Startup and Pilot Testing of MBBR and IFAS Partial Denitrification/Anammox Processes

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Academic Abstract

Partial denitrification/anammox (PdNA) is an emerging biological nutrient removal (BNR) process that can be used to remove ammonia (NH_3) and NO_x from wastewater. This process is a combination of partial denitrification (PdN), which serves to reduce nitrate (NO_3) to nitrite (NO_2), and anaerobic ammonia oxidation, or anammox (AMX), which uses the nitrite as an electron acceptor to oxidize ammonia. PdNA provides significant aeration and external carbon savings when compared to the conventional nitrification/denitrification biological removal process for nitrogen but has been difficult to implement in mainstream treatment conditions due to many factors. One of these factors is the slow growth rate and startup time of anammox bacteria. This research first focused on determining the required startup time and startup optimization methods for a proposed mainstream polishing PdNA MBBR at Hampton Roads Sanitation District's James River Treatment Plant (JRTP). These two MBBRs were started with either virgin carriers or carriers coated with a preliminary biofilm and were fed secondary effluent The MBBRs were dosed with glycerol based on a feedforward carbon control approach and were not seeded with anammox at any point. Anammox activity was detected in the preliminary biofilm and virgin media MBBRs approximately 52 and 86 days after startup, respectively. Based on these results, starting up a mainstream PdNA reactor without seed is possible, and using preliminary biofilm carriers can speed up startup by approximately one month. A second experiment was conducted to determine the carbon demand and nitrogen removal capabilities of a glycerol fed PdNA MBBR and AMX MBBR in series. A nitrifying MBBR was also added to the MBBR train to test how well residual nitrite leaving the MBBRs could be polished off to limit ozone/disinfectant demand downstream. Additionally, a methanol-fed PdNA integrated fixed-film activated sludge (IFAS) reactor was also operated to determine the carbon demand and nitrogen removal capabilities for a PdNA process in an IFAS reactor. The PdNA and AMX MBBRs had average effluent TIN concentrations of 3.75 ± 1.25 and 2.81 ± 1.21 mg TIN/L, respectively, with a COD dosed per TIN removed ratio (COD/TIN) of 2.42 ± 0.77 g COD/g TIN for the entire process. The PdNA IFAS reactor had average effluent TIN concentrations of 4.07 ± 1.66 mg/L and 3.30 ± 0.96 mg/L at hydraulic retention times (HRTs) of 30 and 25 minutes. At these two HRTs, the PdNA IFAS process had a COD/TIN ratio of 1.08 ± 0.38 and 2.18 ± 0.99 g COD/g TIN, respectively. Overall, this indicated that both the PdNA MBBR and IFAS processes could reach low effluent TIN limits in mainstream conditions with low demand for COD, even with relatively low and unstable PdN efficiencies. Additionally, the nitrifying MBBR managed to keep the effluent nitrite concentration consistently below 0.5 mg/L at ammonia and nitrite influent loadings rates of 0.055 ± 0.035 and 0.379 ± 0.112 g N/m^2/day. This research demonstrated that starting a PdNA process in mainstream conditions, without seed, can be accomplished within a reasonable timeframe and provides knowledge that can help engineers better understand the advantages of PdNA and design and startup mainstream polishing PdNA MBBRs and IFAS reactors.

Startup and Pilot Testing of MBBR and IFAS Partial Denitrification/Anammox Processes

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General Audience Abstract

As the human population continues to grow and wastewater discharge requirements continue to become more stringent, researchers and engineers have been exploring new technologies and methods to treat wastewater more efficiently. Once such method that is currently being explored is the integration of anaerobic ammonia oxidation, or anammox (AMX), bacteria with a variety of wastewater treatment technologies to remove nitrogen more efficiently from wastewater. AMX synchronously remove ammonia, which exists naturally in wastewater, and nitrite through an oxidation/reduction reaction in which the nitrogen leaves the wastewater in the form of dinitrogen gas. This process greatly reduces the amount of aeration and external carbon needed for the removal of nitrogen from wastewater compared to the commonly used method of full nitrification and denitrification, which are large operational costs at a wastewater treatment plant. While AMX have found use at full-scale plants in treating concentrated sidestreams with the use of partial nitrification (PN) to produce nitrite for the AMX, little progress has been made to integrate AMX into a full-scale mainstream treatment process where the stream is less concentrated and not ideal for consistent PN. Partial denitrification (PdN), however, has shown some promise in reliably producing nitrite in mainstream conditions for AMX usage. On top of the demand for nitrite, AMX bacteria also grow very slowly compared to most bacteria, which means these processes require relatively large amounts of time to get started. A common strategy for decreasing the startup time of AMX processes has been the addition of AMX biomass to a reactor during startup, but this is not feasible in a full-scale mainstream process due to the large amount of biomass that would be required. Therefore, other methods for startup optimization must be evaluated, which this study sought to do through two startup experiments in separate mainstream polishing moving bed biofilm reactors (MBBRs), which use plastic carriers to develop biofilms of bacteria. These two MBBRs were started with different types of carriers in them, one with carriers coated with a pre-established preliminary biofilm and one with brand-new, virgin carriers, to see what kind of effect these different types of carriers have on AMX startup time. AMX activity was detected in the preliminary biofilm and virgin media MBBRs approximately 52 and 86 days after startup, respectively, which was much quicker than expected. This indicates that starting up a mainstream PdNA reactor without seed is possible and using the preliminary biofilm carriers can speed up startup by approximately one month. After the startup experiment, one of the MBBRs was converted to a PdNA integrated fixed-film activated sludge (IFAS) reactor through the addition of activated sludge. This PdNA IFAS reactor was operated alongside a PdNA MBBR and AMX MBBR to test their nitrogen removal and carbon savings capabilities. Operation of these reactors demonstrated that both a PdNA MBBR or IFAS process are capable of consistently removing nitrogen to low levels with relatively low amounts of external carbon addition, even with inconsistent PdN. Overall, this research provided valuable insight into startup methods and design requirements of PdNA MBBRs and IFAS reactors which will make the implementation of these treatment processes more feasible.

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List of Abbreviations

A/B: Adsorption/Bio-oxidation
AerAOB: Aerobic ammonia oxidizing bacteria
AMX: Anammox
AvN: Ammonia versus NOx (nitrate plus nitrite)
BNR: Biological nutrient removal
CETP: Chesapeake Elizabeth Treatment Plant
COD: Chemical Oxygen Demand
COD/TIN: Carbon dosed to total inorganic nitrogen removed
COD/NO_3: Carbon dosed to nitrate removed ratio
C/N: Carbon dosed to nitrogen removed ratio
CSTR: Continuous stirred tank reactor
DO: Dissolved oxygen
EDTA: Ethylenediaminetetraacetic acid
EQ: Equalization
FdN: Full denitrification
GAC: Granular activated carbon
HRSD: Hampton Roads Sanitation District
HRT: Hydraulic residence time
IFAS/IFFAS: Integrated fixed-film activated sludge
JRTP: James River Treatment Plant
MBBR: Moving bed biofilm reactor
MLSS: Mixed liquor suspended solids
MLVSS: Mixed liquor volatile suspended solids
N_2: Dinitrogen gas
NH_3-N: Ammonia as nitrogen
NOB: Nitrite oxidizing bacteria
NO_2-N: Nitrite as nitrogen
NO_3-N: Nitrate as nitrogen
OHO: Ordinary heterotrophic organisms
OP: Orthophosphate
PdN: Partial denitrification
PdNA: Partial denitrification/anammox
PN: Partial Nitritation
PNA: Partial nitritation/anammox
SBR: Sequencing batch reactor
SOR: Surface overflow rate
sCOD: Soluble chemical oxygen demand
SDNR: Specific denitrification rate
TIN: Total inorganic nitrogen
TSS: Total suspended solids
UASB: Upflow anaerobic sludge blanket
VSS: Volatile suspended solids
WWTP: Wastewater treatment plant

Introduction

With the human population continuously rising and wastewater treatment plant (WWTP) effluent limits becoming more stringent, wastewater treatment research has been placing a greater emphasis on exploring methods of treatment intensification and efficiency. One method being explored is the integration of anaerobic ammonia oxidation bacteria, or anammox (AMX), with wastewater treatment technologies. AMX remove nitrogen from wastewater by oxidizing ammonia (NH_3) to dinitrogen gas (N_2) using nitrite (NO_2) as an electron acceptor. The use of AMX to remove nitrogen from wastewater can provide great operational cost savings, especially in the form of aeration, external carbon, and solids production.

