ABSTRACT

Sensor driven aeration control strategies have recently been developed as a means to efficiently carry out biological nutrient removal (BNR) and reduce aeration costs in wastewater treatment plants. Under load-based aeration control, often implemented as ammonia-based aeration control (ABAC), airflow is regulated to meet desired effluent standards without specifically setting dissolved oxygen (DO) targets. Another approach to reduce aeration requirements is to constantly maintain low DO conditions and allow the microbial community to adapt to the low-DO environment. In this study, we compared the performance of two pilot-scale BNR treatment trains that simultaneously used ABAC and low-DO operation to evaluate the combination of these two strategies. One pilot plant was operated with continuous ABAC while the other one used intermittent ABAC. Both processes achieved greater than 90% total Kjeldal nitrogen (TKN) removal, 60% total nitrogen removal, and nearly 90% total phosphorus removal. Increasing the solids retention time (SRT) during the period of cold (∼12 °C) water temperatures helped maintain ammonia removal performance under low-DO conditions. However, both processes experienced poor solids settling characteristics during winter. While settling was recovered under warmer temperatures, improving settling quality remains a challenge under low-DO operation.

Key words: aeration control, biological nutrient removal, dissolved oxygen, enhanced biological phosphorus removal, nitrification, solids volume index

HIGHLIGHTS

• The performance of two pilot-scale reactors operated under continuous and intermittent low-DO aeration were compared.
• A stepwise increase in SRT led to improved nitrification during cold water periods.
• Intermittent aeration led to improved total nitrogen removal without a loss in EBPR.
• Deteriorated sludge settleability under low-DO remains a challenge to be solved.

INTRODUCTION

Nitrogen (N) and phosphorus (P) are key nutrients that must be removed from wastewater as they can cause eutrophication when introduced into surface waters. Traditional biological nutrient removal (BNR) systems employed at wastewater treatment plants (WWTPs) typically remove N through a two-stage process consisting of aerobic nitrification and anoxic denitrification (Metcalf & Eddy 2003). P is removed under cyclic anaerobic-aerobic conditions through enhanced biological phosphorus removal (EBPR) by polyphosphate accumulating organisms (PAOs) (Barnard 1975). Conventionally, extensive aeration is implemented for both processes, and in some cases, supplemental carbon is added to reach treatment requirements. These requirements are driving efforts to modify operating configurations with the goal of reducing energy and carbon requirements in BNR systems (Zheng et al. 2009; Gao et al. 2014; Keene et al. 2017).

Automated process control through the use of online sensors has gained interest as a means to reduce aeration energy consumption while meeting effluent quality standards (Åmand & Carlsson 2012; Åmand et al. 2013; Rieger et al. 2014). One such control...
strategy is ammonia-based aeration control (ABAC) where airflow is regulated to satisfy oxygen requirements for biological ammonia oxidation. ABAC has been implemented through continuous and intermittent aeration and has been shown to decrease aeration energy demands and increase total N removal in both aeration methods (Regmi et al. 2014, 2015). Intermittent ABAC was originally employed as a strategy to achieve nitrite shunt, which may result in up to a 25 and 40% reduction in oxygen and chemical oxygen demand (COD) requirements, respectively (Regmi et al. 2015). However, recent research has shown nitrite shunt may not be achieved in all intermittently aerated BNR systems (Roots et al. 2019; Klaus & Bott 2020). In general, continuous or intermittent ABAC has been implemented to maintain high dissolved oxygen (DO) conditions during the aerated periods (Regmi et al. 2015). In contrast, we are interested in the use of ABAC in combination with intermittent and continuous aeration in systems aimed at maintaining low-DO conditions (<0.7 mg/L) during aerated periods. This combination of operational conditions could achieve ecological stability and further reduce aeration energy requirements.

In this study we investigated the use of continuous and intermittent ABAC to control aeration in low-DO BNR systems. We used two pilot-scale plants that received primary effluent from the full-scale Nine Springs WWTP at the Madison Metropolitan Sewerage District (MMSD) in Madison, WI, USA. Both of these pilot plants had multiple tanks in series to simulate plug-flow conditions which are not often studied, yet are the most common process used to treat municipal wastewater. We were particularly interested in process performance during the winter period, when wastewater temperature in Madison, WI, USA, can reach below 12 °C and maintaining nitrification during low-DO operation is of concern (Bayer 2018). We were also interested in evaluating pilot plant results with influent flow rates that mimicked full-scale flow variations since most pilot plant research to date does not consider flow variations in the operation of reactors given the intrinsic complexities of implementing this option.

