Evaluation of passive treatment technologies for the treatment of septic tank septage under temperate climate conditions

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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. Decentralized septic tank systems are widely employed as a means of wastewater treatment. The routine collection and management of the septage is required and one approach involves the use of a passive waste stabilization pond systems to treat and dispose of waste and wastewater in an environmentally sustainable manner. Storring Septic, like many other similar wastewater treatment facilities across Canada, has the potential to act as an eco-engineered facility that municipalities and service providers can utilize to manage and dispose of their wastewater emissions. However, with growing demands on existing municipal infrastructure, a concern that the increasing inclusion of third party material may be detrimental to the performance existing pond systems. In order to augment the current facility into a self-sustaining system with the capacity to safely accept septage from other sewage haulers, it was hypothesized that the addition of two commercially available technology solutions, BioDome and BioCord, could be employed to augment the treatment performance of the naturalized system, leading to the reduction of wastewater quality parameters to below the regulatory effluent discharge limits. These systems make use of biofilm technology to improve treatment performance, as well as enhance the robustness of these systems to temperature and hydraulic and constituent loading fluctuations. Pilot scale testing allowed for the investigation of the effects of applying these technologies on effluent quality when operated under temperate climate conditions. This study provides a comparison of these technologies with the aim to understand the important mechanisms involved in biological treatment and the optimizations strategies that could be employed to enhance the robustness of the overall eco-engineered system to ensure its long term sustainability. In doing so, a recommendation matrix was elaborated with the potential to be used as a universal implementation strategy for eco-engineered facilities located in environments of similar climate and ecology.

The pilot-scale testing aimed to identify the biofilm technology that would best enhance the reduction of wastewater effluent parameters. The study also focused on nutrient removals, air cycling and energy consumption, as well as resilience and performance during extended system shutdown periods. During the testing season, reductions from the influent were considered, but a higher importance was placed on the ability of a treatment to significantly outperform the control. This is of particular importance when comparing outcomes to costs of implementation. BioCord was observed to outperform the control for all parameters with the exception of orthophosphate. In the case of orthophosphate, inflow concentrations were not exceptionally high, implying that treatment by BioCord was sufficient for the purposes of the site-specific goals. For implementation into an existing lagoon facility, treatment by BioCord would be optimal when applied in a secondary treatment pond, or prior to discharge into a final, tertiary treatment pond. This would allow for a further uptake of nutrients by pond algae to meet regulatory effluent discharge requirements, and to promote pathogen removal in the polishing pond. BioCord also exhibited the best performance in terms of maintenance and energy requirements. It is likely that BioCord outperformed the similar biofilm technology, BioDome, because of its superior ability to provide the biofilm with optimal contact to both oxygen and substrate. In turn, BioCord was able to develop a more stable, heterogeneous microbial population and maintain high levels of activity in its biofilm, even during periods of extended anaerobic conditions. This also implied that BioCord would require less aeration, and hence a lower energy expenditure, than its competing counterpart. Moreover, BioCord showed the fastest rates of recovery, reaching significant levels of parameter reductions within one week of system re-initiation. In terms of aeration cycling, 24h of consistent aeration was recommended for the first 2-4 weeks of technology implementation, in order to allow for sufficient biofilm development and acclimatization. After this, a 12h on/12h off cycling schedule was demonstrated to be optimal in providing the best overall treatment, particularly for effective total nitrogen and orthophosphate removals.

1. 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[1]. High concentrations of microbial aggregates form stable biofilms, allowing for lower hydraulic retention times while minimizing washout[1]. 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[2]. 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 [3-5]. 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 (18.5-29.5^{o}C) contaminated by domestic, industrial and agricultural wastewater effluents[3]. 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[4].

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[5]. The system was operated from October to February, with the lowest ambient temperature being 0.9^{o}C and the highest ambient temperature being reported as 15^{o}C, 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 ^{o}C – 12.6^{o}C)[6]. 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 [7,8]. 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[3]. 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.

2. 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 1 shows a schematic of the experimental setup implemented for the full operational testing season of the chosen biofilm technologies.