Despite this, full-scale AMX processes have only been applied to sidestream treatment, with most of them being partial nitritation/anammox (PNA) processes. PNA requires that ammonia oxidizing bacteria (AOB) outcompete nitrite oxidizing bacteria (NOB) to accumulate nitrite through partial nitritation (PN), and sidestream conditions, with high free ammonia concentrations and temperatures, make this feasible. Mainstream conditions, however, do not provide a viable environment for PN, so another alternative to produce nitrite is required for mainstream applications. The partial denitrification/anammox (PdNA) process appears to be a viable alternative for AMX implementation in mainstream treatment (Regmi et al. 2015, Campolong et al. 2019, Schoepflin et al. 2020, Fofana et al. 2020, Cui et al 2020, Klaus et al. 2020). PdNA accumulates nitrite through partial denitrification (PdN), where nitrate (NO_3) is only denitrified to nitrite. While the aeration and external carbon savings in PdNA are not as high as those from PNA, the savings are comparable (Zhang et al. 2019, Le et al. 2019_b), and PdNA does not require NOB outselection, making it a promising method for mainstream AMX treatment applications.

An upcoming water reuse facility upgrade to Hampton Roads Sanitation District's (HRSD) James River Treatment Plant (JRTP) required lower nitrogen concentrations in the plant's effluent. A polishing MBBR with four zones was proposed to provide more nitrogen removal at the JRTP to meet the requirements for the future water reuse facility. The MBBR could be operated in two configurations. The first configuration uses full denitrification to polish off any remaining nitrogen in the secondary clarifier effluent. The zone layout for this configuration is displayed in Figure 1.

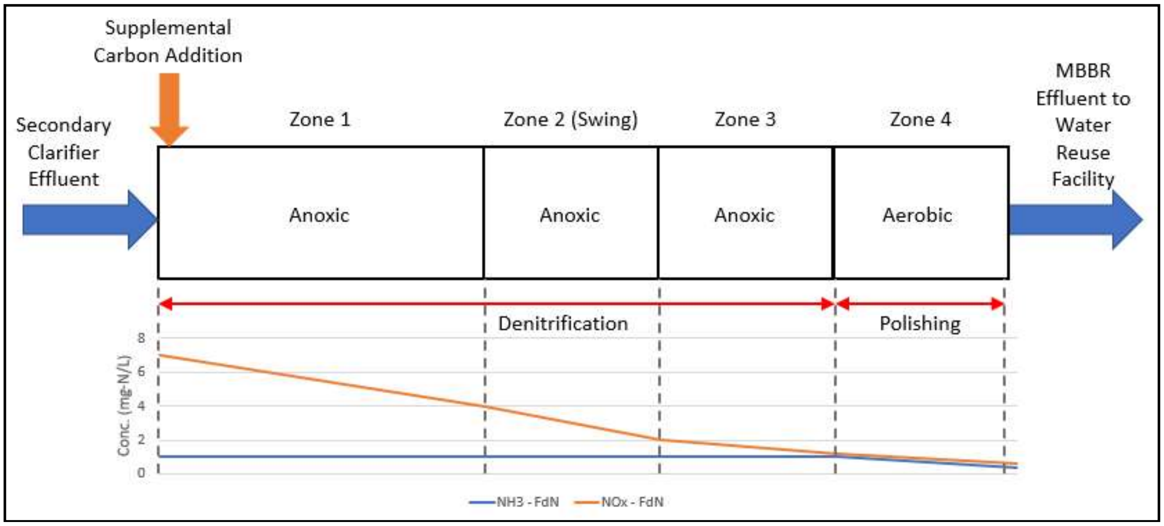

Figure 1. Proposed full-scale, full denitrification polishing MBBR configuration and the theoretical NH3 (blue) and NOx (orange) concentrations in each zone.

The second proposed configuration adapted the first zone from a full denitrification zone to a PdNA zone and changed the second zone from an anoxic denitrification zone to an aerated nitrification zone (Figure 2).

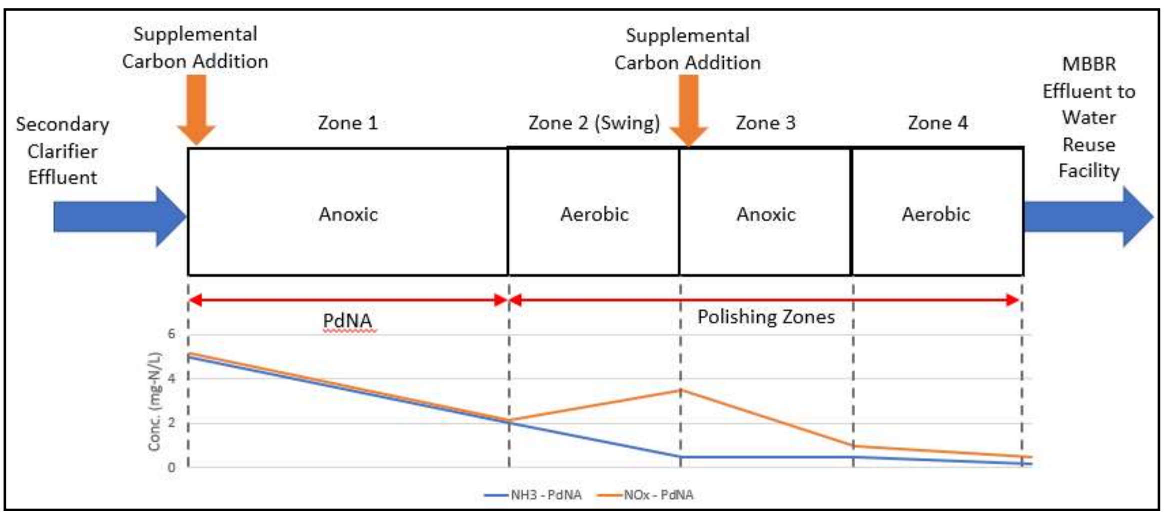

Figure 2. Proposed full-scale, PdNA polishing MBBR configuration and the theoretical NH3 (blue) and NOx (orange) concentrations in each zone.

Despite the promising results from past mainstream anammox research work, there were still concerns about the PdNA MBBR configuration. First, it would be very difficult to acquire enough AMX seed for the MBBR, so the anammox in the PdNA zone would have to be started up by scratch in mainstream conditions. While it was hypothesized that an anammox start-up would be possible in mainstream conditions without seed at the JRTP, the start-up process for anammox was hypothesized to still take a considerable amount of time considering how slow-growing anammox are. Previous research studies demonstrated that modifications to media in a MBBR could lead to a faster anammox start-up process (Klaus et al. 2016, Klaus et al. 2017, Kanders et al. 2019, Kowalski et al. 2019, Tian et al. 2020), particularly media with a pre-established, preliminary biofilm. Therefore, it was hypothesized that using a carriers coated preliminary biofilm would speed up anammox startup time in a mainstream polishing PdNA MBBR. This hypothesis was tested with two anammox startup experiments, one with media containing a preliminary biofilm and one with brand-new, virgin media, to determine the difference in anammox start-up time between these two types of media.

The objectives of these startup experiments were:

1. Determine whether it is possible to startup a polishing PdNA MBBR, without anammox seed, treating mainstream secondary clarifier effluent.
2. Determine the difference in startup times between two PdNA MBBRs with different types of carriers in them: carriers with a preliminary biofilm or virgin carriers.
3. Gain a better understanding of the TIN (total inorganic nitrogen) removal that can be accomplished in a polishing PdNA MBBR treating mainstream secondary clarifier effluent.

A follow-up experiment was then conducted after the startup experiment was successful in quickly establishing AMX activity. Two additional reactors were then added, in series, behind the preliminary biofilm PdNA MBBR, one being an AMX MBBR (no carbon addition) and the other being an aerated nitrifying MBBR. The purpose of the operation of these three MBBRs was to simulate the newly proposed full-scale polishing MBBR zone configuration (Figure 3), which was a modified version of the original 4-zone PdNA configuration shown in Figure 2. The final zone in both configurations was piloted by the aerated MBBR, whose primary purpose was to polish any remaining nitrite to prevent ozone demand spikes downstream in the water reuse facility.