MATERIALS AND METHODS

Pilot-scale reactor operation and control

Anoxic-oxic (AO) reactor with intermittent aeration (AOia)

A pilot-scale treatment train, operated at the Nine Springs WWTP (Madison WI), is configured as an anoxic-oxic with intermittent aeration (AOia) process (Figure 1). The AOia pilot plant was seeded with activated sludge from the full-scale plant

Figure 1 | AOia pilot-scale treatment train configuration showing anaerobic and aerobic tanks, and the clarifier. Return activated sludge (RAS) was pumped from the bottom of the clarifier into the first unaerated tank. Waste activated sludge (WAS) was pumped out of tank AO5. Sensors (NH₄⁺, NO₃⁻/NO₂⁻ and DO) and aeration control details are shown in Figure S1A. (a) During the first 341 days of operation the system included an unaerated tank (AO1), three tanks under the intermittent ABAC regime (AO2, AO3, and AO4), and one high-DO polishing tank (AO5). (b) AOia reconfiguration after day 341. Tank AO1 was replaced by four smaller tanks.
(configured as a University of Cape Town process without internal nitrate recycle) in October 2018 and initially operated at conditions aimed at achieving nitrite shunt. The plant was converted to the intermittent aeration operational strategy on May 1st, 2019 (day 0 in this study) and was evaluated for 483 days, until August 25th, 2020. Between days 0 and 341 the pilot plant system (Figure 1(a)) consisted of a non-aerated tank, three intermittently aerated tanks that maintained low DO conditions (≤0.7 mg/L) when aerated, and a high DO polishing aerated tank with a combined total volume of 2,080 L. On day 342, the system was reconfigured to achieve better plug-flow conditions by replacing the one unaerated 473 L tank with four 140-L tanks (Figure 1(b)). The high DO polishing aerated tank was also replaced by a larger volume tank for a total pilot reactor volume of 2,280 L. Throughout the entire operation the system also included a 1,210 L secondary clarifier. Primary effluent from the full-scale WWTP was used as influent to the pilot-scale reactor. Influent flow rates were aimed to maintain a hydraulic retention time (HRT) comparable to that of the full-scale plant. To achieve this, during the first five months of operation (days 0–174), the influent flow rate was manually controlled and adjusted daily; afterwards, the plant was equipped with a variable speed influent pump that was automatically controlled to mimic real-time changes in full-scale influent flows. During the entire operational period, the daily average influent flow rates were between 2.50 and 6.80 m³/day (Figure S2A), corresponding to daily average HRT of 13.1 ± 5.0 hours (Figure S2B).

Return activated sludge (RAS) was recycled back into the first non-aerated tank at an average of 1.7 m³/day between days 0–158, 3.2 m³/day between days 159–287, and 5.9 m³/day between days 288–483 (Figure S2C). The increase in RAS flow rate throughout the study was implemented in response to the development of poor settling sludge that required faster pumping out of the clarifier to prevent solids overflow into the effluent.

Solids retention time (SRT) variation for winter operation

The SRT was controlled by implementing waste activated sludge (WAS) removal from the last aerated tank and calculating the sludge exiting the plant via the WAS, the effluent, and in any accumulated foam that was manually removed from tank water surfaces. WAS flow rates were adjusted daily using a target SRT and measurements (three times per week) of suspended solids in WAS, effluent, and removed foam.

We implemented a stepwise increase to the SRT starting in fall 2019 by reducing the WAS flow rate (Figure S2E). This was carried out over the course of 6 weeks to bring the SRT from about 10 days to 16 days (day 161–day 203). Then, a stepwise SRT decrease began in February 2020 (day 292) to bring the plant back to 10 days SRT by April 2020, for warmer weather operation.

