NOTE: Published on May 24th, 2021, in Water and Environment Journal, available here: https://doi.org/10.1111/wej.12736

Upgrading municipal lagoons in temperate and cold climates: Total nitrogen removal and phosphorus assimilation at ultra-low temperatures

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Word count: 6408

Abstract

In this study, a municipal lagoon with high wintertime effluent total ammonia nitrogen (TAN) concentrations was upgraded with a pilot-scale nitrifying-nitrifying-denitrifying (NIT-NIT-DENIT) moving bed biofilm reactor (MBBR) treatment train to characterize its effluent over wintertime operation, investigate the feasibility of upgrading lagoons to achieve substantial biological total nitrogen removal across ultra-low temperatures (0.6 – 3.0°C) and investigate nitrification inhibition pathways in facultative lagoon systems at ultra-low temperatures. Throughout the study, it was observed that the system substantially reduced total nitrogen (TN) and total phosphorus (TP) effluent concentrations by an average of 69.8 ± 24.5% and 74.7 ± 20.1%, respectively. Furthermore, it was observed that sulfide toxicity may play an important role in the inhibition of nitrifying organisms in lagoons. Finally, the MBBR treatment technology has emerged as a suitable and sustainable upgrade technology for existing lagoon and waste stabilization pond facilities operating in temperate, northern, and cold climate countries.

Keywords: wastewater treatment, water quality, pollution, seasonal, environmental management

1. Introduction

The impoverishment of surface waters is a global problem that impact many communities around the world. Anthropogenic release of nutrients to natural waters is widely recognized as a leading cause of eutrophication and water toxicity (Smith et al., 1999; Lewis et al., 2011). Furthermore, it has been recognized that the effluent discharge of water resource recovery facilities (WRRF) is a major source of these anthropogenic nutrients (nitrogen & phosphorus) that are released to the natural environment (Preston et al., 2011). As a result of the impact of wastewater discharge, several countries and states have imposed stringent effluent discharge regulations for total nitrogen (TN) and total phosphorus (TP) (Directive Council of the European Union, 1991, 1996; Ministry of Ecology and Environment of the People's Republic of China, 1996; USEPA, 2002; MOE Ontario, 2008).

In many countries, waste stabilization ponds, also named lagoons, remain one of the most common types of WRRFs. Currently, over 8,000, 3,000, 2,500 and 1,200 lagoons are in operation in the U.S., Germany, France and Canada, respectively. (Mara, 2009; USEPA, 2011; Statistics Canada, 2016). Lagoons typically consist of single or multi-celled basins that hold wastewater for extended periods (7 to 30+ days) (USEPA, 2000; Delatolla and Babarutsi, 2005; Bruce, 2009). They can be operated in non-aerated (facultative) or aerated configurations, and lagoon-type WRRF installations often combine several types of cells to achieve the desired treatment level. Lagoons are designed to reduce the concentration of total suspended solids (TSS), carbonaceous biochemical oxygen demand (CBOD) and, at some installations, total ammonia nitrogen (TAN), prior to their discharge to receiving waters (Asano et al., 2007). Due to their simplicity and low operational intensity, lagoons remain one of the preferred methods of treating municipal wastewaters in smaller communities where general land availability allows for their construction (Mittal, 2006; Muga and Mihelcic, 2008).

Despite the extensive installation and use of lagoons in many countries, these systems are subject to several drawbacks and limitations. The nutrient treatment removal capabilities of lagoons situated in northern climates often becomes significantly limited due to temperature-induced decreases in the metabolic activity of the microbial populations (Andreottola et al., 2000; Wessman and Johnson, 2006; Delatolla et al., 2009, 2010, 2012; Hoang, 2013; Hoang et al., 2014; Ragush et al., 2015; Young et al., 2016a). During fall and winter in temperate and northern climates, wastewater temperatures in lagoons can often fall below 1.0°C (Heaven et al., 2003; Krkosek et al., 2012; Ahmed et al., 2019). Cold-temperature driven loss of bacterially-mediated nitrification in lagoons located in northern climates has often been shown to cause effluent TAN concentrations to substantially increase (Painter and Loveless, 1983), leading to the harmful release of ammonia to receiving waters (Painter and Loveless, 1983). Additionally, removal of phosphorus in lagoons is typically performed via the chemical addition of coagulants or lime (Narasiah et al., 1994), as few naturally occurring biological pathways exist within these systems that are capable of effectively reducing the release of phosphorus. Limited overall performance and specifically limited nutrient removal during low temperature operation has created a need to upgrade these systems to maintain compliance with increasingly stringent nutrient regulations, and specifically to remove nitrogenous and phosphorous compounds prior to discharge.

Treatment technologies designed to upgrade lagoons should be appropriate in their design to require similar, low level operational intensity; as lagoon facilities often do not have full-time operators always present. Several technologies have been employed to upgrade lagoons; such as extended aeration of aerated lagoons (Melcer et al., 1995), trickling filters (Archer and O'Brien, 2005; Avsar et al., 2008), rotating biological contactors (RBC) (Hassard et al., 2015), and constructed wetlands (Cameron et al., 2003; Butterworth et al., 2016). In recent years, studies have outlined the use of rock or aggregate-based attached growth systems (Swanson and Williamson, 1980; Mara and Johnson, 2006; Mattson et al., 2018), stationary in-lagoon fixed film media (Shin and Polprasert, 1988; Srinivas, 2007; Gan and Champagne, 2015) and moving bed biofilm reactor (MBBR) systems (Wessman and Johnson, 2006; Delatolla et al., 2010; Hoang, 2013) to upgrade lagoons either by enhancing microbially-mediated nitrification or by increasing the lagoon system's volumetric or loading capacity. Numerous studies have recently demonstrated the effectiveness of nitrifying attached growth technologies such as the MBBR to enhance TAN-removal performance at the end of lagoons to meet stringent ammonia effluent guidelines at ultra-low (0.6 – 3.0°C) temperatures (Delatolla et al., 2010; Hoang, 2013; Almomani et al., 2014; Young et al., 2016b; Ahmed et al., 2019; Patry et al., 2019). Post-carbon nitrifying MBBR systems utilize plastic media with a high specific surface area to encourage the adhesion and attachment of nitrifying bacterial communities. The microbial populations in nitrifying MBBR systems have been shown to be highly diversified (Otawa et al., 2006), allowing nitrifying MBBR systems to operate in a wide range of temperatures (1°C – 20+°C). Biologically active organisms in nitrifying biofilms are ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB)

(Gieseke et al., 2003). In locales where total nitrogen is regulated, nitrifying MBBR systems can be supplemented with a follow-up denitrifying MBBR system to convert produced nitrate to nitrogen gas, effectively removing nitrogen from the aqueous environment. The denitrifying MBBR technology has been utilized for over 20 years (Aspegren et al., 1998)however it is almost exclusively applied in larger, mechanical plants, where mesophilic conditions exist (10°C+). In the context of lagoon facility upgrades and retrofits with denitrifying MBBR technology, current available literature does not address operation below 5°C (Dale et al., 2015). As a result, a fundamental lack of knowledge remains regarding the capacity of utilizing the MBBR technology to perform total nitrogen removal (nitrification and denitrification) as an add-on technology at a municipal lagoon in temperate and cold climates, where water temperatures below 1°C may routinely be encountered during wintertime. In addition, there is a gap of knowledge with respect to biologically mediated upgrade systems for lagoons to enhance total phosphorus removal. Ensuring effective total nitrogen and total phosphorus removal in lagoon treatment systems is necessary to ensure the perenniality of water resources in various countries (Brettle et al., 2016; Statistics Canada, 2018) and as such feasible and appropriate nutrient removal upgrade technologies are urgently needed as low operational intensity add-on technologies for lagoon WRRFs.