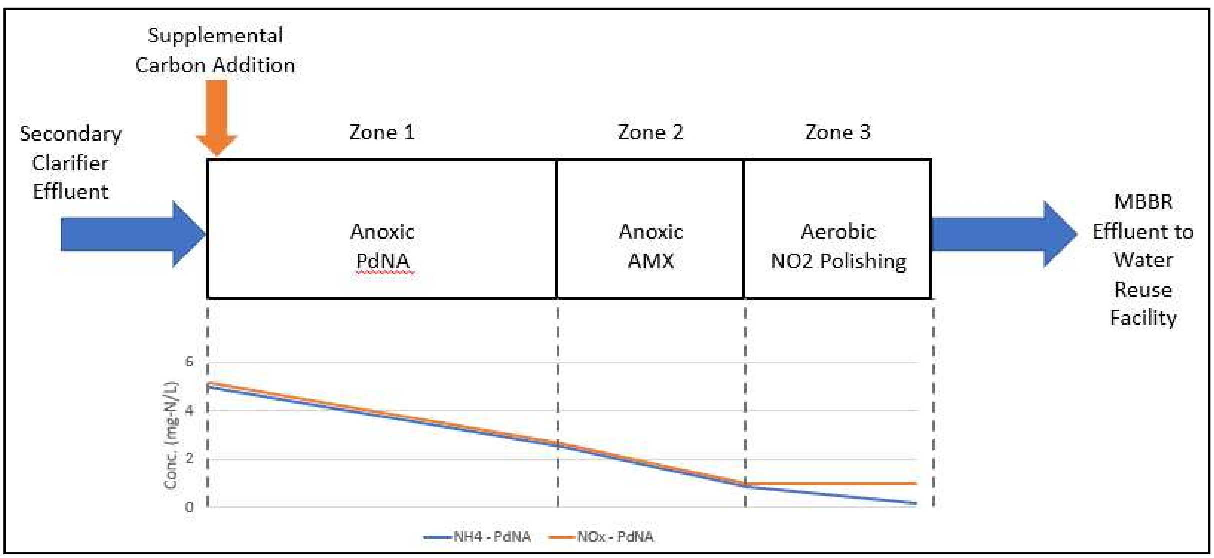

Figure 3. Proposed full-scale, 3-zone PdNA polishing MBBR configuration and the changes in the NH3 (blue) and NOx (orange) concentrations in each zone.

This experiment also involved the operation of a PdNA integrated fixed-film activated sludge (IFAS) reactor, which was intended to explore the possibility of converting JRTP's IFAS reactors underutilized second anoxic zones to PdNA IFAS zones to potentially acquire enough additional nitrogen removal to remove the need of a polishing MBBR completely. Figure 4 shows JRTP's IFAS reactor setup, with the second anoxic zones displayed as R5.

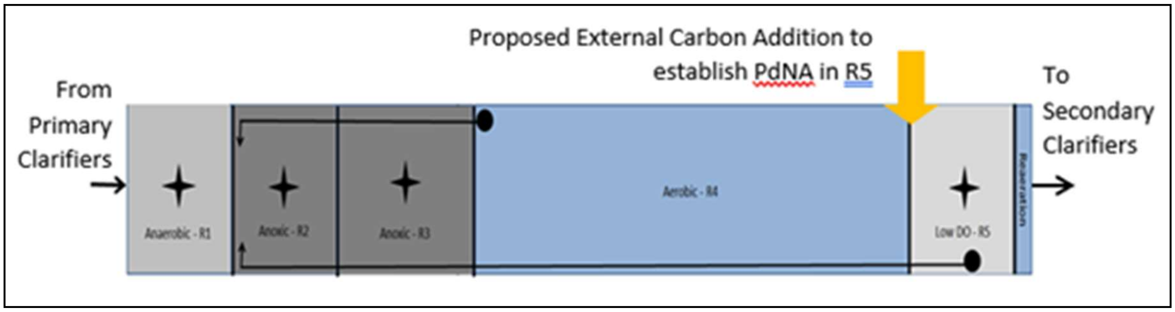

Figure 4. Layout of JRTP's full-scale IFAS reactors and proposed PdNA external carbon addition point.

The objectives of these operational experiments were:

1. Ensure that 3 MBBR zones (2 PdNA, 1 nitrifying) can sufficiently polish effluent from the JRTP's IFAS tanks to keep TIN concentrations below 3 mg N/L and maintain low effluent NO_2 (<0.5 mg N/L).
2. Investigate the nitrogen removal capabilities of a PdNA IFAS second anoxic zone.

The overall motivation behind this research was to determine whether starting a mainstream polishing PdNA process without seed was possible at JRTP and explore potential PdNA integration options for nitrogen polishing at JRTP that could provide both operational and capital savings.

Literature Review

Anaerobic Ammonium Oxidation

Anaerobic ammonium oxidation, otherwise known as anammox (AMX), is a term used to describe a group of bacteria, in the Planctomycetales order, which oxidize ammonia (NH_3) to dinitrogen gas (N_2) in anaerobic conditions by using nitrite (NO_2^-) as an electron acceptor. Along with nitrite, anammox also require a small amount of inorganic carbon and produce dinitrogen gas, anammox biomass, water, and a small amount of nitrate. Equation 1 is the balanced chemical equation for this reaction.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2N_{0.5}O_{0.15} + 2.03H_2O$$

Equation 1

When used in a biological nutrient removal (BNR) process, anammox provide many different advantages compared to the conventional nitrogen removal process which requires full nitrification and denitrification. These advantages include carbon savings, aeration savings, and lower solids production.

While utilizing anammox in a BNR process has many operational advantages, retention of the anammox bacteria in such a process has proven to be difficult due to their slow growth rate. Anammox have a doubling time of 11 days and a maximum specific growth rate of 0.0027 h^{-1} (Strous et al. 1998), which are both relatively slow compared to typical bacteria found in nitrification or denitrification processes.

Along with anammox retention, the other major obstacle to implementing an anammox BNR process is generating nitrite to serve as an electron acceptor for the oxidation of ammonia. Two methods for acquiring the needed nitrite for anammox have been developed: partial nitritation and partial denitrification.

Partial Nitritation with Anammox

Partial Nitritation

Partial nitritation (PN) is the use of aerobic AOB (AerAOB) to only oxidize ammonia to nitrite, which can then be used by anammox to oxidize ammonia anoxically. Equation 2 is the reaction for partial denitrification.

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^- \quad \text{Equation 2}$$

The combination of both partial nitritation and anammox is typically referred to as partial nitritation/anammox (PNA). Since nitrification typically requires no external carbon, the use of this process results in 100% external carbon savings compared to a conventional nitrification and denitrification. Partial nitrification does require some aeration though, although with half of the ammonia only being nitrified to nitrite, rather than nitrate, the PNA process still provides a maximum aeration savings of 60% compared to the conventional nitrification/denitrification BNR process. The full PNA process is illustrated in Figure 5.

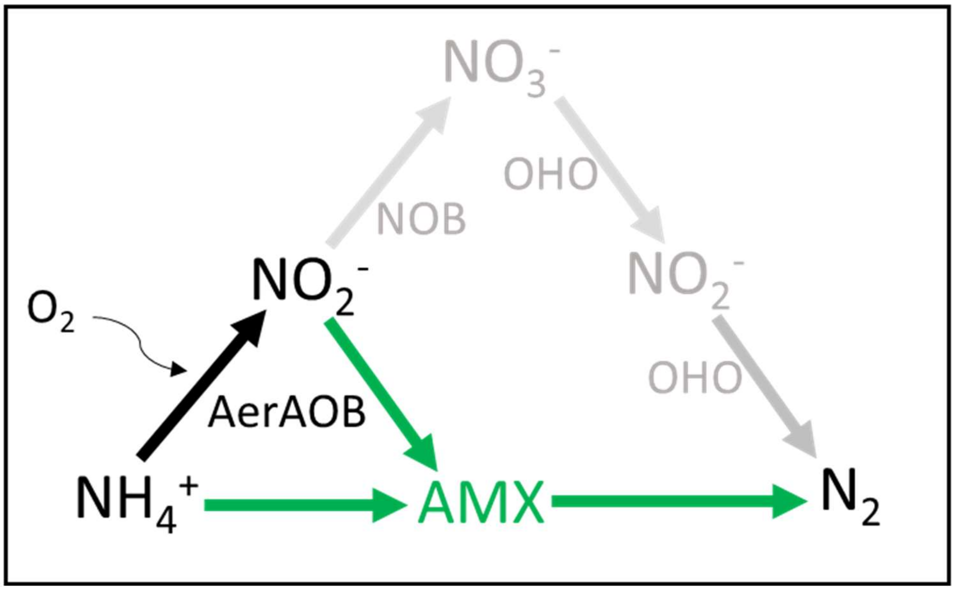

Figure 5. PNA process flow diagram.

The major downside to the PNA process, however, is that AOB must outcompete NOB for nitrite production to occur. This has not been a major issue in sidestream treatment conditions , as the high-water temperatures and ammonia concentrations typically found in sidestream systems create a suitable environment for the AOB to outcompete the NOB (Lackner et al. 2014). These two sidestream conditions that make NOB outselection possible, however, are not common in mainstream treatment conditions, making both NOB outselection and PNA extremely difficult to implement in mainstream treatment systems. Additionally, the nitrate produced by anammox in a pure PNA process could not be removed without some form of a downstream, denitrification polishing step (Bahtiar et al. 2020). Overall, the difficulty of NOB outselection, along with inability to remove the nitrate produced by anammox, make PNA an ineffective process for mainstream treatment applications.