Aeration control loops and sensors

Intermittent aeration in the A0ia pilot reactor occurred in the three middle aerated tanks (AO2, AO3, and AO4 in Figure 1) by using proportional-integral (PI) control according to cascading control loops (Figure S1A). The primary control loop, NH4 Loop 1, determines aerated and un-aerated periods using NH4 and NO3 concentrations as input variables. NH4 Loop 1 initiates ‘air-on’ mode until a minimum operator-specified NH4 concentration within tank AO4 has been reached, at which point the ‘air-off’ mode is initiated by automatically closing the air valve in tanks AO2, AO3, and AO4. The air-on mode resumes once an operator-defined maximum NH4 setpoint has been reached, the NO3 concentration has reached a maximum allowable decrease, or NO3 is at a concentration of zero. By this control logic, nitrification is prioritized over denitrification by setting a maximum allowable NH4 concentration and limiting the unaerated periods based on the amount of NO3 denitrified. The NH4 and NO3 sensors were placed in the last intermittently aerated tank to perform feedback control which enacts system control based on the difference in a measured variable and a desired setpoint (Rieger et al. 2014) For the majority of the operation the high and low NH4 concentration limits were set at 2 and 5 mg NH4-N/L, respectively. These setpoints were established to ensure NH4 removal in the final high-DO polishing tank and to also operate the sensor in an environment with sufficient NH4 to ensure reliable signal. The maximum allowable NO3 concentration decrease was set at 3.5 mg NO3-N/L to achieve optimal conditions for denitrification while also maintaining sufficient aerated periods for nitrification and phosphorus removal. While in air-on mode the control loop selects a user-defined DO setpoint that is to be achieved in each of the intermittently aerated tanks. The DO setpoint was 0.7 mg/L.

The secondary control loops (DO Loop 2, DO Loop 3, and DO Loop 4) are used to maintain the DO setpoint determined from the NH4 Loop 1 by adjusting the air flow rate to each tank using motorized proportional air valves. If the DO concentration is above the DO setpoint, the air valve position is reduced, and vice versa. During aerated periods, compressed air was provided though a fine-bubble membrane diffuser (Sanitaire, Brown Deer, WI) installed at the base of each aerobic tank. The
final high-DO polishing aerated tank, intended to remove any residual NH$_4^+$ from the prior intermittently aerated tank, is aerated to maintain a DO concentration of 2.0 mgO$_2$/L using an on/off air solenoid valve.

Online sensors used for the control loops included an ion selective electrode to record NH$_4^+$ (AmmoLyt Plus 700 IQ, YSI, Yellow Springs, OH), UV-vis sensor for NO$_3^-$ and NO$_2^-$ (NiCaVis 701 IQ, YSI, Yellow Springs, OH), and optical DO sensors (IQ SensorNet FDO 701, YSI, Yellow Springs, OH).

University of Cape Town (UCT)-type reactor with continuous aeration (UCTca)

A pilot-scale system simulating the configuration of the full-scale process at MMSD but operated with continuous low-DO conditions (Figure 2(a)) was also operated, for comparison with the AOia pilot plant. This process, referred to as the UCTca pilot plant, is a modification of the UCT configuration that does not use internal recycle (Park et al. 2006; Keene et al. 2017). The pilot plant was seeded in September of 2018 with activated sludge from the full-scale process. However, most results reported here correspond to May 1st, 2019 (day 0) to August 25th, 2020 (day 483). The anaerobic, anoxic, and aerated portions of this pilot plant initially consisted of five tanks for a combined total volume of 2,180 L. The anaerobic portion of the plant was reconfigured on day 446 to achieve better plug-flow conditions by replacing a 284-L tank with three 140-L tanks (Figure 2(b)). In addition, the volume of the anoxic portion was decreased from 473 L to 140 L for a total pilot reactor volume of 1,980 L. A 1,010 L secondary clarifier was also included for the entire operation. Similar to the operation of the AOia pilot plant, influent flow rates were initially adjusted manually in the UCTca and starting at day 174 they were controlled using speed influent pumping that automatically changed flow rates to mimic real-time changes in full-scale influent flows (Figure S2A). The influent flow rate into the UCTca reactor varied between 2.14 and 7.90 m$^3$/day resulting in an average HRT of 14.0 ± 2.1 hours. As with the AOia pilot plant, the RAS flow rate was increased in response to poor sludge

![Figure 2](http://iwaponline.com/wst/article-pdf/85/2/578/998171/wst085020578.pdf)
settling characteristics (Figure S2B) and stepwise SRT adjustments were implemented to achieve a higher SRT during the low temperature period (Figure S2C) as described above for the AOia pilot plant.

Aeration in this pilot plant was also controlled based on NH$_4^+$ concentration. An NH$_4^+$ online sensor (described above) was installed in the second aerated tank of the UCTca treatment train (tank UCT4 in Figure 2). Each of the three aerated tanks was equipped with a DO sensor (described above).

