EVALUATION OF SEMI-PASSIVE TREATMENT TECHNOLOGIES FOR SEPTIC LAGOON CAPACITY EXPANSION

by

Christine Gan

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Abstract

In Canada, increases in rural development has led to a growing need to effectively manage the resulting municipal and city sewage without the addition of significant cost- and energy- expending infrastructure. Storring Septic Service Limited is a family-owned, licensed wastewater treatment facility located in eastern Ontario. It makes use of a passive waste stabilization pond system to treat and dispose of waste and wastewater in an environmentally responsible manner. Storring Septic, like many other similar small-scale wastewater treatment facilities across Canada, has the potential to act as a sustainable eco-engineered facility that municipalities and service providers could utilize to manage and dispose of their wastewater. However, it is of concern that the substantial inclusion of third party material could be detrimental to the stability and robustness of the pond system. In order to augment the capacity of the current facility, and ensure it remains a self-sustaining system with the capacity to safely accept septage from other sewage haulers, it was hypothesized that pond effluent treatment could be further enhanced through the incorporation of one of three different technology solutions, which would allow the reduction of wastewater quality parameters below existing regulatory effluent discharge limits put in place by Ontario's Ministry of the Environment and Climate Change (MOECC). Two of these solutions make use of biofilm technologies in order to enhance the removal of wastewater parameters of interest, and the third utilizes the natural water filtration capabilities of zebra mussels. Pilot-scale testing investigated the effects of each of these technologies on treatment performance under both cold and warm weather operation. This research aimed to understand the important mechanisms behind biological filtration methods in order to choose and optimize the best treatment strategy for full-scale testing and implementation. In doing so, a recommendation matrix was elaborated provided with the potential to be used as a universal operational strategy for wastewater treatment facilities located in environments of similar climate and ecology.

Co-Authorship

This research was conducted independently by the author and under the supervision of Dr. Pascale Champagne, who has reviewed and edited this thesis. All experimental work and results were obtained through her technical guidance and feedback.

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AOB	Ammonia-oxidizing bacteria
BAF	Biological aerated filter
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DO	Dissolved oxygen
HRT	Hydraulic retention time
IFAS	Integrated fixed-film activated sludge system
MBBR	Moving bed biological reactor
MOECC	Ministry of the Environment and Climate Change
MCRT	Mean cell residence time
N	Nitrogen
NOB	Nitrite-oxidizing bacteria
P	Phosphorus
PAO	Polyphosphate-accumulating organism
RBC	Rotating biological contactor
SBBR	Sequencing batch biofilm reactor
T	Temperature
TF	Trickling filter
TN	Total nitrogen
TSS	Total suspended solids
V	Volume
WSP	Wastewater stabilization pond
ZM	Zebra mussel(s)

Chapter 1

Introduction

1.1 Background information

As both urban and rural communities continue to develop and grow across the globe, so has the need for more efficient treatment and management of septic waste. The disposal of septage and the effective treatment of wastewater is a crucial aspect in the prevention of disease and water borne illnesses (Ashbolt, 2004). Wastewater stabilization ponds (WSPs) are an effective, cost-efficient way to use the natural ability of lagoon systems to improve the quality of wastewater. As the importance of sustainability and long-term performance have continued to evolve and become a more significant public concern, WSPs have become an increasingly favoured—albeit non-conventional—method for natural, de-centralized wastewater treatment. WSPs are easy to implement and maintain, require minimal electrical energy, are proven to effectively reduce solids, biological oxygen demand (BOD), pathogens and nutrients, and provide the possibility of effluent reuse (e.g. for irrigation or agriculture) (Mara, 2009). In Canada, WSPs are often implemented in small, rural and remote communities, as the land requirement for lagoon systems are often quite large and they cannot be easily integrated into cities with dense urban populations and limited land availability. Although these rural and remote communities are smaller in population density, the rural populations of Canada have experienced steady increases in the last 100 years. Figure 1-1 shows the trend of increasing populations of Canada's rural communities.

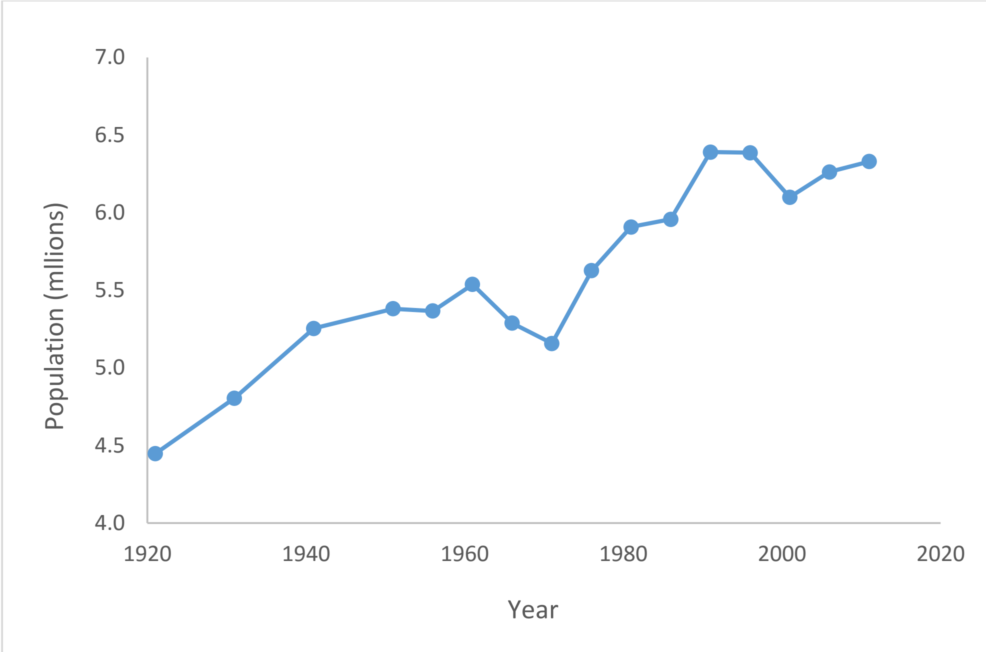

Figure 1-1. Rural growth in Canada. Population, in millions, of Canada's rural regions from 1920 to 2010 (Statistics Canada, 2015).

As this trend continues, the number of rural inhabitants in Canada is expected to continue to grow at a rapid rate. This increase in population, coupled with more stringent wastewater effluent discharge guidelines implemented by the Canadian government, means that rural areas will be faced with larger quantities of waste combined with a higher stringency on wastewater effluent quality (Environment Canada, 2015). Many WSP and lagoon facilities across Canada will be faced with the dilemma of having to upgrade their current treatment approaches in order to conform to the guidelines as outlined in Wastewater Systems Effluent Regulations by the Government of Canada (Canadian Fisheries Act [CFA], 2015). The cold Canadian climate is another challenge for operators, as winter conditions may freeze operation and often lead to significant declines in treatment efficiencies (Metcalf and Eddy, 2003). However, many of these facilities do not have the financial resources or land/worker availability to expand their operations without the addition of significant infrastructure. As such, many sewage haulers and wastewater stabilization pond operators are exploring low-cost, semi-passive treatment technologies as solutions to increase the robustness and efficiencies of their pond systems. Biofilm treatment technologies which help to increase the proliferation and activity of the microorganisms already present in

WSPs, have shown great promise for augmenting existing lagoon system attenuation capacities. The use of other biological organisms, such as zebra mussels—which already have well-established filtration capabilities—may also be an effective method of passively insulating WSPs, allowing them to handle larger hydraulic and constituent loadings in influent wastewater.

1.2 Storring Septic: Site and operation

Storring Septic is a licensed wastewater facility located just north of the rural town of Tamworth, Ontario. They currently operate three, clay-lined wastewater stabilization ponds in series (with a fourth being prepared for future operation) which receive domestic sewage that their company pumps from residential septic and holding tanks. They provide their services to 26 municipalities in parts of the Lennox and Addington, Frontenac, Lanark, and Hasting counties. Figures 1-2 and 1-3 show birds-eye views of the facility and illustrates the site specifications and operational characteristics.

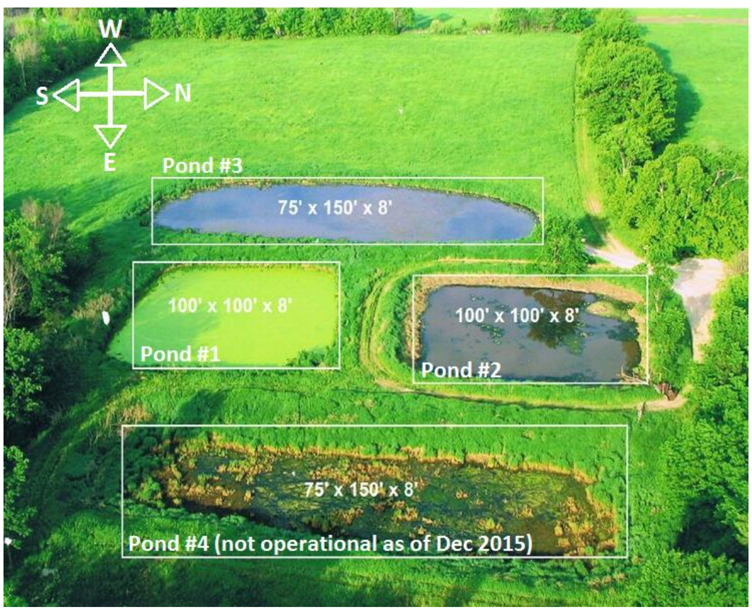

Figure 1-2. Aerial view of the four ponds at Storring Septic's facility, showing the dimensions of each pond. Pond 4 is being prepared for future operation and was not in use for the duration of this project. The site is located at a latitude and longitude of 44°30'45.35"N, 77° 0'19.03"W.

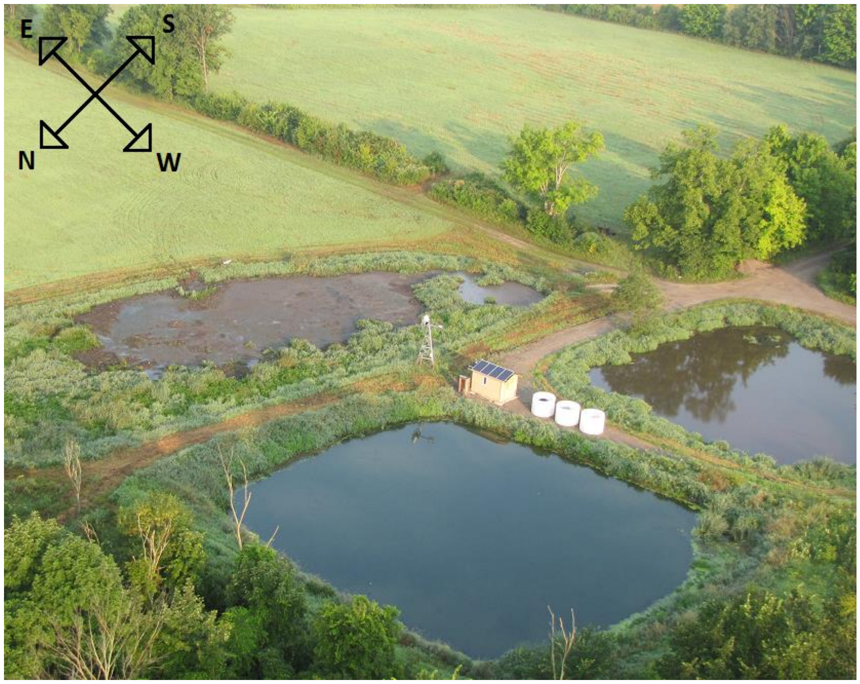

Figure 1-3. Alternative aerial view of the Storring Septic site, with Pond 3 (primary pond) not in view. This view shows the beginning stages of the experimental setup implemented for this research project, with three treatment tanks in view between Ponds 1 (right) and 2 (left). As can be seen, Pond 4 is in the process of being prepared for future use. This figure also shows a fraction of the land area used for land spreading of the final effluent.

Influent wastewater from the Storrings septic trucks are drained into the ponds. Although there is no rigid scheme for the transfer of wastewater between ponds, raw septage is typically dumped into the primary pond, Pond 3. From here, after solids settling, the wastewater from Pond 3 is siphoned into Pond 2, the secondary pond, and allowed to be treated via biological mechanisms. Pond 1 is typically the clarification pond, which receives effluent from Pond 2 and whose final effluent is discharged onto the land for land spreading/distribution and evaporation. Effluent does not discharge into or reach any receiving body of water. A simplified diagram of this process flow is shown in Figure 1-4. Although there is no formal schedule for filling/emptying/dredging the ponds, solids are periodically removed by dredging followed by land application.

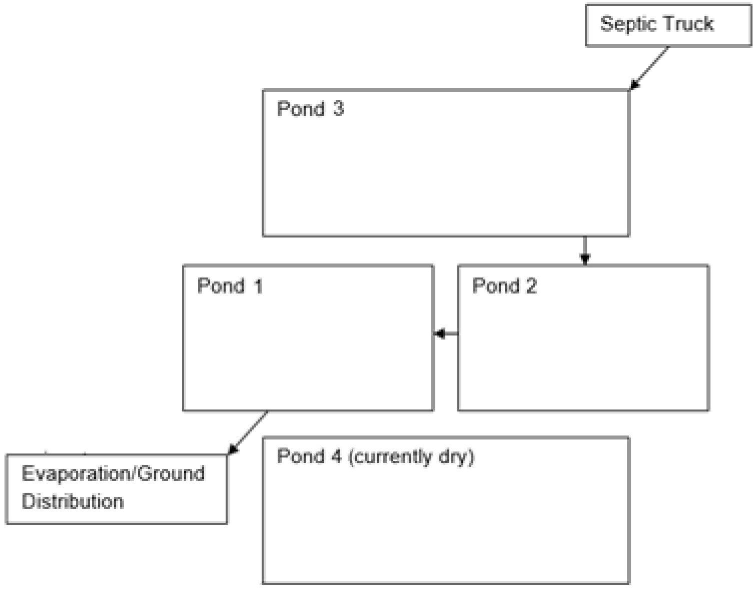

Figure 1-4. Diagram of applicable process flow. Arrows indicate direction of inflow/outflow of wastewater.

The typical characteristics of the inflow septage vary, and depend on the number of septic trucks emptied per day. The average daily inflow to the system is 17.7 m^3/day, with peak inflow to the system being 29.4 m^3/day. Table 1-1 provides typical ranges of the raw septage characteristics entering the Storrings' facility, based on a set of data collected during the beginning of this research project. These data were collected during March 2014.

Table 1-1. Typical characteristics of inflow septage entering the treatment facility.

Wastewater Parameter	Average concentration range in raw septage (mg/L)
BOD_5	200-300
COD	200-500
TSS	~600
Ortho-P	6-25
NH_3/NH_4^+	400-600

This range of values is representative of medium- to high-strength domestic wastewaters, with the exception of ammonia (Spellman, 2014). The concentration of ammonia in the influent stream is much higher than would typically be noted in domestic or municipal wastewaters. The upper limit of the orthophosphate range is also a little higher than would be expected from domestic wastewaters. This is due to the nature of the wastewater entering Storrings' facility, which accepts raw septage from a number of anaerobically-treated septic tanks, resulting in an influent that is highly variable in quality depending on the volume and characteristics of the raw septage collected during that particular day. Thus, raw influent is not typically monitored on a regular basis, as the range of influent wastewater parameters varies too significantly to be representative of average levels of the parameter actually found in the treatment ponds.

The major concern of Storring Septic is that the opening up of the facility to third-party haulers will result in influent volumes and organic loads that too high for their current pond system to efficiently process, leading to pond shock or effluents that do not conform to discharge guidelines. In addition, the lack of regulation over what third-party sewage haulers are bringing into their facility may result in atypical influent loads or compositions that their current pond setup may be unable to handle. It is the intention of this project to assist Storring Septic in increasing the efficiency and robustness of their WSP facility, so that their pond systems may effectively process all regular and third-party/excess sewage without detriment.

1.3 Overview of passive treatment technologies

Three methods of passive wastewater treatment were tested for research purposes. Two of these were systems developed by third-parties that utilize biofilm technologies to reduce contaminants via microbial activity. The third method uses the natural ability of zebra mussels to filter particulates and other contaminants from wastewater. All three methods offer the potential for effective, naturalized treatment and the removal of key wastewater parameters: organic carbon, total suspended solids (TSS), phosphorus (P) and nitrogen (N).

When in contact with wastewater, naturally-occurring aerobic microorganisms can degrade and oxidize influent organic materials and pollutants, nitrify and reduce ammonia, and contribute to disinfection and removal of pathogens (Metcalf and Eddy, 2003). Thus, in the presence of sufficient dissolved oxygen concentrations, these aerobic microorganisms lead to the reduction of currently regulated wastewater effluent quality parameters such as TSS, organic material (measured as BOD/COD), nutrients (nitrogen and phosphorus) and pathogens. The performance of these aerobic processes are dependent on a number of environmental and operational factors, such as temperature, dissolved oxygen content, microorganism-substrate contact time (i.e. solids retention time, retention time), and wastewater characteristics (Metcalf and Eddy, 2003; Snoeyink and Jenkins, 1980).

When microorganisms are provided with a fixed surface upon which they can attach and proliferate, a biofilm is formed, consisting of a high, active concentration of wastewater-treating microorganisms. This increased number of microorganisms allows for more effective treatment in comparison to biological treatment using suspended sludge (Loupasaki and Diamadopoulos, 2013). The biofilm technology systems selected to passively treat the septage wastewater for this study were: BioDome (of Wastewater Compliance System), and BioCord (by Bishop Water Technologies). Figure 1-5 illustrates both the BioDome and BioCord systems' approaches for optimizing biomass growth and proliferation.

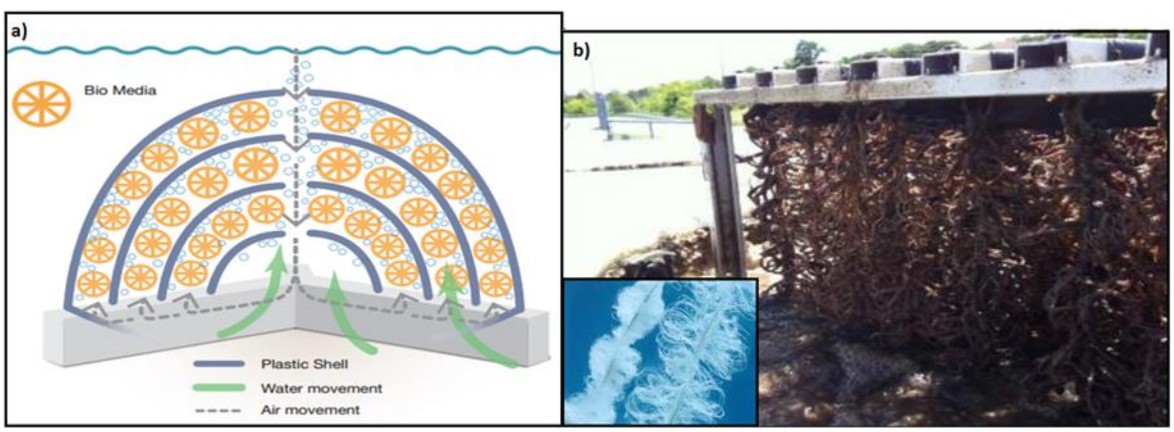

Figure 1-5 a) BioDome system schematic (cross-section). The BioDome treatment technology works to promote the growth of beneficial bacteria by providing biofilms with protection from sunlight (to reduce the growth of competing photosynthesizing organisms), and optimal degrees of aeration and nutrient mixing (Johnson, 2011). b) A mid-sized, half-submerged BioCord module and a close-up of the BioCord system's material. The BioCord system's

man-made polymer substrate is a cord covered with rings of thread, providing a high surface area for biofilm development and multiple layers of cell growth (Bishop Water Technologies, 2012).

The BioDome and BioCord treatment technologies each promote biofilm development by providing a large colonisable surface area, methodical oxygenation cycling, and wastewater mixing to enhance metabolism and proliferation of microorganisms in the biofilm. Aeration must be provided to both of these systems in order to provide the developing biofilms with oxygen and mixing. The media of the attachment surface allows for high biomass retention and therefore a high mass transfer area/conversion capacity.

The BioDome treatment technology consists of four concentrically stacked domes containing the honeycomb-like packing materials; these act as the media upon which the biofilm develops. One BioDome unit contains enough packing material to result in a surface area of approximately 260.13m^2. When air is delivered to the diffuser manifold attached to the bottom of the BioDome system's structure, air is supplied to the packing media via bubble distribution tubes located at the base of each dome (seen as grey arrows in Figure 1-5a). The entire BioDome structure is covered by an opaque outer shell, which provides a biofilm environment that is exposed to minimal sunlight. This increases the amount of nitrifying/denitrifying bacteria in the biofilm and reduces competition from photosynthesizing organisms such as algae and cyanobacteria (Xu *et al.*, 2011). In sunlight-exposed suspended growth form, the water-purifying microorganisms that have the ability to reduce total nitrogen and phosphorus have a tendency to be out-competed by cyanobacteria, nitrogen/phosphorus-consuming algae or other organisms requiring common substrate (Dolman *et al.*, 2012; Oehmen *et al.*, 2006). The BioDome treatment technology has also been reported to have an increased nitrification performance (i.e. ammonia reductions) in cold weather compared to other technologies (Johnson, 2011). The BioDome system used in this study had a bottom diameter of 1.83m and a height of 1.22m, and full-scale implementation of this treatment technology would require the purchase of multiple BioDome units. A full-scale implementation of the

BioDome system would also require a higher aeration demand that is in proportion to the number of modules being implemented.

The BioCord system, although lacking the element of reduced sunlight exposure, uses a similar principles of biofilm technology as the BioDome treatment technology—the formation of a microbial consortium on an attachment surface in multiple layers to provide enhanced biological activity—to improve effluent quality and reduce waste sludge generation (measurable as TSS). The more "open", modular structure of the BioCord system, coupled with a more lightweight frame, allows for easier access and direct biofilm observation in comparison to the BioDome treatment technology. This may be especially important if maintenance of a treatment technology is required during the course of its life. The BioCord unit used in this study was commissioned specifically for pilot-scale testing, and measured 1.3m H x 0.92m W x 0.92m L. Full-scale implementation of the BioCord treatment technology would involve either the purchase of multiple, pilot-scale sized modules, or the commissioning of one, larger BioCord system consisting of a larger frame and a larger length of threaded cord. Aeration to the system would also have to be increased in proportion to the increase in size. Although the exact surface area of the pilot-scale BioCord system used in this study was not known, the specific surface area was estimated by its manufacturers to be approximately $2.4m^2$ per meter of cord. Based on the dimensions of the pilot-scale unit, it was estimated that the amount of cord contained in the BioCord system was approximately 100m. This leads to a surface area estimate of approximately $240m^2$. Thus, the surface area provided by the media of both the BioDome and BioCord systems was similar.

Zebra mussels have a capability to filter water and reduce the amount of particulates present (Effler *et al.*, 1996; Sprung, 1992). This has been well-documented through the clarification of waters in the Great Lakes and other freshwater bodies by this invasive species (Nicholls and Hopkins, 1993; Binding *et al.*, 2007; Fanslow *et al.*, 1995; Fahnenstiel *et al.*, 1995). Because each mussel has the ability to reduce suspended solids in waters at a rate of as much as 1 L/day, it can be hypothesized that they may also possess the ability to reduce wastewater parameters in order for effluent concentrations to comply with discharge standards put in place by the MOECC (Effler *et al*., 1996). However, no previous research has been conducted to test the efficacy of zebra mussels to reduce levels of ammonia, phosphorus or total nitrogen from wastewater in any climate[1]. Hence, the research presented in this thesis hopes to provide a better understanding of the processes involved in their filtering abilities and potential for effective wastewater treatment. In order for zebra mussels to survive in a non-natural environment (e.g. a wastewater treatment tank), they must be provided with oxygen for respiration and energy. Because of this, the implementation of zebra mussels in this study required the presence of external aeration. In order to utilize zebra mussels in full-scale testing or implementation, the number of zebra mussels being utilized would need to be increased appropriately, as well as the aeration required to maintain a thriving population. The addition of zebra mussels to a WSP system would also likely require them to be attached onto a solid surface, such as reef balls or a grid structure, to ensure that they remain above the bottom of the lake and are not suffocated by settling solids, sediments, or the overgrowth of zebra mussels.

When implemented in actual lagoon systems, it is surmised the biofilm systems and the zebra mussel system will enhance treatment efficacy and system robustness. The pond system would be expected to experience an increase in robustness and offer a buffer from potential detrimental effects often associated with shock loading, which could result from a significant increase in raw septage intake, particularly from third party suppliers. In the case of the BioDome and BioCord systems, there is particular emphasis on optimizing proliferation of nitrifying/denitrifying bacteria and phosphorus accumulative organisms (PAOs) in order to reduce the nutrient load of wastewater discharged from regular septage treatment lagoons. Both systems report ease of system installation to optimize existing lagoon system treatment efficiency, through the addition of fixed biomass to increase biological treatment, resistance to toxic

[1] To this author's knowledge, based on extensive research into the literature on *Dreissena polymorpha*

shock, and a proficient capacity for nitrification, reduced ammonia effluent levels, and denitrification of nitrate/nitrites (Johnson, 2011; Bishop, 2012). In the case of zebra mussels, there is a focus on maintaining their survival under severe conditions (wastewater conditions), as well as determining the optimal levels of aeration/inflow rates to maximize their water-filtration capabilities.

1.4 Thesis objectives

This thesis investigates and compares the ability of each treatment technology to effectively reduce targeted wastewater parameters under varying conditions of aeration, temperature, organic loadings and hydraulic retention times. Each technology was assessed for its ability to effectively remove organic matter (in the form of COD), TSS, total ammonia (NH_3/NH_4^+), total nitrogen (TN) and orthophosphate (Ortho-P). The goal was to identify one or more treatment technologies that could significantly improve the performance of the lagoon system, as represented by a control system, and to recommend the best system for full-scale testing and implementation. The condition of "being the most effective technology" was assessed based how well each technology was able to:

1) Handle larger quantities of septage by demonstrating significant reductions in wastewater effluent water quality parameters,
2) Recover from shock conditions (i.e. due to unknown third-party materials or system shutdown),
3) Treat wastewater effectively with the lowest energy and maintenance requirements, and
4) Perform adequately under cold-weather operational conditions.

Once an effective treatment technology has been identified, the intention will be to assess the operational conditions under which it performed optimally. From this information, a recommendation matrix will be provided for use in other pond operational facilities facing treatment challenges under similar environmental and operational conditions. Recommendations for full-scale testing and implementation, including air cycling and possibilities for attenuated pond operation, will be made as a guideline for lagoon operators wishing to employ a biofilm technology as an alternative to upgrading their current facilities.

In addition to determining which treatment technology has the most potential for full-scale testing and implementation, this thesis aims to explore the ability of zebra mussels (*Dreissena polymorpha*) to uptake wastewater parameters—particularly forms of the nutrients nitrogen and phosphorus—from both low-strength and synthetic wastewaters. At the present time, there has not been extensive research conducted on the wastewater-treating potential of these freshwater molluscs. This thesis attempts to answer the question of whether or not there is a filtration or uptake mechanism present in zebra mussels that allows for the reduction of key wastewater parameters (total ammonia, orthophosphate, COD and TSS) from both low-strength and synthetic wastewaters in controlled laboratory conditions.

1.5 Thesis outline

The literature review in Chapter 2 of this thesis serves as an introduction to the main concepts of biological wastewater treatment in WPS systems. It presents an overview of the biological mechanisms of wastewater contaminant reduction in WSPs and lagoon treatment facilities, focusing on the bacterial processes leading to stabilization of nitrogen, phosphorus and organic matter. It then goes on to detail the use of biofilm technologies for passively increasing the performance of stabilization ponds by increasing the concentration and activity of the microorganisms treating wastewater. The literature review concludes with examples of existing biofilm technologies and discusses design and operational considerations of implementing biofilm technologies as a facility upgrade strategy.

Chapter 3 and 4 present the results of cold-weather (fall/winter 2014) and complete operational (summer/fall 2015) testing of each of the three technologies employed for increasing pond efficiency, respectively. Chapter 3 focuses on the overall wastewater parameter reductions in the BioDome and BioCord treatment technologies and the zebra mussels under cold-weather start-up and operation, and focuses on their ability to overcome the negative effect of low temperature of treatment performance. Chapter 4 demonstrates the treatment effects of all three technologies in comparison to a control, and more thoroughly assess the effects of varying aeration cycles and loading rates on the treatment performance and system robustness.

Chapter 5 of this thesis discusses the laboratory zebra mussel (*Dreissena polymorpha*) experiments that were conducted to observe the ability of zebra mussels to uptake contaminants from wastewater in a controlled environment. The results obtained from these experiments lay the groundwork for future studies involving a more in-depth and encompassing approach to nutrient and organic material uptake via zebra mussel filtration and accumulation, and discuss the potential of zebra mussels as a viable approach for upstream passive wastewater treatment.

Chapters 6 and 7 are the concluding chapters of this thesis. Chapter 6 focuses on the possibilities for full-scale design and implementation, and recommendations are made to Storring Septic for operational regimes involving the retrofitting of a biofilm treatment technology to their pond setup. It also discusses the significance of the results obtained, including potential industrial applications and contributions to North America and other WSP facilities, and recommends directions for future research relating to the topic of semi-passive wastewater treatment. Chapter 7 review the results obtained from each study and identifies the treatment technology with the most potential for full-scale testing and implementation, in the context of the information gathered throughout the course of this study.

Chapter 2

Literature Review

2.1 Introduction

In recent years, the importance of sustainability and long-term efficiency has become a more significant public concern and, as such, naturalized wastewater treatment systems have become increasingly popular methods for centralized wastewater treatment. In particular, wastewater stabilization ponds—or WSPs—are attractive options for locations that require lower-cost and lower-maintenance solutions for treating domestic, municipal and industrial wastewaters. This is because WSPs are considered to be both economically and environmentally sustainable: WSPs have low operational and maintenance costs, make use of naturally-occurring biological and physio-chemical processes to treat water, and are not energy-intensive (U.S. EPA , 2011). These systems also have the added benefit of less frequent excess sludge production, relatively little odour emissions, the ability to handle shock loads, and high public acceptance (Mara, 2009). However, WSPs do have relatively large land requirements, making them more ideal for locations where this can be accommodated such as rural communities or developing countries. As these populations continue to grow, treatment efficiencies of implemented WSPs can be improved or maintained through the addition of either passive or mechanically-aerated treatment technologies, which rely on the development of a biofilm to enhance the existing biological processes responsible for reducing wastewater constituents. The overall goal is better wastewater treatment and the ability to continue servicing growing populations and/or influxes in organic and hydraulic loading rates. This chapter will begin with a summary of WSPs and the biological processes responsible for treating wastewater, and then continue with a review of current semi-passive technologies that exist to enhance these processes via biofilm enrichment.

2.2 Wastewater stabilization ponds

Wastewater stabilization ponds (WSPs) are shallow (1-5m deep) ponds that are designed to take in a flow of domestic, municipal or industrial waste (World Health Organization, 1971). WSPs are not vegetated, and therefore differ from treatment wetlands, but can be mechanically or passively aerated and mixed in order to increase treatment performance. Aerated WSPs are called lagoons. The naturally occurring microorganisms present in pond and lagoon environments use a number of processes to decompose, transform, and absorb a variety of pollutants that are typically present in wastewaters (Faulwetter *et al.*, 2009; Wang *et al.,* 2012). There are three kinds of stabilization ponds: aerobic, facultative (consisting of both aerobic and anaerobic environments) and anaerobic. A wastewater treatment lagoon system often consists of three or more types of stabilization ponds in series: a primary pond (typically anaerobic), one or more facultative ponds, followed by a maturation or polishing pond (typically aerobic); although other configurations can be implemented depending on site-specific characteristics and treatment goals (Pescod and Mara, 1988; Phuntsho *et al.,* 2009). Each pond is responsible for a different level of treatment, and the environment of the pond will determine the type of biological processes that will predominate to reduce wastewater constituents.

Primary ponds are the first ponds in the process flow; they are designed to receive the incoming flow of untreated wastewater. Because of their relative depth (2-5m) compared to the subsequent ponds, oxygen transfer is generally limited, and as a result primary ponds are usually anaerobic with limited algae growth (Mara, 2009). They are mainly responsible for removing a large portion of suspended solids (SS) and some organic and inorganic materials by sedimentation and subsequent settling of particulates to the bottom of the pond. This is mainly a physical process and results in the formation of biosolids—otherwise known as sludge—which are then manually removed from the bottom of the pond on a periodic basis (Mara, 2001). The settleable solids and materials that accumulate in primary ponds also provide surfaces for microbial growth (Grady *et al.,* 1999).

Facultative (or secondary) ponds receive the effluent from the primary treatment pond. They are typically aerobic in their upper portions because of surface reaeration and O_2 released from algae. Oxygen does not reach the bottom layers of the pond, resulting in anaerobic lower portions of facultative ponds (Mara, 2006). Treatment occurs via the synergistic activities of algae and bacteria, leading to decreases in organic matter (OM) and nutrients. Figure 2.1 shows the relationship between algae and bacteria in WSPs.

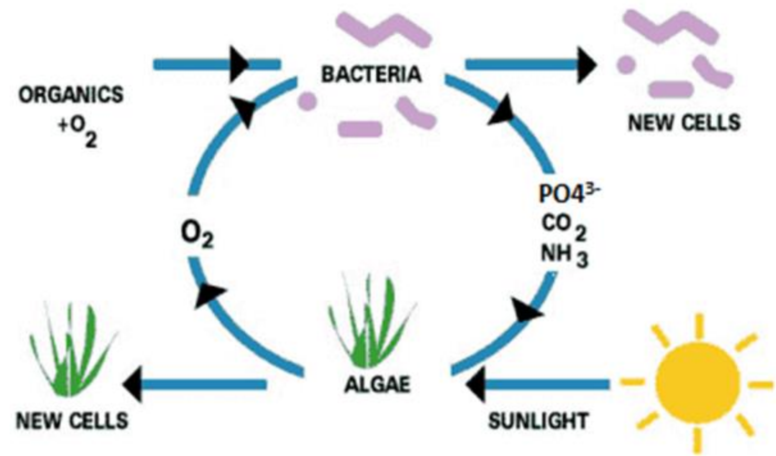

Figure 2-1. Illustration of the symbiotic relationship between algae and microorganisms in wastewater (UNEP, 2002).

Maturation (or polishing) ponds are the final step of wastewater treatment in a WSP system. The main goal of maturation ponds is to further reduce levels of organic matter, SS, and ammonia nitrogen via microbiological processes. Maturation ponds are often effective in removing remaining faecal bacteria and pathogens via photo-oxidation, increased temperatures, and/or increased pH levels (>9.4) (Mara, 2006). If the final effluent is to be discharged into an aquatic receiving environment, and the final maturation pond is not able to adequately disinfect the effluent so that it meets discharge requirements, further disinfection using alternative methods may be required (e.g. UV disinfection, chlorination). This step may be performed offsite and typically involves further removal of pathogens and coliforms.

2.2.1 Biological processes in WSPs

In general, effluents from WSPs must comply with certain wastewater effluent quality regulatory requirements. Although these specific limits vary between countries and depend on the final receiving environment, the main objective is the same: to reduce all wastewater contaminants as much as possible to improve effluent quality. Some important wastewater parameters that are typically targeted for removal include: organic matter (as BOD/COD), ammonia, total nitrogen, phosphorus/orthophosphate, total suspended solids (TSS) and pathogens (Middlebrooks, 1982).

Microorganisms that are naturally present in WSP systems help achieve these targets via aerobic and anaerobic mechanisms, where organically-derived pollutants are broken down into less harmful by-products such as CO_2, nitrogen gas, and water (Faulwetter *et al.*, 2009). Different types of ponds will promote different treatment environments, which in turn determine the type of microorganisms that will proliferate and level of treatment that will occur.

2.2.1.1 Organic matter removal

Reduction of carbon and organic matter is largely achieved in primary anaerobic or secondary aerobic/facultative ponds (Mara, 1986; Libhaber and Orozco-Jaramillo, 2012). Both biochemical oxygen demand (BOD) and chemical oxygen demand (COD) can be used as quantitative expressions of the organic matter present in wastewater. In anaerobic ponds, BOD and COD are reduced by sedimentation of settleable solids followed by subsequent stabilization by anaerobic digestion. Under the low redox conditions generally found at greater depths within primary anaerobic ponds, biodegradable organic matter is converted by anaerobic bacteria into CO_2 and CH_4, in a process called methanogenesis (Mara, 1992).

Heterotrophic microorganisms can oxidize organic soluble matter (i.e. BOD/COD) and convert the carbon into new biomass, H_2O, and CO_2. The carbon dioxide either volatilizes or is assimilated by any algae present in the pond. Excess biomass settles to the bottom of the pond as newly-formed biosolids. An aerobic environment must be present when oxygen is consumed by microbial species to decompose organic matter, as oxygen must be available to act as the electron acceptor (Ateia and Yoshimura, 2015). However, decomposition of organic matter can still occur in the anaerobic zones of a WSP: when oxygen is not present, some microorganisms can utilize a variety of different electron acceptors to drive decomposition, depending upon the physical and chemical conditions of the environment they are present in (Kadlec and Wallace, 2009). Insoluble organic matter is also present in the incoming wastewater and is largely removed by flocculation and sedimentation with other settled particulates. These settled insoluble materials end up in the pond sludge of a WSP. The insoluble matter that is not removed by sedimentation can be biochemically stabilized by both aerobic and anaerobic processes. This occurs when the insoluble organic matter becomes entrapped with soluble organic matter, which is in turn converted into stable end products via microbial processes. The end products of insoluble organic matter stabilization are CO_2, inorganic solids, and insoluble organic residues (Grady *et al.*, 1999).

In anaerobic and aerobic processes, carbon must be readily biologically available to sustain metabolic activity. In the absence of readily biologically available organic carbon, the processes involved in both organic matter and nutrient reductions will slow, and overall treatment efficiencies will decrease (Grady *et al.*, 1999).

2.2.1.2 Nitrogen removal

Nitrogen is a key nutrient that, in excess, can lead to eutrophication in both freshwater and saltwater systems, and thus is an important focus in wastewater treatment. Nitrogen in WSPs is present in both organic and inorganic forms. Figure 2-2 summarizes the nitrogen cycling processes generally occurring in wastewater stabilization ponds.