The aim of this study is to investigate the performance of a nitrifying and denitrifying multi-reactor MBBR system installed as an upgrade system to facultative lagoons operating at ultra-low temperatures (0.6 - 3.0°C), quantify the removal efficiency of TAN and NO_x within the multi-reactor MBBR treatment system at ultra-low temperatures, and to understand the inhibition of nitrification within facultative lagoon systems during ultra-low temperature operation. Furthermore, the study also investigates phosphorus assimilation in a nitrifying-denitrifying upgrade MBBR system as an additional benefit to the operation and as a potentially significant pathway for total phosphorus removal from lagoon effluent.

2. Materials and methods

2.1. Study site

A pilot-scale wastewater treatment plant was installed at the Casselman, Ontario, Canada (45°19'26.3"N, 75°04'48.6"W) municipal facultative lagoon WRRF from Dec. 2017 until Jun. 2018. The facultative lagoon is characterized by high effluent TAN concentrations in wintertime due to seasonal temperature-driven loss of bacterial nitrification. The lagoon system consists of three cells operated in-series. Cells #1 (7.4 ha) and #2 (7.2 ha) are operated as facultative cells, but cell #3 (4.1 ha) is equipped with bottom-mounted aerators and is aerated prior to planned discharge to alleviate sulfides and enhance effluent polishing (Figure 1). Cell #3 was not aerated from day 1-76. Pre-planned aeration in cell #3 began on day 77 of the study, lasting until the end of April (day 125) due to required lagoon discharge. Spring discharge of the WRRF commenced on day 116 of the study and lasted approximately 30 days. The lagoon is typically discharged twice a year, in the fall and in the spring. The study was separated in three distinct periods of comparable lengths: i) ice-forming period (days 1-50), ii) full ice-cover period (days 51-115) and iii) spring thaw period (days 116-165).

2.2. Pilot plant configuration

The pilot plant housed an influent storage vessel and three MBBR reactors in series. The pilot-scale treatment plant was fed with the effluent of the last cell of the three-cell lagoon treatment system in Casselman, Ontario. The lagoon effluent was pumped from the lagoon and into an influent storage vessel in the pilot via a 0.75 kW submersible pump suspended by a buoy approximately 40 cm below the water surface (near to the surface) providing flow to the MBBR treatment train. As such, the treatment train consisted of an influent storage vessel (retention time of less than 10 minutes), followed by three cylindrical 223 L MBBR reactors (total dimensions: 61 cm Ø x 122 cm H) in-series; a nitrifying MBBR reactor followed by a second nitrifying MBBR reactor followed by a denitrifying MBBR reactor, referred to in this manuscript as a NIT-NIT-DENIT configuration (Figure 1). This choice of configuration (NIT-NIT-DENIT) was selected due to concerns of potential sulfide toxicity to the nitrifying microbial communities, and due to intrinsic improvements to nitrifying removal efficiency due to the process being rate-limited at cold temperatures. Sizing of the reactors in the pilot unit was based on the kinetics calculated at projected minimum temperatures, the total flowrate and the TAN loading. Carrier movement in the first and second nitrifying MBBR reactors (R1 & R2) were maintained by supplied aeration. The carriers in the denitrifying reactor (R3) were maintained in motion with a mechanical mixer. The airflow in the reactor was adjusted periodically to maintain adequate carrier motion throughout the experiment. The mechanical mixer speed was also adjusted periodically to maintain adequate carrier motion. The effluent of the pilot-scale treatment system was then recirculated to the first cell of the lagoon treatment system. The carbon source dosed in the third, denitrifying reactors was Hydrex™ 6860 supplied by Veolia Water Technologies (Montreal, Canada). All MBBR carrier media utilized in this study were Anox™ K5 carriers (surface area to volume ratio of 800m^2/m^3). All reactors in this study had a carrier fill percentage of 50% and had an operational HRT of 4.0 hours, based on the design criteria of low temperature nitrifying MBBR systems treating lagoon effluent (Young et al., 2017a; Ahmed et al., 2019).

2.3. Pilot plant start-up

Prior to the beginning of the study, all three MBBR reactors in the pilot plant were seeded with carriers harvested from an integrated fixed-film activated sludge (IFAS) facility in Hawkesbury, ON, Canada, to promote rapid inoculation and growth of the nitrifying community (Young et al., 2017b). The carriers were collected with 20 L HDPE strainers and transported in drained 200 L barrels to the Casselman lagoon facility, approximately 60 kilometres away, and loaded into the MBBR reactors. The three MBBR reactors in the pilot treatment unit were operated in a continuous flow configuration for a

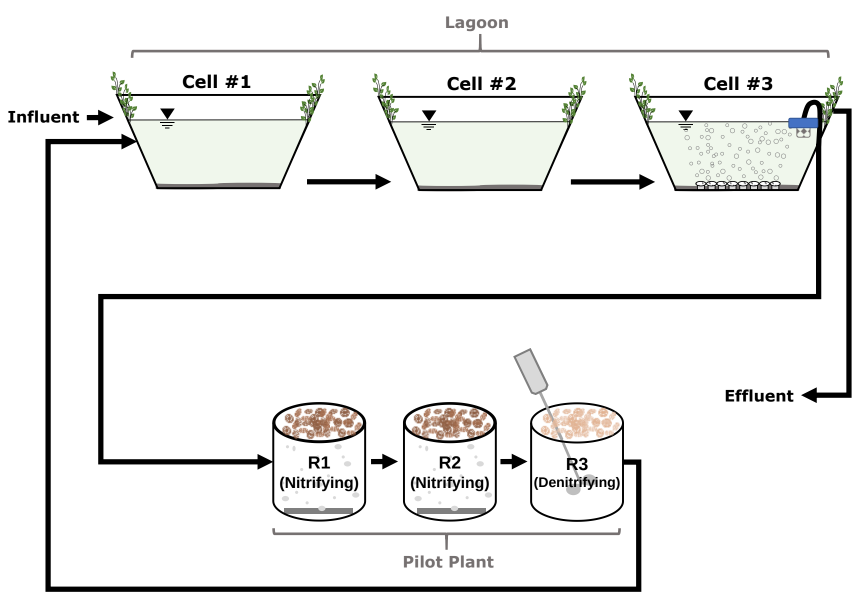

Figure 1: Schematic of the Casselman municipal facultative lagoon WRRF and MBBR nitrogen removal MBBR pilot systems.