PNA Processes and Approaches

PNA can be operated with a variety of wastewater treatment technologies either in a single-stage or two-stage configuration. As a single-stage configuration, both the partial nitritation and anammox activity occur in a single reactor or zone, which requires the NOB and anammox bacteria to coexist. As a two-stage configuration, the partial nitritation and anammox activity occur in separate reactors or zones, which requires the separation of NOB and anammox bacteria. PNA has seen widespread use in sidestream applications, especially in a single-stage configuration, with over 100 full-scale sidestream PNA processes in operation in 2014 (Lackner et al. 2014). Mainstream PNA in both single and two-stage configurations, however, has been difficult to implement, especially in pilot-scale and full-scale processes.

Single-stage PNA has been performed in both MBBRs (Gilbert et al. 2014, Laureni et al. 2016, Veuillet et al. 2015, Trojanowicz et al. 2016) and granular technologies (Winkler et al. 2012, Lotti et al. 2014, Gao et al. 2015, Lotti et al. 2015b, Morales et al. 2016). Due to the slow growth rate of anammox, PNA MBBRs are considered to be quite effective and resilient due to the MBBR carriers promoting anammox retention (Guo et al. 2016), the large amount of interaction that occurs through mixing between the biofilm and substrates (Ødegaard 1999) and how anammox are generally protected from most environmental disturbances, since they reside on the inside of the carrier biofilms (Gilbert 2015). PNA has also been accomplished in an IFAS reactor (Malovanyy et al. 2015, Trojanowicz et al. 2016), which provides similar benefits as the MBBR technology in terms of anammox retention. PNA granular technologies, however, require a strategy to retain the anammox, which typically involves either granular settling and recycle or effluent screens.

Two-stage PNA typically is integrated into one of many kinds of hybrid systems which use different types of technologies to implement the two reactions of PNA, partial nitritation and anammox, separately. A variety of hybrid systems have been able to demonstrate PNA, including the use of granular sludge for partial nitritation and suspended growth for anammox (Cao et al. 2013, Wett et al. 2015, Han et al. 2016), suspended growth for partial nitritation and a MBBR for anammox (Regmi et al. 2015), suspended growth for partial nitritation and a upflow anaerobic sludge blanket (UASB) for anammox (Ma et al. 2011), and separate MBBRs designed to promote different biofilm thicknesses for partial nitritation and anammox (Piculell et al. 2016).

Partial Denitrification with Anammox

Patrial Denitrification

Partial denitrification (PdN) is a biological process in which nitrate is reduced by ordinary heterotrophic organisms (OHOs) to nitrite. The amount of denitrification that occurs during this process is limited so that the denitrification of nitrite to dinitrogen gas is prevented, resulting in overall nitrite production. Equation **3** is the partial denitrification reaction.

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$$

Equation 3

The combination of partial denitrification and anammox is referred to as partial denitrification/anammox (PdNA). When treating typical wastewater influent, a PdNA process follows an aerated treatment step in which the required amount of nitrate is produced from the full nitrification of approximately half of the ammonia present in the influent wastewater. The partial denitrification of the available nitrate produces the nitrite needed by the anammox to oxidize the other half of the ammonia remaining in the wastewater. While PdNA does require more aeration compared to PNA and some external carbon, it can still provide a maximum external carbon savings of 80% and a maximum aeration savings of 50% compared to the conventional full nitrification/denitrification BNR process (Strous et al. 1998, Le et al. 2019b, Zhang et al. 2020). The full PdNA process is outlined in Figure 6.

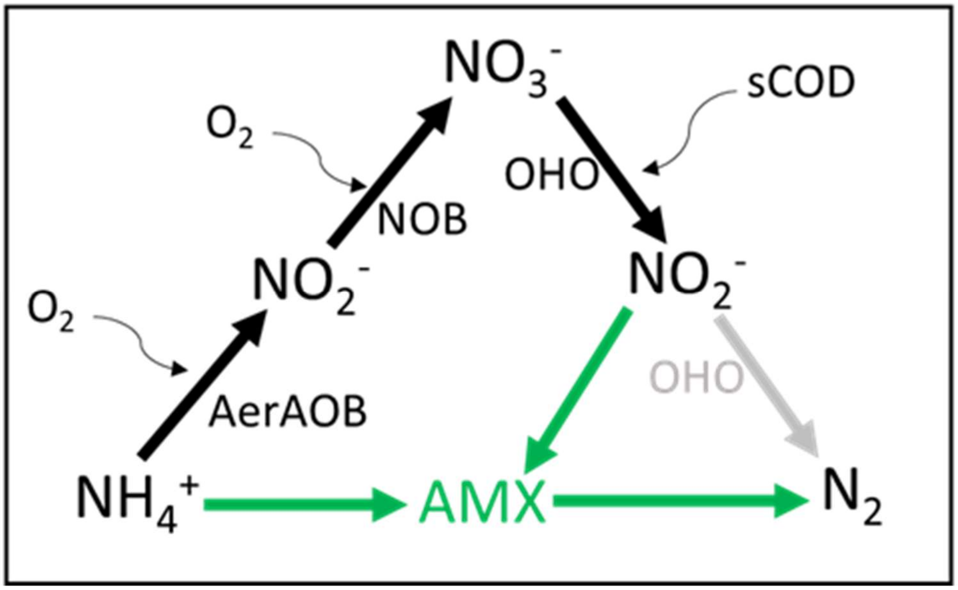

Figure 6. PdNA process flow diagram.

Since the PdNA process allows for nitrite to be oxidized to nitrate, NOB outselection is not required for this process to provide effective treatment. Therefore, PdNA may be more practical for mainstream treatment applications compared to PNA, even though PNA theoretically offers more savings in external carbon and aeration. It is also important to note that the differences between the maximum aeration and external carbon savings between PNA and PdNA are minor (a difference of 10% in aeration savings and 20% in external carbon savings), especially considering how nitrite production through PdN appears to be much more stable than that from PN (Ma et al. 2017). Considering the higher savings and favorable conditions for NOB outselection in sidestream, PNA is usually preferred over PdNA in sidestream applications, although PdNA can still work in sidestream applications (Sharp et al. 2017).

PdN and PN can also be used concurrently in the same process to provide the nitrite required for anammox activity. For example, if nitrite is accumulated upstream in an aeration zone through PN, PdN is not required and can be stopped through pausing the addition of external carbon. If the upstream PN, however, is not able to stably provide the required amount of nitrite, then external carbon addition can be used to provide the additional nitrite needed through PdN (Klaus et al 2020).

PdNA Processes and Approaches

Several different process configurations and types can be used for implementing a PdNA process, including single versus two-stage and a variety of different treatment technologies. Single-stage and two-stage configurations involve the use of either one or two different reactors, respectively, to run the PdNA process in. In a single-stage configuration, both OHOs and anammox reside in the same reactor, causing both the partial denitrification and ammonia oxidation to occur within the same reactor. In a two-stage configuration, two reactors will typically be in series, with OHOs residing in the first reactor and anammox residing in the second one. In this configuration, the partial denitrification will occur in the first, OHO reactor, while the ammonia oxidation will occur in the second reactor where the anammox bacteria reside. Both configurations have been utilized in several different applications and research studies.

By separating the OHOs and anammox, two-stage configurations limit the number of microorganisms, particularly denitrifiers, that the anammox need to compete with for nitrite (Cao et al. 2017, Bahtiar et al. 2020). Two-stage PdNA processes, however, do not provide a way for the nitrate produced by anammox to be removed, which means a nitrate residual will always be present in the process's effluent. Single-stage configurations, however, do not have this issue, since the OHOs present with the anammox can partially denitrify the anammox-produced nitrate, producing more nitrite for the anammox to use to oxidize ammonia. While single-stage PdNA configurations force anammox to compete with OHOs for nitrite, previous studies on single-stage PdNA configurations have also shown that anammox can effectively compete with denitrifiers for nitrite (Le et al. 2019b), showing that limiting OHO competition for nitrite is not necessary to operate an efficient PdNA process. This generally makes single-stage PdNA configurations preferable over two-stage since the only advantage offered by the two-stage configuration is not necessary for effective PdNA. Lastly, single-stage configurations also require a smaller footprint and fewer reactors compared to two-stage configurations, which means they take up less space and are typically less expensive.