Similar to the AOia configuration, aeration control within the UCTca pilot plant was executed using a PI control loop based on NH$_4^+$ concentration. However, aeration was continuous and a single NH$_4^+$ concentration was targeted (Figure S1B). The first loop, NH$_4^+$ Loop 1, adjusts the DO setpoint (allowed to be between 0.1 and 0.6 mg/L) in the two middle aerated tanks to maintain an operator-defined NH$_4^+$ setpoint in the second aerated tank (tank UCT4). If NH$_4^+$ concentration decreases past the setpoint, the DO setpoint also decreases, and vice versa. Throughout the operation described in this study, an NH$_4^+$ concentration of 5 mg NH$_4^+$-N/L in tank UCT4 was targeted. The secondary control loops (DO Loop 2 and DO Loop 3) adjust the position of the proportional air valve to achieve the DO setpoint (in tanks UCT3 and UCT4) defined by the primary NH$_4^+$-based loop. The last aerated tank (UCT5) was operated under a separate control loop to initially maintain a DO of 0.6 mg/L through day 218, at which point it was switched to maintain a DO concentration of 1.0 mgO$_2$/L to slow denitrification in the clarifier.

**Analytical methods**
Filtered influent and effluent grab samples from both pilot plants were collected three and six times per week, respectively. Grab samples from each tank in both pilots were collected once per week. Grab samples from the influent, effluent, AO5, LD1a (or LD1), and LD5 were also collected for solids analysis. For analyses of soluble components, samples were immediately filtered through a 0.45-micron filter (Nitrocellulose Membrane Filters, EMD Millipore Corp., Darmstadt, Germany) and stored at 4 °C until further analysis. Unfiltered samples were used for solids measurements.

Total suspended solids (TSS), volatile suspended solids (VSS), filtered total Kjeldahl nitrogen (TKN), and filtered total phosphorus (TP) were measured according to standard methods (APHA et al. 2005). Nitrite (NO$_2^-$-N) and nitrate (NO$_3^-$-N) were measured using high performance liquid chromatography (HPLC) as described elsewhere (Keene et al. 2017).

**Statistical analysis**
Statistical significance analyses to compare the performance between the AOia and UCTca systems were performed in R (R Core Team 2016). A Shapiro-Wilk test was performed to determine whether a dataset was normally distributed. For normally and non-normally distributed data a $t$ test and a Wilcoxon rank-sum test were performed, respectively. A $p$-value less than 0.05 suggests the difference in variables is statistically significant.

**RESULTS AND DISCUSSION**
Experimentation at the pilot-scale is intended to provide experimental observations to determine whether a process is ready for full-scale implementation. Ideally, wastewater treatment research at the pilot-scale should consider the expected variations in organic and nutrient loadings and the fluctuations in flow rates and temperatures. In addition, testing wastewater treatment processes at the pilot-scale provides opportunities for evaluating other potential challenges to full-scale implementation, such as sludge bulking and foaming. Our pilot-scale research program has considered variations in wastewater characteristics and water temperature by tapping directly into primary effluent from the full-scale WWTP and housing the pilot plants in locations that are not temperature controlled (Kang & Noguera 2007; Keene et al. 2017).

Since the demonstration that nitrification can be successfully implemented using low DO conditions (Park & Noguera 2004) and the recognition that BNR operated under low DO is a feasible approach to reduce energy use during wastewater treatment, approaches to control aeration using sensor technology have emerged (Åmand et al. 2013; Klaus & Bott 2020). Aeration control based on NH$_4^+$ concentrations has gained momentum as a practical approach for automating aeration while maintaining desirable water quality standards (Medinilla et al. 2020). The recent attention to ABAC with continuous or intermittent aeration (Klaus & Bott 2020; Roots et al. 2020) prompted us to perform a comparison of these two aeration control technologies in pilot plants that simulate the general layout of common full-scale plug flow BNR systems operated in a temperate climate and under low-DO conditions. The most important observations in this comparison are described and discussed below.
Nutrient removal was better in the AOia than in the UCTca process

The AOia and UCTca pilot plant performance was assessed over a period of time that included summer and winter water temperatures (minimum below 12 °C and maximum above 25.0 °C) (Figure S2A). Under the different aeration modes employed, both plants exhibited effective nitrification, although the extent of TKN removal was slightly better in the AOia plant than in the UCTca plant (Figure 3(a)). Influent filtered TKN was 34.5 ± 6.5 mg TKN/L. The AOia system achieved an average effluent TKN of 1.82 ± 1.34 mg TKN/L, equivalent to a 95% removal efficiency. The UCTca reactor experienced a higher average effluent TKN of 2.16 ± 1.09 mg TKN/L, a significantly different result ($p = 8.4e-06$) that corresponds to a removal efficiency of 93%. This TKN removal efficiency was consistent throughout the operational period, indicating that the practice of increasing SRT as winter approaches was effective and eliminated earlier problems with nitrification when the SRT was not preventively increased (Figure S3). The better nitrification observed in the AOia plant could be due to the polishing tank in the AOia operated at a higher DO concentration (2 mg/L) than the tank in the UCTca plant (1 mg/L).