period of one month prior to the start of the study to acclimatize the seeded carriers and were fed with TAN-augmented lagoon effluent with the help of a peristaltic pump injecting ammonium chloride solution into the first reactor in-series to stimulate the growth of ammonia-oxidizing bacteria. The water quality parameters of the first reactor in the treatment train during the acclimatization period were as follows: TAN ≥ 25.0 mg-N/L, BOD ≤ 30.0 mg/L, DO ≥ 5.0 mg/L. All three reactors demonstrated steady state operation after one month of operation; with steady state defined as TAN and NO_3^- removal rate fluctuations within a ±10% variation of the geometric mean.

2.4. Water quality analyses

Water samples were collected from the influent holding tank and each of the reactors, and analyzed at the pilot facility every 2-5 days over a period of 165 days. Samples which could not immediately be analysed were stored at 4°C for a maximum of 24 hours prior to analysis in the laboratory. The following constituents were analyzed: biological oxygen demand (BOD) (5210 B) (APHA, WEF, 2012), chemical oxygen demand (COD) (SM 5220 D) (APHA, WEF, 2012), total suspended solids (TSS) (SM 2540 D) (APHA, WEF, 2012), total ammonia nitrogen (HACH Method 8155) (Reardon et al., 1966; Hach Company, 2015), nitrate (SM 4500-NO_3^- B) (APHA, WEF, 2012), nitrite (SM 4500-NO_2^- B) (APHA, WEF, 2012), alkalinity (SM 2320 B) (APHA, WEF, 2012), total sulfide (SM 4500-S^{2-}D) (APHA, WEF, 2012), sulfate (US EPA 375.4) (U.S. Environmental Protection Agency, 1978) and total phosphorus (SM 4500-P E) (APHA, WEF, 2012). Dissolved oxygen (DO) and pH were measured using HACH LDO101 and PHC201 probes paired with a HACH HQ40d meter (Loveland, CO).

2.5. Statistical analyses

Statistical analyses were performed in order to establish statistical significance (*p*-value and Pearson's R) between water quality parameters throughout the study, linear regressions with a 95% confidence interval were performed between parameters of interest. The assumptions associated with a linear regression model were validated.

3. Results and discussion

3.1. Lagoon effluent characteristics

Domestic wastewater entering the Casselman municipal lagoon flows through cells #1 and #2, which are facultative, followed by cell #3, which is intermittently aerated prior to seasonal discharge. The effluent of cell #3 fed the pilot treatment plant and was monitored over a period of 165 days, which encompassed the seasonal spring discharge period from the end of December to early June. The following water quality parameters (COD, TSS, nitrite, alkalinity, sulfate, and pH) were separated in three different lagoon operation periods: the ice-forming period (days 1-50), and the full ice-cover period (days 51-115) and the spring thaw period (days 116-165) (Table 1). BOD (data not shown) and TSS (Table 1) concentrations met or exceeded regulatory standards (<25 mg/L $cBOD_5$ and <25 mg/L TSS) (Canadian Council of Ministers of the Environment, 2009) throughout almost the entire study. During the snowmelt period, COD and alkalinity decreased slightly (Table 1). It is possible that COD and alkalinity concentrations in the third cell of the lagoon may have decreased slightly during the spring thaw period either due to a restart of aerobic carbon removal and nitrifying activity, or potentially due to dilution. Due to the location of the pump used to collect water from the lagoon (near the surface), some biological processes and concentration gradients of different water quality constituents may not have been fully observed, particularly during the full ice-cover period.

Table 1. Average lagoon effluent concentrations of water quality parameters throughout the study period.

Parameter	***Ice-forming period, Average ± SD, (n)***	***Full ice-cover period, Average ± SD, (n)***	***Spring thaw period, Average ± SD, (n)***
COD (mg DO/L)	59.2 ± 5.6, (13)	51.1 ± 9.1, (16)	32.6 ± 5.0, (11)
TSS (mg/L)	13.6 ± 3.2, (5)	11.6 ± 10.7, (18)	12.4 ± 11.7, (10)
NO_2^- (mg-N/L)	0.01 ± 0.01, (13)	0.00 ± 0.01, (17)	0.46 ± 0.73, (11)
Alk. (mg $CaCO_3$/L)	315.3 ± 16.3, (7)	299.0 ± 41.6, (16)	203.1 ± 60.0, (11)
SO_4^{2-} (mg-S/L)	40.7 ± 5.6, (5)	42.6 ± 6.2, (18)	36.9 ± 2.9, (8)
pH	7.9 ± 0.6, (12)	7.6 ± 0.2, (19)	7.6 ± 0.4, (10)

Temperature, TAN, nitrate, total sulfides (TS), TP and DO values in the lagoon system demonstrated distinct concentrations during three distinct periods of operation (Table 2): i) ice-forming period (days 1-50), ii) full ice-cover period (days 51-115) and iii) spring thaw period (days 116-165). During the ice-forming period (Days 1-50), water temperatures in cell #3 were sufficiently low (1.8 ± 0.5°C) to limit benthic nitrification, leading to lagoon-wide increases in TAN concentrations (peaking at 18.41 mg-N/L) and low nitrate concentrations (max. of 2.09 mg-N/L). TAN concentrations in cell #3 during this first period increased from an initial concentration of 14.59 mg-N/L at day 1 of the study to 18.41 mg-N/L around day 50 of the study. Furthermore, the lack of observable nitrite build-up throughout the period is indicative that nitratation was likely not suppressed due to toxic effects.

Table 2. Average ± SD of TAN, nitrate, TS and TP concentrations in cell #3 throughout the study.