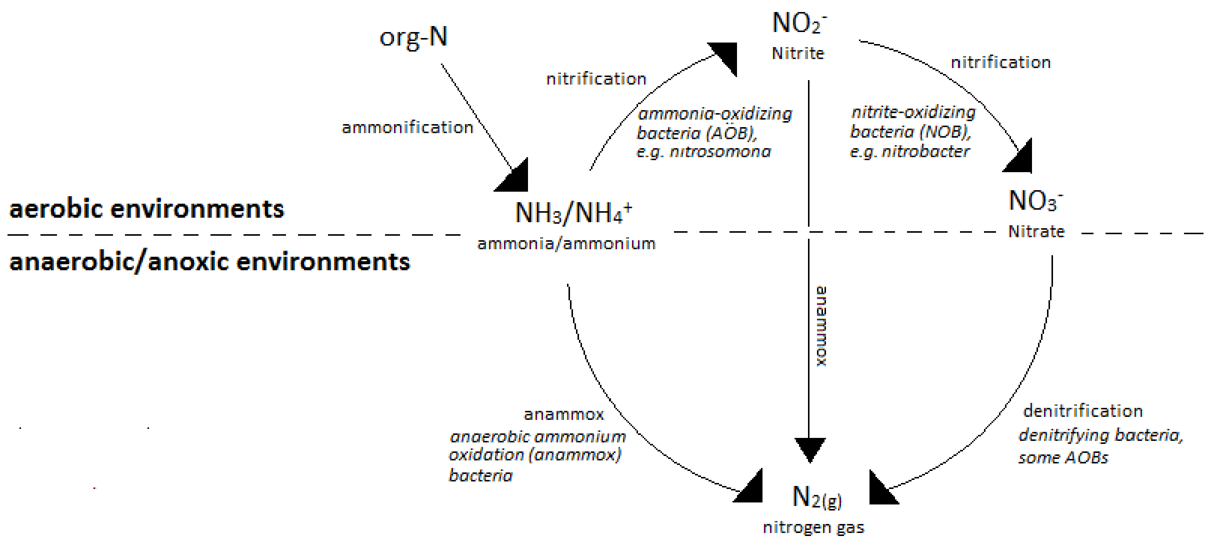

Figure 2-2. A simplified illustration of the nitrogen cycle in biological wastewater treatment, showing transformations of inorganic nitrogen via microbial activity.

Organic nitrogen (org-N), which is the nitrogen originating from plant and animal matter (i.e. decay or waste), is found in amino acids, proteins, and nucleotides, and is typically released as ammonia via ammonification as the organic matter containing them gets degraded by microorganisms (Grady *et al.*, 1999). Both inorganic and organic nitrogen can also be consumed by any algae present in the pond. Sedimentation of the dead algal cells results in the accumulation of this org-N in pond sludge.

Ammonia and total inorganic nitrogen removal is a multi-step process, which begins with the conversion of ammonia to nitrate. This process is called "nitrification", and is performed by autotrophic nitrifiers that utilize either ammonia or nitrite as the electron donor. The organisms that convert ammonia to nitrite—the first and rate-limiting step of ammonia oxidation—are called "Ammonia-Oxidizing Bacteria" (AOBs, *nitroso*-) and "Ammonia-Oxidizing Archaea" (AOAs) (Pester *et al.*, 2012). The bacteria that convert nitrite to nitrate are called "Nitrite-Oxidizing Bacteria", (NOBs, *nitro*-). These microorganisms are slow-growing, and in WSPs are often in competition with heterotrophic bacteria for an organic carbon food source (Witzig *et al.*, 2002). At high pH levels, it is possible for ammonia to be lost via volatilization; however, it has been shown that in typical WSPs, the loss observed due to volatilization is generally relatively small (Epworth, 2004; Camargo *et al.*, 2005). Ammonia is considered harmful to receiving environments, both because of its toxicity to aquatic life, as well as its ability to significantly deplete dissolved oxygen (DO) levels in water via nitrification which exerts an oxygen demand (Guo *et al.*, 2009). In addition, even when not directly discharged into a receiving body of water, soluble nitrogen species can travel considerable distances should they reach the water table (Cole *et al.*, 2006; U.S. EPA, 2002).

The removal of nitrate—denitrification—requires anaerobic/anoxic conditions, and results in the conversion of nitrate to nitrogen gas (N_2) by heterotrophic facultative bacteria using nitrogen oxides as the terminal electron acceptors (Vymazal, 2007). The nitrogen gas subsequently volatilizes, leaving the pond system. It is also possible for some AOBs to perform denitrification anoxically by utilizing organic compounds as their source of cellular carbon, which in turn causes the release of nitrous oxide (N_2O) (Jenkins and Sanders, 2012; Kim *et al*., 2010)

Anaerobic ammonium oxidation (Anammox) is the microbial transformation of ammonium and nitrite directly into nitrogen gas (N_2). The chemoautotrophic bacteria that derive their energy from this conversion are called anammox bacteria, and exist in anoxic and anaerobic ecosystems (Faulwetter *et al*., 2009; Ding *et al*., 2013). They use nitrite as the electron acceptor and do not rely on an organic carbon source, thus enabling them to coexist favourably with heterotrophic bacteria. Although anammox bacteria have been identified in WWTP sludge and have been shown to enhance nitrogen removal with a lower release of greenhouse gas intermediates, their growth rates are extremely slow and are therefore not considered to be the prevalent method of nitrogen removal in WSPs (Ateia and Yoshimura, 2015).

2.2.1.3 Phosphorus removal

Along with nitrogen, phosphorus is another limiting nutrient contributing to eutrophication, and must therefore be sufficiently removed from wastewater prior to discharge into a receiving environment.

Water quality deterioration as a result of eutrophication occurs when effluent levels of phosphorus exceed more than 1 or 2mg/L (Cooper *et al.*, 1994), hence target removals are often aimed to meet these guidelines. In domestic wastewaters often treated by WSPs, the key phosphorus species being targeted is orthophosphate (ortho-P), which is the biologically active form of phosphorus available for microbial metabolism (Spellman, 2014). Condensed phosphates and organic phosphates are also found in the inflowing wastewater; however, these species are eventually metabolized by microbes to become inorganic orthophosphate. The mechanism of biological phosphorus removal (BPR) in WSPs via bacterial metabolism is not completely understood; however, it is known that the removal of reactive phosphorus is largely attributed to the activity of heterotrophic microorganisms called PAOs—polyphosphate-accumulating organisms (Chen *et al.*, 2004). PAOs uptake phosphorus and accumulate it as polyphosphate in granules within the cell. Although other bacteria can store and release phosphate, PAOs have the ability to do so on a much larger scale. The storage of phosphorus by PAOs is generally in response to cyclical environmental conditions; meaning that their ability to store large quantities is dependent on exposure to alternating aerobic and anaerobic conditions (Mara, 2006). Figure 2-3 illustrates how PAOs can reduce overall concentrations of orthophosphate from wastewater by the cycling of anaerobic and aerobic conditions.

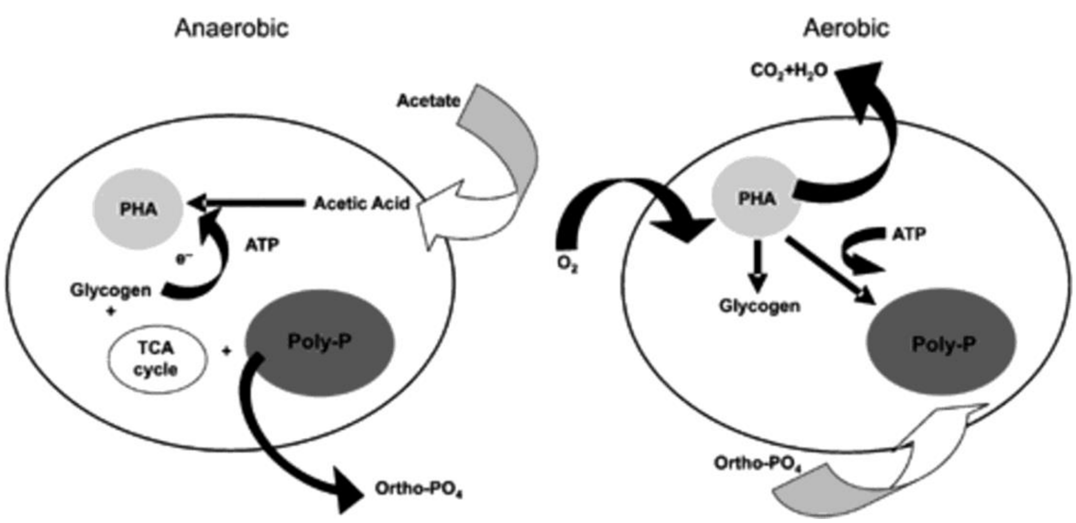

Figure 2-3. The main biochemical features of biological phosphorus removal. In this figure, the carbon and phosphate transformations are shown. In anaerobic environments, ortho-P is released. During aerobic conditions, ortho-P is uptaken by PAOs and stored (Forbes *et al.*, 2009).

The phosphorus is cycled between the two phases. During the anaerobic phase, volatile fatty acids (VFAs) and other organic materials (e.g. acetate) are uptaken by PAOs and stored as intracellular phosphate. Energy from the hydrolysis of intracellular phosphate is used to uptake and store carbon as polyhydroxyalkanoates (PHAs), resulting in the release of phosphorus. Under aerobic conditions, reducing power from the PHAs are used to store more phosphorus than was released during the anaerobic phase (Zhou *et al.*, 2010). Thus, total phosphorus removal occurs via biomass wastage. Aerobic conditions also favour the growth of glycogen-accumulating organisms (GAOs) and as such, competition for organic substrate may affect rates of total phosphorus removal (Zeng *et al.*, 2003; Oehmen *et al.*, 2006).

2.3 Biofilm in wastewater treatment

Although WSPs have been demonstrated to be an effective approach for wastewater treatment, it can often be beneficial to augment pond systems using in-situ naturalized-systems (e.g., in instances where wastewaters of particularly high strength must be treated). Systems that purposefully incorporate biofilm as a method of increased treatment may be called "microbial aggregate", "fixed-film", or "attached growth" systems/bioreactors, and there are many commercially available options that can be retrofitted into an existing WSP *(*Loupasaki and Diamadopoulos, 2013*;* Gao *et al.,* 2014; Gavrilesci and Macoveanu, 2000). A biofilm is the formation of a microbial consortium that grows onto a solid surface or attachment matrix. The microorganisms form a "film" divided into a base film and surface film, which are bound together by a matrix of extracellular polymers (Grady *et al., 1999)*. The surface on which the biofilm grows may be either fixed in space or free-floating, and is usually comprised of a porous material that has a high surface area to volume ratio. Thus, the growth of high concentrations of microorganisms is promoted, resulting in higher removal rates even at colder ambient temperatures (<15ºC), and variations in pollutant loads (Loupasaki and Diamadopoulos, 2013). The attachment of microorganisms onto a solid support also allows for more efficient contact between the wastewater-treating microorganisms and the substrates in the influent stream. In addition, the methodological development of a thick biofilm can be beneficial when considering removal targets and the types of bacteria that help reduce these target compounds. As mentioned previously, competition for substrates between wastewater-treating microorganisms can lead to decreased reductions in certain wastewater parameters (Grady *et al.*, 1999). Although this problem still exists in biofilms, the types of microorganism that predominate a system can be favoured by manipulating operational characteristics such as HRT or aeration; thus, depending on the treatment targets, certain microbial processes can be enhanced (Mamais and Jenkins, 1992; Wang *et al.*, 2009; Loupasaki and Diamadopoulos, 2013). Lastly, the microbial consortium that is incorporated into an attached biofilm is typically more “stable” than microorganisms in suspended growth systems (Shete and Shinkar, 2014). This is important as it implies that slower-growing microorganisms—such as nitrifiers—will be able to withstand washout at comparatively lower retention times and are, overall, present in higher numbers than in suspended growth systems (Wilczak, 2014).

2.3.1 Design and operation of biofilm/passive treatment technologies

Different biofilm technologies will differ in their capacity to augment wastewater treatment. The main factors contributing to enhanced treatment via biofilm systems are the composition, quantity, and activity of the microorganisms making up the biofilm layers (Lazarova and Manem, 1995). These three aspects are, in turn, influenced greatly by the design and operation of the biofilm technology. The media type, level of aeration, and presence of recirculation are design characteristics that influence how well certain microorganisms can thrive in an environment, and the effects of temperature and loading rates on microbial system will influence operational requirements such as optimal hydraulic retention times (Loupasaki and Diamadopoulos, 2013). The selection of these design and operational parameters will determine the type and levels of constituent removals.

2.3.1.1 Biofilm and medium design

The medium, or packing material, is the surface upon which a biofilm will develop. Depending on the method of treatment, this support medium can be either completely submerged in the process flow, or wastewater can be distributed evenly onto the filter through the medium (Lazarova and Manem, 2000).

For example, a submerged fixed-film bioreactor is a type of biofilm treatment technology where the biofilm support medium is completely submerged underwater for the duration of treatment. On the other hand, a trickling filter consists of a bed of support medium over which the wastewater is uniformly distributed and allowed to percolate over and down through the media. These differences are illustrated in Figures 2-3a and b.

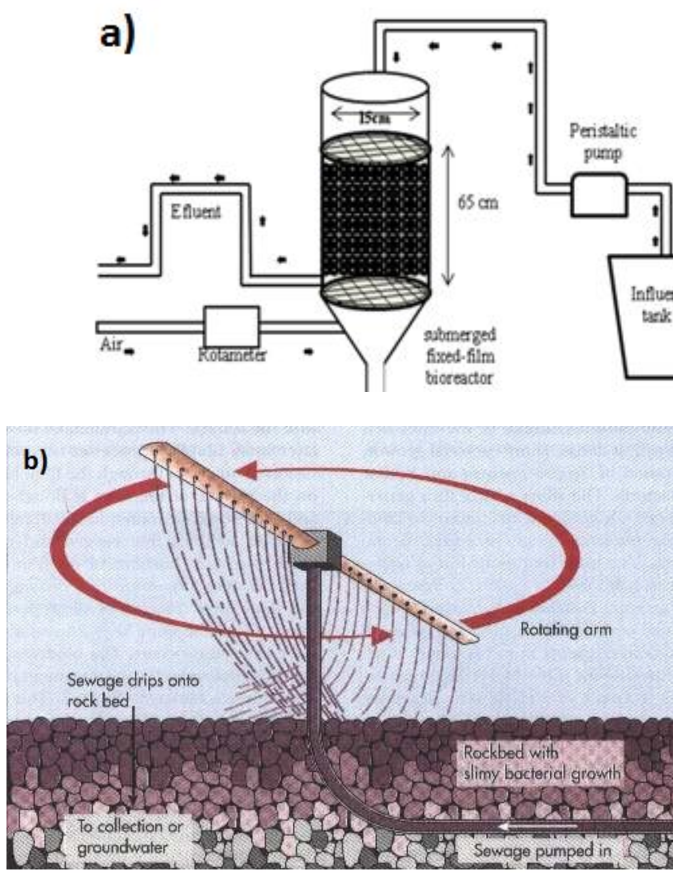

Figure 2-4. a) Submerged biofilm media technology (submerged fixed-bed biofilm reactor) (Rivadeneyra *et al.*, 2014) vs. b) a distribution system (trickling filter) (Toprak, 2000).

In addition, the packing material can be either fixed or suspended in a reactor. Biofilm technologies that utilize fixed media tend to retain biomass more readily and therefore tend to perform more effectively at higher organic loading rates; however, this also means that they are more prone to clogging (Wang *et al.*, 2005). Historically, the implementation of sand, peat or rock filters for use in wastewater treatment has been common due to the widespread availability and low cost of materials, but many other support materials have been tested for better treatment efficiencies under specific conditions (U.S. EPA, 2002). The media used for biofilm development can be natural or synthetic. Some examples of materials that have been previously tested include, but are not limited, to: glass, peat, natural geolite and expanded clay, polyurethane foam cubes, and fibrous carriers (Loupasaki and Diamadopoulos, 2013). These materials differ in their availability, cost, longevity, porosity, and shape. Thus, the material selected can have a significant impact on the subsequent biofilm environment. Waste products such as tire rubber and crushed glass have been developed as biofilm support media in order to capitalize on the use of recycled materials, but these typically have smaller surface areas in comparison to more porous compounds (Zhifei and Graham, 2006; Horan and Lowe, 2007). If total nitrogen is the target parameter for removal, certain polymer matrices as biofilm supports are beneficial because they may act as an external carbon source in wastewaters with low carbon-to-nitrogen (C:N) ratios, thus decreasing competition for soluble substrate and providing simultaneous nitrification and denitrification (Chu and Wang, 2011). However, their biodegradability is a disadvantage because they need to be replaced more frequently than synthetic materials.

Alternatively, natural zeolite provides a favourable environment for nitrifying bacteria because of its ion exchange capacity and increased resistance to ammonia shock loads (He *et al.*, 2007). In the case of environments with variable or low pH, it has been shown that the use of carbonate media as support material can improve the buffering capacity of the wastewater by the release of calcium carbonate (Qiu *et al.*, 2010). In general, packing materials that provide a large surface area will provide more biofilm development per unit volume. Therefore, having a large amount of void space in a particular media is an important factor in maintaining high amounts of diverse microbial populations, but it is not necessarily the primary factor that controls overall performance (Yu *et al.*, 2008).

2.3.1.2 Aeration (mechanically aerated, passively aerated, anaerobic)

Biofilm systems can be mechanically aerated, passively aerated, or anaerobic. The type of aeration employed in a biofilm system will determine the environmental conditions that develop, and therefore, which microbial processes will dominate. For example, aerated environments lead to higher redox conditions, and thus processes such as aerobic decomposition and nitrification will dominate over anaerobic processes like fermentation and methanogenesis (Grady *et al.*, 1999). Depending on the treatment objectives and the capacity/design of the biofilm technology, the on/off cycling of mechanical aeration can be implemented in order to create alternating anaerobic and aerobic conditions. This can help to increase both anaerobic and aerobic processes. How well a biofilm treatment system performs is also dependent on the biofilm media and the method of wastewater application/delivery (Gavrilescu and Macoveanu, 2000).

Mechanically aerated systems employ an external method of aeration (forced aeration) in order to increase oxygen supply to the biofilm and provide the system with adequate mixing for optimal wastewater/biomass contact (Loupasaki and Diamadopoulos, 2013). Aerated systems have the highest energy requirement and operational costs, but result in higher overall reductions and the ability to treat high-strength/primary wastewaters due to the significant increases in oxygen-mediated processes, such as nitrification (Lee *et al.*, 2002).

Passively aerated biofilm units do not require an external oxygen supply to provide aeration; rather, oxygen is supplied to the system via natural ventilation or exposure to atmospheric air (e.g. trickling filters, pumped-flow biofilm reactors, air suction flow biofilm reactors, etc.), allowing oxygen from the atmosphere to diffuse into the wastewater. They have minimal energy requirements, and are most often filtration processes that allow oxygen from the air to diffuse through the biofilm as the intermittently-applied wastewater flows down or through the support media. As such, the limiting factor for treatment in passively aerated units is the amount of oxygen that can be provided to the biofilm via this process. When adequate oxygen is available to the biofilm, passively aerated units have been shown to be successful in treating secondary wastewaters and are effective in the removal of suspended solids, organics, nitrogen and pathogens. Nitrification-denitrification processes can occur in passively aerated filters with intermittent flow due to alternating aerobic/anaerobic environments.

Anaerobic biofilm systems are attractive because of their lower energy requirements in comparison to aerobic treatment processes, which often have high energy demands to mechanically aerate the treatment unit (Loupasaki and Diamadopoulos, 2013). Anaerobic treatment procedures, including anaerobic biofilm reactors, can also produce methane as a potential renewable energy source (Shin *et al.,* 2011). They have been shown to effectively reduce levels of COD by providing good mass transfer of substrate to biofilm and have little potential for clogging and short-circuiting (Rittmann and McCarty, 2001; Switzenbaum and Jewell, 1980). Oftentimes, anaerobic biofilm systems are employed in the pre-treatment of domestic wastewaters (Loupasaki and Diamadopoulos, 2013). These attached growth systems are typically operated as filters under anaerobic conditions and produce less solids residue than other types of biofilm reactors, but have been shown to only produce high COD removal efficiencies (>70%) if the media used has a high porosity (Kennedy *et al.,* 1989). As well, in order to achieve sufficient COD and TSS removals in anaerobic systems, the DO levels must be carefully monitored and controlled in order to ensure that concentrations remain as low as possible. When the ratio of DO to COD exceeds 0.12, the efficiency of organic matter and suspended solids reductions are lessened. This is because higher DO levels in anaerobic biofilm systems can cause the growth of oxygen-consuming organisms and a loss of adequate methanogenic activity (Shin *et al.*, 2011; Whitman *et al.,* 1992). Anaerobic biofilm systems can also effectively reduce ammonia nitrogen if the HRTs are sufficiently long (Bodik *et al.,* 2003; Reyes *et al.,* 1999). However, they are generally not effective in reducing orthophosphate concentrations—regardless of HRT or prolonged substrate-biofilm contact time—and so should not be considered if target removals include high levels of phosphorus (Reyes *et al.*, 1999; Feng *et al.,* 2008).

2.3.1.3 Effects of temperature, loading rates and hydraulic retention times

The microorganisms present in wastewater are influenced by the interplay between temperature, organic/nutrient loading rates, and hydraulic retention times. It is well-known that temperature will have an effect on microbial activity: in general, the optimal temperature for wastewater-treating microorganisms to thrive is between 25-35ºC (Metcalf and Eddy, 2003). Below this temperature range, the growth rates of microorganisms decrease with decreasing temperature, with activity and growth rates becoming fully inhibited in very cold environments. For example, the activity of nitrifying bacteria becomes almost inert at temperatures below 5ºC (Metcalf and Eddy, 2003). In wastewater stabilization ponds, cold weather also adversely affects both the settling characteristics of settleable solids (i.e. biomass, sludge, etc.) and gas-transfer rates (Spellman, 2011). Facultative and aerobic WSPs tend to have large lagoon surface areas, leading to more heat loss and lower overall water temperatures (Grady *et al.*, 1999). As well, the prevalence of WSPs use in cold-weather climates would suggest that freezing/chilling of lagoon systems is a common occurrence, resulting in a significant decline in treatment efficiencies (Grady *et al.*, 1999). Therefore, the addition of a biofilm technology to a pond or lagoon system can be helpful in improving wastewater treatment during cold-weather conditions.

Although biofilms are made up of microorganisms, and their biochemical transformations are affected by low temperatures, it has been shown that biofilm filters are able to dampen these temperature effects to some extent (Loupasaki and Diamadopoulos, 2013). Results of a submerged aerobic biofilm filter showed that, at constant organic loading, BOD removals did not significantly decrease when temperatures decreased from 35ºC to 20ºC, and even when reduced to 5ºC (Hu *et al.*, 1994). In a study conducted by Williamson (2010), it was found that the wastewater effluent temperature had no effect on BOD or TSS concentrations when comparing results from different attached growth biofilm systems, and that the influent wastewater temperature had a negligible effect on BOD within a normal range of 10 to 22ºC. A study conducted by Gray and Learner (1984) suggested that the support medium of a biofilm may help to assuage the negative effects of low temperatures on wastewater treatment. The study found that certain support medium materials, such as slag and plastic, helped to retain heat and maintain a constant temperature in the inner layers of a biofilm, and that heat loss from these inner layers were further balanced by the heat produced during biological oxidation, which is generally exothermic. Hence, little variation in temperature occurred in the inner layers of the media, and metabolic processes—particularly oxidation of organic matter—were allowed to continue at a relatively constant rate.

Attached growth biofilm systems have also been shown to help negate the cold-temperature effects on biological nutrient removals. Christensson and Welander (2004) and Hubbel and McDowell (2003) showed that nitrification rates in biofilm systems were less affected by changes in temperature in comparison to wastewater treatment by suspended bacterial populations, making them a good choice for nitrogen removal in cold climates. In particular, biofilms help to maintain high levels of nitrifier activity, which is of particular importance in the context of cold-weather nutrient removals, as nitrifiers are more sensitive to temperature variability than other nutrient-reducing microorganisms such as denitrifiers and phosphorus-accumulating organisms (PAOs) (Oleszkiewicz, 2015; Metcalf and Eddy, 2003). A study conducted by Oleszkiewicz (2015) showed that in integrated-fixed and activated sludge (IFAS) systems—which incorporate microorganisms in both attached-growth (i.e. biofilm) and suspended growth form—the efficiencies of nitrification by the attached-growth and suspended bacteria were found to change with temperature. During warm temperatures, nitrification was found to occur predominantly by bacteria in suspended form, but as temperatures were lowered, nitrification was predominantly seen in the attached-growth portion of the IFAS. In addition, the higher concentrations of DO at colder temperatures allow for deeper penetration of oxygen within a biofilm, which promote the growth of active nitrifiers despite their low growth rates (Regmi *et al.*, 2011). Biological phosphorus removals can also be aided by the integration of a fixed film media, as attached-growth systems help prevent washout of phosphorus-reducing microorganisms (e.g. PAOs) at low temperatures (Sriwiriyarat and Randall, 2005; Mamais and Jenkins, 1992).

Organic loading rates also have an effect on both nutrient and organic constituent removals. Therefore, there is an interplay between a number of different factors that contribute to wastewater treatment, making it difficult to predict system performance given a limited subset of conditions. Autotrophic nitrifiers and heterotrophic bacteria compete with each other for organic substrates, nutrients and DO; as such, the loading rates (i.e. C/N ratio) will necessarily influence which microorganisms will tend to dominate. Higher concentrations in carbon substrate generally lead to a decrease in competition between heterotrophs and autotrophs, and allows for better accumulation of both types of microorganisms simultaneously. However, a study by Rostron *et al.* (2001) involving the treatment performance of immobilised biomass (biofilm), showed a decrease in nitrification upon an increase in carbon substrate in high-ammonia wastewater, due to the consequent growth of the heterotrophic bacterial population. Wijeyekoon *et al.* (2004) also found that high organic substrate loading led to biofilms with lower porosities and lower specific activities due to the stratification of microbial populations, leading to inhibited mass transport of substrate and DO to the inner biofilm layers.

The hydraulic retention time (HRT) of a biofilm treatment system is an important control parameter to consider when aiming to reach target treatment goals of nutrients. High HRTs typically provide longer contact time between the biofilm and wastewater (substrate), leading to better wastewater contaminant reductions (Loupasaki and Diamadopoulos, 2013). Small retention times decrease the contact time between the microorganisms and substrate. When insufficient time is allotted for microorganisms to stabilize wastewater contaminants and consume organic substrate, their growth rates are stopped, resulting in biomass washout. HRTs that are too low can also cause shear forces that lead to the washout of microorganisms, particularly those which are slow-growing, and especially during the unsteady-state development stages of the biofilm (Wijeyekoon *et al.*, 2004). Feng *et al.* (2008) found that in an anaerobic biofilm bed reactor, when HRT was reduced from 48h to 18h, COD removal efficiencies decreased by 10% due to washed-out biomass. HRTs also influence the organic load being treated by the microorganisms per day. Feng *et al.* (2012) showed that, in a biological aerated filter (BAF) biofilm system, HRT reduced to 1h from 5h resulted in high hydraulic loadings and a decrease in COD removals. However, compared to suspended sludge/continuous-stirred tank reactor (CSTR) systems, biofilm technologies contribute to better removals by decreasing the washout times for nitrifying bacteria, PAOs and heterotrophs. Rostron *et al*. (2001) showed that reactors employing immobilisation media (biofilm) yielded efficient nitrification at HRTs as low as 12h, while nitrifiers in suspended sludge were washed out at a HRT of 1 day. This was likely because, as mentioned previously, the microbial aggregate system allowed for the attachment of microorganisms into a more stable and washout-resilient biofilm layer. However, initial loading rates and retention times (shear rate) can also influence the type of bacteria that will populate the biomass and the subsequent structure of biofilm. High retention times and low shear rates are needed during the initial formation of the biofilm, as a growing biofilm is more susceptible to shear forces and more frequent sloughing and/or detachment. As well, low shear rates are recommended to increase the heterogeneity of the microorganisms populating the biofilm (Loosdrecht *et al*., 1995).

Overall, the effectiveness of a biofilm technology in reducing wastewater constituents is dependent on many factors. In addition to the design and operational characteristics, the growth and activity of microorganisms—and therefore treatment efficiencies—can also be affected by pH, recirculation, and aeration cycling, amongst other variables. The interplay between these factors is complex and at times difficult to predict. As such, system performance must be evaluated on a case-by-case basis.

2.3.2 Examples of existing biofilm reactors/systems

In general, biofilm technologies as a method of enhancing wastewater treatment are advantageous because of their operational simplicity, low energy consumption and associated costs, as well as minimal sludge production (Gavrilescu and Macoveanu, 2000; Gao et al., 2014). There are a variety of existing biofilm system technologies that can be retrofitted into lagoon systems: these employ attached-growth systems to enhance wastewater treatment, but differ in their design and operation. Depending on the removal or effluent requirements, bioreactors design can be tailored based on aeration and sizing requirements, optimal energy consumption, recirculation and temperature (Jenkins and Sanders, 2012). Additional design considerations include: potential for support media clogging, inadequate mixing leading to short-circuiting, and excessive growth (i.e. resulting in the sinking of free-floating media) (Water Environment Federation, 2010). This section will discuss a few well-established designs that have been utilized for wastewater treatment, and detail the conditions under which they each operate.

2.3.2.1 Trickling filters

Trickling filters (TFs) are one of the earliest microbiological methods employed in the treatment of sewage (U.S. EPA, 2000). More recent designs in biofilm technologies use similar treatment principles. It is an aerobic treatment system design to effectively remove organic matter from wastewater via absorption and transformation by the aerobic microbial population attached to the support medium (Molof and Yun, 1992). The wastewater is evenly applied at the top of the medium by a rotating-arm distribution system driven by water jets, similar to a sprinkler system. As the water “trickles” down through the medium (gravity-drained), microorganisms in the wastewater attach to the supporting media, forming the biofilm layer. As more wastewater is applied and the biofilm thickens, anaerobic microorganisms develop in the inner layers of the biofilm due to the inability for dissolved oxygen to penetrate and diffuse through the thickness of the biofilm from the bulk phase without being fully consumed. When the biofilm thickness becomes too large, the outer portions will eventually slough off as the aerobic microorganisms at the biofilm surface lose their ability to remain attached to the medium (Molof and Yun, 1992, Mara, 2003). As such, an underdrain system is needed to collect and remove the solids which have sloughed off. Oftentimes, liquid collected from this chamber is recirculated back to the top in order to optimize removal rates. A diagram depicting a typical trickling filter is shown in Figure 2-4.

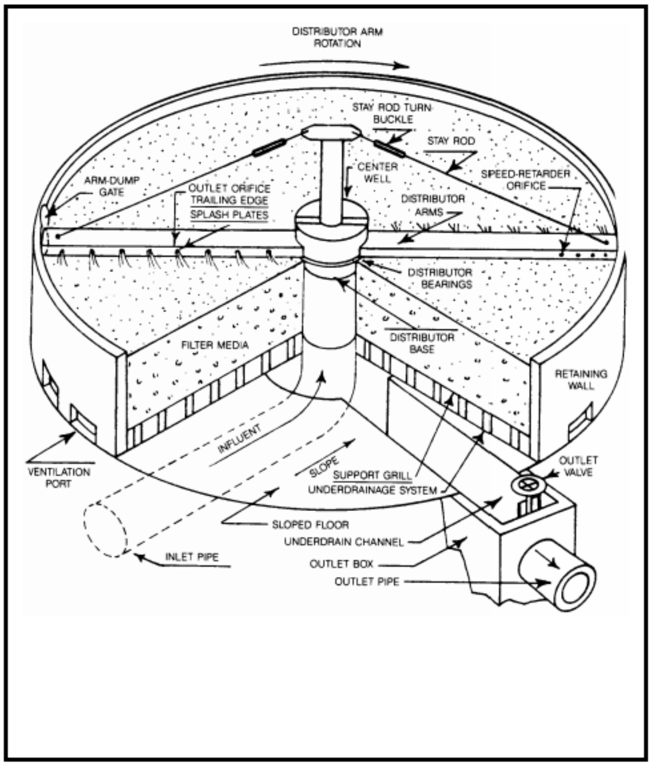

Figure 2-5. Design of a trickling filter (Metcalf and Eddy, 2003).

Sufficient air must be available to provide oxygen to the wastewater-treating microorganisms. Although external aeration is not usually applied in TF systems, ventilation ports are provided which allows wind and natural draft forces to aerate the system and maintain successful operation (U.S. EPA, 2000). The support medium in a trickling filter varies between each individual system. Generally, the filter media is composed of a bed of rock, slag or plastic. Heavier media results in a design with a larger diameter and smaller depth, while lightweight media can be used in trickling filter "bio-towers" that are smaller in diameter and can reach depths of up to 12 m (U.S. EPA, 2000). Synthetic plastics and foams have become the preferred media for biofilm development, as the high void space allows for sufficient airflow throughout the filter, less probability of clogging, and a high surface area for growth (Molof and Yun, 1992). However, crushed rock or gravel are less expensive options and equally as durable (Loupasaki and Diamadopoulos, 2013). Trickling filters can vary in design (low-, intermediate-, or high-rate) depending on the expected organic load of the influent and target BOD removals. TFs can also be used in conjunction with other treatment processes (i.e. activated sludge) to produce a higher-quality effluent and reduce the possibility of shock loading (U.S. EPA, 2000).

2.3.2.2 Biological aerated filters

Biological aerated filters (BAFs) combine physical filtration with organic matter removal, nitrification and/or denitrification (Goncalves and Rogalla, 1992). The packing medium is relatively small in size to provide a high surface area for biofilm development, and is packed in a bed approximately 2-3m in depth (Stephenson, 1997). BAFs differ from trickling filters in that the entire system is submerged and wastewater is pumped through the filter. The applied wastewater can be pumped in an upflow or downflow mode, although upflow BAFs are more common as they typically minimize channeling (U.S. EPA, 1983). Figure 2-5 depicts an upflow BAF.

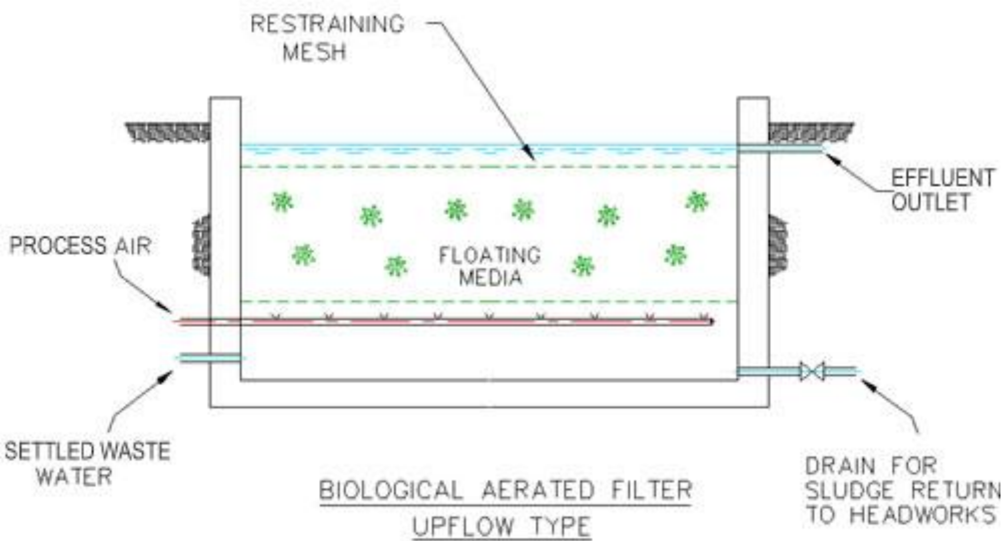

Figure 2-6. Upflow biological aerated filter (Water Maxim, 2007).

The flow of wastewater through the media provides a physical method of filtration, which reduces the need for a separate clarification process. External aeration must be applied via a blower that provides air bubbles and oxygen to the attached biomass. Depending on the variation of organic load in the influent, BAFs can be designed to include different filter “cells” that are used in rotation depending on the strength of wastewater being treated. Cells containing the filter media must be periodically washed in order to remove excess biomass growth and remove trapped solids; this is to prevent clogging of the media and blockage of the filter pathways (Smith, 1998). Washing is performed via high flow-rate backwashing or by using an air scour system to loosen the media bed. If backwashing is used, the waste backwash water is collected and recirculated back to primary treatment.

The operation of BAFs can be configured to treat organics, as well as provide nitrogen removal. In order to achieve the latter, additional filter cells are included in the BAF system design and a supplemental carbon source (e.g. methanol) is added (Pramanik *et al.,* 2012). Total nitrogen removal is also aided by operation in an anoxic mode, where the external aeration to one or more filter cells is discontinued for a sufficient period of time (Zhang *et al.*, 2013).

2.3.2.3 Moving-bed biological reactors

Moving-bed biological reactors (MBBR) are a relatively recent technology, having been established only in the past 25 years (Jenkins and Sanders, 2012). MBBRs are simple and flexible systems that require little space for operation and maintenance, and have been shown to be effective in treating BOD, ammonia and total nitrogen (Ødegaard *et al.,* 1999; Ødegaard *et al.*, 1994). MBBRs utilize biofilm via free-floating media, which is suspended in a reactor by the mixed motion of an aerated wastewater treatment basin. Independently-circulating biofilm carriers allow for better oxygen and substrate transfer to the biomass as well as the development of a highly robust and diverse environment of heterotrophic and autotrophic microorganisms (Ødegaard *et al.*, 1999). External aeration is applied in order to provide oxygen to the media, as well as to impart the turbulence needed to keep the biofilm media in suspension. This turbulence results in shear forces that help to effectively maintain an optimal biofilm thickness and prevent overgrowth. This turbulent energy can also be achieved using liquid recirculation or mechanical mixing (Jenkins and Sanders, 2012). The medium carrier is typically a plastic material with high porosity and surface area, and it takes up around 1/3 to 2/3rds of the available space in the bioreactor. Studies have demonstrated that the shape and size of the media carriers did not have a significant effect on treatment efficiencies as long as the surface area remained consistently high; however, plastic media is preferred due to its longevity (Jenkins and Sanders, 2012; Rodgers and Zhan, 2003).

MBBRs can consist of one or more compartments containing biofilm carriers, depending on the characteristics of the inflowing wastewater. Multiple reactors can be configured in series to achieve a range of treatment goals (i.e. BOD removal, nitrification, and denitrification) by promoting the development of specialized biofilms optimized to achieve specific target removals (Jenkins and Sanders, 2012). Figure 2-6 illustrates a multi-compartment MBBR utilizing free-floating biofilm media.

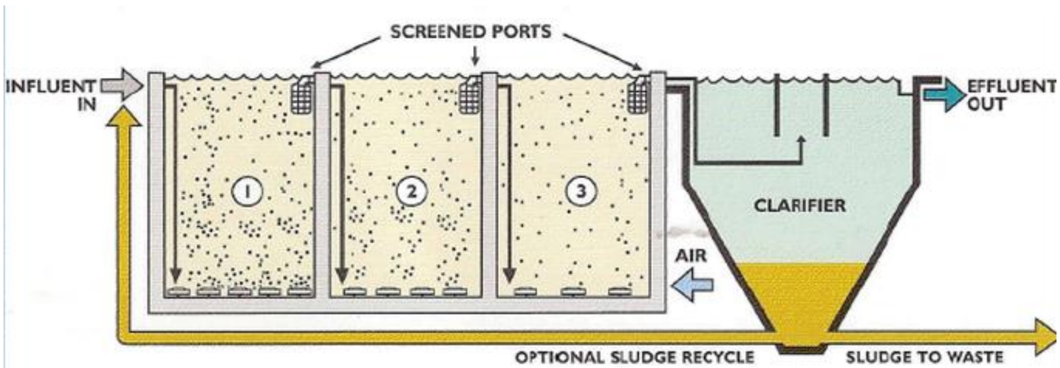

Figure 2-7. Schematic of a moving-bed biological reactor with free-floating biofilm media, followed by a clarifier (EnviroTech, 2014).

Generally, MBBR processes maintain an optimal level of productive biofilm on their own (they have minimal sludge production in comparison to conventional activated sludge processes), and most of its active biomass is retained continually in its reactor (Ødegaard *et al.*, 1999). MBBRs are also continuous flow-through processes, meaning that backwashing and maintenance requirements are minimized. As such, MBBRs are especially beneficial in terms of retrofitting for existing treatment facilities. They do not require an extra stage for solids separation due to the retention of active biomass in the reactor; therefore, MBBRs are compatible with a variety of separation techniques in addition to conventional clarifiers (Jenkins and Sanders, 2012).