Figure 3 | Comparison of nutrient removal performance between the two pilot-scale systems. Blue and red dashed vertical lines indicate the AOia and UCTca configuration change, respectively. The grey dashed vertical line indicates the change to variable influent flows, and the blue and red lines indicate when the reactor configurations were changed in the AOia and the UCTca plants, respectively. (a) Influent and effluent total Kjeldahl nitrogen (TKN) and effluent nitrate (NO₃⁻) in filtered samples. To simplify the figure, nitrite was (NO₂⁻) was not plotted, but concentrations on average were less than 1.0 mg/L. Note that the lack of nitrate data for 36 days after Feb 2020 was due to the inability to perform these tests due to the covid-19 pandemic. (b) Total phosphorus (TP) in filtered samples.
Effluent nitrate concentrations (Figure 3(a)) were also statistically different ($p = 1.7 \times 10^{-5}$) in both plants, averaging 8.60 ± 3.57 mg N/L in the AOia and 10.06 ± 3.09 mg N/L in the UCTca, respectively. While there was not a clear difference in effluent nitrate concentrations during the first half of pilot plant operation, effluent nitrate concentrations in the AOia became consistently lower than in the UCTca after 231 days of operation. Neither pilot plant showed nitrite accumulation in the effluent. In the AOia plant, effluent nitrite was 0.23 ± 0.16 mg N/L, and in the UCTca it was 0.30 ± 0.23 mg N/L, results that are not statistically different ($p = 0.38$).

With better TKN removal and lower effluent nitrate concentrations in the AOia, total nitrogen (Garcia Martin et al. 2006) removal was also statistically different in the two pilot plants. The AOia treatment train achieved an average TN removal efficiency of 68%, compared to 63% in the UCTca train ($p = 0.004$) (Figure 4(a)). With the configuration of these pilot plants, denitrification occurs in two different locations: the unaerated zones at the head of each plant and either the continuously aerated low-DO zone in UCTca or the intermittently aerated zone in the AOia. Using a mass balance approach (i.e. the difference between TN in the influent, unaerated zones, and the effluent), we evaluated the extent of denitrification in the unaerated and aerated zones on a mass per day basis. Total denitrification within the AOia ($105 \pm 34 \text{ gN/day}$) was greater

![Figure 4](image-url)

**Figure 4** | Total nitrogen (Garcia Martgarin et al. 2006) removal efficiency between the two pilot-scale systems reveals the AOia pilot plant achieves better TN removal than the UCTca pilot plant. Dashed vertical lines indicate the change to variable flow rates in both plants (grey) and configuration changes in AOia (blue) and UCTca (red). (a) Comparison of TN removal efficiency, (b) Comparison of denitrification in unaerated and aerated zones. Note that the lack of data for 36 days after Feb 2020 was due to the inability to perform nitrite and nitrate tests due to the covid-19 pandemic.
than in the UCTca (94 ± 33 gN/day) pilot plant, a statistically insignificant difference (p = 0.074). The contribution of unaerated and aerated zones to denitrification was also estimated (Figure 4(b)). In this case, denitrification in the unaerated zones was statistically higher (p = 2.0e-12) in the UCTca pilot (47 ± 14 gN/day) than in the AOia pilot (29 ± 12 gN/day). The difference in the unaerated zone denitrification could be attributed to RAS flow rates, since they were higher in the UCTca pilot system for most of the operational period (Figure S2B). Conversely, the opposite was true for denitrification attributed to the low DO (47 ± 32 gN/day) or intermittent zones (76 ± 32 gN/day), likely because the cyclic anoxic periods experienced during intermittent aeration in the AOia pilot create more opportunity for denitrification than the low-DO conditions in the UCTca. Denitrification in the aerated zones varied widely in both pilots, with minimal to no SND occurring in the UCTca pilot on some days, although denitrification in these zones substantially increased when the flows into the pilot plants were set to mimic the flow variations observed in the full-scale plant (Figure 4(b)).