In-lagoon (Cell #3) water quality parameters	***Ice-forming period, Average ± SD, (n)***	***Full ice-cover period, Average ± SD, (n)***	***Spring thaw period, Average ± SD, (n)***
Temperature (°C)	1.8 ± 0.5, (12)	2.1 ± 0.8, (20)	13.4 ± 6.2, (11)
TAN (mg-N/L)	17.01 ± 1.62, (14)	19.23 ± 3.52, (18)	12.58 ± 5.60, (11)
NO_3^- (mg-N/L)	1.32 ± 0.62, (13)	0.33 ± 0.25, (18)	0.42 ± 0.42, (11)
TS (mg-S/L)	0.14 ± 0.14, (4)	6.26 ± 6.17[α], (10) / 0.07 ± 0.08[β], (9)	0.04 ± 0.04, (11)
TP (mg-P/L)	0.38 ± 0.05, (4)	0.38 ± 0.05, (4)	0.08 ± 0.03, (11)
Dissolved Oxygen (mg O_2/L)	0.8 ± 0.6, (13)	0.3 ± 0.1[α], (7)/ 9.0 ± 5.5[β], (13)	8.9 ± 4.4, (11)

α: concentration prior to start of aeration in cell #3; β: concentration after start of aeration in cell #3

As a result the observed data suggests limited nitrifying and denitrifying microbial activity in the lagoon system in wintertime may occur due to a temperature-induced (<4.0°C) reduction in microbial activity and low-level sulfide toxicity in the benthos, which is known to be inhibitory to the nitrifying and denitrifying microbial communities (Joye and Hollibaugh, 1995; Senga et al., 2006; Bejarano Ortiz et al., 2013). Hypoxic conditions prevailed throughout cell #3 across days 1 to 50. The low DO conditions (0.8 ± 0.6 mg O_2/L) were also potentially exacerbated by the production of hydrogen sulfide in the benthic zone of the lagoon, causing a high sediment oxygen demand (Wang, 1980; Chen et al., 2017; D'Aoust et al., 2018, Alqaralleh et al., 2019). However, TS concentrations in the water column remained low during this period (0.14 ± 0.14 mg-S/L). Meanwhile, TP concentrations were stable and saw little fluctuations, with concentrations of 0.38 ± 0.05 mg-P/L being observed during this period.

During the ice-cover period (days 51-115), ice and snow covered the entirety of the lagoon and water temperatures averaged 2.1 ± 0.8 °C. TAN concentrations further increased during this period, reaching an average concentration of 19.23 ± 3.52 mg-N/L and ultimately plateauing at 20.27 mg-N/L between days 94 to 102 (Supplemental Figure 1). Seasonal increases in TAN concentrations show strong correlation to decreases in water temperature (R=0.651, p<0.02), highlighting the sensitivity of lagoon nitrifying communities to the wastewater temperature (Van Dyke et al., 2003). Ice cover completely inhibited aerobic conditions in the top portion of the lagoon water column (Macdonald et al., 1991; Chen et al., 2017; D'Aoust et al., 2017),

The low DO concentrations of the lagoon during ice-cover and the lack of reaeration processes in cell #3 during wintertime caused widespread anaerobic processes to take hold. Specifically, sulfate-reduction in the lagoon led to an average TS concentration of 6.94 ± 6.15 mg-S/L across the ice-cover period, peaking at 17.11 ± 5.66 mg-S/L (Supplemental Figure 1). As ice-cover forms and becomes covered by snow, light and reaeration processes ceased, causing the death and degradation of most lagoon macrophytes, which could potentially provide carbon to sustain sulfate-reduction. With this onset of TS production, a rapid decrease in residual nitrate concentration (1.98 to 0.09 mg-N/L) was observed to occur simultaneously with increases in TS concentrations (Supplemental Figure 1). This correlation between TS and nitrate concentration is believed to be caused by sulfide toxicity to nitrifying microorganisms (Joye and Hollibaugh, 1995; Senga et al., 2006; Bejarano Ortiz et al., 2013), and may be indicative that sulfide toxicity in the benthic zone further limits nitrification in lagoons at low temperatures, beyond effects caused by low temperatures alone. The identification of sulfide benthic toxicity of nitrifiers in lagoons during low temperatures as a contributing factor to loss of nitrification modifies conventional belief that nitrification in lagoons located in northern or temperature climates ceases solely due to temperature inhibition of nitrifying organisms at temperatures lower than 4°C (Sharma and Ahlert, 1977; Painter and Loveless, 1983). As both nitrification and sulfate-reduction processes occur in the benthos of lagoons, it is plausible that in-sediment sulfide exposure may be a secondary, significant inhibitor of nitrification (Caffrey et al., 2019) in wastewater lagoons. Another possible cause for decreases in nitrate could be due to potential autotrophic denitrification performed by sulfate-reducing bacteria (Shao et al., 2010), but the authors believe this is less likely due to relatively low nitrate concentrations. During this period, TP concentrations did not fluctuate significantly, averaging a concentration of 0.32 ± 0.10 mg-P/L.

Hypoxic conditions in the lagoon continued from day 51 until day 77 (0.3 ± 0.0 mg O_2/L), at which point DO concentrations increased significantly due to the planned lagoon cell mechanical aeration. On day 77 of the study, aeration in cell #3 was activated to polish the lagoon effluent in preparation for seasonal discharge to receiving waters during the thaw period. The start of the aeration was governed by the partner municipality and was required due to the limited allowed discharge period outlined in their Environmental Compliance Approval (ECA). The mechanical aeration had the effect of stripping sulfides out of the water column of cell #3. The average DO concentrations in the cell after aeration commenced increasing slowly to 1.6 mg O_2/L by day 92. Average DO concentrations from day 92 to day 115 were of 12.6 ± 0.6 mg O_2/L, outlining a lag period of approximately 15 days between the beginning of aeration and the cessation of widespread pond hypoxia, possibly due to sulfidic oxygen demand in the lagoon masking immediate effects of aeration on DO concentrations. Meanwhile, after stripping sulfides, TS concentrations decreased significantly to 0.09 ± 0.09 mg/L.

Finally, during the spring thaw period (days 116-165), wastewater temperatures increased rapidly from 1.3°C on day 116 to 10.3°C on day 123 and 15.1°C on day 130. This rapid increase in wastewater temperature led to rapid dilution effects, causing TAN concentrations to decrease to 9.00 mg-N/L. Nitrate concentrations during this period were low (<2% of TN as NO_3^-) until temperatures reached 15°C and above, and subsequently increased to 2.51 mg-N/L (31% of TN as NO_3^-). Meanwhile, TS concentrations (0.04 ± 0.04 mg-S/L) remained suppressed due to sustained aeration in cell #3 of the lagoon. At the same time, TP concentrations significantly decreased, with an average of 0.08 ± 0.03 mg-P/L in the water column of cell #3. The decrease in TP concentration is believed to have occurred due to spring thaw period induced dilution and biochemical effects.