These three wastewater treatment technologies are only a small subset of the multitude of different designs that utilize microbial aggregates to treat wastewater. Other design examples include integrated fixed-film activated sludge systems (IFAS), rotating biological contactors (RBC), sequencing batch biofilm reactors (SBBR), and percolating/sand filters. Generally, these systems have been designed to treat secondary wastewater; that is, to improve upon the quality of effluent provided by a primary treatment system (e.g. a stabilization pond or primary clarifier) via enhanced microbial activity (Loupasaki and Diamadopoulos, 2013; Jenkins and Sanders, 2012). The design and operation of each system must be specifically tailored to site-specific requirements and conditions of the influent wastewater; therefore, these parameters that must be taken into consideration in the selection and modification of fixed-film wastewater treatment systems.

2.4 Conclusion

Wastewater treatment is a complex and highly variable process. The effectiveness of wastewater treatment can be influenced by a number of parameters. Biological treatment promoting microorganisms that can metabolize and transform wastewater contaminants is one of the most effective approaches to reduce wastewater constituents in WSPs and biofilm technologies. The growth and activity of these microorganisms, although relatively well-defined, are sensitive to changes in environmental and operational conditions, and their optimization relies on conditions that are interconnected and, at times, unpredictable. As such, for effective use of biofilm technologies to improve upon existing WSPs, the effects of temperature, aeration, and retention times must be considered in the implementation and design of any such technology. Operation and maintenance conditions can only be determined on a case-by-case basis to adapt system conditions that will be best suited to optimize the growth, activity and proliferation of the developing biofilm.

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Chapter 3

Comparison of semi-passive treatment technologies during start-up conditions and low seasonal temperatures

3.1 Abstract

In Canada, the cold temperatures associated with winter climates can considerably decrease the treatment efficiencies of wastewater stabilization ponds. As such, the addition of semi-passive treatment technologies as a pond or lagoon upgrade may assist in increasing pond capacity in order to achieve higher treatment performance at colder temperatures. The successful addition of these technologies could also potentially increase treatment efficiencies during warmer months and allow for an increase in the amount of septage accepted at a particular facility. In this study, the potential use of three different semi-passive (i.e. aerated) treatment technologies—two third-party developed biofilm systems (the BioCord and BioDome systems) and the application of zebra mussels to filter wastewater—to improve ammonia, total nitrogen (N), phosphorus (P), chemical oxygen demand (COD) and total suspended solids (TSS) removals from septic pond effluent was investigated. Amongst the three technologies, the BioCord biofilm system showed the highest percent reductions of all water quality parameters compared to the others, and maintained significantly lower concentrations of all parameters in comparison to the influent. The results suggested a heterogeneous biofilm population able to sustain relatively high levels of nitrification/denitrification and organic matter reductions. This also indicated that the media employed by the BioCord system allowed for a thicker biofilm development, leading to better insulation of the system to fluctuating temperatures and overall better performance during colder ambient temperatures. In conclusion, the BioCord system performed better than both the BioDome and zebra mussel systems, yielding a better effluent quality of the wastewater, and therefore showing the most potential for full-scale testing and implementation.

3.2 Introduction

Lagoon facilities across Canada experience seasonal changes in temperature, resulting in cold weather constraints during the winter operations. During cold temperatures, WSP treatment efficiencies decrease as a result of reduced bacterial growth and activity, as well as slower settling characteristics of biological solids and gas-transfer rates (Grady *et al*., 1999; Metcalf and Eddy, 2003). Nutrient removals are particularly affected during cold climates; when nitrogen (N) and phosphorous (P)-consuming microbes are slower-growing and often outcompeted by algae and heterotrophic microorganisms. To compensate for these decreases, lagoon facilities may have to increase their retention times up to 30 days (slow pond operation) in order to satisfy discharge guideline requirements (Crites *et al.,* 2010). In addition, premature freezing can require for pond operation to be stopped prematurely for winter operation, leading to an overall substantial decrease in treatment during cold weather conditions. The inability to effectively treat incoming wastewater leads to an excess volume of septage that must be held over the winter season, resulting in a slower startup when pond operation resumes in the springtime. The addition of semi-passive treatment systems to WSP facilities presents an attractive option to enhance the efficiency of lagoon facilities during cold-weather operations. This is especially beneficial for Canadian rural communities, as cold temperatures and decreased pond efficiencies are challenges faced by most facilities operating in North American climates (Gloyna, 1971). The lower cost, energy, and maintenance requirements of these semi-passive technologies are also an added benefit for operations that wish to increase their treatment performance without adding substantial energy- and cost-intensive infrastructure to their existing systems. By implementing such technologies, existing facilities may be able to maintain sufficient levels of treatment without compromising retention times. As well, enhanced parameter reductions during winter seasons suggest better overall treatment during the summer season as well, which can be beneficial for communities that are expected to experience significant population growth (and therefore wastewater loading) in the future.

Biofilm technologies have been reported to increase performance by increasing the overall concentration of bacteria present for wastewater treatment. The biofilm matrix allows for a fixed structure of microorganisms and cellular products, which helps protect the culture from changes in ambient environmental conditions, increases interactions amongst cells, and allows for growth through cell division and adhesion (Lazarova and Manem, 1995; Nicolella *et al.*, 2000). Biofilm technologies have been shown to buffer the negative effects of lower temperatures, likely due to the transport of substrate through the outer biofilm layers to reach the warmer, more insulated environment of the inner layers (Loupasaki and Diamadopoulos, 2012). This transport from bulk liquid to the inner layers of the biofilm is often a limiting factor in achieving effective treatment.

Two third-party-developed, semi-passive biofilm technologies were chosen to observe their potential for improving wastewater treatment during cold weather conditions at a large (pilot) scale. Both systems were submerged biofilm reactors that required mechanical aeration for optimal biofilm development and treatment performance. The on-site experimental setup during cold-weather testing of these biofilm technologies included a solar hybrid power system in order to provide the necessary energy required to aerate the test systems. The first semi-passive biofilm technology, the BioCord system, was developed by Bishop Water Technologies. The second, the BioDome system, was developed by Wastewater Compliance Systems Inc. Both biofilm technologies have been reported to aid in optimizing the conditions for microbial aggregate growth and proliferation, with minor differences in structure and framing. Section 1.2 in Chapter 1 provides a more detailed overview of the two biofilm treatment technologies. Although the BioCord system's effectiveness in treating wastewater has been documented in a number of published studies (Yuan *et al.*, 2012; Ateia and Yoshimura, 2015), the BioDome treatment technology is the only system whose cold-weather treatment efficiencies and ability to treat wastewater in a Canadian climate have been specifically investigated in a case study. In this study, Johnson (2011) showed that the BioDome system could produce significantly higher percent reductions of wastewater parameters in comparison to an aerated control tank, for temperatures as low as 5ºC.

Zebra mussels (*Dreissena polymorpha)* have a filtration mechanism that has been reported to be capable of reducing total suspended solid (TSS) concentrations and increasing the clarity of large bodies of water (Bruner *et al.*, 1994; Noordhuis *et al.*, 1992). They have been shown to bioaccumulate a number of aquatic contaminants, which are then retained in their tissues and shells or deposited in mussel feces and pseudofeces (Kock and Bowmer, 1993; Bruner *et al.*, 1992). Although their optimal temperature range extends from about 20-25ºC, the shell growth of zebra mussels can occur at temperatures as low as 3ºC, and the lower limits of their survivability have not yet been conclusively determined (Molloy, 2002; Molloy *et al.*, 1997). Filtration rates of zebra mussels are also highly variable and dependent on temperature, with rates rising drastically between 5 and 10ºC, levelling off between 10-20ºC and potentially being inhibited at temperatures over 20ºC (Noordhuis *et al.*, 1992). Slightly different temperature effects have been observed, with Fanslow *et al.* (1995) noting maximum zebra mussel filtration rates between 10 and 20ºC, and Reeders and Bij de Baate (1990) reporting maximum filtration occurring in the range of 10ºC to 22ºC. Gossiaux *et al.* (1996) found that aquatic contamination bioaccumulation by zebra mussels was higher at 20ºC than at 4ºC. This suggests that zebra mussels could be effective in filtering/uptaking wastewater constituents in the temperature range of 10ºC to 20ºC. Although filtration may be reduced at temperatures exceeding 20ºC, the reproduction and life cycle of zebra mussels depend on season, with zebra mussel oogenesis typically occurring in the autumn and female eggs being expelled and fertilized in the spring/summer seasons (Fahnenstiel *et al.*, 1995). Larval development is also optimal in the range of 20-22ºC (Sprung, 1993). Thus, although filtration capabilities may not be as efficient during the Canadian summer season, when temperatures can exceed 20ºC, the ability of *D. polymorpha* to maintain a robust and growing population is dependent on warmer environments. In addition, some research suggests that zebra mussels may be able to acclimatize to seasonal temperature variations, allowing them to effectively utilize their filtration mechanisms outside the optimal temperature range (McMahon, 1996). As such, should zebra mussels be utilized for wastewater treatment, the longevity of these organisms would be enhanced by maintaining year-round implementation in wastewater environments. Although their filtration rates during warmer temperatures may not be as efficient, ensuring an adequate growth and reproduction environment for zebra mussels is important so that their population is continually growing and/or maintained, especially partial zebra mussel mortality increases cases of accidental shock or hypoxia. The zebra mussels utilized for wastewater treatment in this study were also provided with mechanical aeration in order to ensure sufficient oxygen levels for viability. The purpose of this study was to investigate the differences in performance between three selected semi-passive treatment technologies during colder fall/winter temperatures.

3.3 Experimental setup and design

This study was conducted at the lagoon facility of Storring Septic, located in Tamworth, Ontario. This remote location experiences weather and environmental conditions representative of rural lagoon facilities across Canada and North America. Hence, the results obtained during this study could presumably be applicable to other WSP operations. The on-site experimental setup is shown in Figure 3-1.

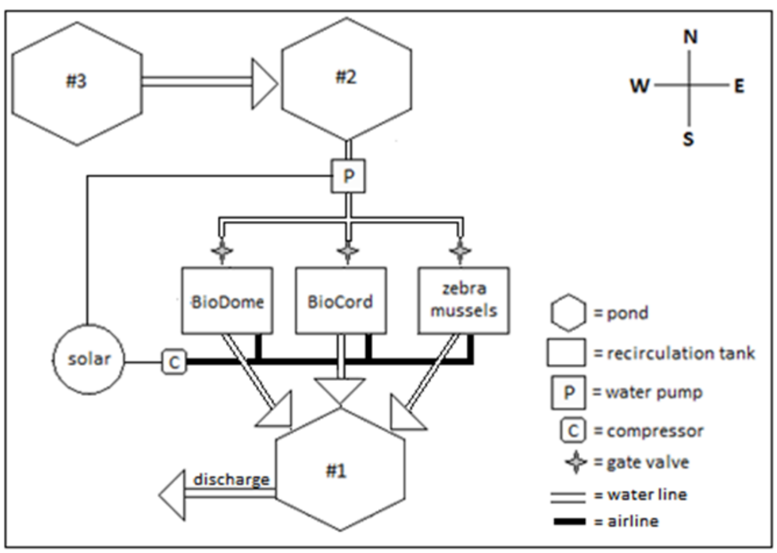


Figure 3-1. Schematic detailing the flow of domestic septage treatment. Influent entering Pond 2 of the Storring Septage site was pumped into each of three tanks, conveyed to one of three treatment technologies, and drained (by gravity flow) into Pond 1. Tank #1 housed the BioDome system, Tank #2 the BioCord system and Tank #3 the zebra mussel treatment. Final effluent from Pond 1 was discharged via land spreading. Aeration was provided to each of the tanks via air compressor.

To test and compare the three treatment technologies, three cylindrical treatment tanks were set up to receive influent from the secondary stabilization pond of the Storring Septic facility ("Pond 2"). Each tank had a volume of approximately 5678L, a diameter of ~2.2m and a height of ~1.7m. As shown in Figure 3-1, each tank housed one of the treatment technologies and received wastewater that was pumped from Pond 2 using a water pump. After treatment at specified hydraulic retention times (HRTs) by the BioCord, BioDome, or the zebra mussel systems, the treated wastewater in the tanks was then discharged to the tertiary treatment (maturation) pond for final treatment of the wastewater. After treatment in the tertiary treatment pond, the final effluent was then discharged for land spreading and evaporation.

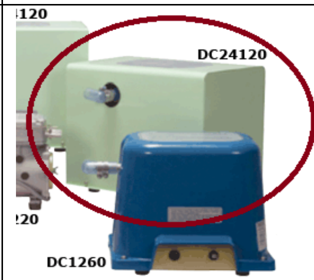
Storring Septic currently uses two hauling trucks to pump and deliver wastewater from domestic septic tanks to the WSP facility. This inflowing wastewater is dumped directly into the Storring Septic primary pond, Pond 3, and the volume of septage added to this pond can fluctuate from 150kL/month to as much as 900kL/month, depending on customer demand and periods of peak activity (June-September). The volume of inflowing wastewater also varies greatly on a day-to-day basis depending on the number of loads delivered that day. The influent composition of each individual load can also fluctuate drastically. Septic tanks of each individual household can differ in quality and quantity, depending on water usage, the size of the household, and the age of septage, amongst other factors. Because of this, it is important that the treatment technologies employed be robust enough to handle these types of fluctuations.

An air compressor with a 4 cubic feet per minute (CFM) capacity was used to deliver aeration to each of the three tanks, with air pressure gauges in place to ensure that the amount of air being delivered was relatively evenly distributed between the tanks. It was made certain that the three tanks were receiving at least 1CFM of air flow during periods of aeration. This minimum airflow was selected based on the requirements for the BioDome system as stated by the manufacturer, Wastewater Compliance Systems, Inc., who specified a minimum of 1CFM to effectively introduce air into the system and achieve a similar performance as conventional aeration systems (Wastewater Compliance Systems, Inc., 2015). Due to the lack of information regarding dissimilarities between the BioDome and BioCord systems (submerged biofilm systems), as well as the resilience of zebra mussels to relatively low oxygen concentrations (Benson *et al.*, 2015), a minimum airflow of 1CFM was determined to be appropriate for each of the three treatment technologies to achieve good treatment levels. All of the energy requirements for this experimental setup were met using power generated by a hybrid solar electricity system installed by the Storrings.

Wastewater flow from Pond 2 to the three treatment tanks was controlled using an above-ground water pump and 1” PVC gate/ball valves. Table 3-1 lists the model/make of the equipment used in the experimental setup, as well as some specifications for each unit. As seen in Figure 3-1, gate valves were placed at the inlets of each treatment tank to control the flow rates (and resulting HRTs) into the three tanks. Loading rates were adjusted between 0.75 - 1.25kg CODm^{-3}d^{-1}, as the goal of testing in the fall season was to observe performance under decreasing temperature conditions. As such, flow rates and retention times were adjusted according to influent COD levels, such that the potential for shock loading and failure of treatment technologies would be minimized.

Table 3-1. On-site equipment used for the experimental setup at Storring Septic.

Equipment/purpose	Model/make	Specifications	Image
Water pump; pump water from Pond 2 into treatment tanks	DC4000 RLSS Waveline™	24V DC water pump Continuous-duty (24h) usage Dimensions: 151mm x 91mm x 127mm Max flow 3997 litres per minute (LPM) 11-speed controller included Inlet diameter: 1-½”, outlet diameter: 1”	

PVC gate/ball valves (x3); control water flow into treatment tanks	T-601 Legend Valve & Fitting, Inc.	1” PVC Threaded FPT x FPT Ball Valve	
Air compressor; provide aeration to treatment tanks	DC24120 Pentair Aquatic Eco-systems®	24V DC air compressor Max flow 120 LPM/4.0 CFM Continuous-duty (24h) usage Steel casing Outlet diameter: ¾”	
Air pressure gauge/flow regulators (x4); monitor and control airflow	MP514803AV Campbell Hausfeld	Regulates and records pressure of 0 to 120 PSI Approximately 15CFM flow capacity at 90PSI 1/4-in. NPT female ports	N/A
Hybrid solar electricity system; provide solar energy to site Solar photovoltaics (PV) array and batteries (x4)	PV Panels Friendly Fires 6CS25PS Rolls Battery by Surrette	Solar PV array: 1kW Batteries: 6V, 1156Ah @100 Hr. rate (x4 = 24V system) Flooded lead-acid Deep cycle, performance over long service life	

Samples were collected approximately twice per week, provided access to the site and weather allowed for sampling. Monitoring was initiated on October 4th, 2014 (Day 1) and continued until November 7th, 2014 (Day 35), at which time, the minimum temperatures fell well below freezing (0°C). Monitoring was initiated once the biofilm technologies had been allowed to acclimatize to the wastewater for a two-and-a-half-week period with aeration and continuous flow, and once the zebra mussels had been allowed to acclimatize to the wastewater for a period of one week. It was assumed that at the start of the testing period, both the BioDome and BioCord systems had established relatively robust biofilms.

The main objective of this study was to assess the ability of each of the three treatment technologies to reduce wastewater constituents of interest under cold-temperature conditions representative of typical Canadian fall/winter. The data collected over the 35-day testing period was separated into two parts (start-up conditions under milder temperatures, and pseudo-steady state conditions under colder ambient temperatures) and analyzed separately, as the temperatures recorded in the first 14 days were clearly higher than those observed in the later part of the study. It was also assumed that the biofilm/zebra mussels were acclimatizing during the first two weeks of operation (Days 1-14), when ambient temperatures were considerably higher and that during this time the biofilm technologies were in the process of reaching a pseudo-steady state. Due to the high variability in the factors influencing biofilm growth and development, and the limited information available pertaining to biofilm development under conditions specific to this study, whether the biofilm treatment technologies had reached steady state during or before this time period could not be ascertained conclusively. However, according to an investigation conducted on behalf of Wastewater Compliance Systems Inc., which outlined lagoon enhancement using the BioDome treatment system, an operational timeframe of four weeks was reported as sufficient in establishing a well-developed population of wastewater-treating microorganisms, and allowed the system to reach steady state (Johnson, 2011). To be conservative, it was assumed that, for this study, the biofilms would have reached a pseudo steady state by Day 14 of sampling (after four-and-a-half weeks total). As such, Days 15-35 of sampling represented the data showing treatment results after start-up of the treatment technologies had reached pseudo-steady state, and the entire testing period (Days 1-35) has been represented as two separate sets of data corresponding to wastewater parameter reductions during milder and colder temperatures. The data are summarized in Table 3-2.

Table 3-2. Maximum, mean maximum, minimum, mean minimum and average temperatures during testing periods 1 and 2 of the treatment season.

Testing period	Time (days)	Max (ºC)	Mean max (ºC)	Min (ºC)	Mean Min (ºC)	Mean Average (ºC)	Details
1st	1-14	21	17 ± 0.66	4.6	9 ± 1.10	13 ± 0.83	Representative of start-up ability of treatment technologies and treatment ability in mild temperature conditions

2nd	15-35	16.5	10 ± 0.70	-3.3	4 ± 0.69	7 ± 0.63	Representative of treatment technologies' performance in cold-weather conditions after biofilm establishment and organism acclimatization

Aeration was supplied continuously for the duration of the testing period (Oct 4 – Nov 7, 2014), to maximize aerobic microbial activity and to assess the performance of each technology under operational conditions where aeration is not limited. In future testing, aeration will be cycled on/off to provide insight into the performance of these treatment technologies under intermittent aerobic and anaerobic operational conditions.

3.4 Methods

The wastewater constituents of interest that were monitored included TSS, organic matter (as COD), ammonia/ammonium (total ammonia), nitrite/nitrate and orthophosphate concentrations, as well as pH. These constituents were analyzed for each of four samples collected on any given sampling day including: the influent, effluent from the BioDome system tank, effluent from the BioCord system tank, and effluent from the zebra mussel tank (see Figure 3-1). Samples were collected approximately two times a week. Influent wastewater into the tanks (wastewater coming from Pond 2, the secondary pond) was sampled using a mixture of grab samples from the inflow points of each tank, for the most accurate representation of inflow composition. Approximately 1L of each sample was collected in five separate 1L polytetrafluoroethylene (PTFE) plastic containers and placed on ice for transport until analysis was able to be carried out. Each sampling container was completely filled with sample, such that there were no visible air bubbles present in each container. Analysis of the samples occurred as soon as possible and typically occurred approximately 1-2h from the time of collection.

Table 3-3 outlines the methods used in the analysis of each of the constituents of interest.

Table 3-3. Methods employed for the analysis of wastewater constituent concentrations.

Wastewater parameter	Method
TSS	Filtration and drying; standard methods (Eaton *et al.*, 1998)
COD	Calorimetric; standard methods ($K_2Cr_2O_7$ digestion) (Eaton *et al.*, 1998)
Ammonia/ammonium ion	mV potential; accumet® electrodes (Fisher Scientific)
Nitrite	Calorimetric; Orion Aquafast Nitrite LR/HR
Nitrate	mV potential; accument® electrodes (Fisher Scientific)
Reactive phosphorus (orthophosphate)	Calorimetric; Orion Aquafast Phosphate LR
pH	Fisher Scientific accumet® pH electrode

Total suspended solids were measured by filtering 100mL of wastewater sample through a pre-weighed glass fiber filter using gravity and vacuum filtration. The residue and filter were then dried at 105°C in a drying oven for 1h, the mass recorded, and the TSS calculated using the mass difference and volume of sample. A sample volume of 100mL was used, except in instances where the suspended solids concentrations were too high to allow for percolation of the sample through the glass fiber filter (i.e. influent samples). In such cases, the sample volume was reduced to 50mL.

COD was measured using the calorimetric, closed reflux method as outlined in section 5220 D of Standard Methods for the Examination of Water and Wastewater (Eaton *et al.*, 1998). The digestion solution used in this procedure was prepared by adding 500mL of distilled water to 10.216g potassium dichromate ($K_2Cr_2O_7$), 167mL concentrated sulfuric acid (H_2SO_4) and 33.3g mercury(II) sulfate ($HgSO_4$). The sulfuric acid reagent was prepared by adding 10.07g of silver sulfate (Ag_2SO_4) to 1L of H_2SO_4 (a rate of 5.5g Ag_2SO_4/kg H_2SO_4) and allowing the solution to stand for 2 days in order for the Ag_2SO_4 to completely dissolve. 1.5mL of the $K_2Cr_2O_7$ digestion solution and 3.5mL of sulfuric acid reagent were consecutively added to each test tube containing 2.5mL of sample wastewater. The test tubes were then capped and placed in a block digester (Hach DRB200) (150°C) for 120 minutes to induce a colour change. After samples had cooled, they were inverted multiple times, and the solids were allowed to completely settle to the bottom of the tube before absorbance readings were taken. These three steps (cooling, inverting, settling) typically took 15-20 minutes in total. Absorbance for COD testing was measured at a wavelength of 600nm.

Ammonia/ammonium ion and nitrate concentrations were determined using ammonia and nitrate accumet® electrodes (Fisher Scientific). Either 2mL of ammonia pH/Ionic Strength Adjuster (ISA) (Thermo Scientific) or nitrate pH/ISA (Fisher Scientific) was added to each volumetric flask containing 100mL of sample being tested for either ammonia or nitrate, and moderately stirred using a magnetic stir bar. The probe was placed in the sample solution and mV (millivolt potential) readings allowed to stabilize before recording the reading. Calibration curves were generated every time a new batch of samples were being tested.

Total nitrogen was calculated by the summation of all nitrogen species tested. It was assumed that the levels of organic nitrogen were low enough to be omitted from the calculation of total nitrogen, as most of the organic nitrogen in untreated wastewater has been reported to be associated with particulate matter, and to readily settle out during the primary treatment phase of a multi-cell WSP operation (Reed, 1985). Organic nitrogen can also be contained in organic matter, but it is released as ammonia when the organic matter is degraded by microorganisms (Grady *et al.*, 1999). In order to ensure that levels of organic nitrogen were not a significant fraction of the total nitrogen, wastewater samples from the infuent were sent to the Analytial Services Unit (ASU) at Queen's University approximately every three weeks to be tested for Total Kjeldahl Nitrogen (TKN), a measure that represents the sum of organic nitrogen, ammonia and ammonium. The value of organic nitrogen was obtained by subracting the value of ammonia/ammonium from the TKN value. The percentage of organic nitrogen in the TKN was found to range between 0.5% to 3% of the TKN composition.

Calorimetry was used for the testing of nitrite and orthophosphate using Thermo Scientific™ Orion™ AQUAfast™ reagent tablets. Either one nitrite low-range (LR) tablet or one orthophosphate LR tablet was placed in 10mL of sample and allowed to dissolve to induce a colour change. The absorbance of the resulting colour was then immediately measured and the concentration of each parameter calculated using a generated standard curve. The absorbance for nitrite was measured at 540nm, while the absorbance for orthophosphate was measured at 880nm.

Standard curves for all parameters using calorimetric methods were generated weekly to determine concentrations from absorbance readings. Each sample was tested in duplicate: for each parameter, two aliquots of sample were taken and tested, resulting in two absorbance values. The two absorbance values of each sample were then averaged. Testing two aliquots of each sample ensured that there was consistency in the measurement and that interference from suspended solids or turbidity did not affect the absorbance readings. For each parameter, a t-test was conducted between all pairs of readings to ensure that the variance between the two was not significantly high (i.e. there was no difference between the means). All t-tests between pairs of readings for all parameters resulted in p values of over 0.05, suggesting that there was no significant variance between the pairs of readings.

Zebra mussels used for this study were collected from Beaver Lake located west of Tamworth, Ontario. Permission to collect these live organisms obtained from the Ontario Ministry of Natural Resources, and a permit (“License to Collect Fish for Scientific Purposes”, License No. 1079875) was issued by the appropriate authorities to certify their approval. Approximately 1000 live, adult zebra mussels were collected by hand from the bottom of the Beaver Lake. They were placed in 6L plastic containers along with sufficient lake water to ensure their survival during transport and storage. They were immediately transported to the Storring Septic site and placed into the treatment tank intended for zebra mussel filtration (Figure 3-1). The zebra mussels were allowed to acclimatize to the influent wastewater by allowing the inflow to drip slowly into the tank, displacing the lake/rain water over a three-day period.

3.5 Results and discussion

The goal of this study and late fall start-up was to identify the treatment technology that showed the most potential for full-scale testing and implementation, by demonstrating the highest reductions in the water quality parameters of interest. The premise was that a technology that exhibited good treatment performance under low temperature conditions and showed resilience to ambient conditions and input variability could have a high potential for full-scale treatment. The data is presented into two testing periods for the purpose of analysis: the first half of the season, when ambient temperatures were warmer and the system was under start up conditions, and a second testing period for when temperatures were relatively low typical of a Canadian rural winter.

3.5.1 Ambient temperature/precipitation accumulation

The daily maximum, minimum and average daily ambient temperatures in Tamworth, Ontario were obtained from Environment Canada and recorded daily throughout the testing season. Precipitation data was also obtained from Environment Canada and plotted in Figure 3.2.

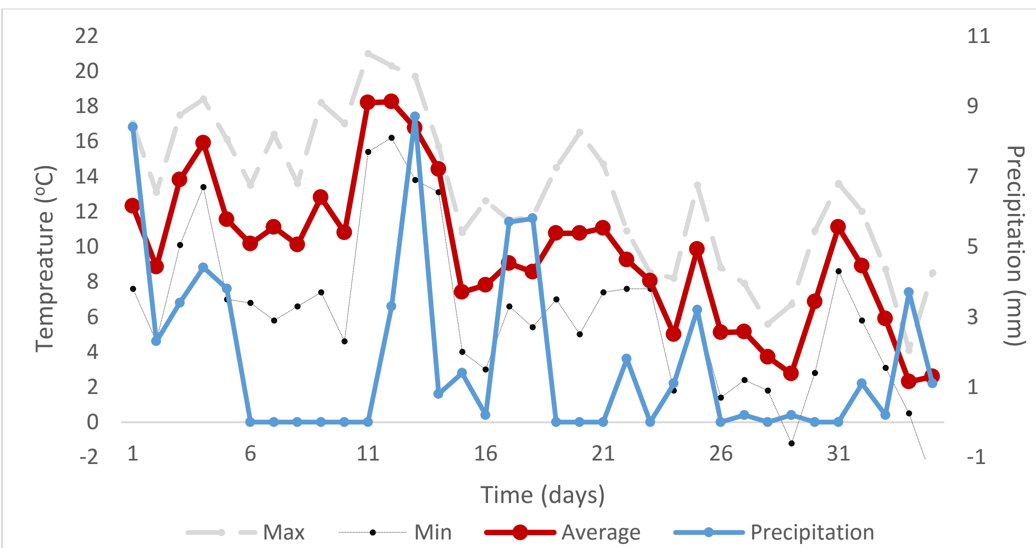


Figure 3-2. Temperature and precipitation data for the entire testing season. Maximum, minimum and average temperatures, as well as precipitation accumulation, per day for the entire testing season (Oct 4th-Nov 7th, 2014) (Environment Canada, 2015).

The highest recorded temperature was on day 11 (21ºC) and the lowest on day 35 (-3.3ºC). As can be seen, the average temperatures during the beginning of the testing season (Day 1-14) were considered to be relatively mild, although the daily temperatures saw large fluctuations (with a minimum of 4.6ºC). As mentioned, the data was separated into two testing periods as detailed in Table 3-2. This allowed for better differentiation between mild (start-up) and cold temperature testing periods, to better assess performance at colder (<10ºC) temperatures.

3.5.2 Nitrogen species and total nitrogen removals

In wastewater treatment, there are three important nitrogen species that are often targeted for removals: ammonia (or ammonium), nitrite and nitrate. Ammonia (NH_3) is the volatile form of ammonium (NH_4^+), the latter of which is predominant in typical wastewater stabilization pond systems due to its predominance at pH levels under 8 (Camargo *et al.*, 2005). Both forms of ammonia are important in wastewater treatment, as ammonia is known to be toxic to aquatic species and environments, and thus are generally targeted for removals in areas where discharge regulations are particularly stringent (Oleszkiewicz, 2015). Because the dominant ammonia species in wastewater is the non-volatile (i.e. ionized and soluble) ammonium, wastewater treatment facilities must often rely on biological activity to reduce levels of ammonia. This nitrification process uses the aerobic activity of ammonia-oxidizing bacteria and archaea to convert ammonia to nitrite and nitrate (Pester *et al.*, 2012). This process relies on the availability of dissolved oxygen (DO) as a substrate by biological organisms. As such, in wastewater treatment, the addition of mechanical aeration and subsequent increases in DO/mixing tend to enhance reductions in total ammonia concentrations (Grady *et al*., 1999; Lee *et al*., 2002). In this study, aeration was continuously supplied to each of the three treatment tanks (minimum of 1CFM) throughout each of the testing periods, with the exception of the days when the total system was shut down. Hence, it was anticipated that for the duration of the testing periods, total ammonia concentrations would remain low in comparison to the influent ammonia levels for the biofilm technologies utilizing biological treatment. Figure 3-3 shows the total ammonia (NH_3/NH_4^+) concentrations from the effluent of each treatment tank, as well as from the influent wastewater, over the entire 35-day testing season. BioCord data is missing for the sample taken on Oct 23rd (Day 20), as accurate testing was compromised by the presence of *Daphnia* fleas in Tank #2. Day 20 also experienced a system shutdown resulting in no flow or aeration for 3 days. During system shutdown, influent samples continued to be collected in addition to samples from each treatment tank. This was done in order to demonstrate the fluctuations in total ammonia concentration seen in Pond 2. Day 35 also experienced a system shutdown resulting in ~18h of no airflow.

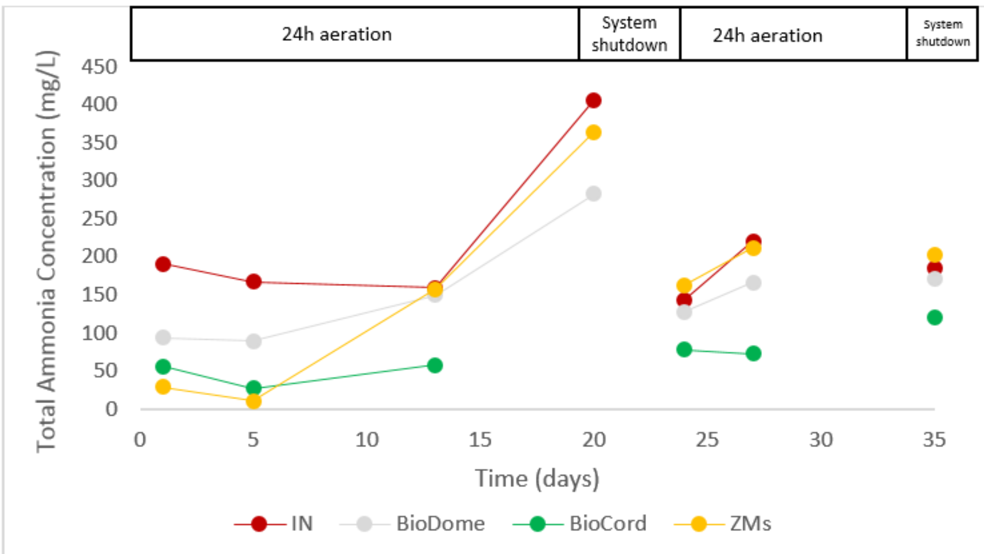


Figure 3-3. Total ammonia (ammonia/ammonium) concentrations in the influent and the BioDome, BioCord and zebra mussel system tank effluents over the 35-day testing season. System shutdown occurred on Days 20 and 35. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

The pH range was recorded and noted to range from 7.12 to 8.18 for all of the treatment systems over the 35-day testing season (i.e. both testing periods). The majority of total ammonia reductions were assumed to be via nitrification rather than volatilization, due to the ranges of pH observed. From the results illustrated in Figure 3-3, it can be seen the BioCord consistently showed the lowest concentrations of effluent total ammonia, with the exception of the first two sampling dates. Although data was not available for BioCord on day 20, it can be inferred that the levels of total ammonia in BioCord's effluent were not drastically high at this time, due to the excessive presence of *Daphnia* in the reactor tank (water fleas, see Figure 3-4). This is because *Daphnia* are organisms that are often used as indicators of toxicity.

They have a low tolerance for unionized ammonia and will not survive in environments containing ammonia levels higher than 0.7mg/L (Hathaway and Stefan, 1995).

Figure 3-4. Photo of the presence of *Daphnia* (appearing as tiny red dots) in the BioCord system treatment tank during Day 20 of testing. A magnified picture of the water flea is shown in the bottom left corner. The presence of these *Daphnia* were nearly undetectable by Day 24.

To compare the total ammonia reductions of the three treatment technologies in the context of cold-weather and pseudo-steady state conditions, the average total ammonia concentrations were analyzed for each testing period. Table 3-4 summarizes the average total ammonia concentrations in the influent and effluent of each treatment technology is shown for both the first (Days 1-14) and second (Days 15-35) testing periods. Because the objective of this study was to observe whether or not implementing a treatment technology would produce significantly lower concentrations of wastewater parameters in comparison to a baseline value (i.e. the influent coming from Pond 2), the average total ammonia concentrations resulting from each treatment technology were analyzed and highlighted in blue if they were significantly lower than the corresponding average influent ammonia level for that testing period.

Table 3-4. Average total ammonia concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean total ammonia concentration in the treatment tank effluent was significantly lower ($p \leq 0.05$) than the mean total ammonia concentration found in the influent, for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Time	Average Temp (°C)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
1st	Weeks 1-2 (Days 1-14)	13	172 ± 7	†111 ± 14 $\eta^2 = 0.77$	†47 ± 7 $\eta^2 = 0.77$	†66 ± 33 $\eta^2 = 0.77$
2nd	Weeks 3-5 (Days 15-35)	7	257 ± 45	192 ± 26	†75 ± 11 $\eta^2 = 0.75$	246 ± 34
All	Weeks 1-5 (Days 1-35)	10	210 ± 34	154 ± 25	†68 ± 13 $\eta^2 = 0.75$	162 ± 45

From Table 3-4, it can be seen that the colder temperatures observed during the second period appeared to have a negative effect on the treatment technologies. During the first, warmer testing period, each of the three technologies showed significantly lower concentrations of total ammonia in comparison to the influent. Although during the first period it was assumed that the biofilm in the BioDome and BioCord systems was still reaching pseudo-steady state, the consistent aeration meant that there was sufficient DO available for nitrification to occur. However, during the second testing period, when temperatures decreased, only the BioCord system showed significantly lower concentrations of total ammonia in comparison to the influent. This indicated that both the BioDome and BioCord systems were likely able to establish populations of ammonia-oxidizing bacteria (AOB), but when temperatures decreased and/or when aeration was stopped during times of system shutdown, the BioCord system appeared to be more robust with respect to sustained total ammonia reduction. Hence, the result would suggest that the BioCord system may be better at insulating the developed biofilm from low temperatures, leading to higher activities of nitrifying bacteria than would typically be observed under cold-weather conditions in an open pond system. The BioCord system was also relatively reliable during short periods without aeration, as it showed significant reductions during the second period when two separate system shutdowns were noted (i.e. periods of no water or airflow being delivered into the tanks). Figure 3-3 also shows that after the first system shutdown (Day 20), the BioCord system was able to recover from the less favourable operating conditions quite quickly, as it was able to achieve a 54% reduction in total ammonia by the next sampling date.

Like the BioDome and BioCord systems, the zebra mussel system showed significant decreases in total ammonia for the first testing period. During this time, the average ambient temperature was approximately 13ºC. This is within the range reported to be optimal for zebra mussel filtration activity, metabolism and tissue growth (McMahon, 1996; Noordhuis *et al.*, 1992; Reeders and Bij de Vaate, 1990). It is likely that the observed decrease in total ammonia concentrations can be attributed to the ability for zebra mussels to filter and bioaccumulate aquatic contaminants. The decrease in treatment observed during the second testing period may have been due to a decrease in filtration rates due to the colder ambient temperatures, or a reduction in the number of viable zebra mussels. The average ambient temperature for the second testing period was approximately 7ºC, with lows sometimes reaching below 0ºC. As this is below the optimal range for zebra mussel filtration, the decrease in total ammonia reductions may be attributed to lowered filtration rates (Reeders and Bij de Vaate, 1990). In addition, although zebra mussels have been found to survive in hypolimnetic zones and at DO levels as low as 0.1mg/L (Benson *et al.*, 2015), the anoxic tolerance of zebra mussels has been shown to decrease with decreasing temperatures (McMahon, 1996). This would imply that a reduction in DO due to system shutdown (Day 20 and Day 35) may have led to anoxic conditions in the tank, which consequently led to zebra mussel death and decreases in total ammonia reductions. It is also possible that zebra mussel death led to the release of ammonia from the tissue of the organisms. Zebra mussels have been reported to release nutrients they have processed upon their mortality and decomposition, resulting in net increases of these dissolved inorganic nutrients in the surrounding water column (Arnott and Vanni, 1996).