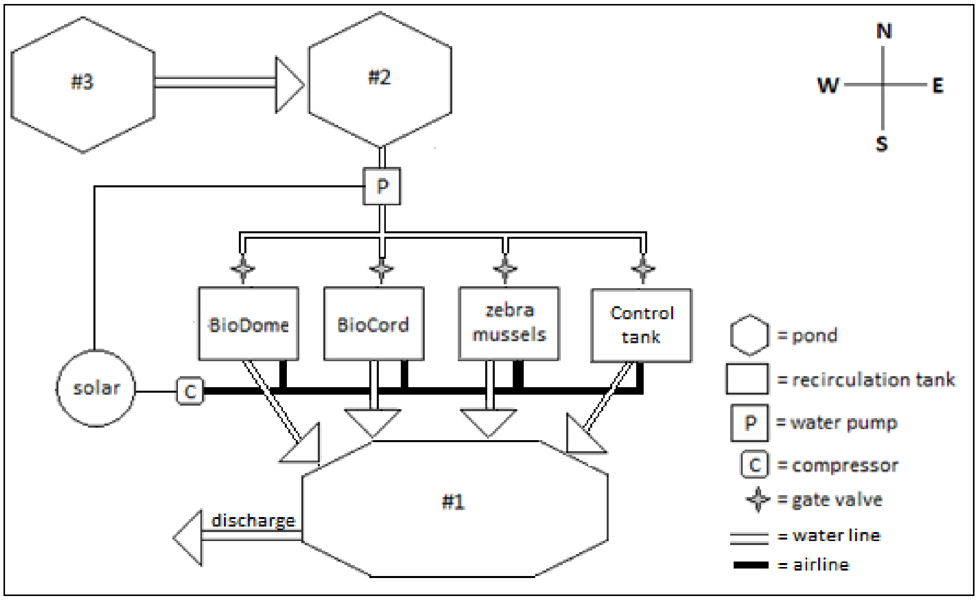


Fig 1. Experimental setup for testing of biofilm technologies at Storring Septic, weeks 1-14. During weeks 15-20 of summer testing, the zebra mussel tank was decommissioned, leaving the three remaining tanks for use in our research. Image not to scale.

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 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.

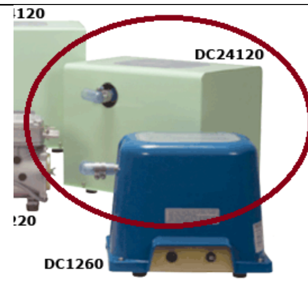
Storring Septic currently uses two hauling trucks to pump and deliver wastewater from domestic septic tanks to the WSP facility. This inflowing wastewater is dumped directly into the Storring Septic primary pond, Pond 3. During the entirety of this testing season, the average volume of wastewater added 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 [9]. 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 [10]. 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 [11], 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 1.

Table 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:

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

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 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 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[12]. 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[13]. 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[14]. 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[15]. 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) [16,17]. 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 [18]. 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.)[13]. 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.

3. 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[19]. 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[20]. Organic nitrogen can also be contained in organic matter, but it is released as ammonia when the organic matter is degraded by microorganisms[9]. 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 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.

4. 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.

4.1 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[2].

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

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 2 shows the fluctuation in ambient temperatures throughout the 140-day summer/fall testing period, as well as the precipitation noted during this time.

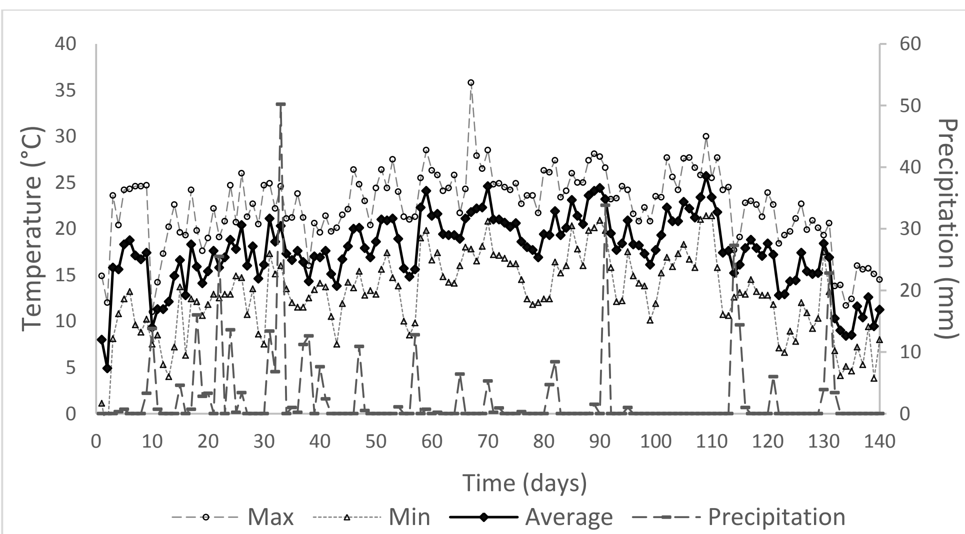


Fig 2. Maximum, minimum, and average temperatures, as well as precipitation accumulation, over the 140-day testing season (May 22^{nd} - Oct 8^{th}, 2015)[21].

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)[22]. The water temperatures were also always consistent between each of the treatment tank (less than 1.5^{o}C 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 3.