Spring thaw caused a reaeration of the lagoon water column due to a significant increase in influent flow rates while simultaneously reallowing wind reaeration pathways (surface oxygen transfer) to increase water column DO. This subsequently caused the cessation of widespread hypoxic conditions in cell #3 of the lagoon. In the presence of oxygen, benthic sulfate-reduction was demonstrated to cease (Atkinson et al., 1995), potentially preventing sulfide-induced dissolution of naturally occurring benthic Fe(III)-(oxyhydr)oxides and the reduction of Fe(III) to Fe(II) (Jansson, 1987; Ding et al., 2012; Kumar et al., 2018), lowering total phosphorus (and specifically orthophosphate) concentrations in the water column. The presence/absence of sulfides has been recognized in many studies to be a major cause of release/adsorption of phosphorus to the water column, due to the interactions between sulfide and other phosphate-containing minerals and compounds (Nürnberg, 1984; Morse et al., 1987; Boström et al., 1988). It is therefore believed that upon reaeration of the lagoon, surface adsorption of phosphate to newly available ferric reaction sites in the benthic zone could potentially be a second, minor pathway for phosphorus removal in the lagoon cells during spring-melt.

3.2. Upgrade MBBR treatment train performance

3.2.1. Nitrifying MBBR reactors

The pilot treatment plant installed at the outlet of a multi-cell facultative wastewater lagoon was operated across a 165-day period spanning winter and spring. During the study, the temperature from the pilot plant's influent wastewater remained below 5°C for a period of 119 days. The performance of the upgrade MBBR pilot system is separated and presented as three distinct operational periods: i) ice-forming period (days 1-50), ii) full ice cover period (days 51-115) and iii) spring thaw period (days 116-165). These periods were selected due to an interest in delineating treatment performance across influent wastewater characteristics entering the pilot system and associating performance of the upgrade pilot treatment train to the lagoon operation. The water quality characteristic results are shown below in Figure 2 across these three periods.

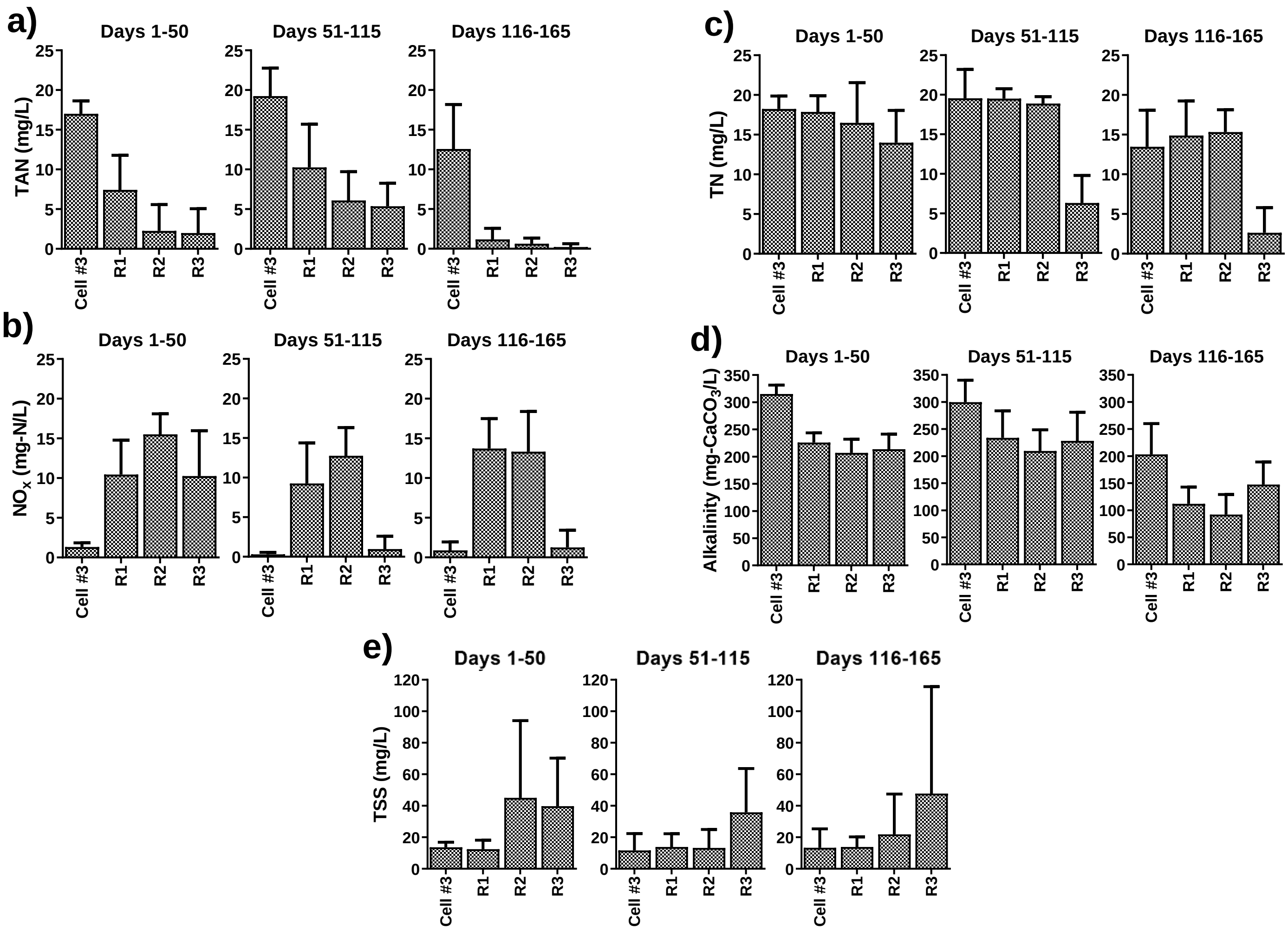

Figure 2. Influent and effluent concentrations across three distinct operational periods: ice-forming period (days 1-50); full ice covered period (days 51-115) and spring thaw period (days 116-165)). a) TAN; b) NO_x; c) TN, d) alkalinity and e) TSS.

Ice-forming period (days 1-50)

The temperatures in the two nitrifying reactors in series, R1 and R2, decreased to an average value of 3.9 ± 1.9°C and 3.4 ± 2.2°C during the ice-forming period of lagoon operation, respectively. The representative period (days 1-50) removal performance in terms of removal percentage and removal rate in R1 and R2 was of 55.6 ± 26.6% (85.0 ± 43.0 g-N/m^3·d^{-1}) and 74.8 ± 29.8% (49.1 ± 44.1 g-N/m^3·d^{-1}). The total treatment train

TAN removal of the nitrifying reactors in-series, R1-R2, was 88.3 ± 18.0% (134.14 ± 35.03 g-N·m^3·d^{-1}) (Figure 2 a, b, c). This performance is similar to results observed in a previous, non-acclimatized ultra-low temperature MBBR nitrification study (Ahmed et al., 2019). Alkalinity consumption in the two nitrifying reactors is observed in this study and is proportional to the oxidation of ammonia in the reactors (Figure 2 d). TSS concentrations did not increase significantly within the nitrification train. This is consistent with previous work, as intrinsic cell yields for nitrifiers are low (Madigan et al., 2012; Forrest et al., 2016; Young et al., 2016a). During this period, NO_2^- accounted for 11.5 ± 14.2% of NO_x in R1 and 57.2 ± % of NO_x in R2. This difference in NO_x is potentially due to the fact that R1 had a slightly lower average operating temperature than R2 (3.9 ± 1.9°C and 3.4 ± 2.2°C respectively), causing nitrite accumulation (Delatolla et al., 2009; Wang and Li, 2015). It has also been hypothesized that sulfides may also have bled through the reactor treatment train inside the pilot treatment plant, which would have caused low-level inhibition of nitrification rates, but this was not verified.