Although ammonia is often a primary constituent targeted for removal in wastewater treatment, it is also important to reduce levels of nitrite and nitrate (and therefore total nitrogen concentrations). Nitrification will generally lead to an increase in nitrate concentrations in wastewater, due to the biological oxidation of ammonia into nitrite and the subsequent oxidation of nitrite into nitrate, with the former being the rate-limiting step (Zhang *et al.*, 2009). Nitrification relies on the sufficient supply of DO. Although not as directly toxic to aquatic environments, nitrite and nitrate are still harmful in high quantities and can be detrimental to human health. For example, nitrate contamination in ground and/or drinking water can cause methemoglobinemia (i.e. blue-baby syndrome) in infants (Majumdar, 2003). Levels above 50mg/L of nitrate in drinking waters have been known to be associated with this disease, which decreases the ability of blood to carry oxygen and can be fatal to newborns (World Health Organization, 1998; Super *et al.*, 1981). Nitrate reductions are facilitated by anaerobic and facultative bacteria that convert nitrate into nitrogen gas (N_2) under anaerobic conditions, which is subsequently removed from the wastewater via volatilization (Vymazal, 2007). This process requires anaerobic or anoxic conditions, although aerobic denitrification is also possible, to a lesser extent, by some microbial organisms present in wastewater (Wang *et al.*, 2007; Miyahara *et al.*, 2010). The total nitrogen compositions for the influent the BioDome, BioCord and zebra mussel systems can be found in Figure 3-5. BioCord data is missing for the sample taken on Oct 23rd (day 20), as accurate testing was compromised by the presence of *Daphnia* fleas in Tank #2. Day 20 also experienced a system shutdown resulting in no flow or aeration for 3 days. Day 35 also experienced a system shutdown resulting in ~18h of no airflow.

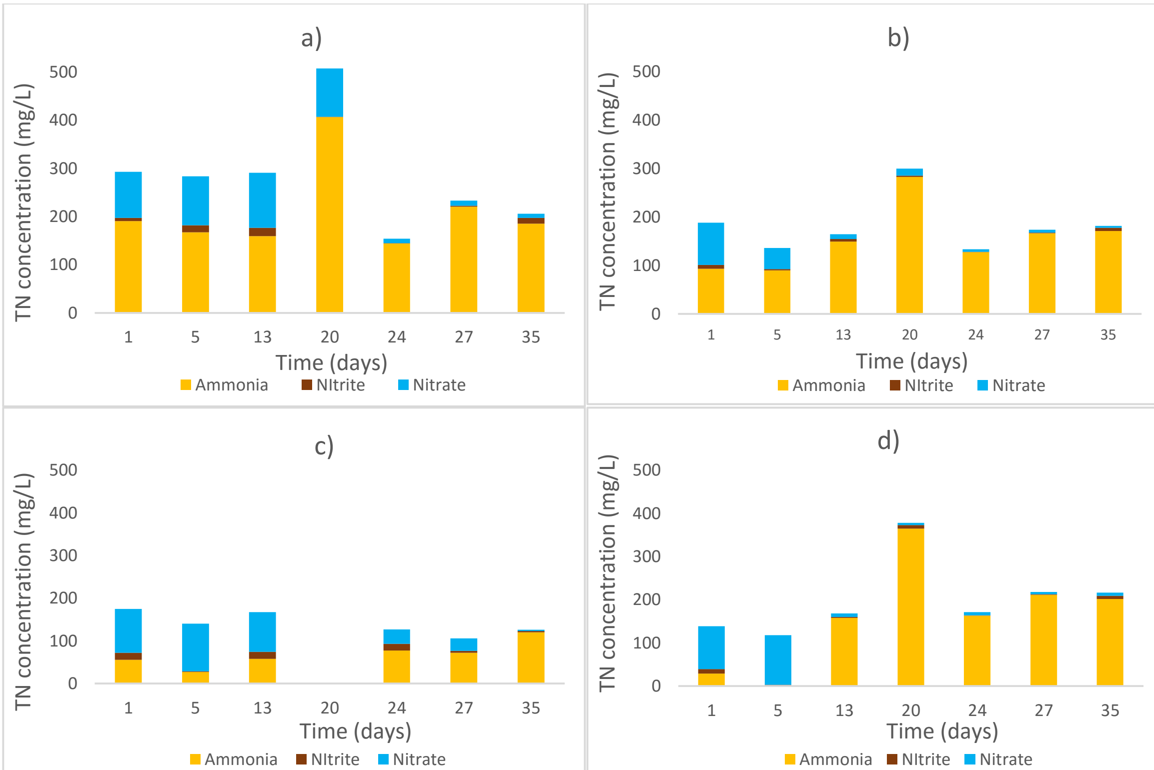


Figure 3-5. Total nitrogen concentrations in a) the influent, b) the BioDome system c) the BioCord system and d) the zebra mussel effluents for the 35-day testing period. System shutdown occurred on days 20 and 35.

Without the implementation of anaerobic cycles to enhance denitrification, effective nitrification of ammonia was expected to yield relatively high levels of nitrate. In wastewater, nitrite (the intermediate species in nitrification) is typically unstable and quickly converted into nitrate by nitrite-oxidizing bacteria (NOB), which are faster-growing than ammonia oxidizers (Sin *et al.*, 2008). As such nitrite concentrations are usually relatively low during biological treatment, which corresponds with the results obtained for the treatment technologies investigate in this study (Figure 3-5). The results would suggest that nitrite was effectively converted into nitrate in each of the treatment systems. Although high total nitrogen removals were not expected during the testing period as air was not cycled on and off to facilitate the denitrification process, quantifying the different nitrogen species present in each of the treatment system effluents can provide valuable information regarding the effectiveness of the total nitrogen removal capabilities of each of the treatment systems. This is because, although air cycling schedules were not regimented during this testing season, anaerobic/anoxic conditions were still experienced by the treatment systems during system shutdown (Day 20 and Day 25), leading to potential denitrification processes during these times.

The effectiveness of nitrification and denitrification was compared for the biofilm treatment technologies. Table 3-5 summarizes the reductions in total nitrogen for each of the treatment technology and testing periods. Although both the BioCord and BioDome systems significantly reduced total nitrogen concentrations during the start up period, Figure 3-5 shows that, during periods of constant aeration, the BioCord system consistently produced lower total nitrogen concentrations in comparison to the BioDome system effluent, despite having higher nitrate concentrations. This indicated that, overall, the BioCord system was better able to nitrify ammonia and reduce overall total nitrogen concentrations.

Table 3-5. Average TN concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean TN concentration in the treatment tank's effluent was significantly lower ($p \leq 0.05$) than the mean TN concentration found in the influent, for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Time	Average Temp (°C)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
1	Weeks 1-2 (Days 1-14)	13	289 ± 3	†163 ± 15 $\eta^2 = 0.77$	†161 ± 10 $\eta^2 = 0.77$	†145 ± 12 $\eta^2 = 0.77$
2	Weeks 3-5 (Days 15-35)	7	275 ± 79	197 ± 36	†120 ± 7 $\eta^2 = 0.75$	246 ± 45
All	Weeks 1-5 (Days 1-35)	10	281 ± 42	182 ± 21	†140 ± 11 $\eta^2 = 0.36$	202 ± 32

The effects of colder temperatures and the two shutdowns (Days 20 and 35) were illustrated during the second testing period, during which lower removals of total nitrogen were observed in the BioDome and the zebra mussel system effluents, but higher removals were noted in the BioCord system effluents, which also showed significant reductions during both testing periods. The lower removals observed in the BioDome system during the second testing period were likely due to the colder ambient temperatures recorded during this time, as nitrification proceeds at a slower rate at temperatures below 10°C (Metcalf and Eddy, 2003). The BioCord system, which showed the highest total nitrogen removals during the second testing period, may have experienced conditions suitable to allow for denitrification during the two periods of site shutdown (i.e. no air flow being delivered to the reactor tanks). These anaerobic periods were not present during the first testing period. From Figure 3-5, it can be seen that the nitrate concentrations in the BioCord system effluent on Day 35 (system shutdown) were very low when considering the corresponding low levels of total ammonia at this time.

The zebra mussels showed similar reductions in total nitrogen as was observed for total ammonia, producing significant levels of total nitrogen removals during the first testing period, but not during the second. It was likely due to the reduced ability of zebra mussels to filter wastewater and bioaccumulate contaminants under colder temperature conditions.

3.5.3 Orthophosphate

Phosphorus, much like ammonia, is a key nutrient that depletes oxygen and causes eutrophication and algal blooms in natural waters (Cooper *et al.*, 1994). Orthophosphate is the biologically active form of phosphorus and is the relevant species targeted for removal in biological wastewater treatment as it is available for microbial uptake (Spellman, 2014). Like total nitrogen, the most efficient removal of orthophosphate in wastewater relies on alternating aerobic and anaerobic environments (Mara, 2006). It is performed largely by heterotrophic microorganisms called polyphosphate-accumulating organisms (PAOs) (Chen *et al.*, 2004). However, unlike nitrogen, the removal of phosphorus from wastewater is a result of uptake from wastewater into microbial cells, and not due to the direct conversion of the nutrient to a volatile species. Biological phosphorus removal occurs when anaerobic environments cause PAOs to uptake organic matter and release orthophosphate; subsequently, aerobic environments are induced and the PAOs then assimilate more orthophosphate into their biomass than was previously released (Zhou *et al.*, 2010). The orthophosphate is stored in their cells as polyphosphate. Thus, total phosphorus removal occurs via biomass wastage, but the timing of phosphorus uptake and release are often difficult to predict as the thorough isolation and characterization of PAOs has not yet been accomplished (Sathasivan, 2009). It is likely that the consortium of microorganisms responsible for biological phosphorus removal require different pathways and hence mechanisms for this process, and that these mechanisms have yet to be fully understood (Sathasivan, 2009). Figure 3-6 shows orthophosphate reduction in each of the treatment systems compared to the influent, over the 35-day testing period. BioCord data is missing for the sample taken on Oct 23rd (day 20), as accurate testing was compromised by the presence of *Daphnia* fleas in Tank #2. Sampling during day 20 also occurred during system shutdown resulting in no flow or aeration for 3 days. Day 35 also experienced a system shutdown resulting in ~18h of no airflow.

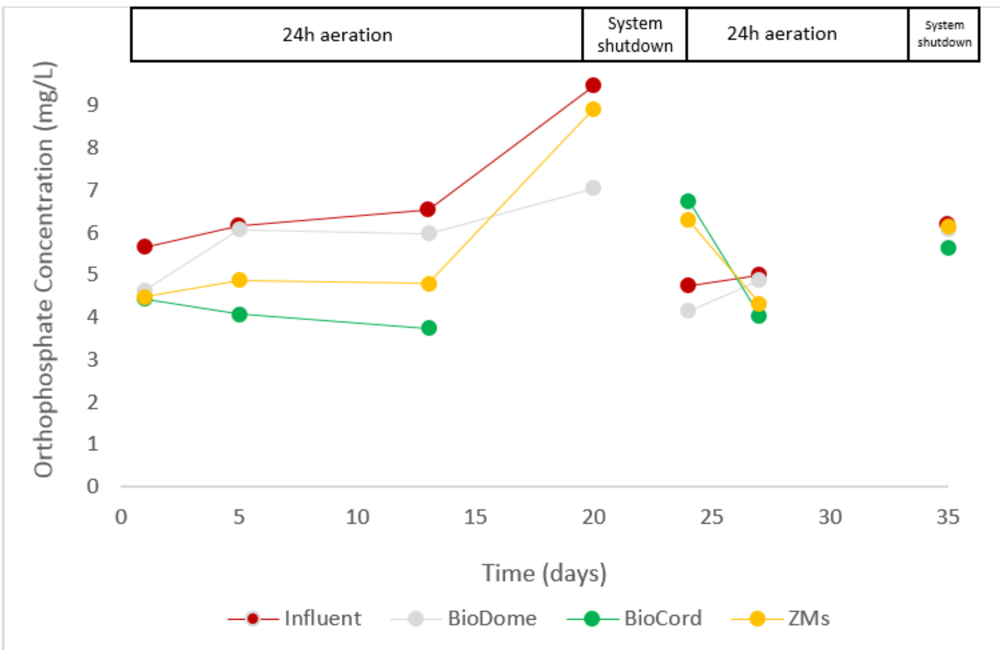


Figure 3-6. Orthophosphate concentrations in the influent and the BioDome, BioCord and zebra mussel system effluents for the 35-day testing season. System shutdown occurred on days 20 and 35. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

Because anaerobic periods were only inadvertently induced on two separate occasions during the testing period (days 20 and 35), it was not expected that large reductions in orthophosphate would be observed in the treatment technologies resulting from PAO removal, as there was little opportunity for the cyclic uptake of orthophosphate by these microorganisms. Although phosphorus uptake can occur during purely aerobic phases, without an initial anaerobic phase to allow PAOs to uptake volatile fatty acids (VFAs) and other organic matter for stored energy, the uptake during the oxygen-rich phase of treatment will generally not be as efficient (Henze *et al.*, 2008). Therefore, orthophosphate removal was not as effective as would be anticipated if air cycling had been implemented. The removals observed during anaerobic conditions were not notably different from those noted under aerobic conditions, and statistical analysis of orthophosphate reductions showed no significant decreases in concentrations in comparison to the influent for any treatment technology during either of the testing periods. It is possible that longer anaerobic times are needed in order to allow for PAOs to store sufficient amounts of energy for more substantial orthophosphate uptake during subsequent aerobic periods. Table 3-6 summarizes the orthophosphate concentration results and related statistics for each treatment technology during the first and second testing periods.

Table 3-6. Average orthophosphate concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean orthophosphate concentration in the treatment system effluent was significantly lower ($p \leq 0.05$) than the mean orthophosphate concentration found in the influent, for the specified testing period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Time	Average Temp (ºC)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
1	Weeks 1-2 (Days 1-14)	13	6.12 ± 0.26	5.56 ± 0.46	4.08 ± 0.20	4.72 ± 0.12
2	Weeks 3-5 (Days 15-35)	7	6.35 ± 1.09	5.54 ± 0.64	5.47 ± 0.78	6.42 ± 0.95
All	Weeks 1-5 (Days 1-35)	10	6.25 ± 0.59	5.55 ± 0.39	4.78 ± 0.48	5.69 ± 0.61

From Figure 3-6 and Table 3-6, it can be seen that influent treated with the BioCord system resulted in the lowest overall concentrations of phosphorus for all testing periods, although these concentrations were not significantly lower than the influent. All treatment technologies showed moderate reductions in phosphorus and lower percent reductions during the second testing period (i.e. the treatment technologies were less effective). This is consistent with anticipated performance during cold-weather conditions as PAOs are slow-growing and are typically present in lower numbers than nitrifiers and other auto/heterotrophic microorganisms (Keller and Zeng, 2004). As such, when conditions become unfavourable, metabolism by PAOs would be expected to be minimal as they would be outcompeted by other microorganisms and/or washed out. PAOs compete with other microorganisms, such as glycogen-accumulating organisms (GAOs) and other heterotrophs, for organic food sources (Zeng *et al.*, 2003; Oehmen *et al.*, 2006). This competition for organic substrate, coupled with lower seasonal temperatures and the relatively low concentrations present in the influent, may be factors that would have contributed to the moderate (but not significant) reductions in phosphorus. Zebra mussels showed some potential in uptaking orthophosphate from wastewater during warmer temperatures, but showed increases in influent concentration during the second testing period. This indicates that zebra mussels may have the ability to both uptake and release nutrients. Further studies should be conducted to observe this more fully (see chapter 5).

3.5.4 Chemical oxygen demand

Chemical oxygen demand (COD) is an indirect measure of the organic matter present in wastewater. High levels of organic constituents can deplete the oxygen supply in water reservoirs and lead to environmental concerns, including the death of aquatic organisms (Pisarevsky *et al.*, 2005). They include fecal matter, food particles, fats, detergents and greases, amongst other compounds. In biological wastewater treatment, aeration is typically applied in order to promote the breakdown of these organic compounds by heterotrophic microorganisms. Aeration provides a supply of oxygen to support the microbial metabolic activity that breaks down organic materials (Peavy *et al.*, 1985; Grady *et al.*, 1999). As oxygen and substrate mixing were provided to the biofilm treatment technologies (BioDome and BioCord systems), it was expected that both of these treatment technologies would show good reductions in COD concentrations. Figure 3-7 shows the reductions of influent COD concentrations as achieved by each of the treatment technologies. BioCord data is missing for the sample taken on Oct 23rd (Day 20), as accurate testing was compromised by the presence of *Daphnia* fleas in Tank #2. Day 20 experienced a system shutdown resulting in no flow or aeration for 3 days. Day 35 also experienced a system shutdown resulting in ~18h of no airflow.

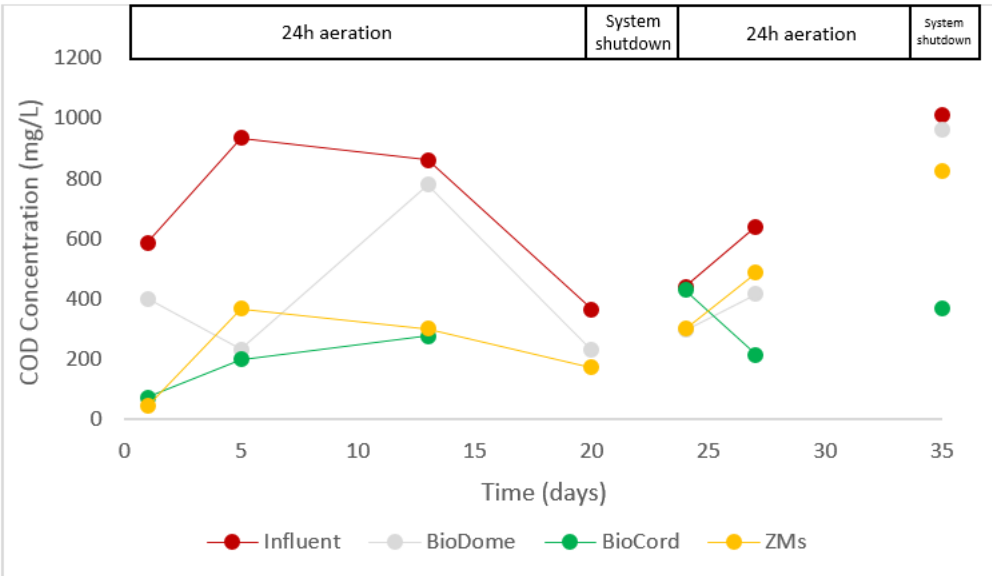


Figure 3-7. Concentrations of COD present in the influent and the BioDome, BioCord and zebra mussel system tank effluents for the 35-day testing season. System shutdown occurred on days 20 and 35. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

As can be seen, the BioCord system exhibited the lowest effluent COD concentrations for both testing periods. Although constant aeration was applied during the majority of the testing period, the BioCord system only showed significant reductions during the first testing period, when ambient temperatures were higher. The BioDome system did not show any significant reductions for either testing periods, while the zebra mussel tank showed significant reductions during the first testing period and overall. These results are tabulated in Table 3-7.

Table 3-7. Average COD concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean COD concentration in the treatment tank's effluent was significantly lower ($p \leq 0.05$) than the mean COD concentration found in the influent, for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Time	Average Temp (°C)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
1	Weeks 1-2 (Days 1-13)	13	794 ± 105	471 ± 162	†184 ± 60 $\eta^2 = 0.77$	†239 ± 98 $\eta^2 = 0.77$
2	Weeks 3-5 (Days 14-35)	7	614 ± 144	477 ± 166	338 ± 64	447 ± 142
All	Weeks 1-5 (Days 1-35)	10	691 ± 94	474 ± 108	†261 ± 52 $\eta^2 = 0.61$	†358 ± 94 $\eta^2 = 0.39$

Significant reductions in the second period were likely not observed due to a combination of low temperature effects and the periods of anaerobic conditions experienced during the second testing period. With respect to the biofilm treatment technologies (the BioDome and BioCord systems), the results would suggest that the COD-reducing organisms in the biofilm technologies were affected by colder temperatures and/or system shutdown. The rate of growth and the metabolic activities of aerobic heterotrophic bacteria are typically temperature-dependent, with biological reaction rates decreasing as temperatures decrease (Grady *et al.,* 1999; Metcalf and Eddy, 2003). In the BioCord system, the biofilm did not appear to insulate the system against colder temperatures as was noted for nitrogen removal. This may be due to the fact that faster-growing heterotrophic microorganisms tend to dominate the outer layers of the biofilm and could, hence, be more susceptible to temperature fluctuations (Nogueira *et al.*, 2002; Benthum *et al.,* 1996). However, the BioCord system was still able to produce significantly lower COD concentrations in comparison to the influent for the overall 35-day treatment period. This would indicate that the BioCord system was better able to establish higher and/or more efficient populations of COD-reducing organisms than the BioDome system. This is particularly notable when considering the results observed for total nitrogen concentrations. Because the BioCord system was able to significantly reduce total nitrogen concentrations for both testing periods, it could be inferred that an active population of nitrifiers was established in the BioCord system biofilm. Heterotrophic bacteria typically outcompete the slower-growing autotrophic nitrifiers for organic substrate when organic carbon concentrations are high, with nitrifiers becoming more competitive when organic carbon concentrations are sufficiently reduced (Michaud *et al.*, 2006). This has been shown to be true in biofilms, with Wijeyekoon *et al.* (2004) showing that nitrification in biofilms are suppressed at high substrate loads, and Satoh *et al.* (2004) showing that, in a membrane aerated biofilm reactor, high levels of nitrification could occur in the inner layers of the biofilm where O_2 concentration was high and the organic carbon concentration was low. This suggests that in the BioCord system, a sufficient amount of COD was being removed from the outside of the biofilm such that the nitrifiers in the protected inner layers were able establish themselves and reach high levels of nitrification. It may also be expected that the slow-growing, orthophosphate-reducing microorganisms present in the system would be similarly protected by the outer bacterial biofilm layers. However, results of orthophosphate removals show that there were no significant reductions from the influent for either biofilm technology. This may be due to the lack of alternating anaerobic and aerobic conditions that are necessary for biological phosphorus removal. Without this air cycling to induce a net uptake of orthophosphate by PAOs, orthophosphate uptake is limited, resulting in less effective reductions in comparison to ammonia reductions by nitrifiers (Gieseke *et al.*, 2002). It may also be possible that the number of nitrifying bacteria outcompeted the number of PAOs in the biofilm system for oxygen. Pastorelli *et al.* (1999) found that stable phosphorus removals in a biofilm system may only be achieved with the addition of an external carbon source. This suggests that, if orthophosphate-reducing microorganisms were present in the inner layers of the BioCord or BioDome systems, then there may not have been enough organic material available as a carbon source for effective PAO metabolism. However, it is hard to make conclusive statements about the composition and spatial arrangement of the biofilms developed in this study, as analysis of the biomass was not conducted, and the microbial populations of the biofilms were not characterized.

The zebra mussel system was able to significantly reduce COD levels during the first testing period as well as for the overall treatment period. As with the other two treatment technologies, significant reductions in COD concentrations were not observed during the second testing period. Similarly to the results obtained for total ammonia, total nitrogen, and orthophopshate, the zebra mussel system was better able to reduce constituent concentrations during the first than the second testing periods. These results would suggests that the zebra mussel system was not as effective in removing COD from the wastewater, and was sensitive to fluctuations in temperature and aeration, leading to decreased treatment performance during colder temperatures and/or environments of prolonged oxygen depletion.

3.5.5 Total suspended solids

The measure of total suspended solids in wastewater effluents is often an indicator of the clarity/turbidity and the overall quality of the treated wastewater. Both organic and inorganic particles can contribute to concentrations of TSS, including silt, clay, bacteria, sediment, algae, both settleable and nonsettleable solids, and other organic particulates (EPA, 2014). High TSS concentrations can be harmful to receiving bodies of water because it reduces sunlight penetration, decreases levels of DO and can affect the growth rates and health of fish and other aquatic organisms (Wetzel, 2001). As well, pathogens, nutrients and other pollutants can become attached to suspended solids (Kemker, 2014). TSS removal in biological wastewater treatment typically takes place via physical filtration and settling, and degradation by microorganisms (Grady *et al.*, 1999; Kadlec and Wallace, 2009). Zebra mussels have also been shown to effectively reduce TSS concentrations and improve the clarity in natural bodies of water (Binelli *et al.*, 2006; Vanderploeg *et al.*, 2001). The changes in TSS concentrations in each of the treatment systems are shown in Figure 3-8 and Table 3-8. BioCord data is missing for the sample taken on Oct 23rd (day 20), as accurate testing was compromised by the presence of *Daphnia* fleas in Tank #2. Day 20 experienced a system shutdown resulting in no flow or aeration for 3 days. Day 35 also experienced a system shutdown resulting in ~18h of no airflow.

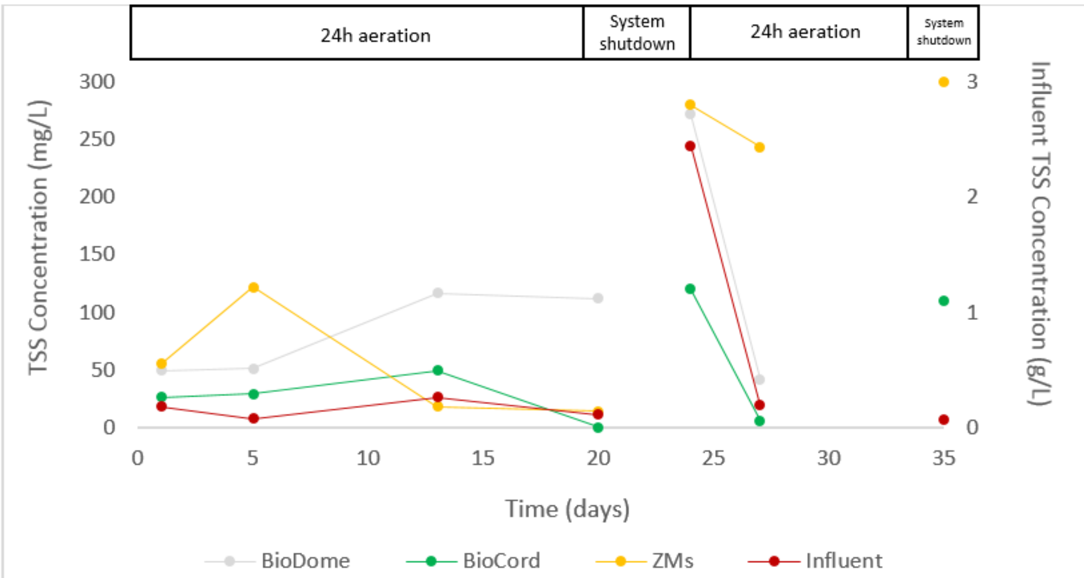


Figure 3-8. Concentration of TSS present in the influent and the BioDome, BioCord and zebra mussel system tank effluents for the 35-day testing season. Influent data is shown on a secondary axis (g/L) due to the extremely high concentrations. System shutdown occurred on days 20 and 35. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

Table 3-8. Average TSS concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean TSS concentration in the treatment tank's effluent was significantly lower ($p \leq 0.05$) than the mean TSS concentration found in the influent, for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Time	Average Temp (°C)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
1	Weeks 1-2 (Days 1-13)	13	1165 ± 730	†72 ± 22 $\eta^2 = 0.77$	†35 ± 7 $\eta^2 = 0.77$	†65 ± 30 $\eta^2 = 0.77$
2	Weeks 3-5 (Days 14-35)	7	7031 ± 5822	†182 ± 63 $\eta^2 = 0.76$	†78 ± 37 $\eta^2 = 0.75$	†209 ± 66 $\eta^2 = 0.76$
All	Weeks 1-5 (Days 1-35)	10	4517 ± 3341	†135 ± 41 $\eta^2 = 0.64$	†57 ± 19 $\eta^2 = 0.75$	†147 ± 47 $\eta^2 = 0.58$

All of the treatment technologies investigated were found to significantly reduce influent TSS concentrations; however, the BioCord system showed the lowest TSS concentrations overall and, overall, reduced TSS concentrations significantly better than both the BioDome treatment technology and the zebra mussels. TSS is related to both microbial activity and the physical filtration of particulates, the latter being less affected by low temperatures, which have contributed to the overall effectiveness of both of the biofilm technologies in reducing TSS. Overall, all the three treatment technologies investigated were not significalty affected by colder temperatures, showing significant reductions in TSS concentrations for all testing periods. The large standard deviation as seen in the influent concentrations of TSS may be attributed to the operational regime of Storring Septic. Though the facility does not have a formal schedule for transfer of wastewater from one pond to another, the levels of TSS in Pond 2 do fluctuate drastically depending on inflow from Pond 3. Based on visual observations at the Storring Septic facility, transfer of wastewater from Pond 3 (the primary pond) to Pond 2 results in exceptionally high TSS increases due to the turbulence imparted by the siphoning (pumping) of Pond 3's effluent into Pond 2. This turbulence was possibly able to dislodge and suspend any settled/accreted particulate matter, and typically resulted in the high turbidity and low clarity of Pond 2's wastewater.

TSS is the only wastewater parameter that has been conclusively shown to be reduced by zebra mussels (Binelli *et al.*, 2006; Vanderploeg *et al.*, 2001). The results obtained in this study were consistent with these findings, showing that the zebra mussels had an ability to significantly reduce TSS levels for both testing periods. Zebra mussel reductions in total ammonia, total nitrogen, orthophosphate and COD did not show any significant reductions for the overall testing season. It is possible that although zebra mussels may have an ability to uptake wastewater contaminants, the reductions in these parameters may largely be due to filtration of suspended solids, as nutrient and pollutants can become attached to suspended solids (Kemker, 2014). It is also likely that, during periods of site shutdown and anaerobic conditions, zebra mussels tend to release soluble wastewater constituents but not filtered suspended solids, leading to significant reductions in TSS but not other wastewater contaminants.

3.6 Conclusions and recommendations

This study involved the assessment in treatment performance of three different treatment technologies under start up and low temperature conditions. An average ambient temperature of approximately 13ºC and unsteady-state (i.e. start-up conditions) were assumed during the first testing period, while pseudo-steady state conditions but lower overall ambient temperatures averaging around 7ºC through the 4 weeks of the second testing period. Two system shutdowns where no water flow or air flow was being delivered into the reactor tanks was also experienced during the second testing period. With the exception of TSS, it was found that, in general, the performance of the treatment technologies was higher during the first testing period. As was expected for the biofilm technologies, reductions in wastewater constituents from the influent appeared to be affected by both lower temperatures and periods of system shutdown. In order to compare the overall efficiency of each of the three treatment technologies, wastewater parameter reductions for each of the treatment technologies and all paramaters were tabulated and are summarized in Table 3-9. Table 3-9 shows the overall average concentrations of total ammonia, total nitrogen, orthophosphate, COD and TSS for the influent, as well as for the effluents from the BioDome, BioCord, and zebra mussel treatment systems.

Table 3-9. Overall mean concentrations (mg/L) of all tested parameters for influent, and effluent BioDome, BioCord and zebra mussel treatment systems.

† = mean concentrations are significantly lower than influent concentrations ($p \leq 0.05$) for Kruskal-Wallis analysis
BioDome, ZMs: N=7
BioCord: N=6

Parameter	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)
Total ammonia	210 ± 34	154 ± 25	†68 ± 13 □□□= 0.75	162 ± 45
Total Nitrogen	281 ± 42	182 ± 21	†140 ± 11 □□□= 0.36	202 ± 32
Orthophosphate	6.25 ± 0.59	5.55 ± 0.39	4.78 ± 0.48	5.69 ± 0.61
COD	691 ± 94	474 ± 108	†261 ± 52 □□□= 0.61	†358 ± 94 □□□= 0.39
TSS	4517 ± 3341	†135 ± 41 □□□= 0.64	†57 ± 19 □□□= 0.75	†147 ± 47 □□□= 0.58

For the entire testing period, the BioCord system showed the best reductions in all parameters and outperformed the BioDome and zebra mussel systems during both treatment periods. The BioCord system was the only treatment technology to show significant reductions in total ammonia and total nitrogen during the second testing period under lower temperature conditions, even in the absence of air cycling to promote denitrification. Without denitrification, it would be expected that the low total ammonia concentrations observed would have resulted in high levels of nitrate. However, the levels of nitrate at this time remained very low, providing evidence that nitrification-denitrification was likely occurring in the BioCord system. Thus, it could be concluded that the BioCord system provided the best overall performance under the operational conditions provided, and that the BioCord system would have the highest potential for successfully increasing the capacity of septic lagoons during low temperature operation.

During the first testing period, when overall temperatures were milder, the zebra mussel system showed lower concentrations than the BioDome system for all parameters tested. During the second testing period, however, the reverse was true and the BioDome system exhibited higher reductions than zebra mussel system, for all constituents of interest except for COD. In the case of COD, the results between the BioDome and zebra mussel systems were similar (22% vs 27% reductions, respectively). This would suggest that, in terms of cold-weather performance, the BioDome system performed more effectively at lower temperatures and under fluctuating environmental conditions and would likely offer a more reliable treatment option during the winter season. The BioCord system also appeared to recover most rapidly from anaerobic conditions, exhibiting the best percent reductions of all parameters after system shutdown by the next sampling date.

Intermittent aeration cycling should be implemented in future testing, such that the effects of anaerobic activity could be assessed during periods of consistent flow. The predominant species/reactions would likely depend on the level of aeration provided to the systems. Thus, a methodical cycling of aeration is needed to optimize the total nitrogen/phosphorus removal in the wastewater, and to allow for the development of a more sophisticated matrix.

On-site testing of dissolved oxygen and pH would prove to be useful for a more accurate discussion and analysis of results. Dissolved oxygen concentrations can have a large impact on the microbial and biological activities occurring in each tank; as such, it must be ensured that a minimum level of oxygen is being delivered to each tank. As well, DO concentrations may give insight into bacterial populations and treatment efficiencies if one biofilm technology is more adept at providing oxygen/circulation to the developed biofilm. On-site pH readings would give us better insight into the composition of nitrogen species present in the effluents and provide us with a more definitive assumption about ammonia volatilization.

Lastly, a control tank should be implemented for warm-weather testing. A tank equipped with only aeration can mimic the treatment effects of a simplified suspended sludge reactor. In terms of cost/outcome benefits, it is important that the chosen treatment technology be able to significantly outperform a control.

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Chapter 4

Comparison of biofilm treatment technologies with the implementation of aeration cycling for targeted nutrient reductions

4.1 Abstract

Two third-party developed biofilm systems and a zebra mussel uptake/filtration process were tested in a pilot-scale investigation to observe their performance, as well as to determine which technology showed the most promise for full-scale testing and implementation with the intent of upgrading an existing wastewater stabilization pond facility. The effluent wastewater quality parameters of interest included COD, TSS, ammonia/ammonium (total ammonia), nitrite/nitrate, total nitrogen and orthophosphate. Different air cycling (on/off) regimes were investigated to observe the effect of alternating aerobic and anaerobic/anoxic conditions on the biological treatment efficiencies of the biofilm systems, as well as to determine the minimum energy requirements—in the form of aeration—for which treatment performance could be sustained. It was found that both biofilm (BioDome and BioCord) and zebra mussel systems were able to significantly reduce all constituents of interest from the secondary wastewater effluent. However, the biofilm technology developed by Bishop Water Technologies, the BioCord system, was able to significantly outperform the control for all parameters, with the exception of orthophosphate. This was hypothesized to be largely due to its increased ability to support oxygen delivery and circulation of substrate to the biofilm, leading to an increased biofilm density and performance of microbial consortium in the biofilm. The BioCord system demonstrated the highest wastewater constituent reductions, most rapid recovery from system shutdown, and required the least maintenance for adequate performance, leading to the recommendation that it should be selected for future full-scale testing and implementation.

4.2 Introduction

The BioCord (by Bishop Water Technologies Inc.) and BioDome (formerly named "Poo-Gloos" by Wastewater Compliance Systems) systems are third-party developed biofilm technologies that have been developed to provide enhanced biological wastewater treatment. These aerated, submerged biofilm reactors are designed to provide conditions that allow for the accumulation of high densities of microbial consortia in a biofilm, allowing for increased reductions in organic matter and nutrients in comparison to traditional suspended growth systems (Guo *et al.*, 2009). High concentrations of microbial aggregates form stable biofilms, allowing for lower hydraulic retention times while minimizing washout (Guo *et al.*, 2009). This implies that—in contrast to microorganisms present in suspended systems—microorganisms in a biofilm will be retained in the system despite relatively high flow rates, hence allowing for a higher mean cell residence time (Metcalf and Eddy, 2004). Although different types of biofilm reactors have been developed, these submerged aerated systems are an attractive option due to their low-cost and maintenance requirements. The effectiveness of the BioCord and BioDome systems have been previously studied in a number of separate case studies, although there has been more published literature on the BioCord system compared to the BioDome system (Yuan *et al.*, 2012; Zhang *et al.*, 2012; Johnson, 2011). These studies have shown that both BioDome and BioCord systems have the potential to enhance the treatment of secondary domestic and/or municipal wastewater in stabilization ponds located in rural Canada.

The BioCord system has been employed in wastewater treatment studies conducted in both Japan and China. Yuan et al. (2012) investigated the ability of the BioCord system to treat upstream river water (18.5-29.5ºC) contaminated by domestic, industrial and agricultural wastewater effluents. They reported that the BioCord system matrix provided a high-porosity and surface area, which enabled suitable conditions for microbial growth, which resulted in increased COD, ammonia nitrogen and total nitrogen removal efficiencies. The microorganisms in the developed biofilm were analyzed to determine whether the composition was stable and high in diversity. The study reported large variations in microbial quantity and diversity between the surface and inner layers of the BioCord biofilm system, as well as various microclimates within the biofilm leading to the formation of aerobic and anaerobic zones. In a study by Zhang et al., (2012) the BioCord system medium was found to be a particularly effective support matrix for organic matter-reducing organisms, and was implemented as an approach the treatment of river water in the heavily polluted Hongqi River watershed in China.

The use of the BioDome system in wastewater treatment has been reported in a collaborative investigation by Wastewater Compliance System, Inc. and the University of Utah (Johnson, 2011). The system was operated from October to February, with the lowest ambient temperature being 0.9ºC and the highest ambient temperature being reported as 15ºC, and demonstrated that the BioDome system could enhance rural wastewater lagoons performance under cold-weather winter conditions. It was found that implementing BioDome units (called "Poo-Gloos" at the time of the study) on a pilot scale led to statistically significant reductions in TSS, COD, ammonia, total nitrogen, alkalinity and total phosphorus in comparison to a control, during the 17-week winter trial. Zabala-Ojeda (2012) also performed a study to assess the carbon and nutrient removal potential of the BioDome system treating municipal wastewater effluent from a primary clarifier relatively low temperatures (0.2 ºC – 12.6ºC). The study demonstrated that the BioDome system could considerably reduce wastewater effluent quality parameters such as COD, TSS and ammonia; and concluded that the BioDome system could be a practical solution for augmenting traditional lagoon treatment systems. However, no statistical analysis was reported to confirm the significance levels of parameter reductions.

Higher removal efficiencies have been reported in the presence of both aerobic and anaerobic cycles, particularly for ammonia, total nitrogen and orthophosphate removals (El-Shafai and Zahid, 2013; Chen *et al.*, 2004). As such, the comparison between the biofilm treatment technologies could allow for the identification of aeration requirements to optimize treatment performance. Although Johnson (2011) and Zabala-Ojeda (2012) examined the effects of air cycling on effluent quality in the BioDome system, the most energy-conservative aeration cycling that was employed was 19h on/5h off. It is possible that less aeration—and therefore energy expenditure—would be required to achieve effective treatment. Studies examining the effectiveness of the BioCord system in wastewater treatment did not consider aeration cycling. As such, it was hypothesized that alternating redox conditions could enhance the performance of the BioCord system. If the primary goal of a treatment facility is to improve wastewater treatment while minimizing energy expenditures, then a biofilm configuration that could produce significant reductions in wastewater constituents of interest while using the lowest daily aeration requirements would represent a desired option for full-scale testing and implementation.