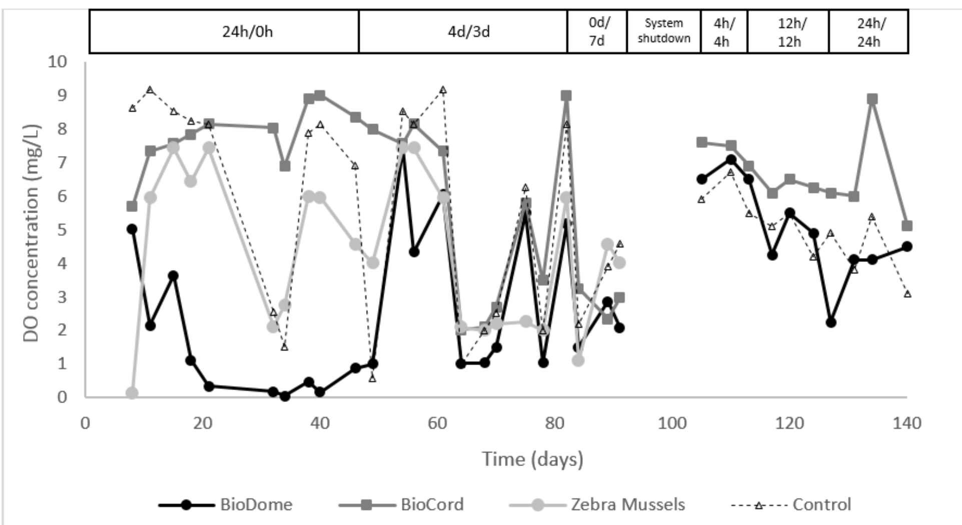


Fig 3. Dissolved oxygen concentrations (mg/L) in each treatment tank during time of sampling. Trend lines in between data points are shown to aid in visualizing the patterns in concentration for each tank over the testing season, but are not necessarily representative of actual values in between data 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[23]. 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[2], although Lindfors (2010) reported that with acclimatization, good nitrification could also be achieved in a pH range of 6.5 to 8.0[24]. 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[25]. Grady *et al.* (1999) reported that all bacteria grow poorly outside of the normal physiological range of 6.0 to 8.0[9]. Zebra mussels have an optimum pH range of about 7.3 to 9.3[26], with some studies noting an upper pH limit of zebra mussel tolerance of 9.3 to 9.6 [27]. 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[28]. This suggests that zebra mussels should be able to survive at pH ranges of 7.0 to 9.6.

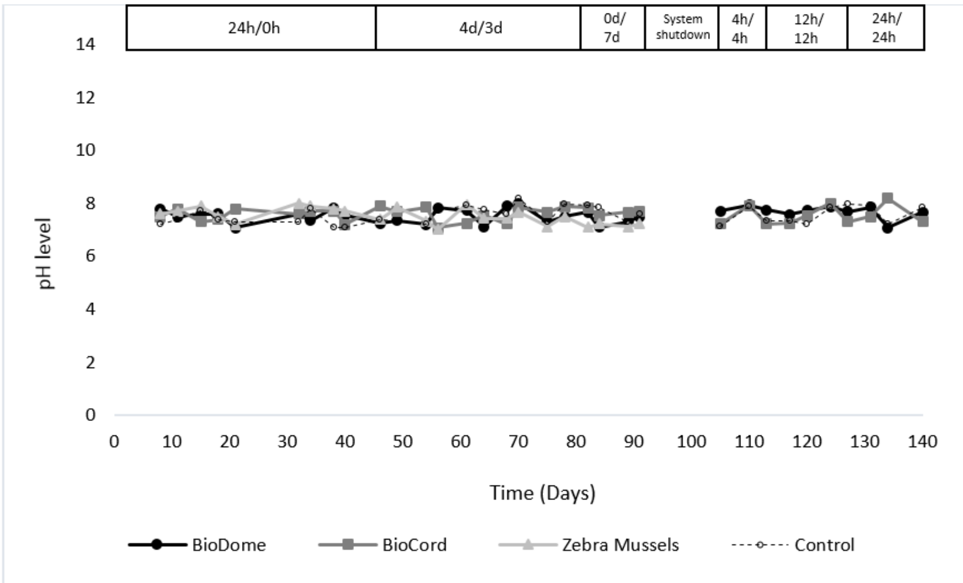


Fig 4. pH levels of each treatment tank over the course of the testing season**.** Trend lines in between data points are shown to aid in visualizing the patterns in in pH for each tank over the testing season, but are not necessarily representative of actual values in between data points.

The pH levels in each of the tanks over the entire treatment season is shown in Figure 4. The pH ranges 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.

4.2 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[29,30]. 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[31]. 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[9,32]. Figure 5 illustrates reductions in total ammonia in each of the treatment systems compared to the influent over the course of the entire testing period.

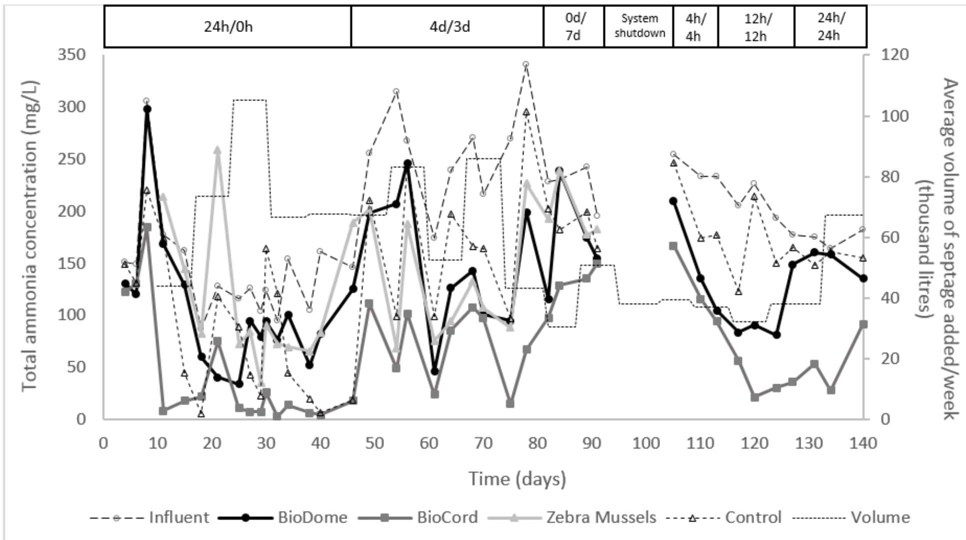


Fig 5. Reductions in total ammonia for each tank (primary axis), compared to the influent total ammonia levels, over a 140-day testing period (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those 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 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 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 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 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 (^{o}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	24h OFF	23	15 ± 10	37 ± 7	10 ± 9	18 ± 1