Full ice cover period (days 51-115)

Full ice-cover operation (days 51-115) did not demonstrate a significant change in average reactor temperature in the two nitrifying reactors in-series, R1 and R2 (2.9 ± 1.0°C and 3.5 ± 1.5°C respectively). The average TAN percent removal and removal rate in R1 and R2 was of 45.8 ± 27.8% (54.2 ± 33.2 g-N/m^3·d^{-1}) and 41.2 ± 27.4.0% (24.7 ± 21.0 g-N/m^3·d^{-1}). The total treatment train performance of both nitrifying reactors in series (R1 + R2) was slightly lower than the ice-forming period (day 1-50), with TAN percent removal of 70.5 ± 20.2% (66.6 ± 43.2 g-N/m^3·d^{-1}). Nitrification in the first nitrifying reactor

(R1) was slightly lower in the full ice cover period (days 51-115) as compared to the ice-forming period (days 1-50). Water temperatures did not change significantly between the ice-forming and full ice cover periods, and it is therefore believed that the slight decrease in observed TAN removal rates in the first nitrifying reactor (R1) may have been caused by hydrogen sulfide bleed-through into R1, impacting the performance of the TAN removal. No notable changes were observed in TSS or alkalinity concentrations within the nitrification train. During this period, NO_2^- accounted for 60.2 ± 21.9% of NO_x in R1 and 57.2 ± 21.4% of NO_x in R2. The nitrite accumulation is believed to occur because of NOB temperature inhibition (Wang and Li, 2015). Another possible contributing factor could be that low-level sulfide bleed-through preferentially inhibits the (NOB) compared to the ammonia-oxidizing-bacteria (AOB) (Erguder et al., 2008) in nitrifying biofilm communities.

Spring thaw period (days 116-165)

The spring thaw operation period of the study was characterized by increasing water temperatures and thawing of lagoon ice cover. Average temperatures in the nitrifying treatment train (R1 + R2) rapidly increased from day 116 to day 125, from 3.1°C to 12.5°C and 3.1°C to 12.7°C, respectively. Temperatures further increased until the end of the spring thaw period, reaching 20.6°C in R1 and 20.7°C in R2. The spring thaw period removal performance increased in the nitrifying treatment train (R1 + R2) as compared to the full ice cover period, demonstrated by higher TAN percentage removal and TAN removal rates of 92.1 ± 7.7% (65.1 ± 16.0 g-N/$m^3 \cdot d^{-1}$) and 46.8 ± 28.7% (3.3 ± 4.1 g-N/$m^3 \cdot d^{-1}$) during the spring thaw period. With increasing temperatures and decreasing reactor loading due to drop in influent TAN concentrations, the majority of TAN was removed by the first nitrifying reactor in-series (R1), with the second nitrifying reactor in-series (R2) not performing significant removal. The spring thaw period was also characterized by icemelt and snowmelt, leading to some potential dilution effects on some of the water quality parameters, including TP and alkalinity. The TAN removal performance of the complete nitrifying treatment train (R1 + R2) was of 95.7 ± 4.2% (65.6 ± 17.8 g-N·m^3·d^{-1}). TSS concentrations in R2 increased throughout the period to reach effluent concentrations of 58.5 ± 16.3 mg/L, likely due to the start of reactor starvation and sloughing of some attached biofilm.

Effects of temperature on nitrifying treatment train performance

Throughout the study, the nitrifying treatment train achieved a TAN percent removal of 82.1 ± 19.6%, with an average TAN effluent concentration of 3.42 ± 3.78 mg/L. The maximum nitrifying removal rate observed during this period was 157.25 g-N/m^3·d. During spring-melt, it is possible that the nitrifying treatment train (R1 + R2) may have been TAN limited, especially during the end of the study. Near complete conversion of TAN to NO_x in the first nitrifying reactor (R1) was possible at higher temperatures

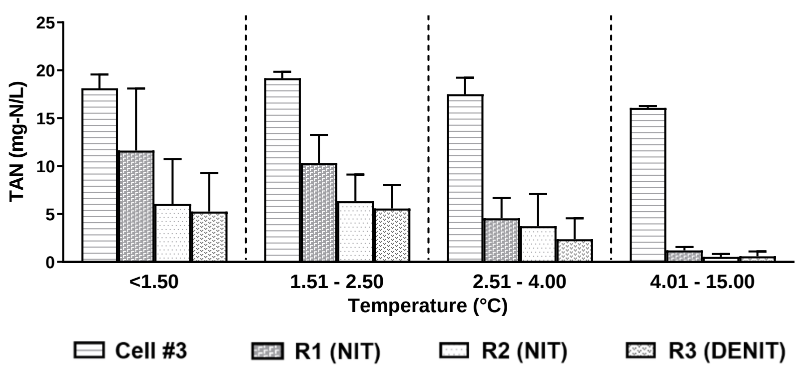

Figure 3. Ranges of TAN concentrations in the MBBR treatment train, by temperature.

observed during the spring thaw period (day 115-165), however, at lower temperatures only partial nitrification was achieved in the first nitrifying reactor (R1), with the remainder occurring in the second nitrifying reactor in-series (R2) (Figure 3). Over the duration of the study, R1's TAN removal performance was on average 15.6% higher at operational temperatures ranging between 1.5 – 2.5°C versus <1.5°C, outlining noticeable operational differences occurring over the span of less than 2.5°C. At temperatures below 1.5°C, the average TAN removal rate of the complete nitrifying treatment train (R1 + R2) was 52.34 ± 0.52 g-N/m^3·d^{-1}.

3.2.2. Denitrifying MBBR reactor

The denitrifying reactor, R3, in the pilot system was installed and operated downstream of the two nitrifying reactors. In an attempt to again identify the effects of temperature on process performance within the MBBR treatment train, results are separated and presented based on the operational conditions of the upstream lagoon system, over the three distinct operational periods: i) ice-forming period (days 1-50), ii) full ice cover period (days 51-115) and iii) spring thaw period (days 116-165).

