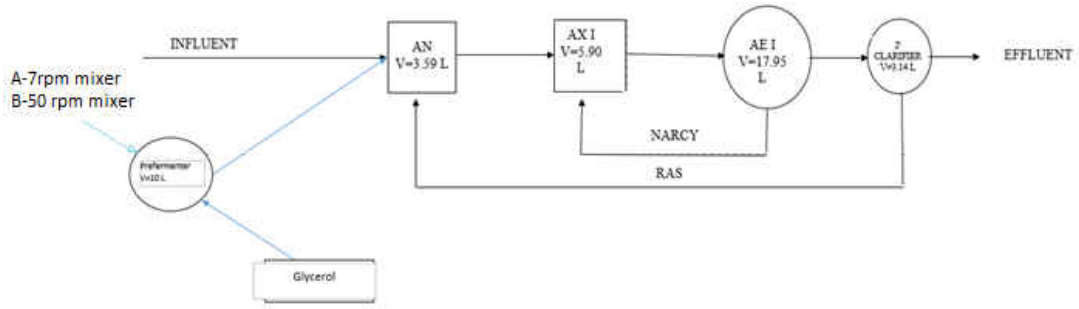

Figure 20 Phase Two schematics for pilot A and B

Phase Three – Chapter: 5 - Pilot A and Pilot B

In Phase Three, two identical 5-stage Bardenpho^{TM} pilot plant systems were used. As can be seen in Figure 21, the 5-stage Bardenpho^{TM} system configuration is anaerobic zone, first anoxic zone, first aerobic zone, second anoxic zone, and second aerobic zone, followed by a secondary clarifier. The RAS recycle flows from the secondary clarifier to the anaerobic zone. The NARCY recycles the nitrate from the first aerobic zone to the first anoxic zone. Both systems were linked to 10 L prefermentation reactors filled with primary solids from Glendale Wastewater Treatment Plant (Lakeland, Florida) at a flow rate of 2 L/day and fitted with 50 rpm mixers. Train A and Train B receive a dose of 3500 mg/day pure glycerol flowing to the second anoxic zone in train A and to the prefermenter in train B. The prefermenters in both systems flowed into the second anoxic zone in order to increase the specific denitrification rate there. This means that the location where the glycerol entered the system was the experimental variable.

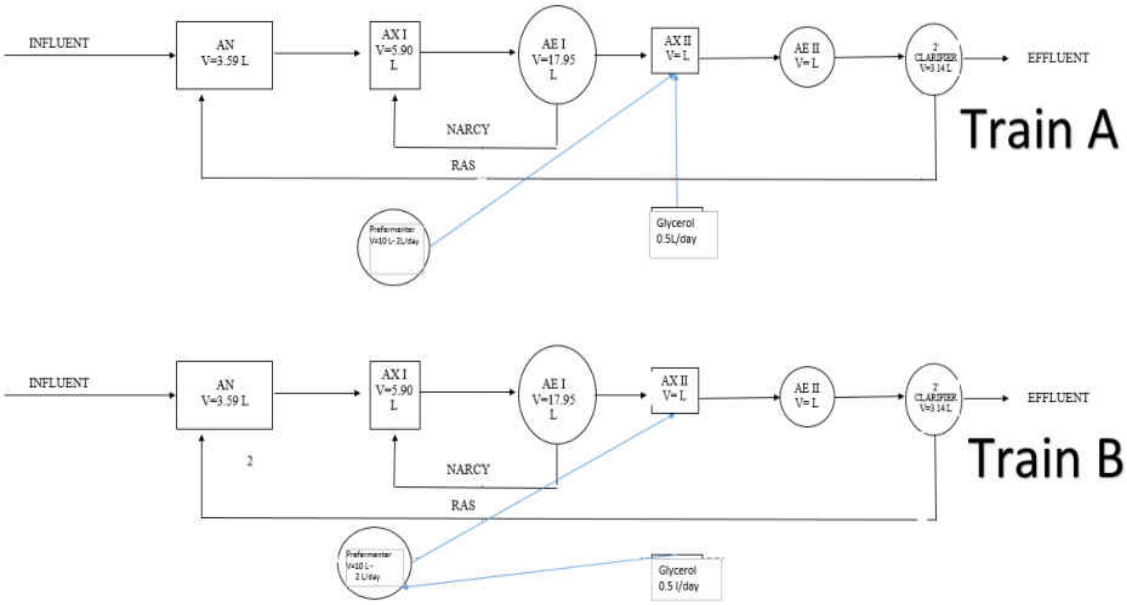

Figure 21 Phase Three schematics for Train A and B

Volatile Fatty Acids (VFAs)

For the purpose of the current study, only Short-chain Volatile Fatty Acids (SCVFAs) were measured using a Shimadzu gas chromatography (GC) 14-A. (SCVFAs) are organic compounds with 2-5 carbon atoms. All samples were filtered immediately on site with a glass fiber filter (Whatman™, 1827-025) before sample transfer to the lab. In the lab, samples were filtered again, this time with 0.45µm membrane filters (Fisherbrand™, SA1J791H5). 1mL sample was transferred into a 1.5 mL GC vials (Shimadzu 228-45450-91). Afterward, samples were acidified to a pH value less than 3 using 0.5 ml of 3% H_2PO_4.

The gas chromatograph was equipped with a flame ionization detector (FID) and Supelco Nukul column (30m × 0.25mm I.D. × 0.25µm; Supelco, St. Louis). The GC also had a Shimadzu auto-sampler AOC-20I. The column temperature was set at 110 °C to 190 °C with an increment rate of 5°C/min until final temperature. The column's final temperature was maintained for 10 minutes. The injection port and the FID temperature were set at 220 °C. The GC used helium at 20cm/min as a carrier gas. After the GC was setup, the sample vials were placed into the auto-injected that injects 2µl from the sample into the injection port. Standard curves were developed using 10mM volatile free acid mix (46975-U; Shimadzu, St. Louis, MO).

Chemical Oxygen Demand (COD)

For the purpose of this study, Lovibond® Tintometer® 2420726 (Sarasota, FL) was used to measure the COD. Lovibond method follows the closed reflux titrimetric method (Standard Methods, Section 5220 C, 1995). Firstly, the sample is homogenized for unfiltered samples. Then the sample along with sulfuric acid and potassium dichromate (a strong oxidizer) is digested for 2 hours at 150 °C in a clear glass vial. The vials also contain a catalyst (Silver), and mercury (for chloride interferences). The reduction reaction from dichromate ion to chromic ion results in a green color. After cooling down to room temperature, the DR5000 (Hach, Loveland) spectrophotometers was used to measure the sample absorbance at 620µm wavelength.

Other Methods

A list of all other analytical methods used in this study is listed in Table 32**.**

Table 32 List of analytical methods

Parameter	Method	Reference
Total Suspended Solids	2540 D	(APHA, 1995)
Volatile Suspended Solids	2540 E	(APHA, 1995)
Ammonia	HACH TNT 10031	HACH Company
Nitrite	HACH TNTplus 839	HACH Company
Nitrate	HACH TNTplus 835	HACH Company
Total Nitrogen	HACH TNT plus 826	HACH Company
Phosphorus	HACH TNT 10127	HACH Company

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