	(days 83-91)						
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 5 and Table 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 6, which is beyond the range noted during this study (Figure 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.

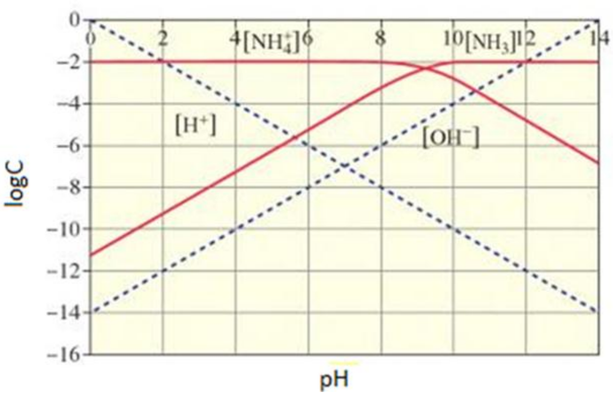
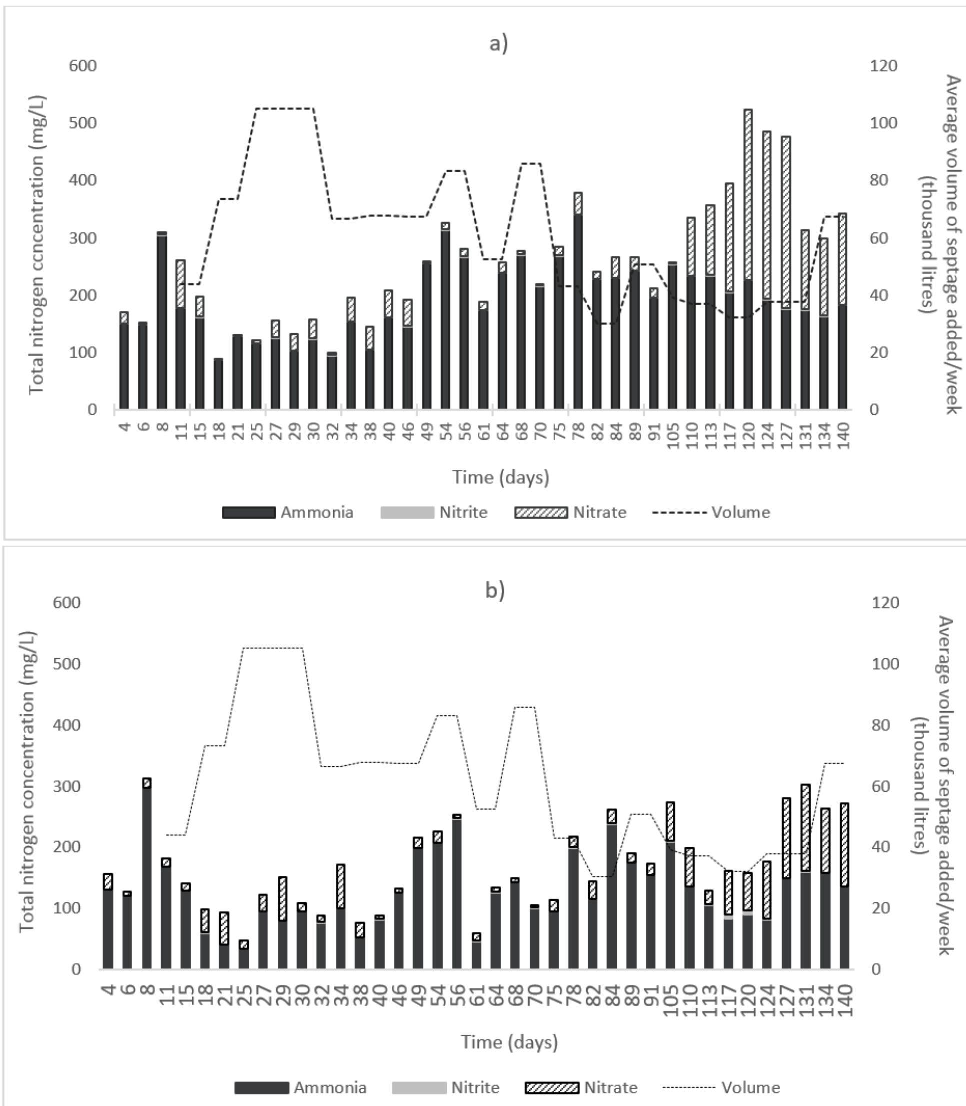


Fig 6. The pC-pH diagram of the NH4+/NH3 system. At a pH of 7-8, [NH4+]>>[NH3] and the dominant species is ammonium (non-volatilizing form). Little nitrogen is present as ammonia and thus, nitrogen removal by volatilization is minimal[18].