The BioDome system has been tested in a number of investigations that are closely aligned with the objectives and experimental design of the research presented in this thesis. The study presented in the current research aimed to compare the potential for wastewater effluent parameter reductions of different treatment technologies with the goal of enhancing the overall treatment performance of an existing wastewater stabilization pond system, with an emphasis on aeration cycling to enhance nutrient removals and energy conservation. In the studies by Johnson (2011) and Zabala-Ojeda (2012), the BioDome system was employed in pilot-scale studies treating wastewater from lagoon facilities, with the implementation of on/off aeration cycles to promote nitrification/denitrification and phosphorus uptake and release. In comparison, the literature involving the BioCord system has focused largely on the utilization of the BioCord system to treat polluted river water and the characterization and analysis of the resulting biofilm. Although these BioCord studies were not conducted in a WSP environment, the results still demonstrated that the BioCord system could significantly reduce the targeted wastewater effluent quality parameters in the present study (Yuan, *et al.*, 2012). To date, these biofilm technologies have not been investigated under similar operating conditions with the aim of comparing their performance in the treatment of a specific wastewater. As such, the BioDome and BioCord systems were selected in this pilot-scale study. The findings will be useful in assessing and comparing the benefits of each technology, as well as their performance with respect to reductions in the wastewater parameters of interest, as well as their robustness under cold-temperature operations. In the future, this study may also assist smaller North American facilities operating under similar climatic conditions in their consideration and pilot scale testing of biofilm technologies to augment wastewater treatment based on their site-specific requirements. This experiment was designed to compare the treatment efficiencies of the BioDome, BioCord, and aerated suspended growth control systems under varying conditions of hydraulic retention times, flow, loading rates, temperature and aeration.

4.3 Experimental setup and design

This study was conducted at the lagoon facility of Storring Septic, located in Tamworth, Ontario in rural Canada. This wastewater stabilization pond facility mimics the climate and service population similar to many lagoon system environments across Canada and North America. Figure 4-1 shows a schematic of the experimental setup implemented for the full operational testing season of the chosen biofilm technologies.

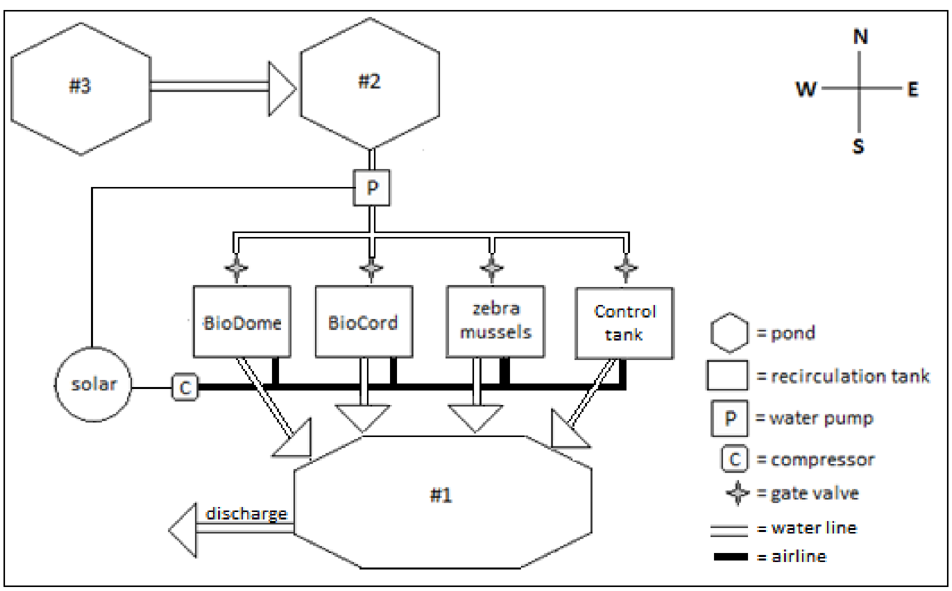


Figure 4-1. Experimental setup for testing of biofilm technologies at Storring Septic, weeks 1-14. During weeks 15-20 of summer testing, the zebra mussel tank was decommissioned, leaving the three remaining tanks for use in our research. Image not to scale.

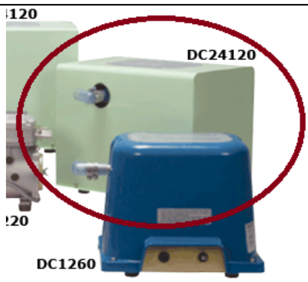
The ponds are numbered in order of when they were implemented. Pond 1 was the first pond to be constructed in 1974, followed by Pond 2 and then finally Pond 3, which was constructed in 1999. Pond 3 in Figure 4-1 represents the first, primary pond in the process flow in the Storrings' lagoon setup. This is the pond in which raw septage is typically dumped, and measures 75' wide x 150' in length x 8' depth. Raw septage is treated anaerobically in this pond and solids are allowed to settle out via sedimentation. From here, the wastewater is siphoned into the secondary pond, Pond 2 (100' x 100' x 8'). For this study, a surface water pump was used to pump effluent from Pond 2 into four separate tanks, with each tank containing one method of treatment. The first tank contained the BioDome technology, the second tank contained the BioCord technology, the third contained approximately 1000 zebra mussels, and fourth and final tank was a control tank.

Storring Septic currently uses two hauling trucks to pump and deliver wastewater from domestic septic tanks to the WSP facility. This inflowing wastewater is dumped directly into the Storring Septic primary pond, Pond 3. During the entirety of this testing season, the average volume of wastewater added to Pond 3 was approximately 57 000L/week. It should be noted that these values are an average, and the volume of inflowing wastewater on a day-to-day basis could vary greatly depending on the number of loads delivered that day. As well, the influent composition of each individual load can fluctuate drastically. Septic tanks of each individual household can differ in quality and quantity, depending on water usage, the size of the household, and the age of septage, amongst other factors. Because of this, it is important that the treatment technologies employed be robust enough to handle these types of fluctuations.

To confirm that the biofilm environment was the main factor enhancing the treatment performance of the systems, a control was introduced to assess whether the BioCord and/or BioDome systems were able to reduce the targeted wastewater effluent parameters more significantly than an aerated system. The addition of aeration alone has been shown to increase the removal of wastewater constituents by providing aerobic microorganisms with the DO required to sustain microbial growth and metabolism (Grady *et al.*, 1999). As such, it may be possible that aeration alone, simulating a simple suspended sludge continuous stirred-tank reactor (CSTR), could significantly reduce the wastewater parameters of interest. Therefore, the addition of the control tank in this study aimed to examine whether the implementation of a biofilm treatment technology would be more effective in improving effluent quality than aeration alone. The control tank contained only four air stones (12-inch Top Fin® air stones) in order to mimic the effects of a simplified suspended sludge reactor. The tanks containing the treatment technologies each contained approximately 5678L (~2.2m D and ~1.7m H) of wastewater, while the control tank was a smaller reactor with an approximate holding volume of 730L (~1.1m D and ~0.71m H). Effluent from each of the four tanks were gravity drained into the final, tertiary pond (Pond 1) at the Storring's facility. From there, effluent from Pond 1 is discharged onto the surrounding environment via land spreading and eventual infiltration and evaporation.

An air compressor (DC24120 from Pentair Aquatic Eco-systems®) with a 4CFM capacity was used in order to deliver aeration to each of the four tanks, with air pressure readers and gauges in place to ensure that the amount of air being delivered was evenly distributed between all of the tanks. It was made certain that the tanks were each receiving at least 1CFM of air flow during periods of aeration. This minimum airflow was selected based on the requirements for the BioDome system as stated by the manufacturer, Wastewater Compliance Systems, Inc., who specified a minimum of 1CFM to effectively introduce air into the system and achieve a similar performance as conventional aeration systems (Wastewater Compliance Systems, Inc., 2015). Due to the lack of information regarding dissimilarities between the BioDome and BioCord systems (submerged biofilm systems), as well as the resilience of zebra mussels to relatively low oxygen concentrations (Benson *et al.*, 2015), a minimum airflow of 1CFM was determined to be appropriate for each of the three treatment technologies to achieve good treatment levels. All of the energy requirements for this experimental setup were met using power generated by a hybrid solar electricity system installed by the facility operators at Storring Septic. The specifications and more detailed information on these pieces of equipment, along with all of the other major pieces of equipment used for this study, are shown in Table 4-1.

Table 4-1. On-site equipment used for the experimental setup at Storring Septic.

Equipment/purpose	Model/ manufacturer	Specifications	Image
Water pump; pump water from Pond 2 into treatment tanks	DC4000 RLSS Waveline™	24V DC water pump Continuous-duty (24h) usage Dimensions: 151mm x 91mm x 127mm Max flow 3997 litres per minute (LPM) 11-speed controller included Inlet diameter: 1-½”, outlet diameter: 1”	
PVC gate/ball valves (x3); control water flow into treatment tanks	T-601 Legend Valve & Fitting, Inc.	1” PVC Threaded FPT x FPT Ball Valve	
Air compressor; provide aeration to treatment tanks In use weeks 1-14	DC24120 Pentair Aquatic Eco-systems®	24V DC air compressor Max flow 120 LPM/4.0 CFM Continuous-duty (24h) usage Steel casing Outlet diameter: ¾”	
Air compressor; provide aeration to treatment tanks In use weeks 15-20	AAPA110L Active Air	Continuous-duty (24h) usage Max flow 110 LPM/3.88 CFM	
Air pressure gauge/flow regulators (x5); monitor and control airflow	MP514803AV Campbell Hausfeld	Regulates and records pressure of 0 to 120 PSI Approximately 15CFM flow capacity at 90PSI 1/4-in. NPT female ports	N/A

Hybrid solar electricity system; provide solar energy to site Solar photovoltaics (PV) array and batteries (x4)	PV Panels Friendly Fires 6CS25PS Rolls Battery by Surrette	Solar PV array: 1kW Batteries: 6V, 1156Ah @100 Hr. rate (x4 = 24V system) Flooded lead-acid Deep cycle, performance over long service life	
Air stones; used in control tank for mixing and delivery of DO (x4)	36-5149681 Top Fin®	12 inch, elongated air stone Submersible, for oxygenation	

Samples were collected on an approximately 2-3 times per week, as the remote location of the facility meant that daily testing was not possible. 1L polyurethane bottles were used to collect effluent from each of the four testing tanks, as well as effluent directly from Pond 2, the latter of which served as a baseline influent reading for all wastewater parameters being tested. Samples were stored on ice in a cooler for no longer than 2h before testing.

Loading rates into each tank were controlled by varying the hydraulic retention times and, hence, flow rates of wastewater entering each of the tanks. Flow rates to all tanks were controlled by changing the power settings on the pump to reduce or increase flow. Flow entering each individual tank was controlled using gate valves. The flow rate was determined based on the influent COD concentration from the previous sampling event and adjusted according to the expected COD and desired loading rate. The flow rate into each tank was measured by recording the time required to fill a 500mL bottle, and the HRT of the tanks were calculated using Equation 1:

$$HRT = \frac{2t*V}{(8.64*10^4)} \quad (1)$$

Where t is the time, in seconds, it takes for the inflow to fill up a 500mL bottle and V is the volume, in liters, of the reactor tank.

The experiment was divided into three testing periods, with each period designed to assess the effects of different water flow/loading rates and aeration cycling. Table 4-2 outlines each of the three testing periods and the main objectives for each. The zebra mussel tank was decommissioned during the third testing period and only the effluents from the BioDome, BioCord and control tanks were sampled.

Table 4-2. Retention times, loading rates, and air cycling regimes for each testing period of the full operational season. The first testing period lasted from May 22, 2015 (Day 1) to July 6th, 2015 (Day 46). The second testing period lasted from July 7, 2015 (Day 47) to August 20, 2015 (Day 91). The third testing period ran from September 3, 2015 (Day 105) to October 8, 2015 (Day 140).

Testing period	Week	HRT (days)	Loading rate(s) (kg CODm^{-3}d^{-1})	Air cycling (on/off)	Rationale
1	1-3 (day 1-25)	3-7	1-1.21	24h/0h	Start-up/establish biofilm, bacteria and zebra mussel acclimatization, let system reach pseudo-steady state **Reach stable biofilm formation** **Maximum biomass density reached**
	4-7 (day 26-46)	7-10	0.57-0.77	24h/0h	Seeing effects HRT/constant aeration; nitrifying and heterotrophic bacteria reach pseudo steady state **Lower loading rates for accumulation of nitrifiers**
2	8-13 (day 47-82)	~7-15	~0.10-0.70	4d/3d	Nitrification/denitrification (goal is TN removal), P removals Observe ability of technologies to buffer **Cycle air 4 days on/3 days off** 2 weeks for acclimatization
	14 (day 83-91)	~9-15	~0.10-0.50	0d/7d	
3	15, 16 (day 105-113)	~7-10	~0.15-0.55	4h/4h	Provide biofilm with enough aeration to rebound from extended anoxic conditions; acclimatization to on/off cycling
	17,18 (day 114-127)	3-5	~0.57-0.87	12h/12h	Nitrification/denitrification, P removals Cycle: 12h on/12h off 24h on/24h off 2 weeks acclimatization each; compare reduction efficiencies and optimal aeration cycles
	19, 20 (day 128-140)	4-7	~0.57-0.87	24h/24h	

The first testing period involved the start-up and conditioning of all treatment tanks. Constant (24h) aeration was delivered to each of the testing tanks to maintain DO concentrations and to facilitate growth and acclimatization of the microorganisms and the zebra mussels in the wastewater, particularly with respect to biofilm formation which can suffer from oxygen diffusion limitations (Trulear and Characklis, 1982). Loading rates were also maintained under ~1.21kg $CODm^{-3}d^{-1}$ during the start-up phase to prevent shock loading of the microbial and zebra mussel populations. High organic loads can result in "unhealthy" biofilm conditions, leading to conditions of nuisance organism overgrowth, deterioration of treatment performance, lowered DO levels, and hindered oxygen transfer to inner biofilm layers (Evans, 1985). According to Mann *et al.* (1999), a loading rate of 1.21kg $CODm^{-3}d^{-1}$ resulted in a stable biofilm with resistance to shear forces when applied during the startup (unsteady-state) phase of a submerged biological aerated filter. Hence, a target of 1.21kg $CODm^{-3}d^{-1}$ was selected as the maximum loading during this phase of treatment. In order to achieve loading rates at or lower than this target, hydraulic retention times were altered appropriately based in the COD values obtained from the previous sampling date. In the context of the biofilm technologies, the goals of Weeks 1-3 were to establish a dense, stable biofilm with a sufficient ability to resist shear forces. Wijeyekoon et al. (2004) found that higher loadings resulted in higher substrate fluxes, denser biofilms and more bacterial growth. Although low enough to prevent organic overloading, the loadings for Weeks 1-3 were maintained considerably higher than in the following weeks in order to achieve these objectives. Following the first three weeks of acclimatization and bacterial growth, the loading rates were decreased to allow for the establishment of a more heterogeneous biofilm consisting of a mixture of both COD-consuming heterotrophs and the slower-growing nitrifiers/denitrifiers/polyphosphate-accumulating organisms (PAOs) (Nogueira *et al.*, 2002; Benthum *et al.*, 1997). The goals of Weeks 4-7 were to achieve a sustainable accumulation of slower-growing bacteria on the BioCord and BioDome biofilm treatment technologies and to allow all the systems to reach pseudo steady state.

The second period of testing introduced air cycling to examine the effects of induced aerobic and anaerobic/anoxic conditions on nitrification and denitrification rates, as well as to assess the lower practical limits of aeration. Reductions in total nitrogen would be expected to be higher than in the first testing period, as denitrification requires anaerobic/anoxic conditions for the conversion of nitrate to molecular nitrogen (Middlebrooks and Abraham, 1982). During air-off periods, the overall DO concentrations present in each treatment tank would be expected to be significantly reduced, which could increase the risk of organic overloading and shocking of the microbial populations in the biofilm treatment technologies, as well as the possibility of an overgrowth of undesirable anaerobic microorganism leading to the formation of an unhealthy biofilms (e.g. filamentous organisms, sulfur-oxidizing bacteria, etc.) (Evans, 1985). Therefore, during these anaerobic conditions, COD loading rates were maintained in the lower range of the suggested 0.10 - 0.70kg $CODm^{-3}d^{-1}$. Hydraulic retention times were also longer than during the previous testing phase, as treatment efficiencies were expected to decrease due to lower DO concentration in the treatment tanks. The long on/off aeration cycles also allowed for the performance assessment of each treatment technology under extended periods of low oxygen conditions, to observe whether any treatment technology experienced shock or overloading, as well as to observe their ability to re-establish their normal activity after extended anaerobic conditions. If a system was able to maintain sufficient reductions in wastewater effluent parameters of interest under these anaerobic conditions, or if it showed rapid recovery to previous reduction levels once aeration was re-established, it would be assumed that the technology would exhibit a good resilience to fluctuations in redox conditions. This would also suggest that a technology that could support a robust biofilm with a high resistance to shock, and/or a good buffering capacity would exhibit the least energy requirements due to its reduced need for continuous aeration. A full week of anaerobic/anoxic cycling was implemented in Week 14 to further observe the buffering capacity of the BioCord and BioDome systems. The schedule for this testing period was maintained for 6 weeks to acclimatize the system to the new air cycling/loading rate regimes.

The third testing period was designed to achieve a balance between overall wastewater effluent parameter reductions and lower energy consumption in the treatment systems. A two-week shutdown of flow and three-week shutdown of air prior to the start of this testing period was induced, both for equipment maintenance and to investigate the ability of each technology to recover after an extended overall system shutdown. These conditions were intended to mimic a potential total system shutdown in the case of pond shocking or treatment technology malfunction, and would allow for observations regarding the ability of each technology to re-establish previous levels of treatment. The zebra mussel system was decommissioned during the third testing period because of suspected zebra mussel death after the 3-weeks of anoxic conditions.

In Weeks 15 and 16 introduced lower organic loading rates (0.15 – 0.55kg $CODm^{-3}d^{-1}$) and aeration cycling regimes of 4h on/4h off. This was to ensure that the biofilm treatment systems were not exposed to extremely high organic loadings or extended anaerobic conditions during the restart of the entire system. The cycling of aeration in a 4h on/4h off regime was implemented to acclimatize the biofilm and microorganisms to an on/off cycling regime, and to allow for the reestablishment of both aerobic and anaerobic microorganisms. This schedule would also allow the systems to recover from the potential shock resulting from the two-week decommissioning. Weeks 17 to 20 also aimed to focus on total nitrogen and orthophosphate reductions in response to different aeration cycling regimes than previously tested. From the second testing period, it was determined that a period of two weeks was sufficient to acclimatize the biofilm technologies to a new air cycling/loading rate schedule.

4.4 Methods

Effluent samples were collected from the outflow of each treatment tank 2-3 times a week. Influent wastewater into the tanks (wastewater coming from Pond 2, the secondary pond) was sampled using a mixture of grab samples from the inflow points of each tank, for the most accurate representation of inflow composition. Approximately 1L of each sample was collected in five separate 1L polytetrafluoroethylene (PTFE) plastic containers and placed on ice for transport until analysis was able to be carried out. Each sampling container was completely filled with sample, such that there were no visible air bubbles present in each container. Analysis of the samples occurred as soon as possible and typically occurred approximately 1-2h from the time of collection.

TSS, organic matter (as COD), ammonia/ammonium ion (total ammonia), nitrite/nitrate, total nitrogen and orthophosphates were tested for each of the five samples collected (influent, effluent from BioDome system/Tank #1, effluent from the BioCord system/Tank #2, effluent from the zebra mussel system/Tank #3 and effluent from the Control Tank #4).

Total suspended solids were measured by filtering 100mL of wastewater sample through a pre-weighed glass fiber filter using gravity and vacuum filtration. The residue and filter were then dried at 105°C in a drying oven for 1h, the mass recorded, and the TSS calculated using the mass difference and volume of sample. A sample volume of 100mL was used, except in instances where the suspended solids concentrations were too high to allow for percolation of the sample through the glass fiber filter (i.e. influent samples). In such cases, the sample volume was reduced to 50mL.

COD was measured using the calorimetric, closed reflux method as outlined in section 5220 D of Standard Methods for the Examination of Water and Wastewater (Eaton *et al.*, 1998). The digestion solution used in this procedure was prepared by adding 500mL of distilled water to 10.216g potassium dichromate ($K_2Cr_2O_7$), 167mL concentrated sulfuric acid (H_2SO_4) and 33.3g mercury(II) sulfate ($HgSO_4$). The sulfuric acid reagent was prepared by adding 10.07g of silver sulfate (Ag_2SO_4) to 1L of H_2SO_4 (a rate of 5.5g Ag_2SO_4/kg H_2SO_4) and allowing the solution to stand for 2 days in order for the Ag_2SO_4 to completely dissolve. 1.5mL of the $K_2Cr_2O_7$ digestion solution and 3.5mL of sulfuric acid reagent were consecutively added to each test tube containing 2.5mL of sample wastewater. The test tubes were then capped and placed in a block digester (Hach DRB200) (150°C) for 120 minutes to induce a colour change. After samples had cooled, they were inverted multiple times, and the solids were allowed to completely settle to the bottom of the tube before absorbance readings were taken. These three steps (cooling, inverting, settling) typically took 15-20 minutes in total. Absorbance for COD testing was measured at a wavelength of 600nm.

Total ammonia and nitrate concentrations were determined using ammonia and nitrate accumet® electrodes (Fisher Scientific). Either 2mL of ammonia pH/Ionic Strength Adjuster (ISA) (Thermo Scientific) or nitrate pH/ISA (Fisher Scientific) was added to each volumetric flask containing 100mL of sample being tested for either ammonia or nitrate, and moderately stirred using a magnetic stir bar. The probe was placed in the sample solution and mV (millivolt potential) readings allowed to stabilize before recording the reading. Calibration curves were generated every time a new batch of samples were being tested.

Total nitrogen was calculated by the summation of all nitrogen species tested. It was assumed that the levels of organic nitrogen were low enough to be omitted from the calculation of total nitrogen, as most of the organic nitrogen in untreated wastewater has been reported to be associated with particulate matter, and to readily settle out during the primary treatment phase of a multi-cell WSP operation (Reed, 1985). Organic nitrogen can also be contained in organic matter, but it is released as ammonia when the organic matter is degraded by microorganisms (Grady *et al.*, 1999). In order to ensure that levels of organic nitrogen were not a significant fraction of the total nitrogen, wastewater samples from the infuent were sent to the Analytial Services Unit (ASU) at Queen's University approximately every three weeks to be tested for Total Kjeldahl Nitrogen (TKN), a measure that represents the sum of organic nitrogen, ammonia and ammonium. The value of organic nitrogen was obtained by subracting the value of ammonia/ammonium from the TKN value. The percentage of organic nitrogen in the TKN was found to range between 0.5% to 3% of the TKN composition.

Calorimetry was used for the testing of nitrite and orthophosphate using Thermo Scientific™ Orion™ AQUAfast™ reagent tablets. Either one nitrite low-range (LR) tablet or one orthophosphate LR tablet was placed in 10mL of sample and allowed to dissolve to induce a colour change. The absorbance of the resulting colour was then immediately measured and the concentration of each parameter calculated using a generated standard curve. The absorbance for nitrite was measured at 540nm, while the absorbance for orthophosphate was measured at 880nm.

Standard curves for all parameters using calorimetric methods were generated weekly to determine concentrations from absorbance readings. Each sample was tested in duplicate: for each parameter, two aliquots of sample were taken and tested, resulting in two absorbance values. The two absorbance values of each sample were then averaged. Testing two aliquots of each sample ensured that there was consistency in the measurement and that interference from suspended solids or turbidity did not affect the absorbance readings. For each parameter, a t-test was conducted between all pairs of readings to ensure that the variance between the two was not significantly high (i.e. there was no difference between the means). All t-tests between pairs of readings for all parameters resulted in p values of over 0.05, suggesting that there was no significant variance between the pairs of readings.

In addition, the DO, pH and temperature in each tank were recorded. During Weeks 1 to 10, these parameters were recorded on site using the Hydrolab DS5, via electrode (pH and temperature) and optical (DO) probes. The pH (model #013410HY) temperature (model #004165HY) sensors were manufactured by Hydrolab®, while the DO sensor was manufactured by Hach® (model # 007460). After week 10, the Hydrolab was unavailable, as such, DO and temperature were measured on-site using a field meter (Yellow Spring Instruments, Model 57; YSI 5739 DO/temperature probe), which was calibrated every day prior to testing. The pH of the samples tested in the laboratory were performed using a Fisher Scientific accumet® pH electrode (#13620112) within 2h. The electrode was placed in a sample of wastewater and the pH reading allowed to stabilize before reporting the data. Although the pH readings were not taken directly from the treatment tanks, the delay did not appear to have a significant effect on the pH levels recorded, as the variance in pH remained quite small. The range of pH values from weeks 1-

10, when pH was recorded on-site, was 7.08 - 7.84. The range in pH values for weeks 10 and onwards, when pH was tested in lab, was 7.10 - 8.01.

Zebra mussels used for this study were collected from Beaver Lake located west of Tamworth, Ontario. Permission to collect these live organisms obtained from the Ontario Ministry of Natural Resources, and a permit ("License to Collect Fish for Scientific Purposes", License No. 1079875) was issued by the appropriate authorities to certify their approval. Approximately 1000 live, adult zebra mussels were collected by hand from the bottom of the Beaver Lake. They were placed in 6L plastic containers along with sufficient lake water to ensure their survival during transport and storage. They were immediately transported to the Storring Septic site and placed into the treatment tank intended for zebra mussel filtration (Figure 4-1). The zebra mussels were allowed to acclimatize to the influent wastewater by allowing the inflow to drip slowly into the tank, displacing the lake/rain water over a three-day period. Approximately 50 zebra mussels were also transported in a 6L plastic container to the laboratory at Queen's University, as permitted by the updated "License to Collect Fish for Scientific Purposes". These zebra mussels were kept for further testing and experimentation (Chapter 5).

4.5 Results and Discussion

The effluent wastewater parameter data for the treatment technologies were analyzed for each testing period, as well as over the entire 140-day treatment season.

4.5.1 Dissolved oxygen, pH and temperature

Temperature, pH and DO concentration of the wastewater in the treatment systems were considered to be important factors that could contribute to the performance of a treatment technology will perform. In the case of biofilm and suspended sludge systems, the ability for the microorganisms to oxidize and metabolize organic material and contaminants could be related to temperature using the Arrhenius relationship, which is shown in Equation 2 (Metcalf and Eddy, 2004).

$$k_T = k_{20} * \theta^{(T-20)} \quad (2)$$

Where T is the temperature (Celsius), k_T is the rate constant of the biochemical reaction at that temperature (day^{-1}), k_{20} is the rate constant of the reaction at standard temperature (day^{-1}), and θ is the temperature coefficient (dimensionless). According to this equation, when all other factors remain constant, the microbial rates of reaction will increase with increasing temperature. Therefore, it would be expected that the biofilm treatment technologies would achieve better reductions of wastewater parameters at higher temperatures, provided the pH and DO concentrations remain relatively constant. Figure 4-2 shows the fluctuation in ambient temperatures throughout the 140-day summer/fall testing period, as well as the precipitation noted during this time.

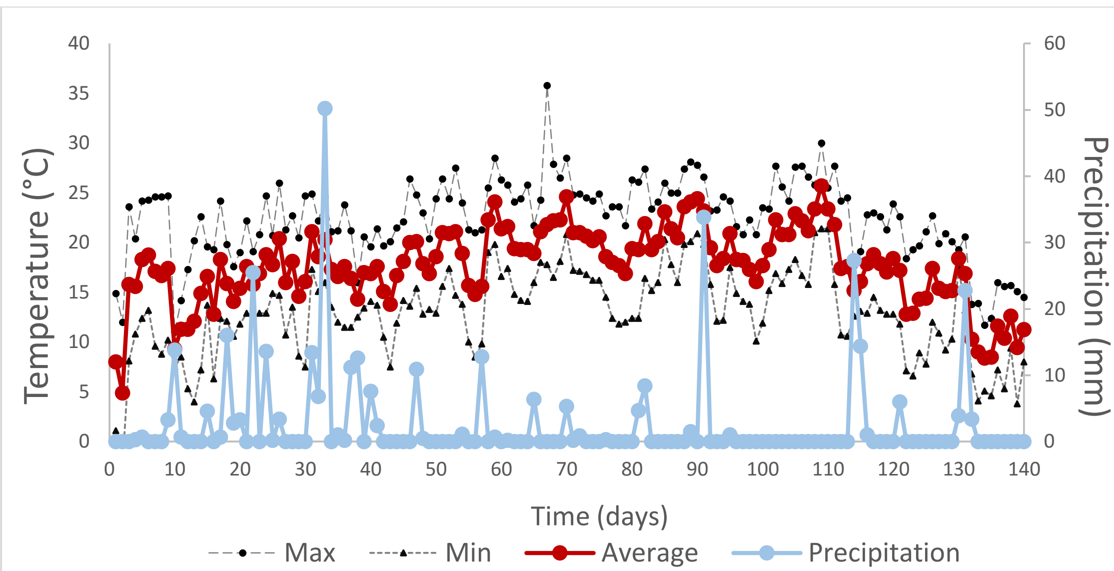


Figure 4-2. Maximum, minimum, and average temperatures, as well as precipitation accumulation, over the 140-day testing season (May 22nd - Oct 8th, 2015) (Environment Canada, 2015).

It was not anticipated that precipitation would significantly affect the performance of each treatment technology, but it is possible that consistently high levels of precipitation could have yielded in diluted concentrations of the wastewater parameters of interest, which could have been misinterpreted as higher treatment performance (or false positive response). The precipitation data was thus included to account for all external environmental variables.

The wastewater temperature in each of the treatment system tanks was also recorded during each sampling day. Correlation analysis indicated that higher average ambient temperatures were highly correlated with higher water temperatures in the treatment tanks ($r > 0.9$ for analysis with average ambient temperature and all treatment tanks) (Evans, 1996). The water temperatures were also always consistent between each of the treatment tank (less than 1.5ºC difference) on any given sampling day ($r > 0.98$ between all treatment tanks). Thus the water temperature data have been omitted for simplicity and ease of analysis, as the day-to-day ambient temperature data is also more comprehensive. Temperatures were, on average, highest during mid-season and lowest at the beginning and end of the overall study period.

The concentration of DO was found to be a large contributing factor affecting treatment performance for each of the treatment technologies. In the case of the system relying on microbial treatment (attached or suspended), sufficient oxygen was needed for the microorganisms to effectively degrade organic wastewater constituents under aerobic conditions. In the case of the zebra mussels, DO was necessary for respiration and to prevent death via asphyxiation. The concentrations of dissolved oxygen in each of the treatment tank throughout the testing season are shown in Figure 4-3.

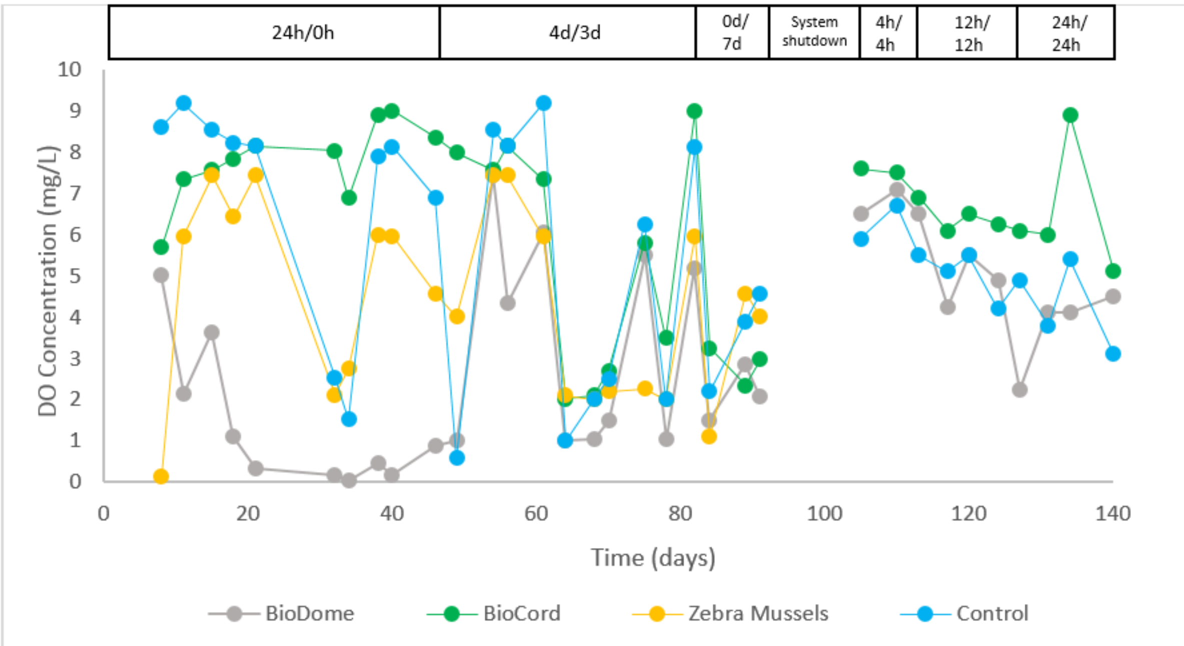


Figure 4-3. Dissolved oxygen concentrations (mg/L) in each treatment tank during time of sampling. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

It was noted that, generally, after the 2/3-week system shutdown (between periods 2 and 3), the BioCord system was the treatment technology that recovered most rapidly from the extended anoxic period, showing the highest percent reductions from the influent immediately after this period, and reaching significant reductions from the influent earlier than both the BioDome system and the control tank (aeration and suspended growth). Moreover, the period of no flow and aeration during the system-off weeks resulted in the death of the zebra mussels. This suggested that, although zebra mussels may have the capacity to treat wastewater to some extent, their ability to thrive depends largely on an adequate supply of DO. Hence, it was concluded that this treatment was limited in its capacity to recover from periods of low DO concentrations and would offer limited protection to shock loading or system shutdown events.

The recorded DO concentrations generally followed the cycles of aeration applied throughout this study, with the exception the BioDome tank during the first testing period, where concentrations were extremely low in comparison to the other treatment tanks. This was due to the clogging of the integrated air diffuser, which impeded water/substrate circulation in the tank as well as oxygen delivery. In such events, the diffuser manifold was blown out using a portable high-pressure air compressor. Once this was accomplished, DO concentrations reached levels that were similar to those of the other treatment tanks. Clogging of the BioDome system air diffuser occurred more than once throughout the testing season, as such maintenance had to be performed periodically (days 49, 82, 117 and 127). The BioDome system manifold was unclogged whenever there was both an apparent loss of aeration/mixing and when the DO concentrations recorded for the BioDome system were notably lower than those of the BioCord system during a sampling event. An apparent loss of aeration was observed as a noticeable decline in bubbling and movement in the wastewater of the BioDome treatment tank. Even with periodic maintenance of the BioDome system air diffuser, the DO concentrations in the BioDome treatment tank never reached those observed in the BioCord treatment tank, suggesting that achieving effective air circulation and mixing in BioDome could present a challenge in applications in more remote or accessible sites. As such the BioDome system was deemed to have the highest maintenance requirements of the three treatment technologies.

Lastly, wastewater pH can have a considerable effect on microbial growth and metabolism in biofilms, and therefore on biological wastewater treatment (Babu, 2011). Each microbial species has an optimal pH range for which their growth rates are maximized. For example, nitrifiers and denitrifiers can grow well in pH ranges from 7.2 to 9.0 and 7.0 to 8.0, respectively (Metcalf and Eddy, 2003), although Lindfors (2010) reported that with acclimatization, good nitrification could also be achieved in a pH range of 6.5 to 8.0. Lessard and Bihan (2003) reported the optimal pH for oxidation of carbonaceous compounds by heterotrophic organisms to be in the range of 6.5 to 8.5, which corresponds to the pH of typical domestic wastewaters. Grady *et al.* (1999) reported that all bacteria grow poorly outside of the normal physiological range of 6.0 to 8.0. Zebra mussels have an optimum pH range of about 7.3 to 9.3 (Alexander and Thorp, 1997), with some studies noting an upper pH limit of zebra mussel tolerance of 9.3

to 9.6 (Bowman and Bailey, 1998). Another study investigating the lower pH limits of zebra mussels observed significant mortality of zebra mussels only at pH levels below 6.9, and only after having been exposed to this pH for a period of 10 weeks (Claudi *et al.*, 2012). This suggests that zebra mussels should be able to survive at pH ranges of 7.0 to 9.6.

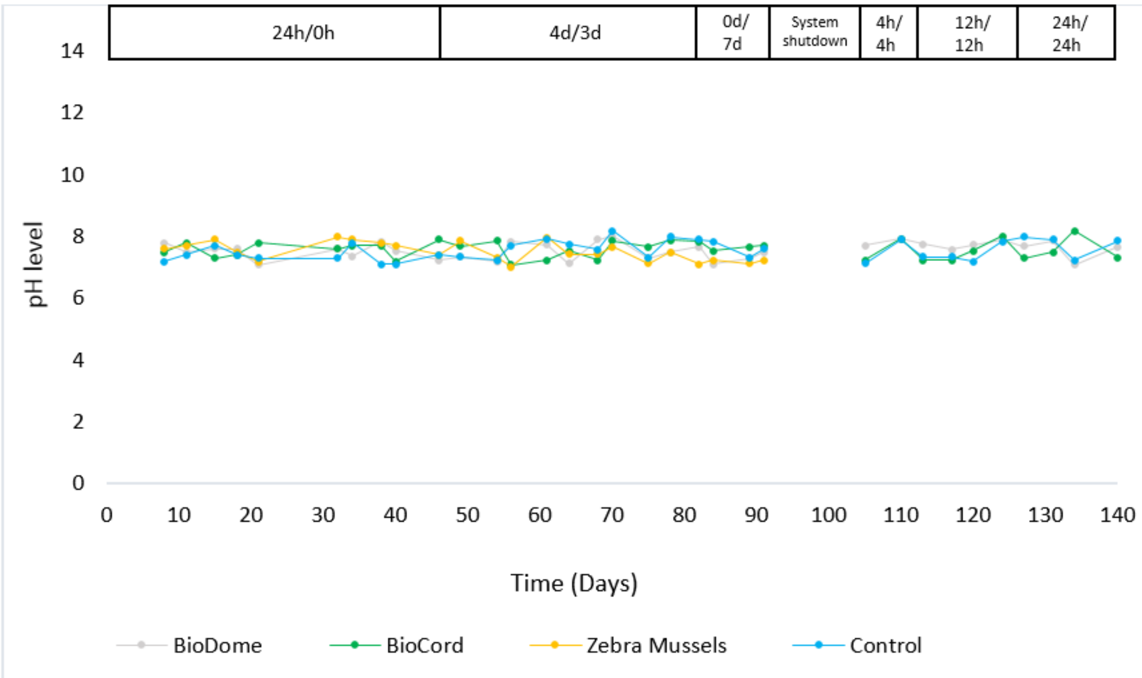


Figure 4-4. pH levels of each treatment tank over the course of the testing season**.** Trend lines in between data points are shown to aid in visualizing the patterns in in pH for each tank over the testing season, but are not necessarily representative of actual values in between data points.

The pH levels in each of the tanks over the entire treatment season is shown in Figure 4-4. The pH range in all treatment tanks ranged from 7.0 to 8.2. As such, it was concluded that the pH should not have had a significant effect on the treatment levels of either the biofilm, suspended growth or zebra mussel treatment technologies, as the pH levels remained largely within the range for both zebra mussel survivability and microbial growth.