Ice-forming period (days 1-50)

The ice-forming period was characterized by low wastewater temperatures. The period average temperature was of 3.2 ± 2.6°C. The ice-forming period NO_x removal performance in the denitrifying reactor, R3, was 20.8 ± 23.2% (38.0 ± 49.9 g-N·m^3·d^{-1}). During this period, R3 produced alkalinity at an average rate of 1.97 mg as $CaCO_3$/mg of N denitrified. This is slightly lower than values observed by other in studies of 2.85 – 3.93 mg-$CaCO_3$/mg-N (Jeris and Owens, 1975; Hamlin et al., 2008), but may be explained by the partial denitrification of nitrite and nitrate, hence producing less alkalinity to the system (Asano et al., 2007; Chung et al., 2014). TSS concentrations increased slightly in the denitrifying reactor (R3) due to the relatively high cell yield of denitrifiers compared the nitrifiers (Strohm et al., 2007), leading to ice-forming period-average effluent TSS concentrations of 39.8 ± 30.5 mg/L.

Full ice cover period (days 51-115)

The full ice cover period (days 51-115) was characterized by no significant change in average reactor temperature in the denitrifying reactor, R3, (4.4 ± 2.0°C). The period NO_x removal performance in R3 was 90.8 ± 18.9% (66.9 ± 26.8 g-N·m^3·d^{-1}). During this period, the denitrifying reactor (R3) produced alkalinity at an average rate of 2.01 mg as $CaCO_3$/mg of N denitrified. This is slightly lower than values observed by other in studies of 2.85 – 3.93 mg-$CaCO_3$/mg-N due to the relatively large proportion of NO_x being in the NO_2^- instead of the NO_3^- form in R2 (57.2 ± 21.4% for NO_2^-). Similarly to what was observed during the first period, TSS concentrations increased slightly in the denitrifying reactor (R3), leading to a period-average effluent TSS concentration of 36.8 ± 28.3 mg/L.

Spring thaw period (days 116-165)

The spring thaw period of the study was characterized by increasing water temperatures and thawing of lagoon ice cover. The average temperature in the denitrifying reactor (R3) rapidly increased from 1.6°C to 13.0°C between days 116-125. Temperatures further increased until the end of the period, reaching 20.6°C by the end of the study. The period NO_x removal performance in the denitrifying reactor (R3) was 91.4±

14.7% (62.2 ± 18.3 g-N·m^3·d^{-1}). Lagoon icemelt led to a global dilution effect of most water quality parameters, including nitrogen and alkalinity. During this period, the denitrifying R3 produced alkalinity at an average rate of 3.68 mg as $CaCO_3$/mg of N denitrified. Stoichiometrically, heterotrophic denitrification theoretically yields approximately 3.57 mg-$CaCO_3$/mg-N (Jeris and Owens, 1975; Asano et al., 2007). The closeness of observed alkalinity yield to theoretical values is explained by the near total proportion of NO_x as NO_3^- (82.9 ± 28.4%). TSS concentrations in the denitrifying reactor R3 increased throughout the period to reach effluent concentrations of 60.9 ± 78.0 mg/L TSS. TSS concentrations were notably higher than in the first two periods (ice-forming and full ice cover), likely due to biofilm thinning in warmer operational temperatures, causing sloughing (Bjornberg et al., 2012). It is important to note that the denitrifying reactor removal rate was limited by the system's influent TAN concentration, and therefore no optimization (increase in removal rate) could be performed. The authors also wish to indicate that denitrifying reactor were dosed with COD in excess, to ensure that the system was never COD limited. It is also noted that with such an approach, an additional reactor might need required for polishing and prevent bleed-through of COD.

Effects of temperature on denitrifying reactor performance

Throughout the study, there was significant removal of NO_x in the denitrifying reactor (R3), with an average removal efficiency of 94.3 ± 0.1% of NO_x at temperatures from <1.5°C to 4°C. At higher temperatures (4.0°C to 15.0°C), removal efficiencies decreased to 80.3 ± 19.0% (Figure 4). This decrease in removal efficiency was due to an upset in the operation of the denitrifying reactor over a period of approximately one and a half weeks (failure of a carbon source pump tubing). At temperatures below 1.5°C, the average NO_x removal rate of R3 was of 69.75 ± 24.49 g-N/m^3·d^{-1}.

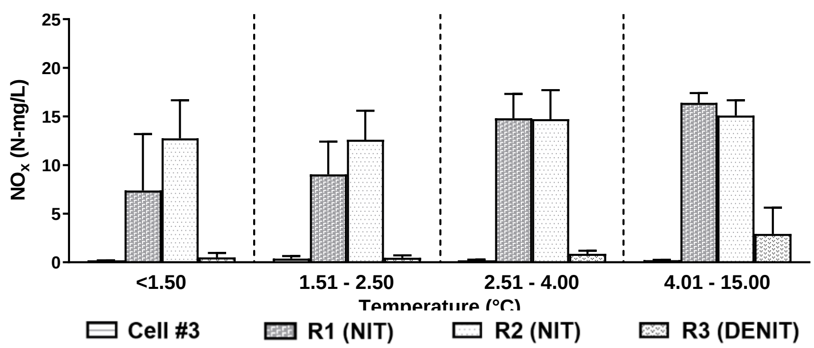

Figure 4. Ranges of NO_x concentrations in the MBBR treatment train, sorted by temperature.

3.2.3. Phosphorus cycling within reactors

TP concentrations within the lagoon's cell #3, the nitrifying reactors in-series (R1 + R2) remained similar throughout the ice-forming (day 1-50) and full ice cover (day 51-115) periods, with average TP concentrations of 0.33 ± 0.09 mg-P/L, 0.29 ± 0.08 mg-P/L and 0.29 ± 0.02 mg-P/L, respectively (Figure 5). The denitrifying reactor, R3, exhibited an interesting trend throughout the study, as the denitrifying organisms assimilated TP, substantially decreasing R3's TP concentrations, rarely exceeding 0.10 mg P/L. TP concentrations within the denitrifying reactor (R3) remained relatively constant throughout the study, with the denitrifying reactor maintaining an average TP removal efficiency of 76.6± 6.2% in reference to R2. This behaviour is well understood and common for post-nitrification denitrifying treatment (Boltz et al., 2012; Mases et al., 2012; Wilson et al., 2012), outlining an additional benefit of deploying denitrifying MBBR systems as an end-of-pipe phosphorus polishing system in constant-discharge or seasonally-discharged lagoon treatment systems due to the phosphorus requirements of heterotrophic denitrifying bacteria to sustain metabolic activity (Kuba et al., 1996; Aspegren et al.,