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 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[33,24,5]

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[34]. This disease, also termed blue-baby syndrome, decreases the ability of blood to carry oxygen and can be fatal to newborns[35]. 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[36]. Denitrification is largely performed by anaerobic and facultative bacteria, which require anaerobic or anoxic conditions[36]. Aerobic denitrification has also been reported to be possible by some microbial organisms present in wastewater, but to a lesser extent[37,38]. As such, reductions in total nitrogen were expected to be higher during periods when cycles of both aerobic and anaerobic conditions were implemented. Figures 7a to 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.



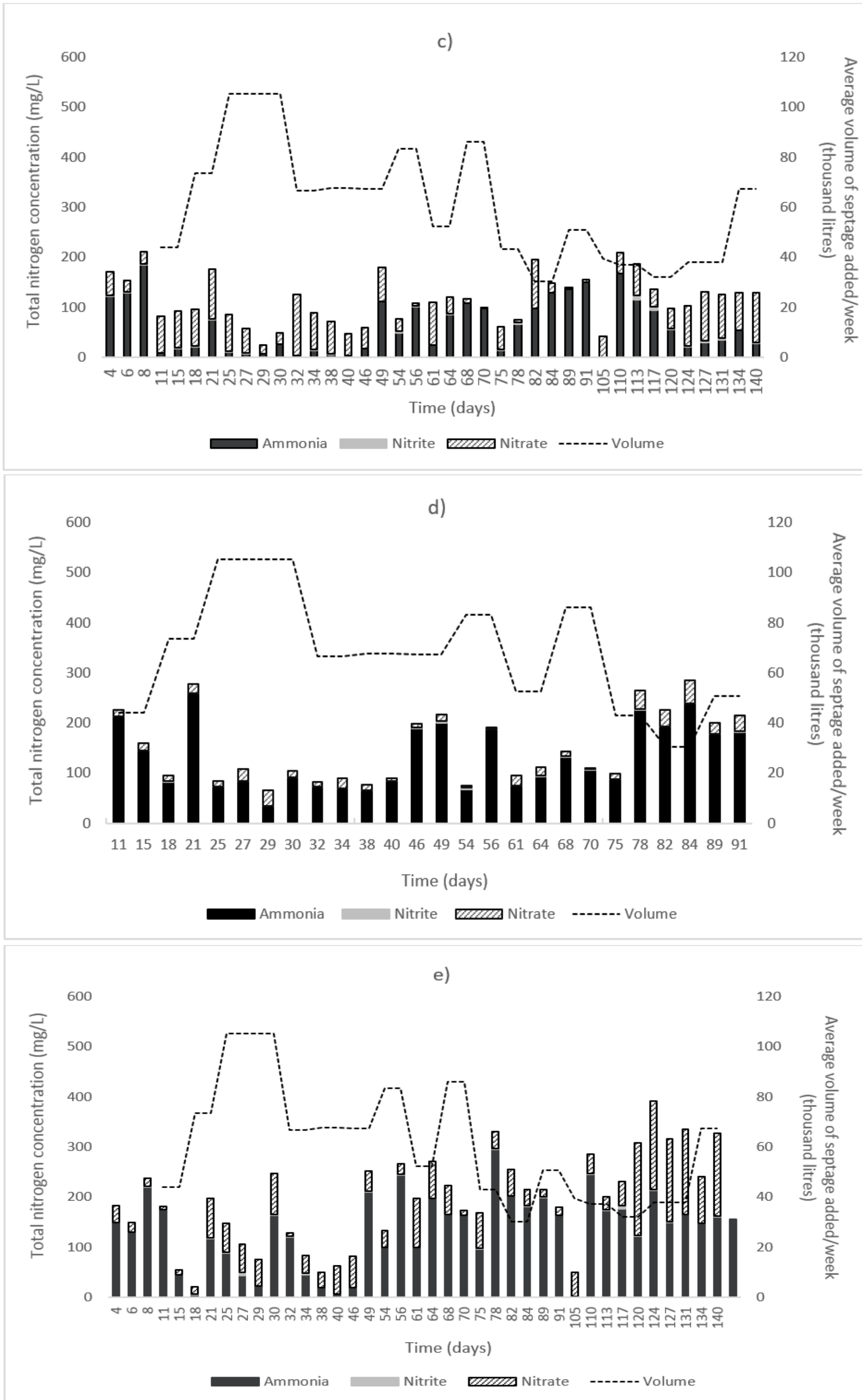


Fig 7. Total nitrogen and nitrogen compositions of a) the influent, b) the BioDome system effluent, c) the BioCord system effluent, d) the zebra mussel tank effluent and e) the control tank effluent. The scale of total nitrogen composition (mg/L) is kept constant to better illustrate the difference in 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). Table 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. 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 (^oC)	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 η^2 = .033	†55 ± 6 η^2 = 0.48	†43 ± 7 η^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 η^2 = 0.58	†78 ± 4 η^2 = 0.76		35 ± 7
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	12 ± 5	†55 ± 6 η^2 = 0.77		7 ± 5
1-3 (overall)	Weeks 1-20 (days 1-140)		18	31 ± 4	†47 ± 5 η^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 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 7c, Table 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 7b, Table 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 7d, Table 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). However, the 12h on/12h off aeration cycling (Table 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.