4.5.2 Nitrogen species and total nitrogen removals

Ammonia (NH_3) and ammonium (NH_4^+) make up the total ammonia concentration of the wastewater. They are both targets for removal in wastewater treatment because of their toxicity to aquatic species and their contribution to eutrophication (oxygen depletion) in natural bodies of water (Oleszkiewicz, 2015; Chu and Wang, 2011). In biological treatment processes, such as processes that use biofilm to reduce wastewater parameters, the presence of ammonia-oxidizing bacteria (AOB) has been reported reduce total ammonia levels via metabolic conversion to nitrite (Pester *et al.*, 2012). This process is dependent on the availability of DO as the electron acceptor for the microbial organisms. Hence, the addition of mechanical aeration in wastewater treatment and subsequent increases in DO/mixing can greatly enhance the reduction of total ammonia concentrations (Grady *et al*., 1999; Lee *et al*., 2002). Figure 4-5 illustrates reductions in total ammonia in each of the treatment systems compared to the influent over the course of the entire testing period.

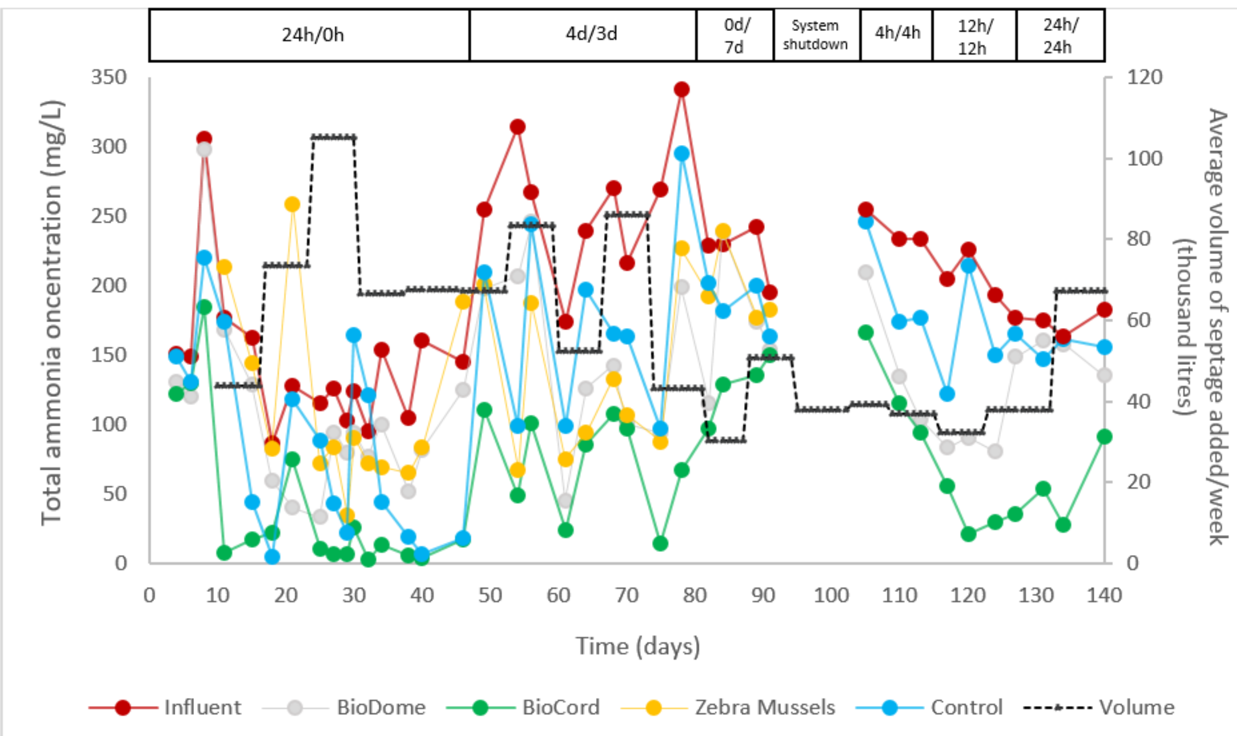


Figure 4-5. Reductions in total ammonia for each tank (primary axis), compared to the influent total ammonia levels, over a 140-day testing period (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

In addition to the effluent concentrations of total ammonia over the course of the testing period, a secondary axis is included in Figure 4-5 to illustrate changes in influent total ammonia concentrations in response to septage loading to the Storring Septic ponds each week. These values represent the total average volume of wastewater added per week to the Storring Septic primary pond, Pond 3, although the day-to-day loads dumped into Pond 3 may have varied drastically depending on the number of customers serviced during that time. These daily volume fluctuations, as well as fluctuations in the composition of each individual septic tank serviced, contribute to the necessity of a robust treatment technology to handle such extreme variations in loads. Based on a visual analysis of Figure 4-5, it can be noted that peaks in influent total ammonia concentrations tended to correspond with septage additions to the wastewater stabilization pond system with a gap of approximately 1-3 weeks. As the pond system is a passive system operated without pumping or HRT control, this trend was likely influenced by the variable retention times throughout the treatment season. However, it can be noted that volumetric loading had an effect on influent--and therefore effluent— total ammonia concentrations. Table 4-3 summarizes the effect of aeration cycling on the overall performance of each treatment technology in comparison to the control during each of the individual testing period. Cells highlighted in blue indicate that the treatment technology produced significantly higher percent reductions of total ammonia than the control.

Table 4-3. Average percent reductions (%) of total ammonia from the influent for each treatment tank.

† = indicates that average percent reductions were found to be significantly higher ($p \leq 0.05$) than the control for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Timeframe	Aeration	Average Temp (°C)	Percent reductions from influent (%)			
				BioDome	BioCord	Zebra Mussels	Control
1	Weeks 1-7 (days 1-46)	24h ON	16	23 ± 3	†75 ± 7 $\eta^2 = 0.22$	15 ± 12	41 ± 11
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	44 ± 6	†70 ± 5 $\eta^2 = 0.46$	47 ± 7	31 ± 7
2b	Week 14 (days 83-91)	24h OFF	23	15 ± 10	37 ± 7	10 ± 9	18 ± 1
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	38 ± 11	48 ± 7		18 ± 7
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	48 ± 11	†82 ± 4 $\eta^2 = 0.76$		19 ± 8
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	13 ± 7	†68 ± 10 $\eta^2 = 0.77$		11 ± 5
1-3	Weeks 1-20 (days 1-140)		18	31 ± 3	†69 ± 4 $\eta^2 = 0.32$	26 ± 7	30 ± 5

As can be seen from Figure 4-5 and Table 4-3, the BioCord system exhibited the highest overall average percent reductions in total ammonia and was the only treatment system to show significant reductions in total ammonia compared to the control (during testing periods 1, 2a, 3b, 3c and overall). The BioCord system also consistently showed the highest percent reductions than any other treatment throughout the entire summer/fall testing season. In wastewater, could also potentially be removed from a system via volatilization. However, volatilization only occurs significantly at pH levels above 9.3, as illustrated in Figure 4-6, which is beyond the range noted during this study (Figure 4-4). Therefore, ammonia volatilization was considered to be negligible during this study, and the reductions in total ammonia observed in the treatment system effluents were presumed to be primarily attributed to biological nitrification.

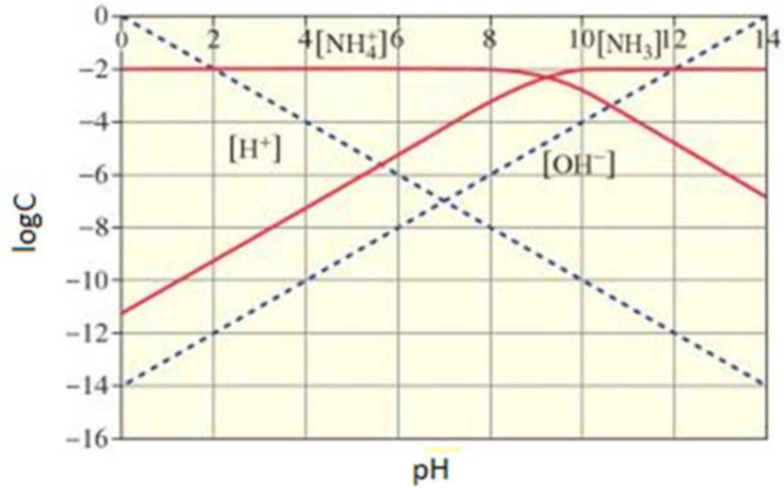
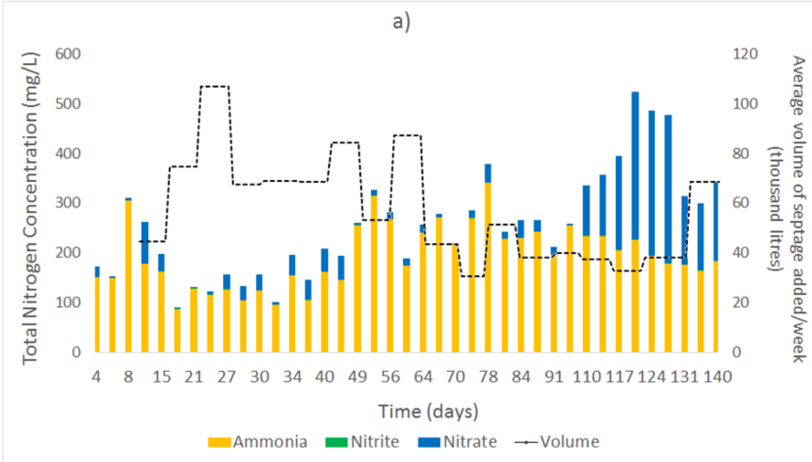
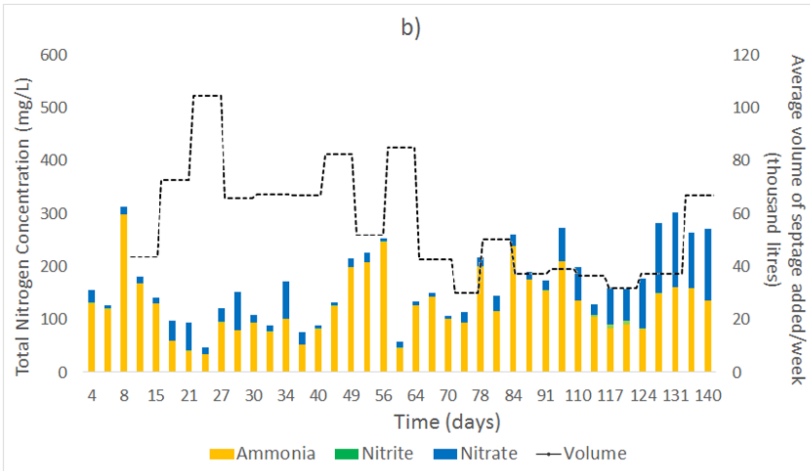


Figure 4-6. The pC-pH diagram of the NH4+/NH3 system. At a pH of 7-8, [NH4+]>>[NH3] and the dominant species is ammonium (non-volatilizing form). Little nitrogen is present as ammonia and thus, nitrogen removal by volatilization is minimal (Middlebrooks and Abraham, 1982).

Because the rate of ammonia oxidation by microorganisms is largely dependent on DO concentrations, it might have been expected that the first testing period, which employed 24h of constant aeration, would have exhibited the highest reductions in total ammonia for the biofilm treatment technologies. As can be seen from Table 4-3, this was not the case. Rather, testing period 3b (12h on/12h off aeration cycling) resulted in the highest percent reductions for all of the treatment technologies and in comparison to the control, followed by the first testing period (24h on). This may be attributed to the fact that the first testing period represented start up conditions and was largely aimed at biofilm establishment and organism acclimatization. As such, a lower treatment performance would be anticipated because 'pseudo steady state' microbial growth conditions had not yet been achieved. Based on literature involving similar biofilm treatment technologies, it was estimated that a four-week period would be required for the biofilm treatment technologies to reach their maximum growth phase and pseudo-steady state conditions (Bolton *et al.*, 2006; Lindfors, 2010; Johnson, 2011).

To assess the role of nitrification and denitrification in nitrogen removal, the composition of nitrogen species of the influent and each of the treatment systems was also analyzed. Nitrite and nitrate concentrations typically increase after the biological conversion of ammonia by AOBs. Both of these nitrogen species can be harmful in high quantities, and their presence in water can cause detrimental health effects in humans such as methemoglobinemia in the case of nitrate (Majumdar, 2003). This disease, also termed blue-baby syndrome, decreases the ability of blood to carry oxygen and can be fatal to newborns (Super *et al*., 1981). To reduce total nitrogen concentrations in wastewater, denitrification must take place to convert the end-product of nitrification, nitrate, to nitrogen gas (N_2), which subsequently volatilizes out of the system (Vymazal, 2007). Denitrification is largely performed by anaerobic and facultative bacteria, which require anaerobic or anoxic conditions (Vymazal, 2007). Aerobic denitrification has also been reported to be possible by some microbial organisms present in wastewater, but to a lesser extent (Wang *et al.*, 2007; Miyahara *et al.*, 2010). As such, reductions in total nitrogen were expected to be higher during periods when cycles of both aerobic and anaerobic conditions were implemented. Figures 4-7a to 4-7e show the distribution of total ammonia, nitrite, nitrate and total nitrogen species for the influent and the effluent of each of the treatment systems.


a)
Total Nitrogen Concentration (mg/L)
Average volume of septage added/week (thousand litres)
Time (days)
Ammonia
Nitrite
Nitrate
Volume


b)
Total Nitrogen Concentration (mg/L)
Average volume of septage added/week (thousand litres)
Time (days)
Ammonia
Nitrite
Nitrate
Volume

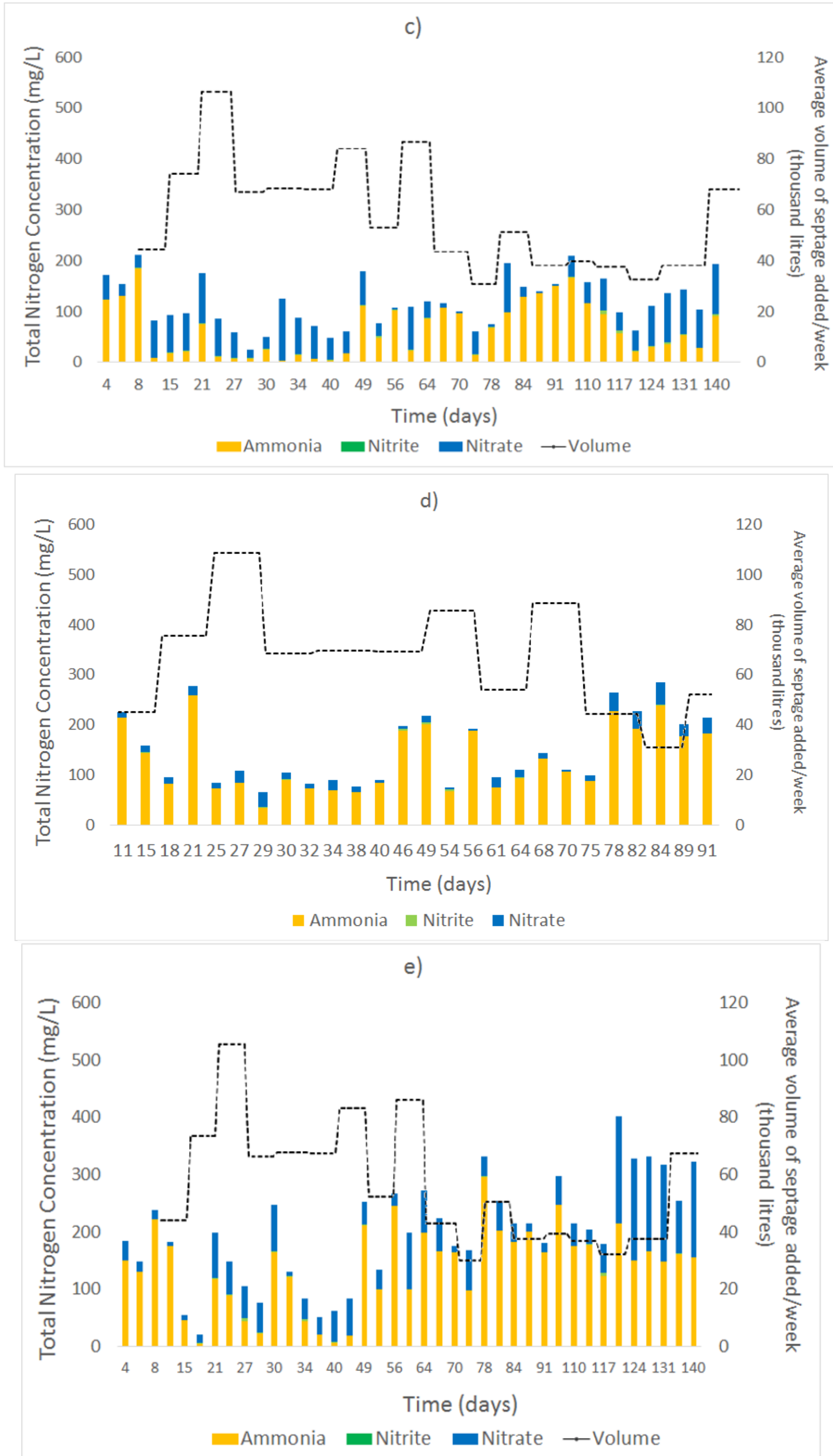


Figure 4-7. Total nitrogen and nitrogen compositions of a) the influent, b) the BioDome system effluent, c) the BioCord system effluent, d) the zebra mussel tank effluent and e) the control tank effluent. The scale of total nitrogen composition (mg/L) is kept constant to better illustrate the difference in total nitrogen reductions between each treatment technology.

The BioCord system showed the highest overall reductions in total nitrogen and nitrification during periods of aerobic activity (air-on periods), suggesting that its biofilm was composed of a robust consortium of both ammonia and nitrite oxidizers, as well as denitrifiers. The BioDome system showed moderate total nitrogen reductions over the course of the study, and particularly during periods on on/off aeration cycling. The low total nitrogen removals observed in the BioDome system during period 1 coincided with to the low DO concentrations measured in the system during the startup period (Figure 4-4). Table 4-4 shows the average percent reductions of total nitrogen noted for each system during the specific aeration cycles employed during summer/fall testing. Cells highlighted in blue indicate that the treatment technology produced significantly higher percent reductions of total nitrogen than the control.

Table 4-4. Average percent reductions (%) of total nitrogen from the influent for each treatment tank.

† = indicates that average percent reductions were found to be significantly higher ($p \leq 0.05$) than the control for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Timeframe	Aeration	Average temp (°C)	Percent reductions from influent (%)			
				BioDome	BioCord	Zebra Mussels	Control
1	Weeks 1-7 (days 1-47)	24h ON	16	23 ± 5	36 ± 10	14 ± 10	23 ± 11
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	†42 ± 6 $\eta^2 = .033$	†55 ± 6 $\eta^2 = 0.48$	†43 ± 7 $\eta^2 = 0.17$	14 ± 7
2b	Week 14 (days 83-91)	24h OFF	23	16 ± 8	40 ± 6	5 ± 10	18 ± 2
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	33 ± 21	42 ± 11		21 ± 18
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	†58 ± 6 $\eta^2 = 0.58$	†78 ± 4 $\eta^2 = 0.76$		35 ± 7
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	12 ± 5	†55 ± 6 $\eta^2 = 0.77$		7 ± 5
1-3 (overall)	Weeks 1-20 (days 1-140)		18	31 ± 4	†47 ± 5 $\eta^2 = 0.12$	17 ± 5	20 ± 5

Overall, the BioCord system showed the most significant total nitrogen reductions in comparison to the control (2a, 3b, 3c and overall). The nitrogen species composition in the effluent of the BioCord system indicated that the system was able to nitrify ammonia, leading to increases in nitrate during consistent (24h) periods of aeration (Figure 4-7c). When air cycling was implemented, the BioCord system showed the best ability to reduce total nitrogen concentrations, implying that the system was able to establish a good balance of both nitrification and denitrification activity (Figure 4-7c, Table 4-4). The BioDome system showed significantly higher average percent reductions than the control during testing period 2a (4d on/3d off aeration cycles) and 3b (12h on/12h off aeration cycles) (Figure 4-7b, Table 4-4). The zebra mussel system showed significantly higher average percent reductions in comparison to the control during testing period 2a (4d on/3d off aeration cycles) alone (Figure 4-7d, Table 4-4). The application of 4d on/3d off and 12h on/12 off aeration cycles to the biofilm technologies allowed the BioCord and BioDome systems to significantly outperform the control (Table 4-4). However, the 12h on/12h off aeration cycling (Table 4-4) contributed to the highest removals of total nitrogen, which would suggest that an optimal aeration cycle could be achieved for balancing nitrification/denitrification within these systems.

4.5.3 Orthophosphate

Phosphorus is a key nutrient that can lead to algal blooms and eutrophication, followed by oxygen depletion, in natural waters (Cooper *et al.*, 1994). As such, it is important to reduce concentrations of biologically available phosphorus in discharge effluents to prevent the negative impacts associated with high phosphorus concentrations on receiving environments. The biologically active form of phosphorus is orthophosphate, which is available for microbial metabolism and is the relevant species targeted for removals in biological wastewater treatment (Spellman, 2014). Microorganisms called polyphosphate-accumulating organisms (PAOs) uptake orthophosphorus into their cells, and require alternating anaerobic and aerobic environments to efficienctly remove orthophosphate from wastewater (Chen *et al.*, 2004; Zhou *et al.*, 2010). The concentrations of reactive phosphorus (orthophosphate) for the influent and all treatment systems throughout the 140-day study period are shown in Figure 4-8.

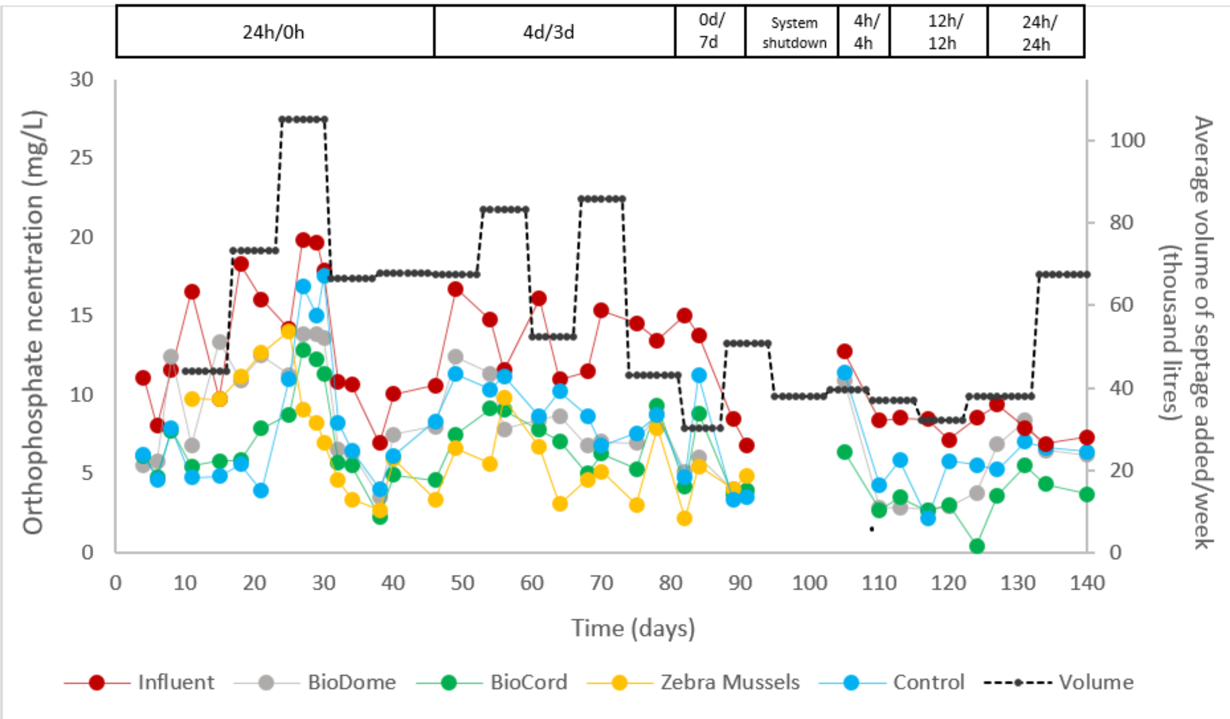


Figure 4-8. Orthophosphate concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

As can be seen from Figure 4-8, moderate decreases in orthophosphate concentrations were observed for the BioCord, BioDome, zebra mussel and control systems in comparison to influent concentrations. When Kruskal-Wallis nonparametric analysis was conducted considering average percent reductions, the results showed no significant differences between the control and any treatment systems for any testing period. This is likely because, although cycling of air to induce aerobic and anaerobic conditions have been reported to enhance orthophosphate treatment by microorganisms, the release of orthophosphate from the biofilm during anaerobic periods could yield lower overall average percent reductions (Wu *et al.*, 2012). The exact moments of orthophosphate uptake and release are difficult to ascertain because a comprehensive characterization of these polyphosphate-accumulating organisms (PAOs) has yet to be completed, and as such, there could be a number of possible phosphorus assimilation mechanisms taking place that are not yet fully understood (Mara, 2006; Sathasivan, 2009). Although none of the treatment technologies significantly outperformed the control tank, all three systems showed good reductions of orthophosphate in comparison to the influent. To further assess orthophosphate reductions for each treatment system, orthophosphate concentrations were compared to influent concentrations for each treatment period. The results are summarized in Table 4-5. It should be noted that cells highlighted in blue indicate that the treatment technology significantly reduced orthophosphate concentrations from the influent for that testing period. This is in contrast to previous tables in this chapter, which showed percent reductions in comparison to the control.

Table 4-5. Average orthophosphate concentrations, in mg/L, of the influent wastewater and each treatment tank effluent for each specific testing period.

† = indicates that the mean orthophosphate concentration in the treatment system effluent was significantly lower ($p \leq 0.05$) than the mean orthophosphate concentration found in the influent, for the specified testing period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Timeframe	Aeration	Average temp (°C)	Influent (mg/L)	BioDome (mg/L)	BioCord (mg/L)	Zebra Mussels (mg/L)	Control (mg/L)
1	Weeks 1-7 (days 1-47)	24h ON	16	13 ± 1	†9 ± 1 $\eta^2 = 0.15$	†7 ± 1 $\eta^2 = 0.44$	†8 ± 1 $\eta^2 = 0.32$	†8 ± 1 $\eta^2 = 0.31$
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	14 ± 1	†8 ± 1 $\eta^2 = 0.64$	†7 ± 1 $\eta^2 = 0.75$	†5 ± 1 $\eta^2 = 0.75$	†9 ± 1 $\eta^2 = 0.69$
2b	Week 14 (days 83-91)	24h OFF	23	10 ± 1	†5 ± 1 $\eta^2 = 0.77$	5 ± 2	†5 ± 0 $\eta^2 = 0.77$	6 ± 3
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	10 ± 1	6 ± 3	†4 ± 1 $\eta^2 = 0.77$		7 ± 2
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	8 ± 0	†4 ± 1 $\eta^2 = 0.76$	†2 ± 1 $\eta^2 = 0.76$		†5 ± 1 $\eta^2 = 0.76$
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	7 ± 0	7 ± 1	†5 ± 1 $\eta^2 = 0.77$		7 ± 0
1-3 (overall)	Weeks 1-20 (days 1-140)		18	12 ± 1	†8 ± 1 $\eta^2 = 0.26$	†6 ± 0 $\eta^2 = 0.48$	†6 ± 1 $\eta^2 = 0.38$	†8 ± 1 $\eta^2 = 0.29$

The results indicate that all treatment systems showed significantly lower orthophosphate concentrations than the influent (baseline) for testing periods 1, 2a, 2b and 3b. In addition, all treatment systems also showed significantly lower orthophosphate concentrations than the influent when considering the average over the entire testing season (weeks 1-20). Orthophosphate concentrations in the treatment systems were lowest during the 12h on/12h off aeration cycle, followed by the 4d on/3d off cycle and the 24h of constant aeration. However, the differences in concentrations in the latter two testing periods were quite small, and the higher orthophosphate reductions observer during period 2a (4d on/3d off) could be attributed to differences in temperature and its influence on microbial activity. It is speculated that, although each treatment technology was able to significantly reduce orthophosphate concentrations from the influent, the different technologies were unable to significantly outperform the control because influent orthophosphate concentrations were relatively low. More research should be conducted into the mechanisms of enhanced biological phosphorus removal in these types of eco-engineered or naturalized systems, as phosphorus is generally a targeted parameter of concern for treatment and concentrations often exceed those observed in this study. The zebra mussel system showed consistently lower average concentrations than both the control and influent baseline concentrations for each of the tested period. This would suggest that zebra mussels may have the capacity to uptake or store orthophosphate, although their sensitivity to system shutdowns is a drawback when considering their potential for wastewater treatment. As well, the less predictable cycling of phosphorus/orthophosphate by zebra mussels may present challenges if the control or accurate prediction of uptake or release of orthophosphate from wastewater is not possible.

4.5.4 Chemical Oxygen Demand

Chemical oxygen demand (COD) is an indirect measure of the organic matter present in wastewater. COD removals are important in wastewater treatment, because the release of high concentrations of organic constituents in wastewater effluents can lead to the death of aquatic organism and oxygen depletion in receiving water bodies (Pisarevsky *et al.*, 2005). As well, organic matter includes a wide range of pollutants such as fecal matter, detergents, greases, and food particles, which are compounds typically associated with unsanitary and low-quality effluents. COD removal is largely dependent on a number of fast-growing heterotrophic bacteria that are able to mineralize organic carbon into water and carbon dioxide, utilizing oxygen in the process. Therefore, it is an aerobic process; however, anaerobic digestion of organic constituents can also take place via a number of different bacteria and archaea, although at a much slower rate (Grady *et al.*, 1999). Therefore, aeration can greatly assist in reducing COD concentrations by providing an adequate source of oxygen to support the microbial metabolic activities that break down organic materials (Peavy *et al.*, 1985; Grady *et al.*, 1999). Periods when more aeration was provided and higher DO concentrations were present in treatment systems were expected to yield higher percent reductions in COD concentrations. Figure 4-9 shows COD concentrations in each of the treatment technologies throughout the entire testing season.

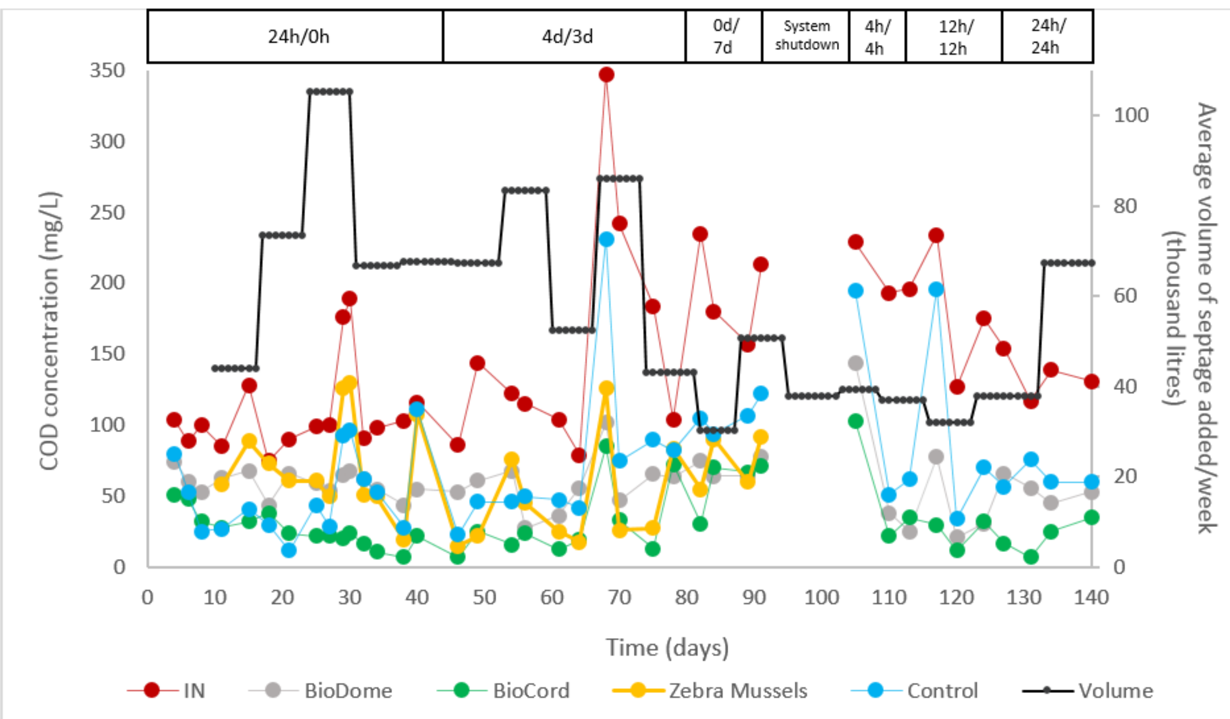


Figure 4-9. COD concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

Overall, the BioCord system produced the lowest average COD concentrations in its effluent. The BioCord system was able to significantly decrease COD concentrations from the influent for all testing periods, even during periods where anaerobic conditions were predominant and during periods of high organic loading. This would suggest that the implementation of a BioCord system in a WSP could result in more efficient processing of wastewater, and an ability for Storring Septic to safely accept higher volumes of septage and organic loads. Table 4-6 summarizes the average ability of each treatment technology to reduce COD concentrations during each of the testing period.

Table 4-6. Average percent reductions (%) of COD from the influent for each treatment tank.

† = indicates that average percent reductions were found to be significantly higher ($p \leq 0.05$) than the control for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Timeframe	Aeration	Average temp (°C)	COD reductions from influent (%)			
				BioDome	BioCord	Zebra Mussels	Control
1	Weeks 1-7 (days 1-47)	24h ON	16	43 ± 3	74 ± 4	39 ± 7	56 ± 6
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	59 ± 5	†78 ± 6 $\eta^2 = 0.51$	67 ± 7	52 ± 5
2b	Week 14 (days 83-91)	24h OFF	23	†62 ± 2 $\eta^2 = 0.77$	†62 ± 3 $\eta^2 = 0.77$	†56 ± 3 $\eta^2 = 0.77$	41 ± 5
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	68 ± 16	75 ± 10		52 ± 19
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	72 ± 6	†87 ± 2 $\eta^2 = 0.76$		53 ± 13
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	60 ± 4	†83 ± 6 $\eta^2 = 0.77$		49 ± 7
All	Weeks 1-20 (days 1-140)		18	55 ± 3	†77 ± 2 $\eta^2 = 0.38$	52 ± 5	53 ± 3

These results showed that the BioCord system outperformed all treatment systems and showed significant reductions in comparison to the control for all testing periods except periods 1 and 3a (before pseudo-steady state was reached and after system shutdown). This would suggested a good proliferation of stable heterotrophic bacteria in the BioCord system biofilm, yielding an ability to reduce organic matter constituents even during periods of non-aeration. It would also indicate that the BioCord system had a buffering capacity that allowed for COD removal even during periods of extended anoxic conditions and a rapid recovery allowing temporaty system shutdown. Testing period 3b (12h on/12h off) showed the highest percent reductions for the biofilm technologies as indicated by overall magnitude of percent reductions during these weeks. However, during the one week air-off regime (period 2b), all of the treatment technologies showed significantly better percent reductions than the control. This was unexpected since airflow was not being delivered to the tanks during that week, leading to low DO concentrations (Figure 4-3) and minimal mixing. The percent reductions observed during this week could also be due to the precipitation levels during Week 14, which totalled 35.3mm, but this is speculative and strictly based on observation. High levels of precipitation may also have induced mixing/aertion of the treatment tanks. In addition, the higher average temperatures recorded during this week (23ºC) may have contributed to the higher treatment by the biofilm and zebra mussel systems as both of these types of organism exhibit optimal growth in this temperature range. Overall, the 12h on/12h off aeration cycle showed the best percent reduction in COD concentrations, after the initial start-up period and appropriate establishment of a stable, dense biofilm.

4.5.5 Total Suspended Solids

Total suspended solids (TSS) is a general water quality parameter. It is related to the clarity and turbidity of a wastewater and the amount of suspended particulates present, with lower levels of TSS indicating higher effluent qualities. High TSS concentrations in discharge effluents can reduce sunlight penetration in receiving bodies of water, reducing sunlight penetration for photosynthetic activity and disinfection, which in turn can also deplete DO concentrations and affect the health of aquatic organisms (Wetzel, 2001). TSS can also be associated with pollutants such as pathogens and other organic materials, can contaminate the receiving water bodies (Kemker, 2014). Because TSS concentrations represent a wide range of compounds and materials, many of which are associated with organic matter, TSS removal in biological wastewater treatment generally involves physical filtration and settling, as well as via degradation by microorganisms (Grady *et al.*, 1999; Kadlec and Wallace, 2009). The results for TSS concentrations and the percent reductions in each treatment technology are shown in Figure 4-10 and Table 4-7, respectively.

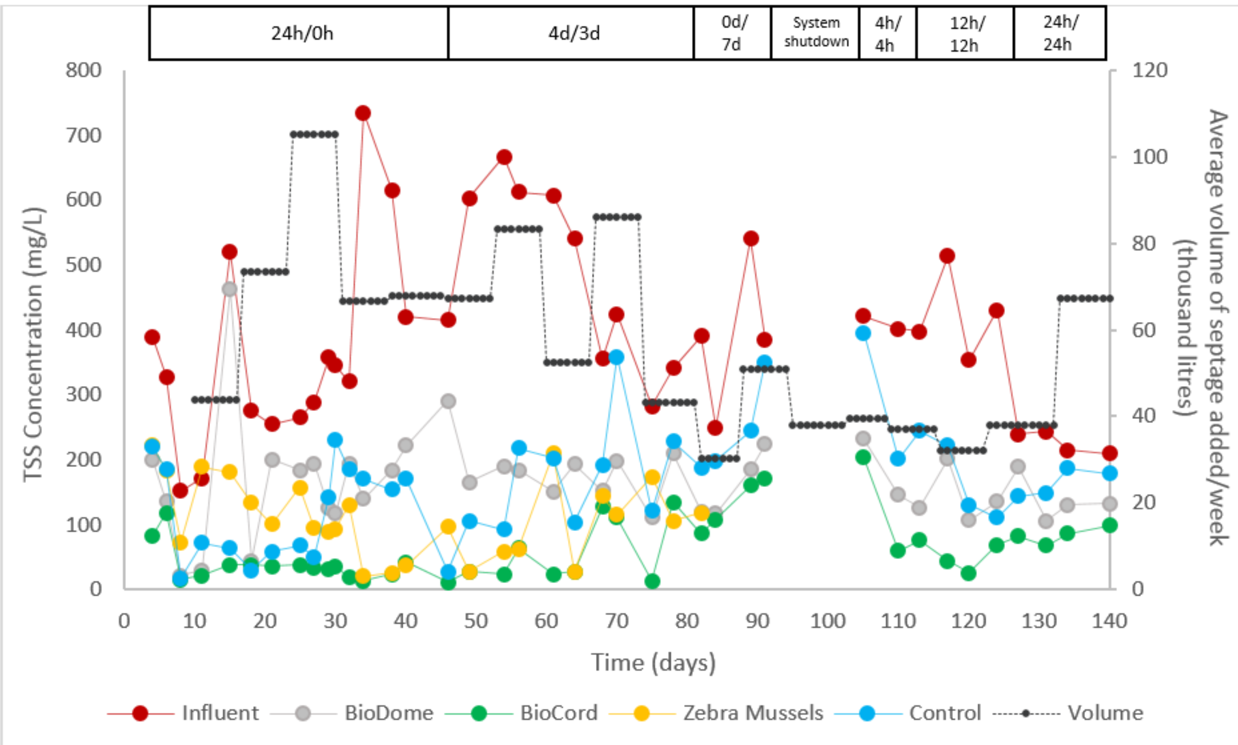


Figure 4-10. TSS concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data points.