Biological P removal, which is known not to be affected by low-DO conditions (Keene et al. 2017), was statistically similar in both plants (Figure 5(b)). Influent filtered TP throughout the course of operation was 3.8 ± 0.7 mg/L. Effluent filtered TP for the AOia and UCTca reactors were statistically similar, at 0.4 ± 0.4 mg TP/L and 0.3 ± 0.3 mg TP/L, respectively (p = 0.24).

A final observation regarding nutrient removal is that the implementation of flow variability in the middle of the research period did not appear to negatively affect N or P removal efficiencies. Pairwise comparisons of TKN, TN, and P removal efficiencies before and after implementation of flow variability shows no significant statistical decrease in efficiency after flow variability implementation (Table S1). Rather, the comparisons show either no statistically significant differences, or statistically significant improvements in efficiency. This result is reassuring since most pilot plant research to date does not consider the flow variations in the operation of reactors given the intrinsic complexities of implementing this option.

**Oxygen, nitrogen species, and phosphate dynamics during intermittent aeration**

Online chemical analysis sensors were used to carry out intermittent ABAC and gather additional high-frequency data in the AOia pilot. A plot of sensor recordings taken from day 273 to day 279 is shown in Figure 5, which is typical of daily trends after the flow rate was switched to mimic flow changes in the full-scale plant. Several important characteristics of the

![Figure 5](http://iwaponline.com/wst/article-pdf/85/2/578/998171/wst085020578.pdf)

**Figure 5** | Sensor recordings on days 273–279 in the AOia pilot plant. (a) HRT variation in both pilot plants implemented to mimic flow variability in full-scale plant. (b) Sensor recordings of NH₄⁺, NO₃⁻, and NO₂⁻ in tank AO4 show NH₄⁺ oscillates between the allowable limits without a diurnal pattern. NO₃⁻ fluctuates in response to air-on/air-off periods, diurnal flows, and periods of high and low loadings. Significant NO₂⁻ accumulation was not observed.
intermittent ABAC control strategy are depicted in Figure 5. First, the reported HRT from the sensor control unit represents the fluctuations in flow experienced by the pilot plant including the expected diurnal cycles and sudden flow fluctuations when pumping stations turn on and off (Figure 5(a)). Second, the sensor readings for NH$_4$ in tank AO4 show consistent cyclic NH$_4$ concentrations between the setpoints of 2 and 5 mg/L (Figure 5(b)), which effectively sets the aeration on/off cycles in tanks AO2, AO3, and AO4. Throughout the entire operation the average DO during air-on periods in tanks AO2, AO3, and AO4 were 0.55, 0.58, 0.64 mgO$_2$/L, respectively. The final high-DO polishing tank maintained average DO concentration of 1.78 mg/L throughout the operation. Third, nitrate concentrations fluctuate not only in response to the air-on/off pattern, but also experience diurnal cycles (Figure 5(b)), with higher concentrations during the evenings each day. The figure shows that during high loading periods NH$_4$ concentrations remain controlled, but nitrate concentrations tend to increase as the plant spends more time in the air-on mode than the air-off mode. The latter is evident on the slopes and length of the NH$_4$ cycles. During the nitrate peaks, the average durations of air-on and air-off periods were 84 and 59 min respectively, whereas during the nitrate valleys, the averages were 64 and 61 min, respectively (Figure S4).

Fourth, during the periods of low nitrogen loading into the plant, nitrate concentration can be remarkably low, as observed for day 276. Finally, small fluctuations in nitrite concentration are observable, which increase in magnitude during high loading periods, but in general they remain low throughout the different cycles.

It is noteworthy that the sensor-measured nitrate concentrations in the AOia process exhibited the described superimposed fluctuations. Although these measurements were taken before a continuously aerated tank (i.e. a polishing AO5 tank), some of these fluctuations may carry on and affect effluent characteristics at a level of resolution that was not captured with the schedule used for collecting effluent grab samples. Nevertheless, effluent nitrate concentrations in the AOia plant were consistently lower than the effluent concentrations from the UCTca, especially after implementing the flow variations that mimic flow variations in full-scale. Thus, from a perspective of TN removal, our results show that intermittent ABAC is better than continuous ABAC because the intermittent aeration created the anoxic periods that strongly favored denitrification.