4.3 Orthophosphate

Phosphorus is a key nutrient that can lead to algal blooms and eutrophication, followed by oxygen depletion, in natural waters[39]. 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[40]. Microorganisms called polyphosphate-accumulating organisms (PAOs) uptake orthophosphorus into their cells, and require alternating anaerobic and aerobic environments to efficienctly remove orthophosphate from wastewater[8,41]. The concentrations of reactive phosphorus (orthophosphate) for the influent and all treatment systems throughout the 140-day study period are shown in Figure 8.

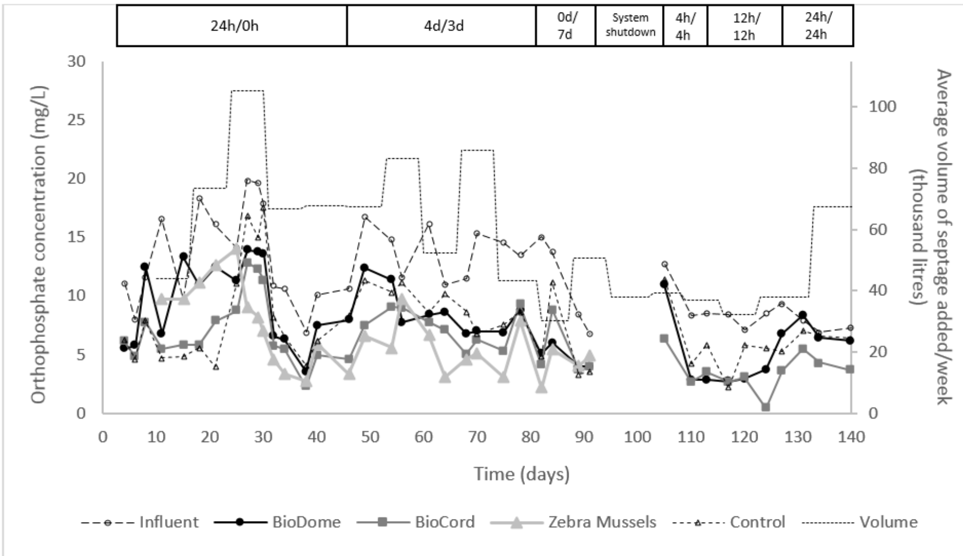


Fig 8. Orthophosphate concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend 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 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[42]. 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[43,44]. 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 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 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 η^2 = 0.15	†7 ± 1 η^2 = 0.44	†8 ± 1 η^2 = 0.32	†8 ± 1 η^2 = 0.31
2a	Weeks 8-13 (days 47-82)	4d ON/ 3d OFF	20	14 ± 1	†8 ± 1 η^2 = 0.64	†7 ± 1 η^2 = 0.75	†5 ± 1 η^2 = 0.75	†9 ± 1 η^2 = 0.69
2b	Week 14 (days 83-91)	24h OFF	23	10 ± 1	†5 ± 1 η^2 = 0.77	5 ± 2	†5 ± 0 η^2 = 0.77	6 ± 3
3a	Weeks 15/16 (days 105-113)	4h ON/ 4h OFF	22	10 ± 1	6 ± 3	†4 ± 1 η^2 = 0.77		7 ± 2
3b	Weeks 17/18 (days 114-127)	12h ON/ 12h OFF	16	8 ± 0	†4 ± 1 η^2 = 0.76	†2 ± 1 η^2 = 0.76		†5 ± 1 η^2 = 0.76
3c	Weeks 19/20 (days 128-140)	24h ON/ 24h OFF	12	7 ± 0	7 ± 1	†5 ± 1 η^2 = 0.77		7 ± 0
1-3 (overall)	Weeks 1-20 (days 1-140)		18	12 ± 1	†8 ± 1 η^2 = 0.26	†6 ± 0 η^2 = 0.48	†6 ± 1 η^2 = 0.38	†8 ± 1 η^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.

4.4 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[45]. 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[9]. 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[46,9]. 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 9 shows COD concentrations in each of the treatment technologies throughout the entire testing season.