Table 4-7. Average percent reductions (%) of TSS from the influent for each treatment tank.

† = indicates that average percent reductions were found to be significantly higher ($p \leq 0.05$) than the control for that time period. Statistics were performed using Kruskal-Wallis post-hoc analysis.

Testing Period	Timeframe	Aeration	Average temp (°C)	TSS reductions from influent (%)			
				BioDome	BioCord	Zebra Mussels	Control
1	Weeks 1-7 (days 1-47)	24h ON	16	51 ± 6 $\eta^2 = 0.12$	86 ± 3 $\eta^2 = 0.36$	53 ± 10	66 ± 5
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	63 ± 4	84 ± 4 $\eta^2 = 0.35$	81 ± 5 $\eta^2 = 0.31$	58 ± 7
2b	Week 14 (days 83-91)	24h OFF	23	54 ± 7	61 ± 5 $\eta^2 = 0.77$	60 ± 15	28 ± 14
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	59 ± 7	73 ± 11 $\eta^2 = 0.77$		32 ± 13
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	55 ± 12	85 ± 6 $\eta^2 = 0.58$		58 ± 7
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	44 ± 6	62 ± 6 $\eta^2 = 0.77$		22 ± 8
All	Weeks 1-20 (days 1-140)		18	55 ± 3	81 ± 2 $\eta^2 = 0.32$	64 ± 6	54 ± 4

The effluent TSS concentrations and percent reductions from each of the treatment systems indicated that the BioCord system exhibited the highest capacity to improve wastewater quality from the influent coming from the Storring Septic secondary pond. The BioCord system consistently produced low TSS concentrations in its effluent throughout the study, and showed significantly higher reductions in TSS in comparison to the control over all testing periods. This suggests that the biofilm composition of the BioCord system may be flexible and robust enough to adapt to extreme and/or prolonged fluctuations in environmental conditions, and corroborates the fact that it is able to improve the overall quality of influent wastewater.

4.6 Conclusions

The results of this study showed that the BioCord system was as the treatment technology with the most potential for full-scale testing and implementation at the Storrings Septic lagoon facility. Previous publications have shown that the BioCord system media is able to sustain a microbial community that is stable in composition and high in diversity (Yuan *et al*., 2012). The results of this study corroborate these findings as they showed that the BioCord system was able to produce significantly higher percent reductions of wastewater constituent concentrations in comparison to the control, for all parameters with the exception of orthophosphate. When strictly considering its ability to reduce contaminants from the influent, the BioCord system consistently produced significantly lower concentrations of all parameters, even during periods of high loading and influent levels. This would suggest that the proliferation of a diverse, stable biofilm was achieved in the BioCord system, with concentrations of bacteria much higher than that of a simplified suspended sludge reactor (i.e. the control). This also allows for the speculation that, in a full-scale study, the BioCord system would help to increase the efficiency of Storring Septic WSP operation by enabling their facility to effectively process higher volumes of wastewater. The BioCord systems offered significant reductions in total ammonia and total nitrogen suggesting a capacity for nitrification/denitrification and implying that there was a relatively heterogeneous population of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and denitrifiers. Resistance to shear/washout was observed by the high treatment efficiencies observed during periods of shorter retention times. The BioCord system performance during periods of extended anaerobic/anoxic conditions and after a three-week system shutdown suggested that the biofilm system was robust enough to withstand periods of variable redox conditions, and that its buffering capacity would likely be beneficial for a lagoon facility intending to increase its intake of septage. In addition, the drastic fluctuations in daily flow rate and wastewater quality indicated that the biofilm of the BioCord treatment technology was robust enough to handle fast-changing variances in both volume and influent compositions (wastewater strength).

The BioCord system also required less maintenance in comparison to its biofilm counterpart, the BioDome system. The BioCord system did not require maintenance or servicing at any time during the testing season, and did not show signs of shock or clogging of the aeration system. Signs of biofilm shock would include: decreased performance due to undesirable microorganism species dominating the media, anaerobic conditions despite appropriate aeration, the presence of septic odors and clogged media (Evans, 1985). On the other hand, the diffuser on the BioDome system had to be serviced periodically throughout the season, and was not as effective in supplying DO to the entirety of the reactor tank. This inability to reach the DO concentration and substrate mixing capacity observed in the BioCord system was likely a contributing factor to the lower performance of BioDome system in this study. It is also possible that the microorganisms responsible for wastewater treatment were better able to attach onto, and populate, the biofilm media of the BioCord system. Bolton *et al.* (2006) found that media surface properties, such as surface roughness and specific surface area, can strongly influence the accumulation and activity of a biofilm. Therefore, the inherent differences in the composition and surface of each of the attachment media could have contributed to how quickly and firmly microorganisms were able to attach onto the media and form a biofilm. Some of these differences may include the surface energy, hydrophobicity, surface charge and pore size of the media. The zebra mussels died after a two-week period without aeration, suggesting that a stock of zebra mussels and a backup aeration system would be required in the case of full-scale zebra mussel system implementation.

The aeration cycle that showed the most promising results was the 12h on/12h off regime. This cycle showed the highest percent reductions for all treatment systems in comparison to the other air cycling regimes, and can be implemented as a less energy-intensive option in comparison to air cycles that have been tested in the past (20h on/4h off, 24h on, etc.). This 12h on/12h off aeration cycle would be implemented after the biofilm has been allowed to develop, reach pseudo-steady state and acclimatize to the wastewater (i.e. after 2-4 weeks of constant 24h aeration).

The overall results—corroborated by Kruskal-Wallis statistical analyses—of the summer/fall testing period can be summarized as follows: the control tank, which emulated a simplified suspended sludge reactor, did not did not show significantly lower concentrations of any parameter in comparison to the influent, with the exception of orthophosphate. The control tank did not perform better than any treatment technology for the entire duration of the testing season (May 22nd – Oct 8th, 2015). The BioCord system was able to produce significantly higher percent reductions than the control for total ammonia, total nitrogen, COD and TSS. The BioCord system also produced significantly lower concentrations of all parameters than the influent concentrations and maintained the lowest levels of all parameters after a 3-week system shutdown. The BioCord system also showed the best percent reductions in all parameters after aeration was re-established after an extended anaerobic period. It was the most promising treatment system for full-scale testing and implementation in terms of performance and ease of scale-up. The BioDome system did not statistically outperform the control in any parameter, but consistently showed significant reductions in all parameters in comparison to influent. The BioDome system demonstrated the highest maintenance requirements. It was speculated that the performance of the BioDome system in this study was due to the inability for its diffusers to distribute air/oxygen adequately and induce effective substrate mixing, which would infer that a higher aeration flow rate would be needed to achieve reductions that were significantly better than the control. This increase in aeration would, in turn, increase the energy requirements of the BioDome system. The zebra mussel system did not outperform the control for any parameter, but showed significant reductions in all parameters in comparison to influent. This would indicate that the zebra mussel system had a capacity to remove constituents from the wastewater influent, but failed to perform significantly better than a control. Further investigations should be conducted, in a more highly controlled environment, to assess the cycling of wastewater contaminants in zebra mussels to corroborate their ability to assimilate nutrients and organics into their tissues. The zebra mussel system was also highly sensitive to system shutdown. The death of the zebra mussels in this study indicated that they had a low tolerance for highly variable redox conditions and had only a small capacity to buffer the system in the event of system shutdowns.

Taking into account energy requirements and the reduction efficiencies of all parameters, the 12/12h cycling approach should be implemented for full-scale testing following a 2-4 week start-up phase using constant (24h) aeration. Continued testing is also required to further optimize and provide a detailed study of the effects of aeration cycling on wastewater treatment and nutrient cycling.

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Chapter 5

Reductions of phosphorus, TSS, COD and total ammonia in diluted and synthetic wastewaters by the filtration and uptake by *Dreissena polymorpha* (zebra mussels)

5.1 Abstract

The ability for zebra mussels, *Dreissena polymorpha,* to reduce wastewater parameters was observed in a controlled laboratory setting. Reductions in chemical oxygen demand (COD), total ammonia (NH_3/NH_4^+), orthophosphate (ortho-P) and total suspended solids (TSS) were observed over a period of time to determine whether nutrient and pollutant uptake by zebra mussels could contribute to wastewater treatment. Reductions in wastewater contaminants by zebra mussels were observed for both synthetic and diluted wastewaters from the secondary effluent of a waste stabilization pond. In the diluted wastewater experiments, the zebra mussels showed an ability to reduce TSS and phosphorus concentrations, and a limited ability to reduce total ammonia and COD. The strength of the diluted wastewater affected their performance. In the synthetic wastewater experiments, zebra mussels showed a limited ability to reduce all parameters, with low confidence due to the high mortality rates of the organisms. The survival of zebra mussels was the found to be the limiting factor in the treatment efficiency in both the diluted and synthetic wastewater treatment testing.

5.2 Introduction

Zebra mussels (*Dreissena polymorpha*) are freshwater, filter-feeding organisms. Adult zebra mussels can grow to be up to 2 inches in length, and have striped outer shells (hence the layman term, “zebra mussels”) (Minnesota Department of Natural Resources , 2016). Figure 5.1 depicts a typical zebra mussel.

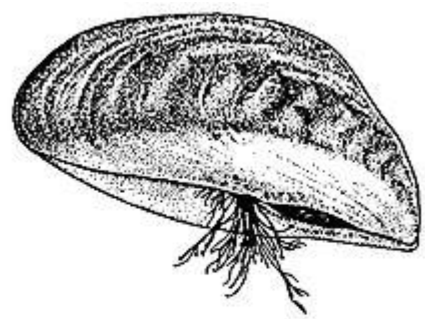

Figure 5-1. Left: an adult zebra mussel, underwater. When feeding, inhalant and/or exhalent siphons emerge from the shell and are visible to the human eye (Wolfe, 2015). Right: drawing of a zebra mussel showing the byssus for attachment to substrate (NOAA, 2016).

As zebra mussels mature, they produce strong byssal threads that allow them to attach onto a solid substrate—typically rocks or clams in a lake environment. These mussels have the ability to filter out particles from the water column with a reported filtering capacity of up to one liter of water per day per mussel (Benson *et al.*, 2015). They feed primarily on algae and phytoplankton, but can also filter a diverse range of suspended materials including bacteria, protozoans, zebra mussel veligers, other microzooplankton and silt. (Berg *et al.*, 1996) Although their ability to filter particulate matter is dependent on a number of variable factors—such as temperature, suspended solids concentration, phytoplankton abundance, and mussel size/maturity— some studies have suggested that zebra mussels have the potential to improve effluent quality in wastewater treatment (Noordhuis *et al.*, 1992; Binelli *et al.*, 2006; Bruner *et al.*, 1994; Goedkoop *et al.*, 2011).

Graczyk, et al. (2004) showed that zebra mussels were able to efficiently accumulate human waterborne pathogens from polluted river water, indicating that the abundance of parasites in zebra mussel tissues can be used as an indicator of poor water quality. Zebra mussels have been shown to reduce levels of TSS, uptake pharmaceuticals (and other drugs), and significantly increase water clarity in fresh water bodies (Binelli *et al.*, 2006; Vanderploeg, *et al.*, 2001). Because zebra mussels have the ability to accumulate environmental contaminants in their tissues, they have been used in the biomonitoring of organic pollutants which would suggest that they are potentially able to reduce BOD/COD in wastewaters

(Binelli *et al.*, 2007). This can be further supported by their reported ability to utilize dissolved organic carbon (DOC) as an alternative food source to phytoplankton biomass (Roditi *et al.*, 2000).

Nutrient (phosphorus and nitrogen/ammonia) uptake and assimilation by zebra mussels could also have a variety of implications for wastewater treatment and prevention of eutrophication in lakes and rivers. These nutrients are often targeted for removal in wastewater treatment facilities (tertiary treatment), as discharge guidelines have become more stringent in terms of effluent water quality concentrations (UNEP, 2015). In addition, conventional microbiological mechanisms may not be sufficient in mitigating wastewaters with especially high concentrations of phosphorus and total nitrogen (e.g. wastewaters that have been contaminated with fertilizer runoff). However, the use of zebra mussels to reduce wastewater effluent quality parameters could be a challenge given the invasive nature of zebra mussels in the Great Lakes and freshwater bodies spanning North America. Previous studies have indicated the potential for zebra mussels to reduce nutrient concentrations and affect the nitrogen and phosphorus budgets of aquatic ecosystems through nutrient retention. Reeders and Bij de Vaate (1990) showed that zebra mussels could reduce phosphorus levels through biodeposition in lake sediments as faeces and pseudofaeces. Goedkoop et al. (2011) showed that, in lake environments, these organisms could also retain nitrogen and phosphorus in their tissues and relatively low concentrations in their shells. A study by Fahnesnstiel et al. (1995) reported an overall decrease of 43% in total phosphorus levels in Saginaw Bay after zebra mussel colonization. However, a number of studies have also indicated that, in freshwater environments, zebra mussels can excrete nitrogen and phosphorus via nutrient cycling (Arnott and Vanni, 1996; James *et al.*, 1997). Despite evidence suggesting the potential for zebra mussel to reduce nutrients in aquatic environments, extensive research examining the ability of zebra mussels to uptake phosphorus and nitrogen from wastewater in a controlled laboratory setting[2] as yet to be reported, and studies investigating the effects of using zebra mussels as a part of a treatment unit on nutrient levels have

[2] To this author's knowledge, based on extensive research into the literature on *Dreissena polymorpha*

presented conflicting results. In contrast to the studies involving the role of zebra mussels in reducing nutrient levels in lakes and rivers, some studies have shown that zebra mussels can actually release of high levels of phosphorus (Bykova *et al*., 2006), and others showed no change in levels of total dissolved nitrogen in lakes between the pre- and post- periods of zebra mussel invasions (Higgins and Vander Zanden, 2010).

The goal of the zebra mussel study was to investigate the ability of *Dreissena polymorpha* to reduce TSS, COD, ortho-P and total ammonia concentrations from septic and synthetic wastewaters, with significant decreases in parameters indicating a potential for improving effluent quality.

5.3 Experimental design and methods

The experiments were conducted in the laboratories of the Department of Civil Engineering at Queen's University. Three separate trials were conducted successively in order to test the ability of zebra mussels to reduce selected wastewater parameters (COD, TSS, NH_3/NH_4^+ and ortho-P) for varying wastewater strengths. The first trial was conducted using a 1:9 dilution of secondary septic wastewater taken from a wastewater stabilization pond facility in Tamworth, Ontario (Storring Septic). The second trial was conducted using secondary septic wastewater taken from the same wastewater stabilization pond, but in a 1:1 dilution instead of a 1:9 dilution (higher strength). Figure 5-2 shows the layout of the Storring Septic facility and indicates where in the process flow the wastewater was collected for these first two experiments.

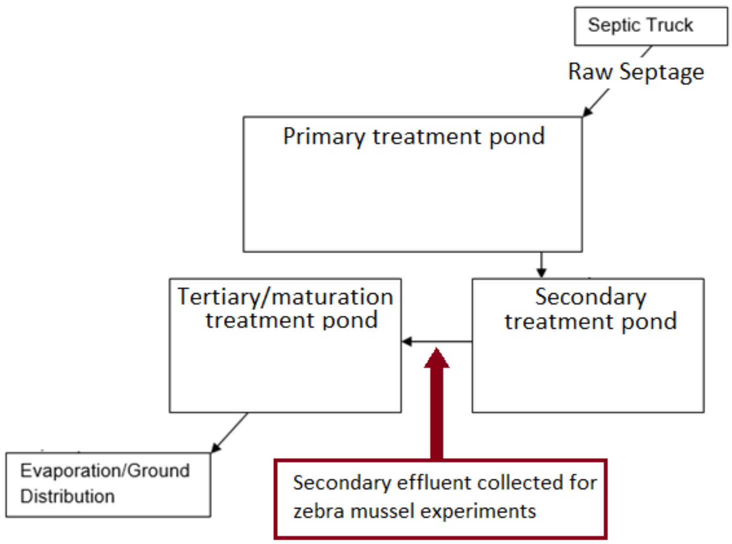

Figure 5-2. Schematic of the Storring Septic waste stabilization pond facility located in Tamworth, Ontario. For trials 1 and 2 of zebra mussel experiments, effluent from the secondary treatment pond was collected (as indicated by the red arrow) and diluted.

In the third trial, the ability of zebra mussels to uptake COD, orthophosphate and total ammonia from a low-strength synthetic wastewater was investigated. Low strength, glucose-based synthetic wastewater was employed and aimed to represent typical values of tertiary domestic septic wastewater. The characteristics of the prepared wastewater were modified from that of a previous study, and was developed to represent a pre-settled domestic wastewater (Chang and Lee, 1998). The synthetic wastewater used during Trial 3 did not contain particulate matters and, hence, TSS reductions were not expected or monitored during this experiment. The characteristics of the synthetic wastewater are presented in Table 5-1.

Table 5-1. Composition of synthetic wastewater used in Trial 3.

Composition	Concentration, mg/L
Glucose	50
Peptone	20
Yeast Extract	5
NH_4Cl	25
KH_2PO_4	10
$MgSO_4^- \bullet 7H_2O$	10
$MnSO_4 \bullet 4H_2O$	1
$FeCl_3 \bullet 6H_2O$	0.02
$CaCl_2 \bullet 2H_2O$	10
$NaHCO_3$	5

A schematic of the experimental design is shown in Figure 5-2.

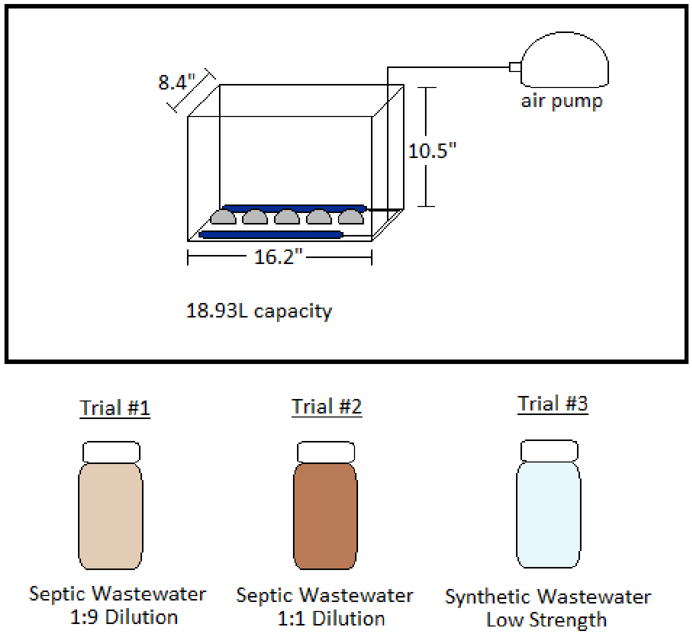

Figure 5-3. Experimental design of the zebra mussel experiments.

Zebra mussels were collected from Beaver Lake as per the protocol outline in Chapter 4.4. Approximately 50 zebra mussels were harvested and brought back to the laboratory at Queen's University and stored in an aerated holding tank until the completion of the study. For each trial, five previously untested adult zebra mussels of approximately equal size (roughly 1.5cm lengthwise) were taken from the holding tank and placed at the bottom of a ~19L aerated aquarium. The number of zebra mussels per trial was chosen based on their suspended solids filtration rates of approximately 1L per day per mussel. If the zebra mussels were able to uptake some wastewater parameters at the same rate as they are able to uptake particulate matter, the gradual decrease in wastewater parameters from the aquarium tank would be difficult to monitor if too many zebra mussels were added to the tank at once. The aquarium was then filled with either (diluted) septic or synthetic wastewaters.

Zebra mussels in both the testing aquarium and the holding tank were fed daily using KENT Marine Phytoplex® (unicellular phytoplankton feed for filter-feeding invertebrates) to provide a nutrient-rich food source and to ensure viability, as well as to induce filtration by the mussels. All zebra mussels were target-fed daily using a feeding dropper (KENT Marine Nautilus Sea Squirt) to dispense the food directly over the mussel siphons (approximately 1 tsp per five mussels).

Levels of TSS, COD, ammonia/ammonium (total ammonia) and phosphorus (orthophosphate) were monitored from samples taken directly from the aquarium approximately 3 times per week. These parameters were selected as they represent the typical targets for reduction in wastewater treatment facilities (CFA, 2015). Dissolved oxygen (DO) and pH levels of the tank were also measured to ensure that they were within an adequate range for zebra mussel viability. Water quality parameters were measured in duplicate for each sampling day using the methods outlined in Table 5-2.

Table 5-2. Methods of measuring water quality parameters.

Wastewater parameter	Method
TSS	Filtration and drying; standard methods (Greenberg et al., 1985)
COD	Calorimetric; standard methods ($K_2Cr_2O_7$ digestion) (Greenberg et al., 1985)
Ammonia/ammonium ion	mV potential; accumet® electrodes (Fisher Scientific)
Reactive phosphorus (orthophosphate)	Calorimetric; Orion Aquafast Phosphate LR
Dissolved oxygen	DO probe (field meter)
pH	Fisher Scientific accumet® pH electrode

More detailed experimental procedures can be found in the “Methods” section of Chapter 4 (section 4.4).

5.4 Results and discussion

Three different trials were conducted to test the ability for zebra mussels to reduce total ammonia, orthophosphate, TSS and COD from wastewater. These parameters were chosen because they are important parameters in wastewater treatment and are all regulated in Canada by the wastewater treatment effluent discharge guidelines put in place by the Ministry of Environments and Climate Change (MOECC) (Environment Canada, 2015). Each trial was initiated with the addition of zebra mussels to either a low-strength diluted wastewater or low-strength synthetic wastewater, and continued until the death of all zebra mussels being tested. As such, the duration of each trial varied from 5 to 25 days. Table 5-3 outlines the timeline for each of the trial and incudes initial concentrations for the constituents of interest.

Table 5-3. Starting concentrations (mg/L) of tested wastewater parameters for Trials 1, 2 and 3.

Trial	Dates	Time (days)	Total ammonia (mg/L)	Orthophosphate (mg/L)	TSS (mg/L)	COD (mg/L)
1	July 6 – 31, 2015	26	18.89	1.25	9.034	2.3
2	Aug 3 – 23, 2015	20	47.61	3.43	22.65	14
3	Sept 7 – 11, 2015	5	25	10	N/A	50

On Day 1 of Trial 1, the zebra mussels were placed in an aquarium containing a 1:9 ratio of septic wastewater:deionized water and observed for 26 days until the death of all zebra mussels. On Day 24, three out of the five zebra mussels died, with the remaining two surviving until Day 26. Changes in the wastewater constituents over the 26-day testing period are illustrated in Figure 5-4. Fluctuations in dissolved oxygen and pH levels in the aquarium over this testing period were also monitored. These results are shown in Figure 5-5.

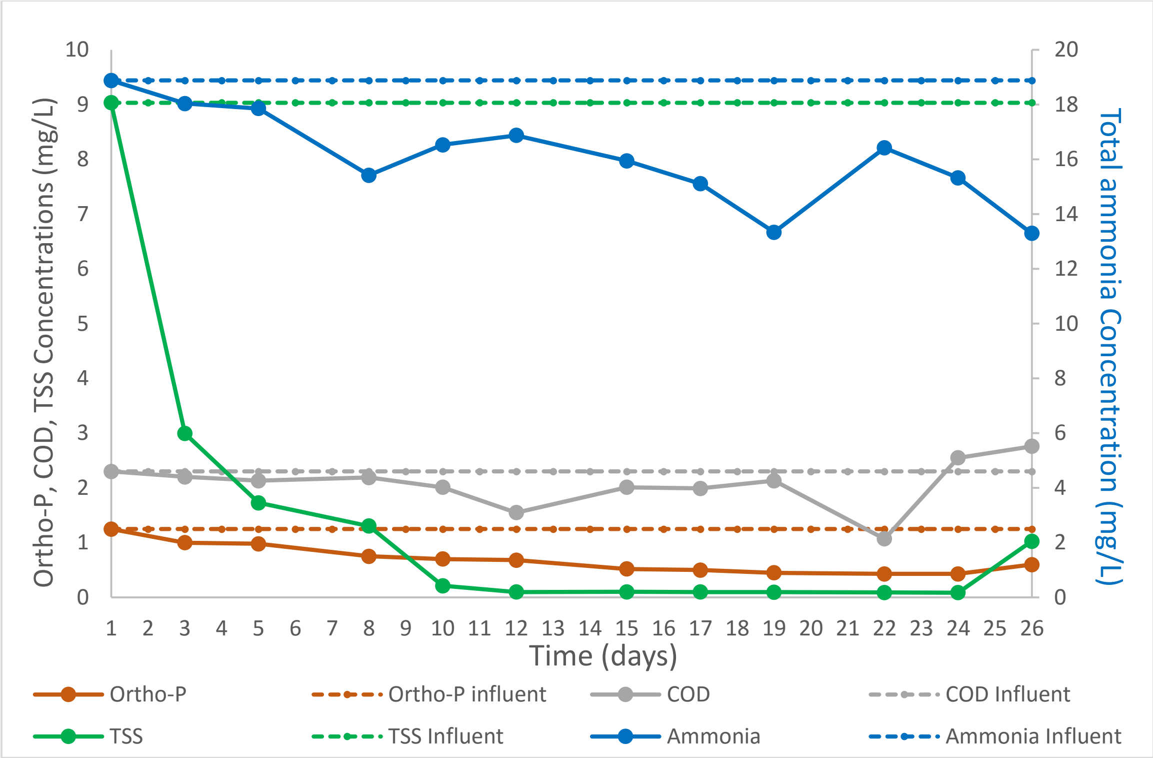

Figure 5-4. Wastewater parameter reductions by *Dreissena polymorpha*; diluted (1:9) secondary septic wastewater (Trial 1). Reductions in orthophosphate, COD, TSS and total ammonia by zebra mussels over a 26-day period. Zebra mussel deaths noted at Day 24 (3 mussels) and 26 (all).

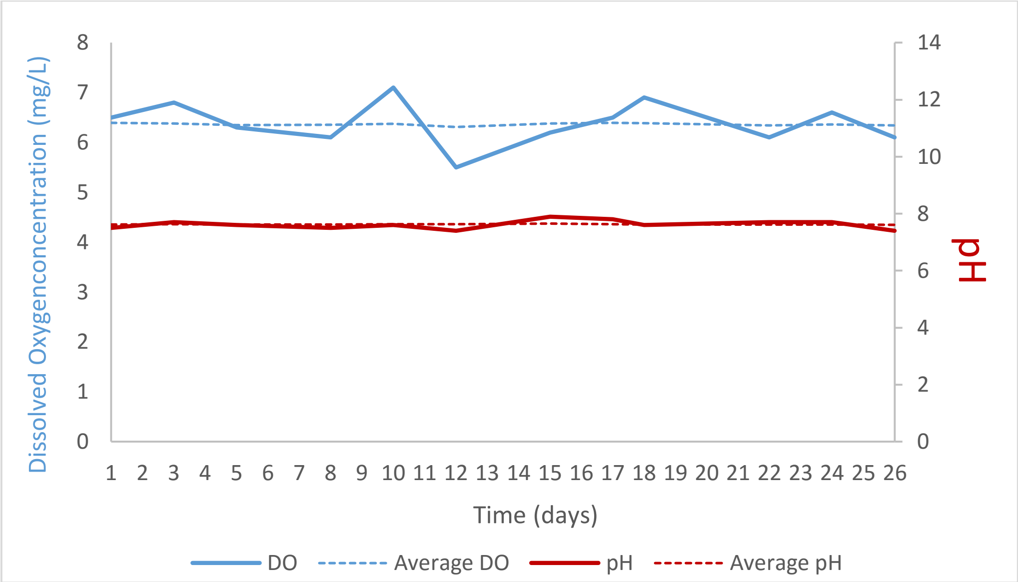

Figure 5-5. Dissolved oxygen concentration and pH Levels; diluted (1:9) secondary septic wastewater (Trial 1). Fluctuations in DO concentration and pH levels over a 26-day period. Zebra mussel deaths noted at Day 24 (3 mussels) and 26 (all).

Dissolved oxygen (DO) and pH levels remained relatively constant throughout the testing of Trial 1, which would suggest that changes in wastewater parameters were not directly affected by fluctuations in these parameters. DO concentration were measured to fluctuate between 5.5 and 6.8mg/L, while the pH ranged from 7.4 to 7.9. This is consistent with other studies where adult zebra mussels have been reported to need only about 25% oxygen saturation in order to ensure survival (i.e. 2-3mg/L DO at 10^{o}C to 25^{o}C), and an optimum pH range of 7.3 to 9.3. (Karatayev *et al.*, 1998; Alexander and Thorp, 1997; Claudi *et al.*, 2012). As such, the DO concentrations and pH levels were considered to be within the optimal ranges required for zebra mussel viability in typical freshwater environments.

Figure 5-3 shows that zebra mussels were moderately able to reduce the wastewater constituents of interest. However, the death of 3/5 zebra mussels during Day 24 and 5/5 mussels during day 26—despite the fact that DO concentration and pH level were within the optimal range for zebra mussel viability—would indicated that other factors affected their ability to survive under extended laboratory wastewater treatment conditions. Because the growth, morphology, and colonization behaviours of zebra mussels are influenced by a number of external environmental factors such as wind currents, calcium availability, byssal thread formation, salinity and temperature (Riessen *et al.*, 1993; Eckroat *et al.*, 1993), it is possible that changes in any of these variables would affect the ability of zebra mussels to maintain livelihood when submerged in a wastewater environment. It is possible that there are other, unknown constituents, specific to the wastewater obtained from Storring Septic that contributed to the mortality of zebra mussels after 26 days, despite their average lifespan being approximately 3-5 years (Ludyanskiy *et al.*, 1993; Chase and Bailey, 1999).

All wastewater constituents, with the exception of total ammonia, appeared to increase after the death of 3/5 of the zebra mussels on Day 24. This would suggest that the zebra mussels had the capacity to uptake these constituents, which may have been released from zebra mussel tissue upon death.

Overall, the largest percent reductions seen during this trial (1:9 dilute secondary wastewater) occurred on Day 22, the last sampling date before the first zebra mussel deaths were observed. Table 5-4 summarizes the percent reductions seen in each of wastewater parameters during this time.

Table 5-4. Percent reductions of each wastewater parameter tested during Day 22 of Trial 1 (1:9 dilute secondary wastewater). Overall, the highest percent reductions were seen on Day 22.

	Percent reduction
Total ammonia	13%
Orthophosphate	66%
COD	53%
TSS	99%
Average	58%

These results indicated that in the presence of 1:9 diluted wastewater, the zebra mussels were able to make considerable reductions in TSS and orthophosphate and moderate reductions in COD and total ammonia over a 3-week period.

Trial 2 was initiated at the end of Trial 1, to determine whether zebra mussels could exhibit similar constituent reductions and viability in a higher strength wastewater (1:1 dilution of wastewater:deionized water), which would be more representative of a typical septage influent. Trial 2 was completed after a period of 10 days, at which time each of the five adult zebra mussels had died. Changes in wastewater constituents during the course of Trial 2 are illustrated in Figure 5-6. The results for DO and pH are shown in Figure 5-7 and were found to be consistent with those observed during Trial 1, where levels remained fairly constant and within the range optimal for zebra mussel viability, even at the time of zebra mussel death (Karatayev *et al.*, 1998; Alexander and Thorp, 1997; Claudi *et al.*, 2012).

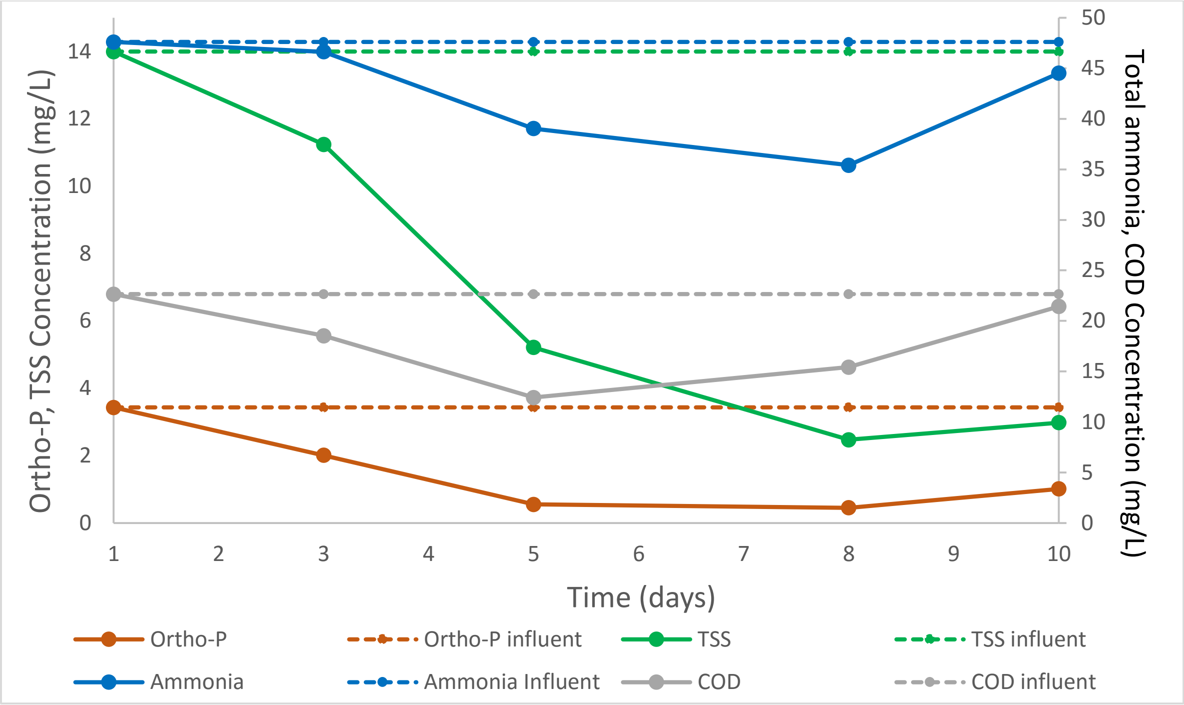

Figure 5-6. Water parameter reductions by *Dreissena polymorpha*; diluted (1:1) secondary septic wastewater (Trial 2). Reductions of total ammonia, orthophosphate, TSS and COD by zebra mussels. The first day of testing, Day 1, corresponds to August 3rd, 2015. Testing lasted until August 12, 2015 (Day 10). Total zebra mussel death was observed during Day 10. Total ammonia and COD concentrations are shown on a secondary axis due to concentration discrepancies.

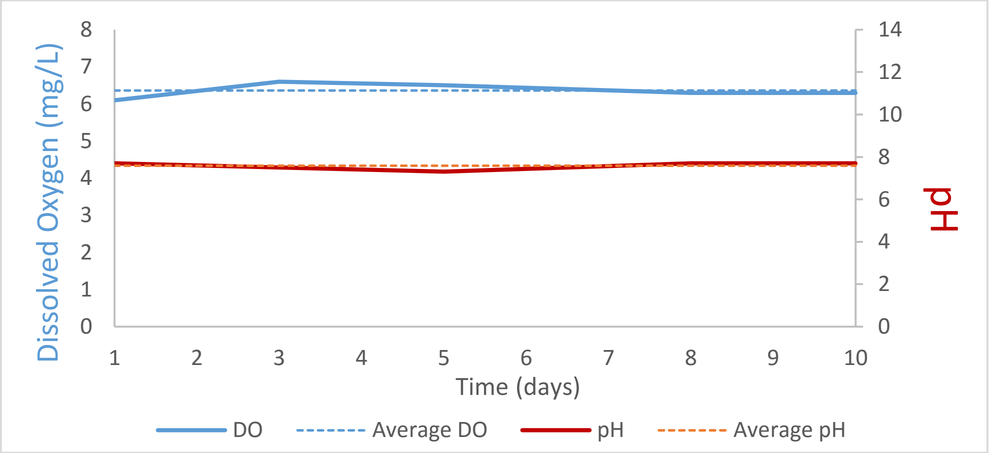

Figure 5-7. Dissolved oxygen concentration and pH Levels; diluted (1:1) secondary septic wastewater (Trial 2). Fluctuations in DO concentration and pH levels over a 10-day period. Total zebra mussel death was observed during Day 10.

From Figure 5-6 it can be seen that the zebra mussels could reduce wastewater constituents from a 1:1 dilution of secondary wastewater. However, the death of all zebra mussels occurred at a faster rate (10 days) than during Trial 1, indicating that wastewater strength likely had an influence on zebra mussel survivability.

Overall, the largest percent reductions seen during this trial (1:1 dilute secondary wastewater) occurred on Day 8, the last sampling date before zebra mussel mortality was observed. Table 5-5 summarizes the percent reductions seen in each of wastewater parameters during this time.

Table 5-5. Percent reductions of each wastewater parameter tested during Day 8 of Trial 3 (1:1 diluted secondary wastewater). Overall, the highest percent reductions were seen on Day 8.

	Percent reduction
Total ammonia	26%
Orthophosphate	87%
COD	32%
TSS	82%
Average	57%

These results would suggest that the zebra mussels had some capacity to reduce wastewater parameters. Aside from TSS, which has been shown to accumulate in zebra mussels, orthophosphate concentrations rapidly decreased in the first 5 days of testing. After this, concentrations of orthophosphate did not significantly change. Following zebra mussel death (Day 10) increases in each of the constituents of interest were noted, which might suggest a release of constituents following zebra mussel death. These findings are consistent with literature which confirms that zebra mussels can release contaminants that have been previously bioaccumulated in their tissues (Arnott and Vanni, 1996; Bykova *et al.*, 2006).

Trial 3 was conducted largely to test the viability of zebra mussels in a synthetic wastewater and to monitor constituent reductions. It was hypothesized that the previously observed zebra mussel mortality may have been due to some unmeasured wastewater constituents present in the wastewater taken from the Storring Septic facility. As such, Trial 3 was conducted to determine whether the zebra mussels would exhibit a better viability in a synthetic wastewater, and to further observe wastewater parameter reductions by zebra mussels under these conditions.

A first run of Trial 3 was attempted on August 24th, 2015. However, the results are not presented due to zebra mussel death after the first day of testing. Aeration was not supplied to the experimental tank for a 24-hour period due to a laboratory power outage, leading to a decrease in aquarium DO levels to about 2mg/L. All five zebra mussels subsequently died. This may suggest a higher sensitivity to environmental changes in synthetic wastewater, as zebra mussels are often found in hypolimnetic zones of freshwater lakes and have been found to survive at oxygen concentrations as low as 0.1mg/L (Benson, et al., 2015). As well, 15 of the 35 remaining zebra mussels in the holding tank (containing a mixture of lake and tap water) survived the aeration shutdown, suggesting that zebra mussels in freshwater environments may be more resilient to low DO conditions. Figure 5-8 shows the wastewater parameter concentrations throughout Trial 3, which lasted five days before the death of all zebra mussels was observed.