The reduction in aeration requirements during the intermittent ABAC operation can be assessed at a larger scale by calculating the amount of time that the plant was in air-on mode versus air-off throughout the 483-day operation (Figure S5). Overall, the AOia pilot plant spent 42% of the time in the air-off mode (i.e. a cumulative total of 202 days out of 483 days). However, the amount of time spent in air-off mode fluctuated widely throughout the operation, with some periods being as low as 28% of the time, and other periods as high as 61% throughout the operation (Figure S5). Using a standard aeration design guide (Sanitaire) an estimate of energy savings can be calculated. Under a volume-weighted average DO concentration across all aerated zones measured in the AOia pilot process of 0.6 mg/L and the average time spent in air-on mode, an energy saving of 51% can be realized when compared to a continuously aerated process achieving a DO concentration of 2.0 mg/L, the current conditions at Nine Springs Wastewater Treatment Plant. It should be noted that actual energy usage to turn on/off high-power blowers used to provide aeration full-scale is not captured in this estimate, and operating blowers in such a manner may not be practical given constraints of current aeration technology.

We were particularly interested in EBPR performance in the intermittently aerated pilot plant, since the cyclic aeration creates anoxic conditions in a section of the conventional anaerobic/aerobic EBPR process where phosphorus uptake is expected to take place because oxygen is present and can serve as the external electron acceptor. In the absence of a sensor to detect the P concentration dynamics during air-on and air-off periods, we measured PO$_4$-P concentrations every 10 minutes during an intermittent ABAC aeration cycle in tank AO2 (Figure S6A). P increases during the air-off periods and decreases during the air-on periods, consistent with P removing activity by PAOs when oxygen is available. Samples for this test were taken early in the treatment train (tank AO2) to best capture P dynamics since similar measurements in downstream tanks (e.g. AO4) show very low P concentrations (Figure S6B). During the air-on period in tank AO2, P decreased at a rate of 21.7 mgPO$_4$-P/L-hr, while P increased at a rate of 16.0 mgPO$_4$-P/L-hr during the air-off period. To determine if the observed rate of P increase was due solely to advection or included P release from PAOs during intermittent aeration, the expected rate of P increase due to advection alone was calculated to be 12.9 mgPO$_4$-P/L-hr using the monthly average PO$_4$-P from the upstream tank (AO1) and the total flowrate (i.e. influent and RAS flowrates). Because the observed P increase during the air-off period was greater than the predicted P increase due to advection, the results provide evidence that cyclic P release and uptake is occurring during the air-off cycles of intermittent aeration.

These results do not provide any evidence of denitrification being associated with phosphorus uptake, as would be expected if denitrifying PAO activity was taking place (Zeng et al. 2003b). Instead, the data support the hypothesis that under the rapid air-on/off cycles that are established with intermittent aeration, PAOs may quickly shift to P release during air-off periods. The
ability of PAOs to use nitrite or nitrate as the electron acceptor (i.e. denitrifying PAO, DPAO) has been investigated (Zeng et al. 2003a; Camejo et al. 2016; Gao et al. 2019) and existing literature suggests that some PAO strains are able to use one or both of these nitrogen species as electron acceptors, while other strains lack specific enzymes to perform full denitrification. In an intermittently aerated sequencing batch reactor (SBR) with aerobic/anoxic interval lengths of 10–20 minutes, Roots et al. (2020) reported that DPAO activity was insignificant due to a combination of low nitrite concentrations and DO inhibition. However, with longer anoxic intervals, such as those seen in this study (~61 min), others have reported DPAO activity (Lee et al. 2001). In the absence of a P sensor, our discrete measurements of P fluctuations during intermittent aeration in the AOia pilot were not supportive of DPAO activity in the AOia pilot plant. Further analysis of P cycling will be necessary to ascertain any potential role of DPAO activity in the AOia process.