During past research studies, both single and two-stage configurations have been utilized with a variety of wastewater treatment technologies. A single-stage polishing PdNA system successfully set up to remove nitrogen using a combination of suspended growth and granular sludge but did require bioaugmentation and screened effluent to retain the anammox (Le et al. 2019b). PdNA has also been implemented using UASB (Xu et al. 2020) and IFAS (Forney et al. 2020) technologies as well. Single-stage PdNA MBBRs (Regmi et al. 2015, Campolong et al. 2019) and single-stage PdNA filters (Fofana et al. 2020, Cui et al 2020) have also been successfully started and used to efficiently remove nitrogen with biofilms containing OHOs, nitrifiers, and anammox, including a full-scale mainstream polishing PdNA deep-bed denitrification filter at HRSD's York River WWTP (Klaus et al. 2020). The PdNA filters in these studies used sand or activated carbon as the filter media. The full-scale mainstream PdNA filter reported by Klaus et al., (2020) is the first reported full-scale mainstream PdNA process.

PdNA Process Control

One of the most critical components of optimal PdNA operation is balancing the amount of ammonia and nitrite present for the anammox to remove. For PdNA processes, this is often done by targeting a user set effluent AvN (NH_3-N versus NO_x-N) ratio through aeration control in the nitrification reactors that are upstream of the PdNA process (Regmi et al. 2014, Regmi et al. 2015, Fofana et al. 2020, Klaus et al. 2020). This form of aeration control adjusts the amount of aeration based on the amount of ammonia present in the effluent over the amount of nitrite and nitrate present. Adjusting the aeration adjusts the amount of nitrification that occurs. The aeration is then turned up when the detected ratio is higher than the set target ratio and turned down when the detected ratio is lower than the set target ratio. Basing aeration control off the AvN ratio, rather than just the ammonia concentration, allows for a set ratio of NH_3 and NO_x to be produced for removal via PdNA downstream. The exact AvN ratio that can be used, however, depends on how efficient the partial denitrification is in the PdNA process, because most of the NOx present in effluent from aerated reactors is usually in the form of nitrate. This nitrate must be converted to nitrite by OHOs before it can be removed by anammox. If PdN efficiency is high, the AvN ratio can be higher, but if PdN efficiency is low, the AvN ratio likewise will need to decrease, which also means more aeration is required.

Another factor that can affect operation of a PdNA process is phosphorus requirement. Both OHOs and anammox require some phosphorus to function and grow, so limited phosphorus concentrations in a PdNA process can limit activity and nitrogen removal. Previous studies have found 0.2 mg P/L to be a non-limiting phosphorus concentration for denitrification in MBBRs (Peric et al. 2009).

PdNA Carbon Source Selection

Multiple organic carbon sources have demonstrated the ability to accumulate nitrite when used as the electron source for the denitrification of nitrate to nitrite, or PdN, by OHOs. Known PdN supporting organic carbon sources include acetate, glycerol, methanol, glucose, ethanol, propionate (van Rijn et al. 1996, Bill et al. 2009, Le et al. 2019$_a$).

Effective and efficient treatment in a PdNA process is highly dependent on PdN efficiency, or the percentage of nitrate that is only reduced to nitrite. Any nitrate that is removed, but not partially denitrified, is likely removed through full denitrification, which minimizes the amount of ammonia that can be removed anaerobically, increases the process's external carbon demand, and can destabilize the anammox activity and PdNA process if it is not mitigated. Glycerol and acetate have generally been found to have higher PdN efficiencies compared to methanol (Le et al. 2019$_a$, Campolong et al. 2019). Methanol's lower PdN efficiency could be attributed to how methanol is only used by a specialized group of bacteria, methylotrophs, instead a wide range of OHOs like the other carbon sources (Akunna et al. 1993, Sperl and Hoare 1971), or due to its electron transport route not being favorable for PdN (Van Verseveld and Stouthamer 1978, van Rijn et al. 1996). Carbon sources that do produce low amounts of PdN, like methanol, may donate their electrons further downstream on the electron transport chain compared to other sources (van Rijn et al. 1996), which means those carbon sources are more likely to have their electrons sent to nitrite reductase rather than nitrate reductase. This is because nitrate reductases are positioned upstream on the electron transport chain compared to nitrite reductases (Almeida et al. 1995, van Rijn et al. 1996, Baideme 2019). If the nitrite reductases receive the electrons from a carbon source, this leads to the denitrification and removal of nitrite.

On top of its generally lower PdN efficiency, many batch experiments have shown that dosing methanol can be inhibitory to anammox (Güven et al. 2005, Isaka et al. 2008), but recent experiments where a pilot-scale PdNA MBBR or PdNA activated sludge reactors with anammox granules were dosed with methanol showed that methanol may not actually be inhibitory to anammox (Le et al. 2019$_a$, Campolong et al. 2019). While it is not entirely clear as to why methanol was not inhibitory to anammox in these experiments, it could potentially be due to the low bulk methanol concentrations in the MBBR and the anammox being in the interior of the media and granule biofilms where it would have limited contact with the methanol. Based on these findings, methanol could still serve as a potential external carbon source in PdNA processes and is cheaper compared to glycerol and acetate. Methanol's lower PdN efficiency and high flammability do provide some downsides to the carbon source, but its low cost could still make it the cheapest carbon source alternative for PdNA (Campolong et al. 2019).

Methods for Promoting PdN

Many different strategies for promoting PdN have been explored and discovered, including maintaining a high pH, limiting the HRT, controlling for a low carbon to nitrogen ratio (C/N), and controlling external carbon dosage to maintain a nitrate residual (Le et al. 2019_b, Ma et al. 2020). While maintaining a high pH, ranging from 7.0-9.0, has proven to be a reliable way to promote PdN and accumulate nitrite (Ma et al. 2017, Si et al. 2018, Shi et al. 2019), doing so in most full-scale applications would require a large amount of chemical addition. Additionally, controlling the HRT would be difficult to implement reliably in a full-scale application.

Maintaining a low C/N ratio promotes PdN by limiting the amount of electron donors, in the form of organic carbon, available for denitrification. The organic carbon is used first to reduce nitrate to nitrite and is then used to reduce nitrite to dinitrogen gas. If the amount of carbon being added is controlled through the C/N ratio though, the carbon will get used up and will not be available to reduce nitrite, which has shown to be effective at promoting PdN (Ma et al. 2017).

Recently, maintaining a nitrate residual has also been found to serve as a very effective method of promoting PdN and nitrite accumulation for anammox (Le et al. 2019_b, Campolong et al. 2019). While this method does require for some nitrate to be present in the effluent, past studies have been able to effectively use this strategy for PdN promotion with nitrate residual concentrations as low as 1 mg NO_3-N/L (Campolong et al. 2019), but others have also shown for it to work at nitrate concentrations at 2-3 mg NO_3-N/L (Le et al. 2019_b). Le et al. (2019_b) showed that nitrite continued to accumulate until the nitrate concentration reached a certain limiting condition. Once the nitrate reached that limiting condition though, the nitrite concentration would begin to decrease as it was removed through the FdN pathway. This change from nitrite accumulation to removal was noticed at low nitrate concentrations with the use of two different C/N ratios, demonstrating that PdN efficiency may be more dependent on the nitrate concentration, rather than the C/N ratio.

Methods for Speeding up Anammox Startup

Due to the slow growth rate of anammox, a limitation on the use of anammox in mainstream treatment is the slow startup speeds of anammox processes. Therefore, strategies for speeding up anammox attachment, increasing anammox retention, and decreasing anammox startup time requirements have been explored and studied to make this process more feasible in mainstream treatment applications. Most of these strategies involve biomass augmentation (seeding), carrier surface modifications, or using carriers with a preliminary biofilm composed of OHOs (Kanders et al. 2014, Klaus et al. 2016, Klaus et al. 2017, Kanders et al. 2019, Kowalski et al. 2019, Tian et al. 2020, Liu et al. 2021).

Many PNA startup experiments have successfully demonstrated the use of anammox biomass seeding to speed up the process startup time (Klaus et al. 2017, Kowalski et al. 2019, Tian et al. 2020). Tian et al. (2020) also found that seeding anammox onto carriers by dosing a reactor with detached anammox biofilm, rather than adding carriers with attached biofilms containing anammox, led to faster startup times. The use of anammox seeding, however, would not be feasible in most full-scale mainstream applications, due to the large amount of anammox seed that would be needed for such a large-scale process. Kanders et al. (2014), however, conducted a startup experiment comparing two MBBRs, one with and one without anammox seeding. The experiment showed that anammox seeding in a MBBR does not lead to faster startup times but can provide process stability and more nitrogen removal during the earlier stages of startup. Additionally, another pilot-scale mainstream anammox startup experiment also demonstrated that seeding anammox was not necessary for startup (Schoepflin et al. 2020).