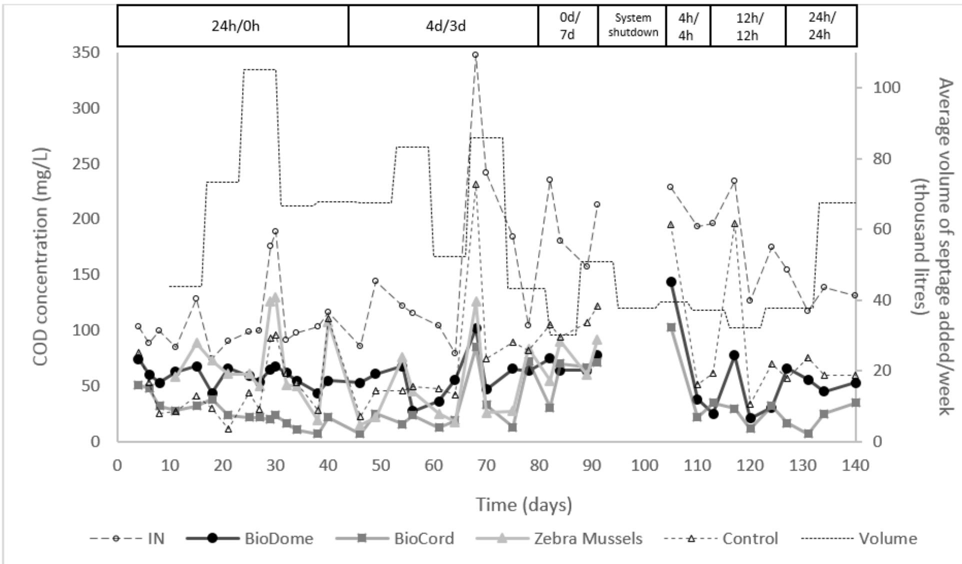


Fig 9. COD concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between 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 6 summarizes the average ability of each treatment technology to reduce COD concentrations during each of the testing period.

Table 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 (^oC)	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 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 (23^oC) 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 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[48]. 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 [9,48]. The results for TSS concentrations and the percent reductions in each treatment technology are shown in Figure 10 and Table 7, respectively.

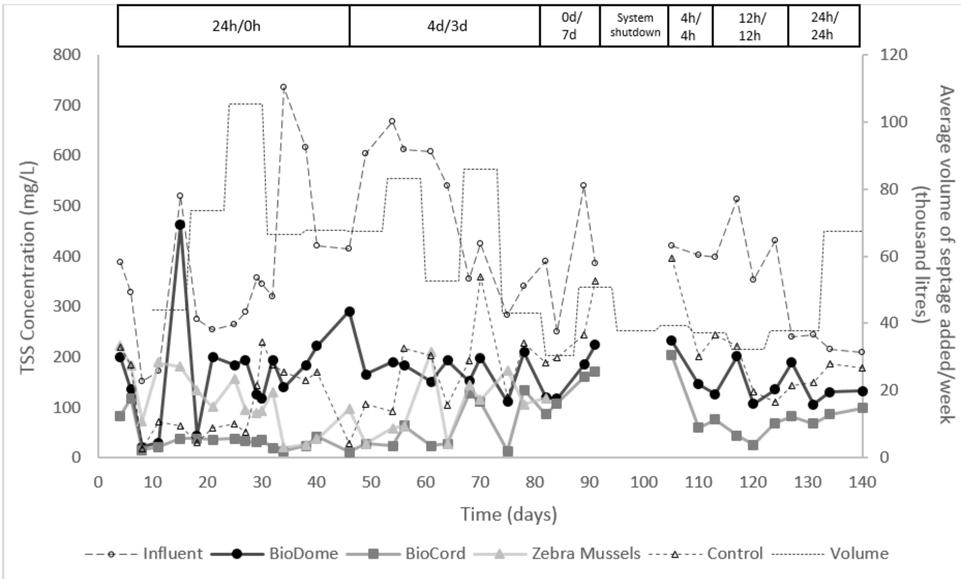


Fig 10. TSS concentrations (mg/L) in the influent and for all treatment technologies over the entire testing season (May 22nd to Oct 8th, 2015). The secondary axis shows the average amount of raw wastewater that was dumped into the Storring Septic facility (Pond 3) during those days. Trend lines in between 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 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.

6. Conclusions and recommendations

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[3]. 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[13]. 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[33]. 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.

6.1 Full-scale 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 11 illustrates the spatial arrangement and dimensions of the four ponds, and allows for better visualization of prospective full-scale applications.

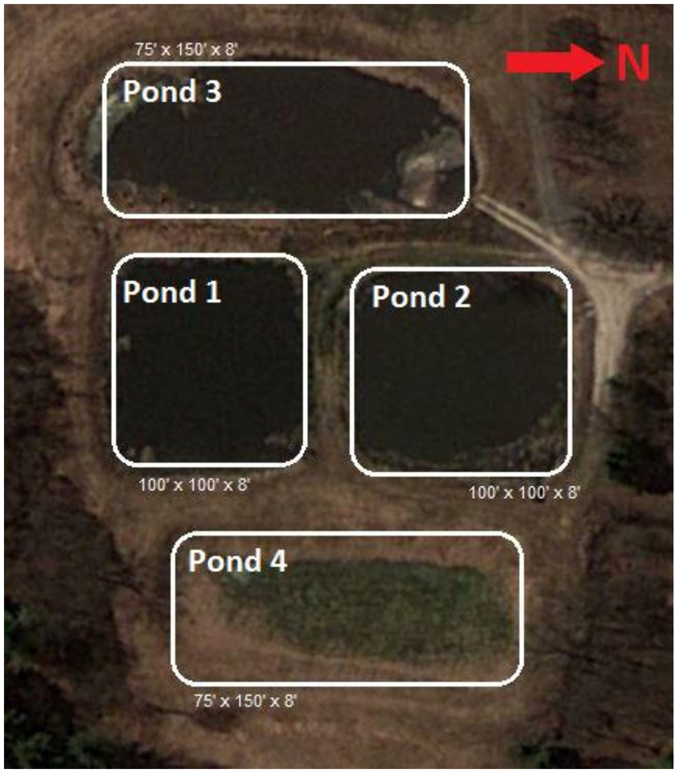


Fig 11. Aerial-view of the Storring Septic facility, including Pond 4 (not commissioned during study).