**Oxygen and ammonium dynamics during continuous aeration**

Online ammonium, \( \text{NH}_4^+ \), and DO sensors were used to carry out continuous ABAC aeration in the UCTca pilot. Sensor data trends are shown in Figure 6 for day 273 thru day 279, which is the same period shown in Figure 5 for the AOia plant and typical of daily trends after the flow rate was switched to mimic flow changes in the full-scale plant. Ammonium in the UCT4 tank (Figure 6), where the sensor was located, fluctuated around the set point of 5.0 mgN/L and was generally stable regardless of the diurnal flow variations (Figure 5(a)). In this control system, a DO set point was not selected *a priori* for tanks UCT3 and UCT4. Rather, DO was allowed to fluctuate in the tanks between 0.1 and 0.6 mg/L, in response to the deviation between measured \( \text{NH}_4^+ \) concentration and the \( \text{NH}_4^+ \) setpoint. In general, during the entire operation DO concentrations in tank UCT3 and UCT4 averaged 0.38 and 0.35 mg/L, respectively. The DO concentration in tank UCT5, independently controlled to a setpoint of 0.6 mg/L (day 0–217) and 1.0 mg/L (day 218–483), exhibited average DO concentrations of 0.73 mg/L throughout the entire operation. Under the average volume-weighted DO concentration experienced in the UCTca pilot of 0.5 mg/L, a 16% in energy savings may be realized when compared with a DO concentration of 2.0 mg/L under continuously aerated conditions.

**Sludge settling characteristics**

Our prior observations from the UCTca pilot plant operating at low-DO conditions showed that efficient TKN removal is difficult to achieve when the temperature decreases during the winter and the SRT is not preemptively increased (Figure S3), whereas nitrification in the full-scale treatment plant is not affected during winter (Park et al. 2006), likely because it operates with higher DO concentrations. One option that we explored to maintain TKN removal during low temperature conditions was to preemptively increase the SRT to accumulate more nitrifying biomass (Supplementary document). The stepwise SRT changes influenced both the suspended solids concentration and the solids volume index (SVI) (Figure 7). Although the SRT
increase was indeed effective at maintaining nitrification efficiency throughout the winter (Figure 3(b)), the SVI in both plants increased at similar rates reaching nearly 300 mL/g in the UCTca pilot and above 400 mL/g in the AOia pilot, exceeding the desired full-scale SVI values of less than 120 mL/g (Martins et al. 2004). This degradation in sludge settling capacity necessitated an increase in RAS flow rates to much higher values than recommended for activated sludge operation (Metcalf et al. 2014) in both pilot plants to prevent biomass loss in the effluent. Foaming on the clarifiers also became a recurrent problem with the high SRT operation. Although, the solids concentrations decreased during the stepwise SRT decrease after the winter period (Figure 7(a)), a recovery in SVI was not observed in either plant until the end of the study period, and sludge settleability was worse in the AOia than in the UCTca plant. This limits the applicability of full-scale implementation of the tested stepwise SRT increase, which brought up the SRT to a value as high as 16 days and resulted in total suspended solids concentrations as high as 5,000 mg/L, which are too high for conventional activated sludge processes.

The challenge of increased SVI has been recognized for operating activated sludge processes under low-DO conditions, which results in SVIs that are generally higher than those in conventional BNR processes (Huang & Ju 2007). For instance, Han et al. (2018) reported SVIs between 120 and 220 mL/g at DO between 0.15 and 0.65 mg/L and Martins et al. (2003) reported SVIs over 250 mL/g at DO < 1.1 mg/L. The main problem with increased SVI is the requirement for additional
surface area of clarifiers, which in practice will require the construction of additional clarifiers when retrofitting a high-DO process into a low-DO process. Alternatively, should SVI levels reach such high values during full-scale operation, other corrective actions may need to be implemented, such as polymer dosing, RAS chlorination, tertiary filtration or selective sludge wasting, that will increase operational costs. Thus, methods to improve sludge settleability through selective sludge wasting (Liu et al. 2005, 2012; Sun et al. 2019), selector zone design (Daigger et al. 2018), or other factors that may influence dense floc formation and retention during low-DO BNR operation remains an issue that requires further investigation.

CONCLUSIONS

- Pilot-scale treatment trains operated using intermittent and continuous ABAC were both effective at ammonia oxidation, total nitrogen removal, and phosphorus removal.
- A statistically significant difference in the nitrification and denitrification performance was observed when comparing both pilot plants, with the AOia plant performing better than the UCTca plant. The location where denitrification occurred was different, with most denitrification occurring in AOia in the intermittently aerated zones and in UCTca in the unaerated zone.
- Intermittent and continuous ABAC were effective at controlling aeration and maintaining the operation of the pilot scale reactors at low-DO conditions and efficient nutrient removal.
- Stepwise increases to SRT proved effective at sustaining nitrification throughout the winter, although the increase led to increased SVI.
- EBPR was not inhibited by continuous or intermittent aeration at low DO. There was no evidence of DPAO activity under intermittent aeration in the AOia pilot plant.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

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