Klaus et al. (2016) investigated using carriers that were coated with preliminary biofilm or had chemically modified surfaces in a sidestream deammonification MBBR, and the affect these surface modifications had on the growth of anammox and anoxic NH_3 and NO_2 removal in the MBBR. The preliminary biofilm coated carriers displayed a significantly larger amount of anammox activity than any of the other carriers, including the unmodified, virgin media carriers. Liu et al. (2021) and Kanders et al. (2019) also conducted similar experiments. Liu et al. (2021) ran a startup experiment with iron-based modified carriers, and found that the iron carriers had a greater amount of anammox gene copies and removed more TIN compared to polyethylene carriers in a integrated floating-film activated sludge (IFFAS) process. This was believed to primarily be due to how the iron-based carriers were less hydrophobic, more electropositive, and had a larger amount of free surface energy (Liu et al. 2021). Kanders et al. (2019) ran a startup experiment on carriers with a preliminary biofilm and carriers coated with granular activated carbon (GAC), which found that the preliminary biofilm and GAC-coated carriers increased the initial, seeded anammox activity by 400% and 50% respectively, while no activity was detected on the control virgin carriers. Besides Klaus et al. (2016) and Kanders et al. (2019), many other startup experiments have demonstrated that carriers with a preliminary biofilm can be attributed to faster anammox startup times (Klaus et al. 2017, Kowalski et al. 2019, Tian et al. 2020). All these startup experiments, however, were seeded with anammox for startup. Based on this literature review, no previous research has proven that MBBR carriers with a preliminary biofilm led to faster startup times without anammox seeding under mainstream conditions, which is vital since anammox seeding will not be viable for mainstream anammox treatment applications in full-scale processes.

Temperature Effect on Anammox

The rate of anammox activity, like in most biological processes, is affected by temperature. At lower temperatures anammox activity decreases, even to the point where a process's stability can be lost at temperatures of 15°C (Dosta et al. 2008). Anammox have been shown to acclimate to cold temperatures though when the temperature change is made gradually (Nifong 2013, Lotti et al. 2015$_a$, Tomaszewski et al. 2017, Wang et al. 2021) similarly to the rate of temperature changes in wastewater throughout a year of operation. Decreases in anammox activity caused by decreases in temperature are also reversible when the temperature is increased, even when the initial decrease in temperature occurs quickly (Nifong 2013, He et al. 2018).

As the wastewater temperature changes throughout the year, the change in temperature must be accounted for to properly compare anammox ammonia removal rates over time and determine whether the amount of available anammox has changed. This can be done by using the Arrhenius equation, which is shown in Equation 4 (Grady et al. 2011). In this equation, R is the gas constant, A is a constant, u is a temperature coefficient, and T is the absolute temperature.

$$\mathrm{k} = \mathrm{A}e^{(-u/RT)}$$

Equation 4

This equation can be adapted into Equation 5 to make direct rate conversions between two different temperatures. In this equation, q_0 is the unadjusted growth/removal rate, θ is the Arrhenius coefficient, q is the adjusted growth/removal rate, T is the temperature the rate is being adjusted to (in degrees Celsius), and T_0 is the temperature the rate was at originally (in degrees Celsius).

$$q = q_0\theta^{(T-T_0)}$$

Equation 5

This equation requires an Arrhenius coefficient, which is represented by "θ" in Equation 5. This value is dependent on the type of biological process, technologies, and organisms involved. Table 1 shows Arrhenius coefficient values on anammox that were collected from a variety of studies (Strous, Kuenen, and Jetten 1999, Dalsgaard and Thamdrup 2002, Rysgaard et al. 2004, Dosta et al. 2008, Isaka 2008, Guo et al. 2010, Nifong 2013).

Table 1. Reported anammox Arrhenius coefficients from the literature.

Temperature Range (°C)	Arrhenius Coefficient	Source
10-45	1.10	Dosta et al. 2008
Not provided, 43 optimum	1.11	Strous, Kuenen, and Jetten 1999
15-37	1.10	Dalsgaard and Thamdrup 2002
-1.8-13	1.08	Rysgaard et al. 2004
22-28	1.15	Isaka 2008
28-37	1.05	Isaka 2008
15-35 (short-term change)	1.10	Nifong 2013
15-35 (long-term change)	1.09	Nifong 2013
5-20, 20-35	1.172, 1.062	Guo et al. 2010

In many scenarios, it was found that changes in anammox activity rates due to temperature had to be described with the Arrhenius equation using more than one Arrhenius coefficients for different temperature ranges (Guo et al. 2010, Lotti et al. 2015_a).

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Manuscript 1: Startup of Mainstream Polishing Partial Denitrification/Anammox MBBRs without Anammox Seeding

Abstract

One barrier to implementing anammox into mainstream processes are the long start-up times due to the slow growth rate of anammox, so increasing anammox attachment and retention are key towards decreasing start-up times. The purpose of this study was to investigate potential start-up optimization factors and the maximum TIN removal possible in a mainstream polishing partial denitrification/anammox (PdNA) moving bed biofilm reactor (MBBR). Two pilot-scale mainstream PdNA MBBRs were started-up and tested, one containing media with a pre-existing, fully nitrifying biofilm and one containing virgin media. The partial denitrification efficiencies and ammonia removal rates of these two MBBRs were monitored throughout the startup process. Anammox activity was confirmed in the preliminary biofilm MBBR through a maximum anammox activity test 52 days after start-up, and anammox activity was also confirmed in the virgin media MBBR through testing 86 days after start-up. After anammox activity was initially detected, the activity continued to increase throughout the remainder of the experiment. Throughout operation, the average effluent NH_3 and NO_2 concentrations for the preliminary biofilm MBBR were 1.32 ± 0.93 and 1.06 ± 0.60 mg N/L, respectively, and the average effluent NH_3 and NO_2 concentrations for the virgin media MBBR were 2.01 ± 1.09 and 1.78 ± 1.01 mg N/L, respectively. The maximum total inorganic nitrogen (TIN) removal detected in the preliminary biofilm MBBR was 0.752 g/m^2/day when adjusted to 20˚C, with an average effluent TIN concentration of 3.36 ± 0.94 mg/L after the detection of anammox activity. The maximum TIN removal detected in the virgin media MBBR was 0.666 g/m^2/day at 20˚C, with an average effluent TIN concentration of 4.42 ± 1.39 mg/L after the detection of anammox activity. This study concluded that PdNA could be started-up in a mainstream polishing MBBR without anammox seeding within 50-90 days, even without high NH_3 or NO_2 concentrations. Using MBBR carriers with a preliminary biofilm can speed up a PdNA MBBR startup by approximately one month. Lastly, TIN removal in both MBBRs could be further increased by increasing the influent nitrogen loading and refining the upstream aeration control.

Introduction

While anammox has been used in full-scale sidestream wastewater treatment processes to remove nitrogen at high temperatures (Lackner et al. 2014), little progress has been made towards implementing anammox in a mainstream treatment process. This is because sidestream conditions are conducive to partial nitritation/anammox (PNA) systems, where aerobic ammonia oxidizing bacteria (AerAOB) are used to partially nitrify ammonia to nitrite, which is then used by anammox to oxidize the remaining ammonia to dinitrogen gas. PNA can provide a maximum theoretical savings of 100% for external carbon and 60% for aeration but requires nitrite oxidizing bacteria (NOB) out-selection, which is possible in sidestream conditions due to the high ammonia concentrations and temperatures present there (Lackner et al. 2014).

Partial denitrification/anammox (PdNA) has proven to be an efficient method for removing nitrate and ammonia from mainstream wastewater in an economical and environmentally sustainable way, even in cold temperatures (Bahtiar et al. 2020). As a nitrogen removal process, PdNA can theoretically provide up to 80% savings on external carbon and 50% savings on aeration compared to the conventional nitrification/denitrification nitrogen removal method (Strous et al. 1998, Le et al. 2019$_b$, Zhang et al. 2020). PdNA also provides these savings without having to achieve NOB out-selection, since it involves using ordinary heterotrophic organisms (OHOs) and anammox in an anoxic environment to both reduce nitrate (NO_3) to nitrite and convert nitrite and ammonia to dinitrogen gas through the anammox metabolism.

Establishing a reliable and adequate balance of ammonia and NO_x going into a PdNA process is necessary for optimal nitrogen removal to be obtained consistently through the anammox pathway. This is commonly done via ammonia-versus-NOx (AvN) based aeration control in a nitrification zone/reactor upstream of the PdNA process (Regmi et al. 2015, Fofana et al. 2020, Klaus et al. 2020). This aeration control requires a user-set AvN ratio and attempts to reach that ratio by controlling the rate of nitrification by increasing or decreasing the amount of aeration. The optimal AvN ratio for a PdNA process can vary from based on how efficiently the process conducts PdN and converts nitrate to nitrite for anammox usage.