From the results of the pilot-scale investigations presented in this paper, 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 the testing season, as well as each of the parameters tested, are shown in Table 8. 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 8. 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 12, and the new possibilities for scale-up using the BioCord system are shown in Figures 13a to 13d.

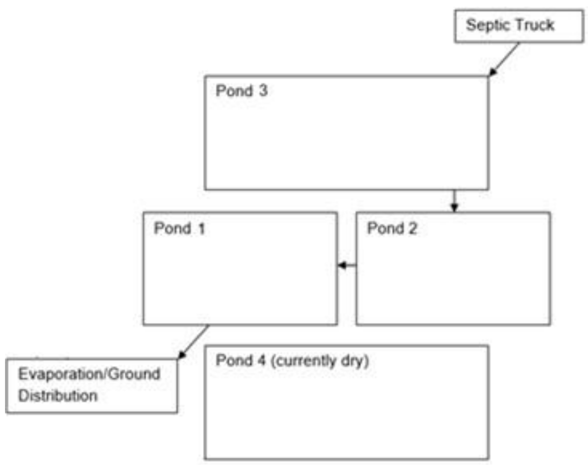


Fig 12. Diagram of the typical process flow currently in use. Arrows indicate direction of the inflow/outflow of wastewater.

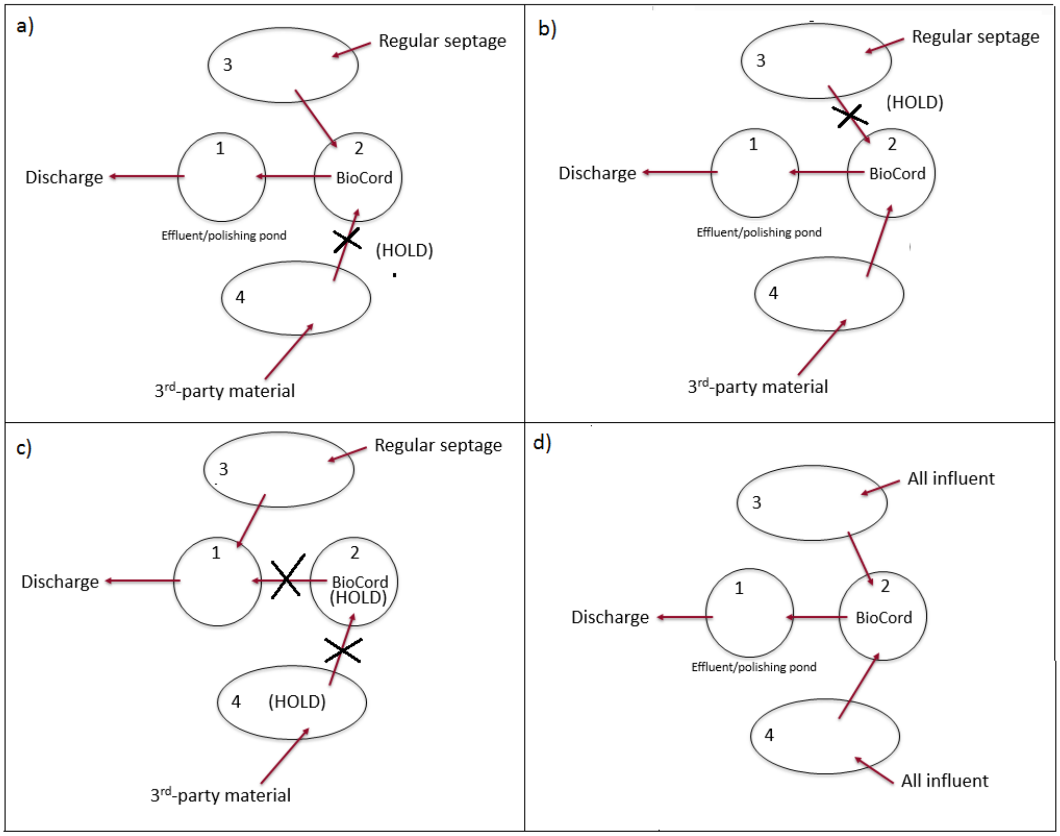


Fig 13. Split-pond operation, with the BioCord system implemented on a full-scale level in Pond 2. Arrows indicate the direction of wastewater flow, and overlaid crosses represent a halt in flow. a) Regular septage is dumped and treated as per the typical process flow, with materials from 3rd party sewage 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 13 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 13a and 13b), "regular septage"[1] 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 (13a), Pond 4

[1] In this paper, "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

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 (13b), 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 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 13c). Alternatively, Figure 13d 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 9.

Table 9. 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[49].

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 10.

Table 10. 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 10, 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 8, 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 9, 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, 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.

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