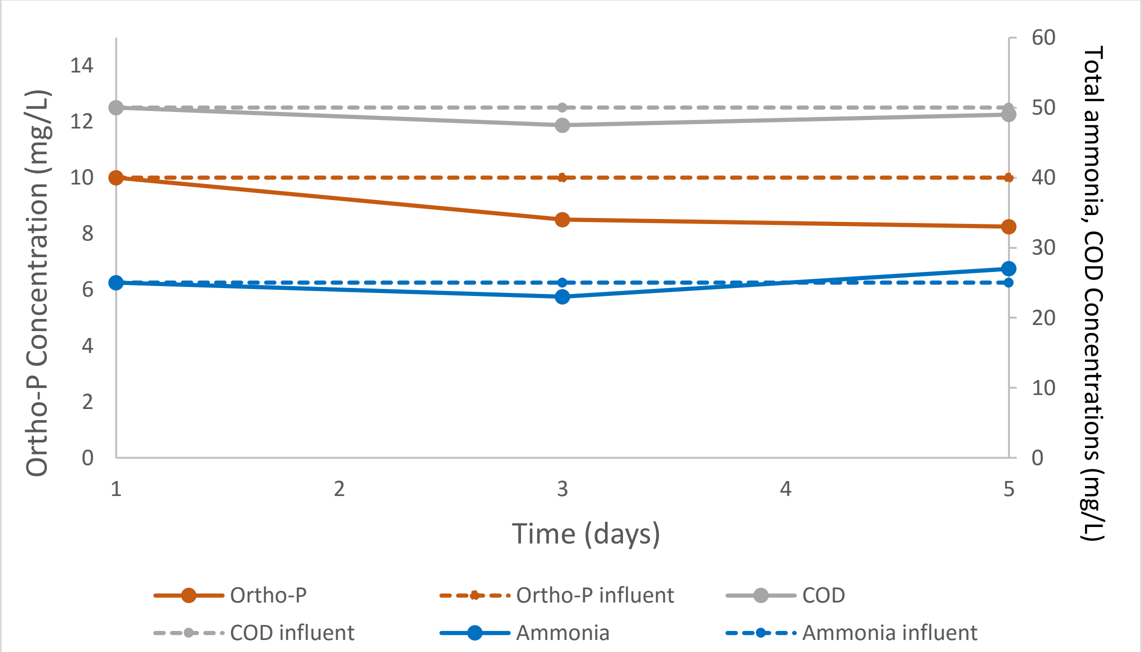

Figure 5-8. Water parameter reductions by *Dreissena polymorpha*; synthetic wastewater (Trial 3). Reductions of total ammonia, orthophosphate, and COD by zebra mussels. The first day of testing, Day 1, corresponds to September 7th, 2015. Testing lasted until September 11, 2015 (Day 5). Total zebra mussel death was observed during Day 5. Total ammonia and COD concentrations are shown on a secondary axis due to concentration discrepancies.

DO and pH levels also were monitored to ensure that these ranges were kept within an appropriate range for zebra mussel viability. These are shown in Figure 5-9.

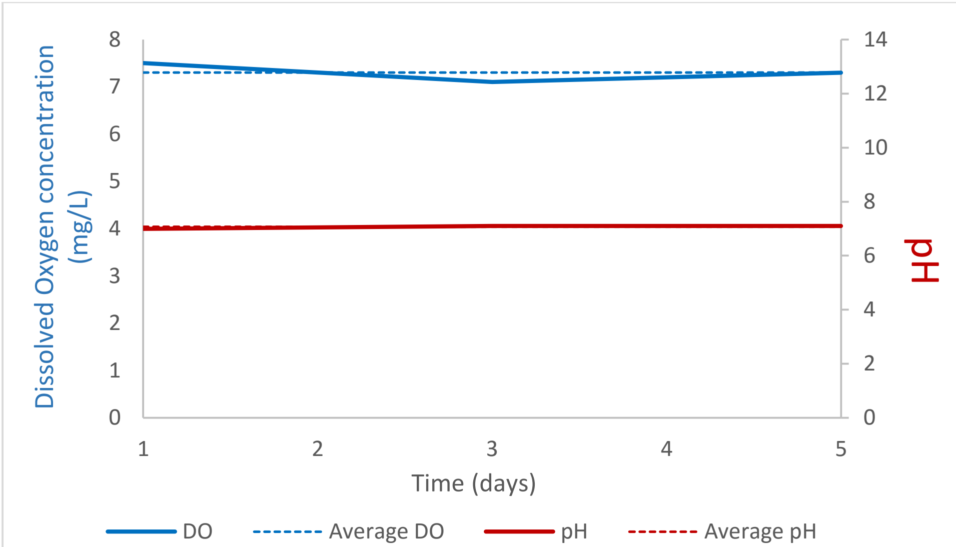

Figure 5-9. Dissolved oxygen concentration and pH Levels; synthetic wastewater (Trial 3). Fluctuations in DO concentration and pH levels over a 5-day period. Total zebra mussel death was observed during Day 5.

Trial 3 lasted for 5 days before the death of all 5 of the zebra mussels was observed. Overall, the largest percent reductions seen during this trial (synthetic wastewater) occurred on day 3, the last sampling date before complete zebra mussel mortality was observed. Table 5-6 summarizes the percent reductions seen in each of wastewater parameters during this time.

Table 5-6. Percent reductions of each wastewater parameter tested during Day 3. Overall, the highest percent reductions were seen on Day 3.

	Percent reduction
Total ammonia	8%
Orthophosphate	15%
COD	5%
Average	9%

The early mortality of the zebra mussels in synthetic wastewater provided would suggest that zebra mussels may require a natural environment to thrive for long periods of time. The pH and DO ranges were not out of a typical range for zebra mussel proliferation. However, although not conclusive due to the short experimental period, the results of Trial 3 showed small reductions in total ammonia, phosphorus and COD.

5.5 Conclusions and recommendations

The results of these experiments show that, in a laboratory setting, the presence of zebra mussels in diluted or synthetic wastewaters can cause reductions in certain wastewater parameters, but their capacity for treatment is limited by their sensitivity to external stressors. Lack of oxygen and a higher wastewater strength are two variables that may contribute to zebra mussel death in a laboratory setting. It was hypothesized that the robustness and invasive nature of zebra mussels are not observed in laboratory settings involving wastewater because these organisms may require characteristics of natural aquatic ecosystems that are not easily reproducible in a controlled setting. The distribution, morphology, growth and colonization of zebra mussels are all influenced by a number of different environmental factors; in the absence of these variables it may be the case that zebra mussels have a more difficult time thriving in harsh environments (i.e. in wastewater).

The highest percent reductions were seen in TSS and orthophosphate, and the results show a promising ability for zebra mussels to uptake phosphorus from wastewater into their tissues. In order to corroborate these findings, further, more complex experimentation or studies should be conducted involving phosphorus uptake by zebra mussels and subsequent analysis of tissue composition.

The results of this study also showed a small to moderate capacity of zebra mussels to reduce COD and total ammonia. Again, this potential is limited by the ability to prolong the life of the zebra mussels. It may be beneficial to perform or conduct studies involving the ability of zebra mussels to uptake one specific wastewater parameter at a time. As well, it would be useful to measure levels of all nitrogen species during analysis of parameter uptake.

In terms of potential for wastewater treatment, provided that methodology is developed that ensures the survival of the zebra mussels, these organisms are a considerable prospective for treating wastewater, particularly if target removals include phosphorus. However, there is risk to such implementation, as wastewater parameters—particularly nitrogen and phosphorus—have the potential to be released from zebra mussel tissue upon their death. Further studies should be conducted in order to determine the extent of contaminant release after death in order to determine the potential risk of utilizing zebra mussels for wastewater treatment. If preventing significant zebra mussel death is a viable option, zebra mussels show potential to act as front-end wastewater treatment for high reductions of TSS and phosphorus.

5.6 References

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Chapter 6

Potential Design Implications for Industrial Applications

The purpose of this thesis project was to carry out both pilot-scale and laboratory-scale investigations with the intent of identifying the means by which biofilm technologies and zebra mussels could be applied to significantly improve the effluent quality of wastewater stabilization ponds (WSPs) system treating septage in facility operated under temperate climate conditions in North America. From the experimental studies and the analysis of the results a number of conclusions can be drawn that could be of use to other WSP operators considering expansion of their facilities. Specifically, this project provided useful information for Storring Septic Service Limited, such that they may further consider expansion of their WSP system and potentially conduct full-scale testing and implementation of one of the semi-passive technologies investigated. This expansion could allow them to reach a higher quality effluent and enable them to safely accept excess wastewater from third-party sewage haulers. As well, the results obtained from these studies provided valuable information that may form the basis of future studies exploring the potential for these biofilm technologies and zebra mussels to successfully treat wastewater in eco-engineered systems.

6.1 Full-scale study and implementation at Storring Septic

At present, the facility at Storring Septic operates three WSPs, with a fourth pond currently being dredged and prepared for future use. Figure 6-1 illustrates the spatial arrangement and dimensions of the four ponds, and allows for better visualization of prospective full-scale applications.

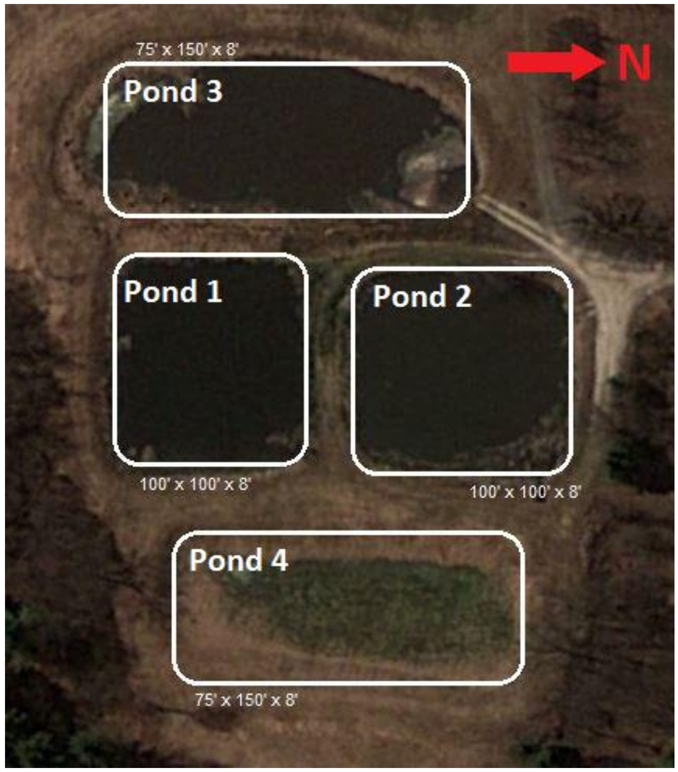

Figure 6-1. Aerial-view of the Storring Septic facility, including Pond 4 (not commissioned during study).

From the results of the pilot-scale investigations presented in Chapters 3 and 4, it was concluded that the BioCord system (by Bishop Concord) showed the highest potential for improving WSP treatment efficiency at the Storring Septic facility on a full-scale level. In pilot-scale testing, the BioCord system was able to successfully, and on a statistically significant level, outperform the control (i.e. aeration alone) when treating wastewater effluent from Pond 2. As such, it was surmised that the implementation of the submerged BioCord system in a pond receiving wastewater quality influent similar to the concentrations observed during pilot-scale testing could be beneficial. The average influent water quality parameters entering the reactor tanks for both testing seasons, as well as each of the parameters tested, are shown in Table 6-1. However, the effectiveness of the BioCord system in reliably and consistently improving effluent wastewater quality in extremely high- or low-strength wastewaters remains to be demonstrated.

Table 6-1. Average influent concentrations, including the standard deviations (**±)**, for two testing seasons, entering the reactor tanks. These values represent the average strength of wastewater typically being treated by the BioCord system.

Parameter	Mean concentration (mg/L)
Ammonia/ammonium	197 ± 10
Nitrite	2 ± 1
Nitrate	61 ± 12
Total Nitrogen (TN)	259 ± 16
Orthophosphate	11 ± 1
Chemical oxygen demand (COD)	228 ± 33
Total suspended solids (TSS)	1015 ± 525

As can be seen, the average concentrations of wastewater quality parameters treated by the BioCord system would be considered characteristic of medium- to high-strength wastewaters (Pescod, 1992). As such, it would be suggested that the BioCord system be utilized as a front-end treatment at Storring Septic—or other WSP facilities that have multiple ponds systems —to ensure that 1) the BioCord system is treating similar-strength wastewater in full-scale testing as in pilot-scale testing, and 2) there is at least one maturation pond available downstream for final effluent polishing and disinfection, and 3) to provide a buffer for upstream ponds in the case of shock loadings. When considering prospective full-scale implementation, other important considerations include: the final cost/size of the BioCord system required (i.e. more material may need to be used if implementation was integrated in Pond 3 or 4), limitations due to energy and aeration requirements, ease of the process flow, and operational alternatives in the case of shock loading to the pond. Given these considerations, some possible options for scale-up at the Storring Septic site have been postulated for full- scale testing. A schematic diagram illustrating the current pond process is shown in Figure 6-2, and the new possibilities for scale-up using the BioCord system are shown in Figures 6-3a to 6-3d.

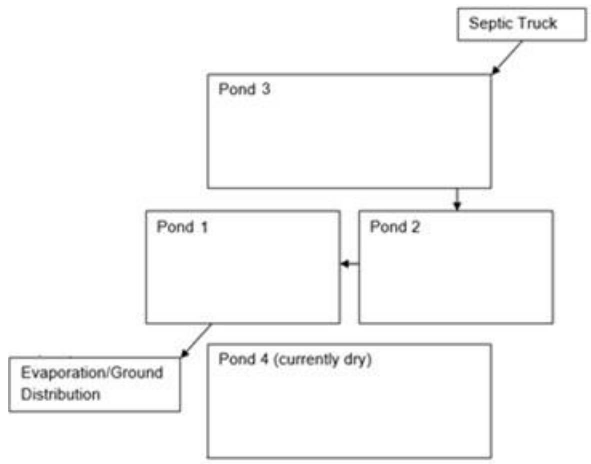

Figure 6-2. Diagram of the typical process flow currently in use. Arrows indicate direction of the inflow/outflow of wastewater.

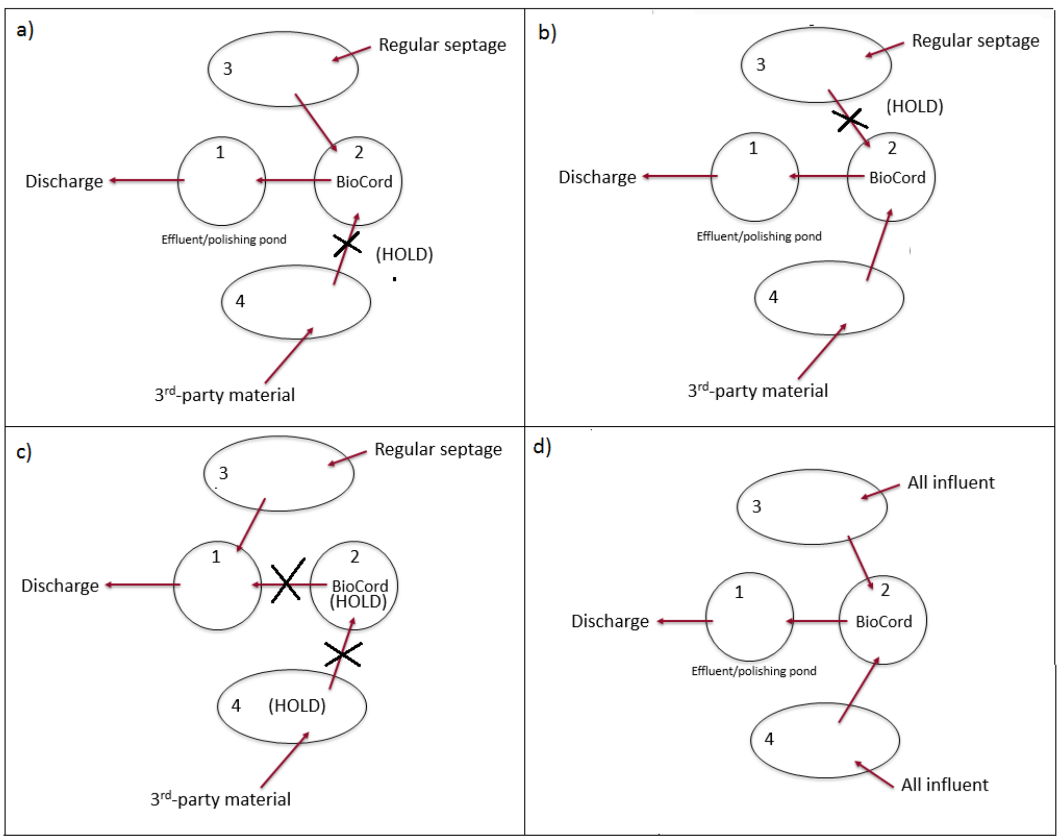

Figure 6-3. Split-pond operation, with the BioCord system implemented on a full-scale level in Pond 2. Arrows indicate the direction of wastewater flow, and overlaid crosses represent a halt in flow. a) Regular septage is dumped and treated as per the typical process flow, with materials from 3rd party sewage haulers being held in Pond 4. b) Regular septage is being held in Pond 3, with wastewater from Pond 4 (containing primary-treated materials from 3rd party sewage haulers) continuing in the process flow for further treatment. c) In the case of pond shock, flow of regular septage is diverted directly into Pond 1 (tertiary treatment pond), while flow from Ponds 4 and 2 are halted to allow for recovery. d) If the BioCord system is able to safely process all 3rd-party materials, all incoming wastewater can be dumped into either pond.

Figure 6-3 illustrates various potential split-pond operational scenarios for the treatment of septage, with full-scale BioCord modules implemented in Pond 2. In the proposed operational configuration for full-scale testing and implementation, primary treatment and the settling of solids would be allowed to occur prior to entering the pond containing the BioCord system. This configuration will prevent large particulates and solids from clogging the BioCord media prematurely, as well as allow for the breakdown and/or removal of some organic matter and suspended solids. As well, in comparison to implementation in Pond 3 or 4, fewer BioCord modular units would be required per unit volume of wastewater, as the wastewater being treated would be lower strength.

In the first stages of this scale-up scenario (Figures 6-3a and 6-3b), "regular septage"[3] and septage being received from 3rd-party sewage haulers (or any "new", uncharacterized wastewater) would be separated into two separate primary stabilization ponds. Regular septage would be conveyed to Pond 3, while 3rd-party septage would be conveyed to Pond 4. Either Pond 3 or Pond 4 (but not both) would be part of the regular process flow at any given time. In the event that Pond 3 was part of the process flow (6-3a), Pond 4 would act as a holding/processing pond for the 3rd party materials until sufficient wastewater from Pond 3 would have been processed. In the case where Pond 4 was part of the regular process flow (6-3b), Pond 3 would act as the holding/processing flow. This operational design would allow for uninterrupted flow into Pond 2 (such that the BioCord pond is always being utilized), while ensuring that any less controlled materials or substances present in Pond 4 were isolated, and in the case of shock loading of the pond shock, they could be retained separately from the regular septage being processed. This would also allow for Storring Septic to more accurately estimate the composition of the 3rd party influent and readily determine whether the composition of the new influent was the source of shock loading or microbial death in the pond. Assuming differences in both the volume and composition of the regular and 3rd-party

[3] In this chapter, "regular septage" is defined as the wastewater collected in the area typically serviced by Storring Septic, or any waste/septage regularly collected and treated by Storring Septic

wastewater entering the Storring Septic facility, this design would also allow for retention times and aeration cycles to be specifically tailored to these, resulting in better and faster treatment overall.

In the case of shock loadings or pond shock, flow could be diverted from either Pond 3 or 4 directly into Pond 2, such that treatment continues while the remaining ponds recover from the overload and/or microorganism death due to unknown harmful substances (Figure 6-3c). Alternatively, Figure 6-3d shows another possible scheme where, given sufficient periods without significant differences between the loading, treatment and effluent quality of typical and 3rd-party wastewaters, both ponds could be utilized for both types of incoming wastewater. This could be implemented after a period of full-scale testing, and would be beneficial in simplifying pond operation in terms of controlling all the variables associated with a split pond operation (i.e. holding times, aeration/flow schedules would be more consistent) employing parallel ponds.

In order to estimate the number of BioCord modules/units needed for full-scale testing and implementation, the expected volume and composition of the wastewater entering Pond 2 must be predicted. The dimensions and holding volume of the Storring Septic ponds are shown in Table 6-2.

Table 6-2. Dimensions (length, width, depth) and holding volumes (L) of the four ponds at Storring Septic. The holding volume of each pond is as reported by the industry partner (Storring Septic, 2015).

Pond	Dimensions (m); L x W x D	Approximate holding volume (thousand L)
Pond 3 (primary pond)	22.86 x 45.72 x 2.44	3785
Pond 4 (primary pond)	22.86 x 45.72 x 2.44	3785
Pond 2 (secondary pond)	30.48 x 30.48 x 2.44	1703
Pond 1 (tertiary/polishing pond)	30.48 x 30.48 x 2.44	1703

The busiest months of operation at Storring Septic are from June to September. This is typically when the facility receives the most amount of septage per month. The volumes of wastewater entering the ponds at Storring Septic for the peak operation months in 2013 and 2015 are shown in Table 6-3.

Table 6-3. Inflow volume of wastewater per month entering the Storring Septic facility for 2013 and 2015. The average volume for both years is also shown.

Month	Volume of wastewater entering facility per month (thousand L), 2013	Volume of wastewater entering facility per month (thousand L), 2015	Average
June	686	318	502
July	737	313	525
August	912	193	553
Sept	550	234	392

From Table 6-3, it can be seen that the largest volume of wastewater entering the Storring Septic facility was approximately 912kL per month. In the future, Storring Septic plans to open up their facility to third-party sewage haulers, as well as increase their service area to accommodate the growing rural population. Future peak loads may be up to double their previous inflows. To be conservative, a hypothetical estimate using twice their highest volume (912kL) can be used. This results in a peak monthly inflow of approximately 1824kL of wastewater per month. This estimated peak inflow is approximately half the holding volume of each primary pond (Ponds 3 and 4), which are to be utilized in an alternating fashion.

This allows for retention times of 30-60 days, which is currently the typical operational scheme employed by Storring Septic.

During pilot-scale testing, the BioCord system (1.3m H x 0.92m L x 0.92m L) was placed in a treatment tank with a holding volume of approximately 5678L. According to Table 6-1, the BioCord system was able to treat an average COD concentration of 228mg/L (an average load of 0.65kg $CODm^{-3}d^{-1}$ at an average HRT of 2 days). The BioCord system was able to significantly reduce wastewater parameters from the influent at this COD loading rate. As such, there should be approximately 1 BioCord module of similar size as the used in pilot-scale testing for every 0.65kg $CODm^{-3}d^{-1}$. At 1824kL of inflowing wastewater into Ponds 3 or 4 per month, it is suggested that an HRT of 30 days be employed for Pond 2, such that the flow into Pond 2 is approximately 60.8kL/day. At this flow rate, the COD loading would be approximately 14kg $CODm^{-3}d^{-1}$. Based on these conservative calculations, approximately 22 units of the pilot-scale sized BioCord system would be needed to achieve appropriate treatment during full-scale testing and implementation, given that the volume of septage entering the Storring Septic facility is doubled (or that volume remains constant, and the COD loadings are doubled). Alternatively, one large-sized BioCord system may be commissioned, resulting in a BioCord system with dimensions of approximately 20.24m (L) x 20.24m (W) x 1.3m (H). According to Table 6-2, this size is within the size constraints of Pond 2.

Full-scale testing of the BioCord system for use at the Storring Septic facility would also consist of conducting one more alternative aeration cycling schedules to optimize for energy-efficiency in achieving consistent wastewater quality parameter effluent concentrations on the order observed during the pilot-scale testing. When air cycling was implemented during the full operational cycle (Chapter 4), it was noted that, after a start-up period of four weeks, an air cycle regime of 12h on/12h off per day was sufficient in significantly reducing concentrations of all wastewater parameters. In the full-scale test of the BioCord system, less energy-intensive schedules (e.g. 16-off/8-on) could be implemented and compared to the 12on/12 off cycle to determine whether a more energy-efficient method of treatment is possible. 24h of consistent aeration would be required during the first four weeks of operation/implementation, in order to allow the biofilm on the BioCord modules to develop appropriately and reach pseudo-steady state. 24h of consistent aeration may also be employed during the colder fall/winter months, when average ambient temperatures are low (<13^{o}C).

Overall, pilot-scale testing of the three treatment technologies contributed information for Storring Septic to make an evidence based decision for the operation of their treatment facility and future plans regarding the increase of their service area to accept more wastewater and to open up their facility to become a commercially available service for third-party sewage haulers. A biofilm treatment technology, BioCord, was identified as the most effective and cost- and energy-conserving treatment of those tested. By implementing this treatment technology on a full-scale basis, and by making slight changes in the operational design of their WSP facility, Storring Septic could improve their lagoon system which will in turn allow them to service a greater amount of and/or a more diverse range of clients.

6.2 Importance to Canada other WSP facilities

This thesis, and the results obtained from the studies conducted throughout the testing periods of this project, will assist smaller and/or rural/northern communities in Canada facing growing populations and more stringent wastewater effluent standards. Due to continued rural development, current wastewater treatment systems are reaching capacity while discharge guidelines have become more stringent (Environment Canada, 2015). Although future rural growth may be dependent on the ability to process the consequential rise in waste materials, building entirely new wastewater treatment plants are not typically economically feasible. As well, the implementation of new infrastructure can be unfavourably considered considering the ecological footprint associated with wastewater treatment and discharge. Hence, existing wastewater treament plants and WSP operators may be searching for opportunities to increase treatment efficiencies without significant additional cost- and energy-intensive expenditures. This project details the benefits of naturalized wastewater treatment technologies that could be retrofitted to existing lagoon or WSP treatment systems. It provides an option for increasing treatment efficiencies at existing treatment facilities across Canada, which could then more effectively treat higher volumes of wastewater, in a manner that is environmentally responsible and economically sound. As well, because the project was carried out in a typical rural Canadian climate, the results obtained from this study can be applicable to communities across Canada that may experience similar challenges facing wastewater treatment in colder environments.

6.3 Future work

Results from the studies conducted throughout this project—in addition to providing valuable insight into naturalized wastewater treatment attenuation—have led to some interesting inquiries that may be answered with further studies and experimentation. As noted, determining the lower limit of air cycling in the context of a full-scale BioCord system implementation would be a simple and effective way to reduce energy consumption (via the air compressor) over time. Through the implementation of an air flow/aeration schedule, it was found that certain wastewater parameters (i.e. total nitrogen and phosphorus) were more effectively reduced by the biofilm technology during periods of alternating aerobic and anaerobic conditions, while other parameters (i.e. TSS and COD) were more effectively reduced in consistent oxygen-rich environments. However, in analyzing the results of the study and comparing the treatment technologies, it was found that phosphorus was the only parameter to follow a less predictable pattern of percent reductions in response to aeration cycling, and was the only parameter that the BioCord system (the most effective biofilm treatment technology) was not able to significantly reduce in comparison to the control. It is suggested that a peripheral study may focus specifically on phosphorus reductions using the BioCord system or a similar biofilm treatment technology, to demonstrate a more defined relationship between phosphorus uptake/release and aeration/oxygen levels, temperature, and hydraulic retention times (HRT). This would be useful to wastewater treatment facilities experiencing difficulties in meeting discharge limits with respect to phosphorus concentrations, and wishing to implement a biofilm treatment technology to improve treatment performance at their facility.

Further testing may also be conducted involving the testing of the BioCord system to efficiently process or buffer against other contaminants not investigated in the scope of this study. Although this project was focused on the ability of a semi-passive treatment technology to assist WSP systems in processing higher volumetric and organic loads, another issue facing biological treatment facilities involves the presence of harmful substances that reduce the number or efficiency of wastewater-treating microorganisms. It may be useful to Storring Septic, as well as other facilities looking to expand their services, to characterize any harmful materials (e.g. detergents, antibiotics) found in the third-party influent and monitor how well the implemented BioCord system is able to resist the detrimental effects of these substances. The results of such a study would indicate how tightly any facility implementing a similar biofilm treatment technology would have to regulate the inflow of wastewater entering their systems, as well as help to determine any future changes involving filtration or pre-treatment that may be important for maintaining efficiency in wastewater treatment.

Characterization and analysis of a fully-formed biofilm would be an alternative method of demonstrating the presence and prevalence of wastewater-treating microorganisms, including phosphorus-accumulating organisms (PAOs) or other microorganisms involved in reducing phosphorus levels in wastewater. An in-depth analysis of the resulting biofilm composition would lead to a better understanding of how certain bacteria and archaea respond to changes in their environments (e.g. dissolved oxygen concentrations, flow rates, temperature, competition with other microorganisms), and allow for definitive statements regarding the presence and/or absence of key microorganisms. Obtaining information about the microbial composition of a biofilm over the course of a treatment season would also allow for further insight into the mechanisms of wastewater parameter reductions by microorganisms—for instance, the ratio of carbon-reducing heterotrophs or glycogen-accumulating organisms to nitrifiers, denitrifiers and PAOs. Such ratios, as well as an analysis of how the microbial composition of a biofilm changes with biofilm depth, would allow for a better understanding of the competition between these microorganisms in wastewater treatment, and allow for more conclusive statements about how the distribution of microorganisms in a biofilm (aerobes, anaerobes, heterotrophs, autotrophs) contribute to better reductions of certain parameters. Including an aspect of modelling and optimization may also be a useful in demonstrating relationships between each of the variables, although this may prove to be challenging due to the sheer number of variables impacting treatment, biofilm development and microorganism activity/growth.

Laboratory-scale experiments were conducted using zebra mussels to examine their ability to reduce wastewater quality parameters, although to a limited extent. The purpose of this study was to perform some preliminary testing to determine whether the presence of zebra mussels in wastewater, would reduce levels of wastewater contaminants. Concentrations of total ammonia, phosphorus, TSS and COD were found to be reduced in aquariums filled with wastewater and inoculated with zebra mussels; however, the death of the zebra mussels after a relatively short period of time implied that their potential to mitigate wastewater constituents could be limited. It is recommended that, firstly, more studies be undertaken involving the zebra mussel filtration and assimilation of wastewater parameters. Conducting a tissue analysis after observed reductions in wastewater contaminants could provide information regarding whether reductions were due to assimilation and to what extent. It would also help determine whether zebra mussels preferentially uptake certain contaminants over others. As well, it was hypothesized that zebra mussel death in a laboratory setting was more prevalent than would be observed in their natural habitats, due to the fact that ecological factors present in their natural environments may be necessary for their survival, but are not easily replicated in a laboratory setting. Hence, it is suggested that further studies be conducted that would involve wastewater treatment by zebra mussels in conditions more akin to those of the organism’s natural habitats. This would be more representative of their potential performance in WSP system attenuation, and may lead to better insights to prevent excessive zebra mussel death in a laboratory setting.

6.4 References

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Chapter 7

Conclusions and recommendations

7.1 Conclusions

Effective wastewater treatment and the management of septic waste are important to smaller and more remote communities. The implementation of wastewater stabilization ponds (WSPs) to treat domestic and municipal sewage represents an effective, low-cost, and environmentally-sustainable manner to treat large volumes of wastewater. Given the spatial requirements needed for successful operation of these pond systems, North American WSPs are generally more common in rural, remote or northern communities where land availability is less restrictive. With increases in rural populations and more stringent effluent discharge guidelines, as outlined by Environment Canada's Wastewater Systems Effluent Regulations, it is anticipated that existing lagoon facilities will face challenges related to effective treatment performance and the handling of influent wastewater (Environment Canada, 2015). This may be especially true during the cold weather operation, characteristic of Canada's winter climate. As such, the aim of this research was to investigate the capability of three different, low-energy treatment technologies to semi-passively increase the treatment efficiencies and robustness of an existing pond facility in Tamworth, Ontario (Storring Septic, Inc.) under operation during both the startup/colder-weather conditions during a fall season (Chapter 3), as well as throughout the duration of a typical full operational cycle (Chapter 4). Two of the implemented treatment technologies relied on the development and activity of a diverse and stable biofilm in order to reduce contaminants in the wastewater. These two technologies were each developed by third-party manufacturers: The BioCord treatment technology was developed by Bishop Water Technologies and the BioDome treatment technology was developed by Wastewater Compliance Systems, Inc. A third treatment technology was investigated and involved submerging zebra mussels (*Dreissena polymorpha)* in wastewater in order to utilize their filtration capabilities to reduce wastewater contaminants. The results obtained from this study provided information regarding the performance and potential of each of these technologies to treat wastewater. They allowed for recommendations to be made that could be applicable to other existing WSP facilities wishing to upgrade their systems using the operational and design characteristics of one or more of the technologies tested (either the BioCord or BioDome treatment technologies or zebra mussels).

In Chapter 3, pilot-scale testing focused on the abilities of the BioDome treatment technology, the BioCord system, and zebra mussel filtration to improve wastewater effluent quality under both start-up conditions (i.e. while the technologies were still in unsteady-state conditions) and lower temperatures conditions typically observed during the Canadian fall and winter months. The duration of this testing period was from October to November of 2015. The first two weeks of testing represented treatment during start-up conditions and milder ambient temperatures (average temperature of 13ºC), while the second half represented treatment during pseudo-steady state conditions and during conditions of colder ambient temperatures (average temperature of 7ºC). The results showed that, during the overall testing period, the BioCord system outperformed the other two treatment approaches, and consistently produced significantly lower concentrations of all wastewater quality parameters (with the exception of phosphorus) in comparison to the influent. When analyzing treatment during the second half of the testing season, when the biofilm was assumed to have reached pseudo-steady state and the ambient temperatures began to decrease, the BioCord system showed a considerable ability to decrease concentrations of the influent wastewater entering the reactor tanks, despite the decreases in effluent quality often noted during the fall season of WSP operation (Grady *et al.*, 1999; Metcalf and Eddy, 2003). As such, the BioCord system showed an ability to attenuate the negative effects of low temperature often observed in wastewater treatment systems involving biological activity. For lagoon or WSP facilities experiencing decreases in pond performance during the winter months, employing the BioCord system as a front-end technology (treatment of secondary effluent) would be an effective approach to maintaining treatment efficiency throughout an extended treatment season. This system could also assist in increasing treatment rates during start-up operation in the spring, as the BioCord system was shown to significantly improve effluent quality during the start-up period of pilot-scale testing. Ideally, 24h of aeration should be provided at this time to maximize biological treatment given the effects of low temperatures, but full-scale winter testing of the BioCord system may be beneficial in order to determine a lower limit of aeration required for sufficient winter treatment. The BioCord system showed an ability to buffer periods of system shutdown and non-aeration, leading to the conclusion that it may operate satisfactorily even when exposed to short periods of anaerobic conditions.

In Chapter 4, testing was conducted in order to identify a treatment technology that was most suitable to effectively reduce all wastewater quality parameters under a full WSP operational cycle (i.e. after reaching pseudo-steady state and during warmer ambient temperatures than those observed in chapter 3). During this time, an air cycling schedule was implemented to observe the effects of alternating aerobic and anaerobic conditions on the overall performance of the treatment technologies to reduce all wastewater quality parameters. This is because some of the microorganisms that reduce target nutrients require both oxygen-rich and oxygen-poor environments to effectively reduce concentrations of total nitrogen and orthophosphate. A regime of air-on and air-off cycling also allowed for lower energy consumption. During this field season, a fourth aerated tank was implemented as a control, to determine whether the BioCord or BioDome treatment technologies, and/or the zebra mussel system could statistically significantly outperform the control, or whether aeration alone was enough to significantly improve wastewater quality. Lastly, a two-week period of system shutdown (e.g. no water flow or air flow into the reactor tanks) was implemented to simulate conditions of pond shock, where the biofilms and/or zebra mussels were exposed to unfavourable conditions for survivability, growth and proliferation. This test was performed to determine the resilience of each treatment technology to a system shutdown, and to document performance of the systems under extended periods of oxygen- and substrate-poor conditions. Of the three treatment technologies investigated, the BioCord system was the only technology to successfully outperform the control for all wastewater quality parameters with the exception of orthophosphate. In the case of orthophosphate, influent concentrations were not exceptionally high, and effluent remained significantly lower than influent concentrations. Hence, the treatment performance of the BioCord system was deemed to be sufficient for the purposes of this study. For implementation into an existing WSP facility, implementation of the BioCord technology would be optimal as a front-end technology, with one or more ponds in the downstream process flow for further maturation and disinfection of wastewater effluent. In addition, the BioCord system required the least maintenance of the three treatment technologies. Monitoring of the dissolved oxygen (DO) levels in each of the treatment tank showed that the BioCord system was most effective in delivering and circulating oxygen throughout the tank. It is likely that the BioCord system outperformed the similar biofilm technology, the BioDome system, because of its ability to more effectively provide the biofilm with optimal contact to both oxygen and substrate under the conditions tested. In turn, the BioCord system likely developed a more stable, heterogeneous bacterial population and maintain high levels of activity in its biofilm, even during periods of extended anaerobic conditions. These findings were corroborated by the results of the implemented system shutdown, during which the BioCord system maintained effective levels of treatment during extended periods of anaerobic conditions and total system shutdown. Moreover, the BioCord system showed the fastest rates of recovery, where significant reductions of most wastewater parameters were noted within one week of system restart. The results of this testing season also showed differences in effluent quality in response to air cycling. Air cycling had an effect on both the amount of DO being delivered to each of the treatment technologies, as well as the amount of substrate-organism contact as a result of adequate mixing. For the first 2-4 weeks of technology implementation, 24h of consistent aeration would be recommended, to allow for sufficient biofilm development and acclimatization. After this start up period, a 12h on/12h off cycling schedule would be recommended as it was shown to provide the best overall treatment, especially when targeted compound removals include total nitrogen and orthophosphate.

A study involving zebra mussels (Chapter 5) was conducted to obtain supplementary information relating to the potential for zebra mussels to treat septage wastewater. Field testing showed that zebra mussels could reduce some wastewater constituents, but current strategies for larger-scale implementation are limited due to their susceptibility to mortality when exposed to periods of extended anaerobic conditions. As well, direct observation of the zebra mussels during the pilot-scale testing was difficult due to the nature of the experimental setup and the turbidity of the wastewater at the facility. As such, it was unclear whether the reductions noted in the zebra mussel reactor tank were a direct result of assimilation/filtration by the zebra mussels, or whether other mechanisms also contributed to the wastewater treatment, which is highly likely as treatment in these eco-engineered systems can generally be attributed to synergistic biological, physical and chemicals processes. The uptake/assimilation of TSS, COD, ammonia and phosphorus by zebra mussels in a controlled laboratory setting was investigated to verify whether zebra mussels alone could contribute to decreases in wastewater constituents. Levels of DO and pH were measured on a regular basis to ensure that they were within the appropriate range for zebra mussel survivability. The results indicated that, while alive, zebra mussels could consistently decrease COD, TSS, total ammonia and phosphorus concentrations while in a low-strength wastewater, for up to 22 days. It was noted that zebra mussel death may contribute to the release of certain constituents from their tissues.

7.2 Recommendations

Recommendations for future studies involve more in-depth analyses of how biofilm and zebra mussels can contribute to wastewater treatment in WSP attenuation. In particular, it would be useful to design future studies aimed at better defining the relationship between targeted contaminants (total ammonia, nitrite, nitrate, phosphorus, total suspended solids, chemical/biological oxygen demand) and changes in either biofilm compositions or zebra mussel activity. Chapter 6 outlines recommendations for some specific studies that could strengthen the understanding between wastewater treatment and semi-passive treatment technologies, but it is important to keep in mind that there will always be potential for more research involving aspects of wastewater treatment not discussed in this thesis. Some suggestions for future studies include: modelling and optimization of biofilms in WSPs, finding the lower limit of aeration and the most energy-conservative operational regime when implementing the BioCord system on a full-scale. Finally, more rigorous studies on zebra mussels and their ability to mitigate wastewater contaminants in a larger scale/field-study setting.

7.3 References

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