Efficient and stable partial denitrification in a PdNA anoxic bioreactor is essential to accumulate nitrite, which can then be used to promote anammox growth and activity. Several different strategies for encouraging partial denitrification have been explored in past studies, which have found that raising the pH, controlling the hydraulic retention time (HRT), diminishing the amount of external carbon dosed per nitrate removed (C/N ratio), and/or using organic external carbon sources, including glycerol, acetate, and ethanol, can lead to an increase in partial denitrification and more nitrite accumulation (Ma et al. 2020). Additionally, leaving a nitrate residual in the effluent of an anoxic bioreactor has also proven to be an effective control strategy for accumulating nitrite and establishing an anoxic MBBR (Le et al. 2019$_b$, Campolong et al. 2019).

While methanol has been shown to not be inhibitory to anammox as previously thought (Campolong et al. 2019), when compared to glycerol and acetate, methanol has repeatedly displayed the lowest rates of partial denitrification efficiency, which could limit anammox growth and TIN removal (Campolong et al. 2019, Le et al. 2019$_a$). Among these three organic carbon sources, glycerol has consistently displayed the highest partial denitrification efficiency in the literature (Campolong et al. 2019, Le et al. 2019$_a$).

Additionally, both OHOs and anammox require phosphorus for growth, so limited phosphorus concentrations in a PdNA process can slow both denitrification and anammox activity rates. Previous studies have found that a phosphorus concentration of 0.2 mg P/L to be non-limiting for denitrification in MBBRs (Peric et al. 2009).

One barrier to the implementation of PNA or PdNA is the long start up time required due to the slow growth of anammox. This has been alleviated in past start-up experiments by adding anammox biomass (Tian et al. 2020) or carriers containing anammox (Klaus et al. 2016, Tian et al. 2020), which is also referred to as seeding. For full-scale mainstream processes, however, seeding is not a viable option for speeding up anammox start-up, due to the large volume of carriers that would be required for seeding in that scenario. Previous studies have shown though, that an anammox process can be started-up without seeding (Kanders et al. 2014, Schoepflin et al. 2020). However, none of these past PdNA startup experiments have successfully attempted to grow anammox from scratch without seeding.

A previous study investigated using carriers with preliminary biofilm or had chemically modified surfaces in a sidestream deammonification MBBR, and the affect these surface modifications had on the growth of anammox and anoxic NH_3 and NO_2 removal in the MBBR (Klaus et al. 2016). The preliminary biofilm coated carriers displayed a significantly larger amount of anammox activity than any of the other carriers, including the unmodified, virgin media carriers (Klaus et al. 2016). Many other studies have also compared carriers with a preliminary biofilm on them to other types of modified carriers and found that the preliminary biofilm carriers increase anammox attachment and retention and decrease the required startup time for anammox processes more than any other type of modified carriers and virgin carriers (Klaus et al. 2017, Kanders et al. 2019, Kowalski et al. 2019, Tian et al. 2020).

The objectives of this experiment were:

1. Determine whether it is possible to startup a polishing PdNA MBBR, without anammox seed, treating mainstream secondary clarifier effluent.
2. Determine the difference in startup times between two PdNA MBBRs with different types of carriers in them: carriers coated with a preliminary biofilm or virgin carriers.
3. Gain a better understanding of the TIN removal that can be accomplished in a polishing PdNA MBBR treating mainstream secondary clarifier effluent.

Materials and Methods

Pilot Setup

Effluent from the aerated, integrated fixed-film activated sludge (IFAS) tanks at HRSD's JRTP was pumped from the full-scale aeration basins using a Godwin GSP10 submersible pump (Xylem, Rye Brook, NY). The IFAS effluent flow was regulated using a progressive cavity pump (Seepex, Enon, Ohio) before going through both a 1000-gallon clarifier with a surface overflow rate (SOR) of 260 gallons-per-day/ft^2 and a 120-gallon equalization tank with a HRT of 30 minutes. Wastewater in the EQ tank was then pumped to two separate MBBRs with two progressive cavity pumps (Seepex, Enon, Ohio) at a rate of 1.7 gallons per minute (gpm) each. Settled solids from the clarifier were pumped from the bottom of the clarifier to a drain using a progressive cavity pump (Seepex, Enon, Ohio) at a rate of 3.5 gpm. The process flow diagram for the pilot setup is displayed in Figure **7**.

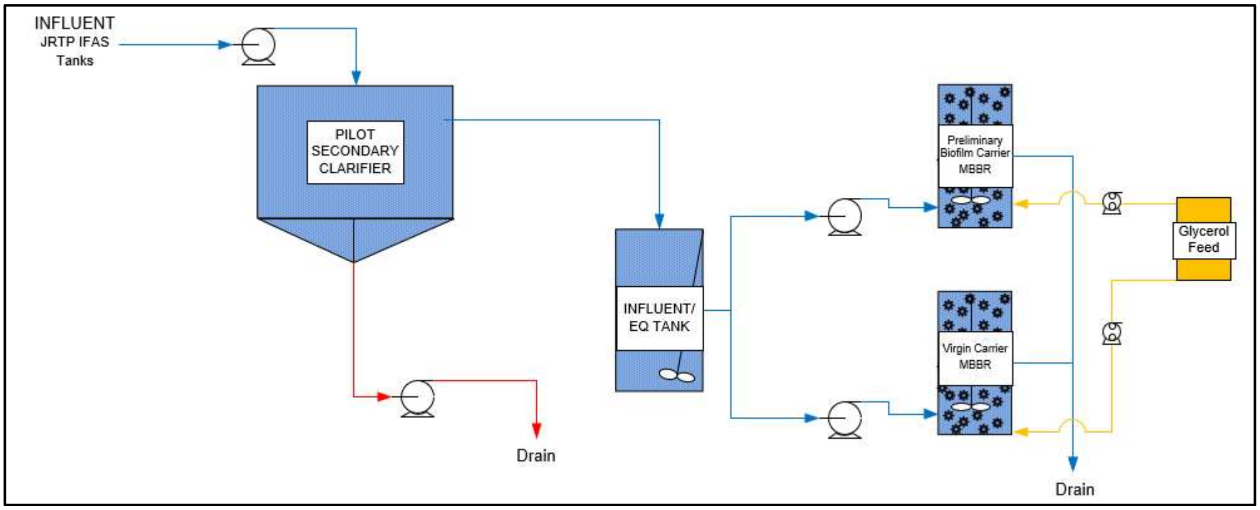

Figure 7. Process flow diagram for PdNA MBBR startup experiment.

Each MBBR had different carriers in them, one with carriers coated with a preliminary biofilm and the other with virgin carriers. The MBBR with the preliminary biofilm K3 media (Anoxkaldnes, Lund, Sweden), with a lower media surface area of 500 m^2/m^3, had a fill fraction of 50.0%, while the MBBR with the virgin WW1 media (World Water Works, Oklahoma City, Oklahoma), with a media surface area of 650 m^2/m^3, had a fill fraction of 38.5% (Figure 8). Both MBBRs had a total media surface area of 94.6 m^2.

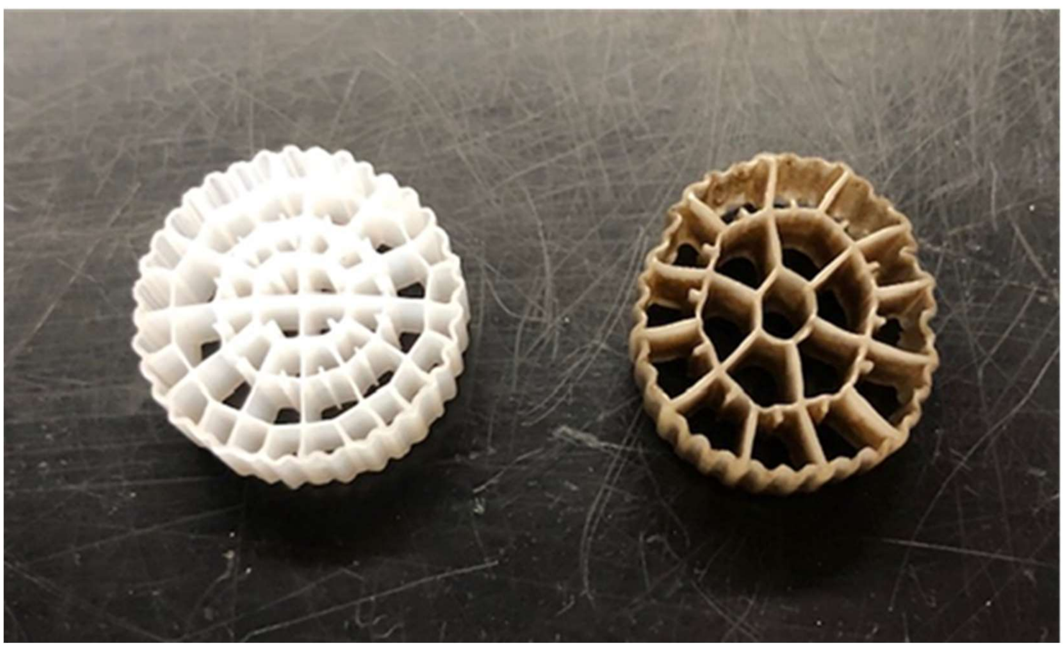
Figure 8. Virgin carrier (left) versus IFAS carrier with a pre-existing biofilm (right).