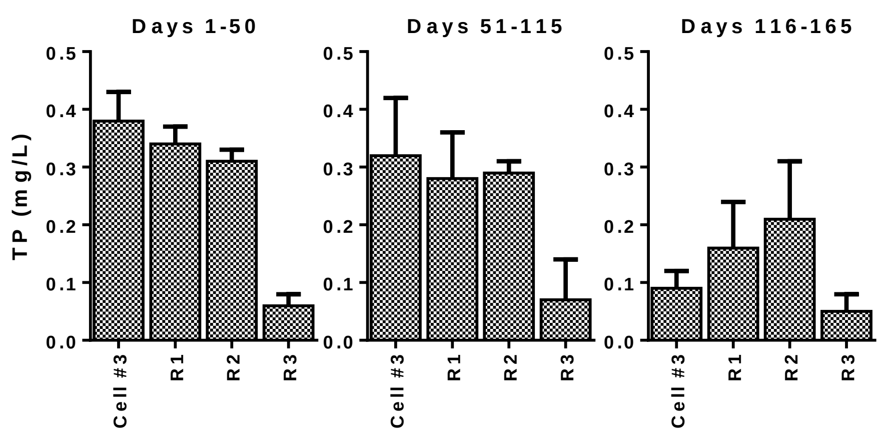

Figure 5. Progression of influent and in-reactor TP concentrations throughout the ice-forming period (days 1-50), full ice cover period (day 51-115) and spring thaw period (116-165).

1998). Meanwhile, minimal decreases in TP were observed in the two nitrifying reactors (R1 + R2), likely due to the intrinsically low cell yield of autotrophic nitrifying organisms (Gee et al., 1990).

As water temperatures increased during spring-melt, influent TP concentrations decreased significantly. It is believed that the decrease was due to reaeration of the lagoon cells, the inhibition of sulfate-reduction and strong dilution effects. Simultaneously, TP concentrations in the nitrifying reactors in-series (R1 + R2) increased. TP concentrations in the first nitrifying reactor in-series (R1) increased to 0.15 ± 0.08 mg-P/L, peaking at 0.36 mg-P/L. Simultaneously, TP concentrations in the second nitrifying reactor (R2) increased to 0.22 ± 0.09 mg-P/L, peaking at 0.47 mg-P/L. It is hypothesized that the increase of TP concentrations in R1 and R2 may have been caused by a biofilm response to changes in ambient TP concentrations. This "homeostasis" response had previously been reported in eukaryotic cells (Wild et al., 2016) via inositol polyphosphate signaling molecules (InsPs). A similar pathway was recently demonstrated for *E. coli* (McCleary, 2017; Chande and Bergwitz, 2018). A similar response may have occurred in the nitrifying and denitrifying biofilm in this study. The release of phosphorus initiated in the nitrifying treatment train (R1 + R2) is thought to have occurred due to a stress response to a rapid decrease in influent phosphorus concentrations. It is therefore suggested that nitrifying communities within biofilms may be capable of regulating bulk liquid phosphorus concentrations during stress events (Nordeidet et al., 1994). It has been shown in previous studies that nitrifying bacterial communities are regarded as phosphorus limited at concentrations below 0.10 mg-P/L (Helness et al., 1999). Studies have also demonstrated a similar trend in denitrifying bacterial communities, where they are considered phosphorus limited at concentrations between 0.03 to 0.10 mg-P/L. Heterotrophic denitrification in attached-growth systems has a relatively high cell yield and requires a minimum TP concentration to occur uninhibited (Nordeidet et al., 1994; deBarbadillo et al., 2014).

4. Conclusions and recommendations

Following the completion of this study, the conclusions outlined are as follow:

1. The MBBR treatment technology has emerged as a suitable and sustainable upgrade technology for existing lagoon and waste stabilization pond facilities operating in temperate, northern, and cold climate countries.
2. Implementation of nitrifying and denitrifying MBBRs in-series at the outlet of a lagoon in a NIT-NIT-DENIT configuration provided treatment robustness in terms of both total nitrogen and phosphorus removal at temperatures as low as 0.6°C.
3. Throughout the 165-day study, the lagoon effluent treated by the pilot treatment plant demonstrates that TN concentrations decreased by an average of 69.0 ± 24.5%, where the average TN concentration exiting the pilot treatment plant were 7.60 ± 5.70 mg-N/L.
4. The nitrifying MBBR reactors achieved TAN removal of 82.1 ± 19.6%, reducing TAN concentrations to an average of 3.42 ± 3.78 mg/L. The denitrifying MBBR reactor achieved NO_x removal of 69.5 ± 37.8%, reducing NO_x to an average of 3.63 ± 5.44 mg/L.
5. It is hypothesized that sulfide toxicity in lagoons might be another important limitation to cold-temperature wintertime nitrification, leading to the hypothesis that nitrification in lagoons situated in northern or temperate climates may not only be inhibited by temperature effects, but also by significant sulfide inhibition events.

6. The use of the denitrification technology treat lagoon effluent demonstrates significant phosphorus removal due to phosphorus assimilation concomitantly occurring with total nitrogen removal through denitrification.
7. Throughout the 165-day study, the lagoon effluent treated by the pilot treatment plant saw TP concentrations decrease by 74.7 ± 20.1%. In addition, the average TP concentrations exiting the pilot treatment plant were 0.05 ± 0.02 mg-P/L.
8. It is hypothesized that the embedded biofilm microorganisms can possibly perform phosphorus homeostasis and increasing bulk-water phosphorus concentrations in the reactors, as a response to low influent phosphorus stress induced by cessation of in-lagoon sulfate-reduction and dilution effects.
9. The results of the study suggest that nitrifying + denitrifying MBBR systems can be installed at the outlets of traditional facultative lagoons to achieve reliable TAN, TN and TP treatment, thereby providing a pathway for substantially reducing the environmental impact of municipal wastewater lagoon discharge on receiving waters in Canada and other northern communities during wintertime operation.

Declaration of competing interests

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

The authors acknowledge the support of the Municipality of Casselman, Veolia Water Technologies Canada and the Natural Sciences and Engineering Research Council of Canada (NSERC) for their support. The authors also thank Mr. Alain Castonguay and Dr. Bradley Young for their continued aid in field operations and logistical support.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Captions for Figures and Tables in text (as ordered).

Figure 1: Schematic of the Casselman municipal facultative lagoon WRRF and MBBR nitrogen removal MBBR pilot systems.

Table 1. Average lagoon effluent concentrations of water quality parameters throughout the study period.

Table 2. Average ± SD of TAN, nitrate, TS and TP concentrations in cell #3 throughout the study.

Figure 2. Influent and effluent concentrations across three distinct operational periods: ice-forming period (days 1-50); full ice-covered period (days 51-115) and spring thaw period (days 116-165)). a) TAN; b) NO_x; c) TN, d) alkalinity and e) TSS.

Figure 3. Ranges of TAN concentrations in the MBBR treatment train, by temperature.

Figure 4. Ranges of NO_x concentrations in the MBBR treatment train, sorted by temperature.

Figure 5. Progression of influent and in-reactor TP concentrations throughout the ice-forming period (days 1-50), full ice cover period (day 51-115) and spring thaw period (116-165).