During the startup experiment, the aeration control for the full-scale JRTP IFAS tanks that the pilot influent was being fed from was operated in AvN mode. AvN-based aeration control requires the user to set a target AvN (NH_3-N versus NO_x-N) ratio for the effluent. The aeration is then adjusted so that enough ammonia is nitrified to nitrite or nitrate to reach the set ratio. The AvN setpoint for the plant was adjusted to help better tune pilot MBBRs' influent AvN throughout the experiment and ranged between 0.7 and 1.0.

The MBBR influent was also dosed with sodium nitrate to add 1.5 mg NO_3-N/L to substitute for any NO_x that was lost due to denitrification occurring in between the end of the JRTP's full-scale IFAS tanks and the pilot MBBRs. The NH_3, NO_2, NO_3, and TIN loading rates were all dependent on the influent coming into the JRTP and the plant's performance, and the pH, temperature, and dissolved oxygen (DO) were monitored daily. Neither of the MBBRs were seeded with anammox at any point during the study. Glycerol was stored in a refrigerator and added as external carbon source using Pro-Series M-2 peristaltic pumps (Blue-White Industries, Huntington Beach, CA). Both the carbon control program and pilot pump flow rates were controlled with a distributed control system (DCS).

Both MBBRs were dosed with glycerol at a rate that was determined by a feedforward control program based on the NO_3-N concentration measured by a Nitratax probe in the equalization tank (HACH, Loveland, Colorado). This program had a nitrate effluent setpoint to ensure a nitrate residual was maintained and COD/NO_3-N ratio setpoint to allow for the feed rate to be adjusted by the user. The Nitratax probes from HACH do read some NO_2 when measuring the NO_3 concentration, so the error produced by the NO2 had to be accounted for in the carbon control program through adjustment of the COD/NO_3 setpoint. The volumetric flow rate of the glycerol stock to the MBBRs that was dosed was calculated using Equation 6.

$$Stock\ Solution\ Flow\ Rate = \frac{(NO_{3in} - NO_{3ef})(Flow\ Rate\ to\ MBBR)(Set\ COD/NO_3\ Ratio)}{(Stock\ Solution\ COD\ Concentration)}$$

Equation 6

Potassium phosphate monobasic was also added to the glycerol stock to ensure that the MBBRs had an effluent reactive phosphorus concentration of 0.2 mg OP-P/L. The complete pilot setup for the startup experiment is displayed in Figure 9.

Figure 9. Pilot setup for PdNA MBBRs startup experiment.

Process Sampling and Analysis

Five times per week, composite samplers (Teledyne ISCO, Lincoln, Nebraska) collected hourly aliquots over a twenty-four-hour period from the two MBBRs and the equalization tank, which served as a representation of the influent. These composite samples were then run through a 0.45 µm filter. The NH_3, NO_2, NO_3, and COD and OP concentrations in these samples were then measured with HACH tubes (HACH, Loveland, Colorado), and read with a HACH DR-2800 spectrophotometer, Sulfamic acid was added to samples that had a NO_2 concentration greater than 1.0 mg/L to counter any nitrite interference in the NO_3 and soluble chemical oxygen demand (sCOD) measurements. For these samples, approximately 50 mg of sulfamic acid was dissolved into 5 mL of sample and left to sit for 10 minutes before evaluation.

The loading rate for each nitrogen species was calculated for the different reactors using Equation 7, which requires the influent concentration, flow rate through the reactor, and total media surface area in the reactor.

$$Loading\ Rate = \frac{(Influent\ Concentration)\times(Flow\ Rate)}{Total\ Media\ Surface\ Area} \qquad \text{Equation 7}$$

The different nitrogen species' concentrations were then used to calculate a removal rate in $g/m^2/day$. This was done using Equation 8.

$$Removal\ Rate = \frac{(Influent\ Concentration - Effluent\ Concentration)\times(Flow\ Rate)}{Total\ Media\ Surface\ Area}$$

Equation 8

To accurately determine the amount of *in situ*, or regular operation, ammonia removal occurring due to anammox activity, the ammonia removal from heterotrophic organisms had to considered. This was done by calculating the average amount of total ammonia removal that occurred in an MBBR before anammox activity was detected, which could be entirely attributed to heterotrophic ammonia removal, or assimilation. This average value was then subtracted from the total ammonia removal to determine the amount of ammonia removal from anammox activity (Equation 9).

$$Removal\ Rate = \frac{\left(Influent\ Conc - Effluent\ Conc - \frac{Avg\ NH3\ Removed\ Before\ AMX}{COD\ Used} \times Glycerol\ Dosed\right) \times (Flow\ Rate)}{Total\ Media\ Surface\ Area}$$

Equation 9

The partial denitrification efficiency (PdN%), or the amount of nitrate that was only denitrified to nitrite (and not to nitrogen gas), was determined using Equation 10.

$$\text{PDN\%} = \frac{NO2out - NO2in + 1.32\ x\ (NH3in - NH3out - NH3assimilated)}{NO3in - NO3out + 0.26\ x\ (NH3in - NH3out - NH3assimilated)}$$

Equation 10

This calculation for partial denitrification efficiency accounts for anammox activity by factoring in the amount of NO_2 removed by anammox and NO_3 produced by anammox. These amounts were determined based off the measured NH_3 removal that could be attributed to anammox and the anammox reaction stoichiometry. The values used for NH_3 assimilated were based on the average amount of NH_3 removal in the MBBRs before anammox activity was detected.

The total suspended solids (TSS) concentrations of the composite samples collected from these three tanks were measured on a weekly basis using 2540D in the standard methods.

Media Biofilm Solids Measurements

The mass of the biofilm on the two media types was measured about every three weeks after anammox activity was detected in one of the MBBRs. This process involved collecting samples of media from both MBBRs, placing the collected media in an oven at approximately 100°C for at least 8 hours, allowing the media to cool, measuring the mass of the media samples, scraping the biofilm off of the media using brushes and by soaking the media in a solution containing 25 g/L of ethylenediaminetetraacetic acid (EDTA), placing the media back into the oven for at least 8 hours, letting the media cool again, and measuring the mass of the media samples without biofilm. The mass of the media without biofilm was then subtracted from the mass of the media with biofilm to calculate the amount of biofilm on the collected media samples. This biofilm mass was then divided by the amount of media samples collected and the surface area of a single piece of media to calculate the mass of biofilm per square meter of media surface area (see Equation 11).

$$\frac{Mass\ of\ Biofilm}{m^2\ of\ Surface\ Area} = \frac{(Mass\ of\ Media + Biofilm) - (Mass\ of\ Media)}{(Piece\ \ of\ Media)\ x\ (Surface\ Area\ per\ Media\ Piece)} \quad \text{Equation 11}$$

Maximum Anammox Activity Rate Testing

The presence of anammox in the MBBRs was confirmed through maximum anammox activity rate tests. These tests were conducted by cutting off the flow to the MBBRs, leaving the MBBRs to sit for approximately 1 hour to ensure all available COD was used up, spiking the MBBRs with 20 mg NH_3-N/L as NH_4Cl and 25 mg NO_2-N/L as $NaNO_2$, and collecting samples to measure the concentrations of NH_3-N, NO_2-N, and NO_3-N in the MBBRs over time. These tests involved the collection of at least 5 samples spread out over a minimum of 2 hours. The nutrient concentrations from these samples were measured with HACH TNT tubes and then plotted against the time the samples were taken. A linear slope that best fit the relationship between the different nutrient concentrations and time was derived for each nitrogen species (in mg/L/hour) to determine the current maximum anammox removal rates for NH_3-N, NO_2-N, and TIN and the maximum anammox production rate for NO_3-N at the time of the test. These removal/production rates were then converted to g/m^2/day, using Equation 12 below, based on the amount of media surface area in the MBBR.

$$Max\ Activity\ Removal\ Rate = \frac{Linear\ Removal\ Rate\ (in \frac{mg}{L}\ per\ hour)}{Total\ Media\ Surface\ Area} \quad \text{Equation 12}$$

The pH, COD concentrations, and OP concentrations of the MBBRs were measured at the beginning and end of the tests. The DO and temperature in the MBBRs were also measured throughout the test to ensure the MBBRs remained anoxic and to have a proper representation of the average MBBR temperature during the test. Once anammox activity was detected in the MBBRs, these tests were conducted on a bi-weekly basis. Equation 13 was used to adjust these rates for temperature changes and is known as the Arrhenius Equation. In this equation, q_0 is the unadjusted growth/removal rate, θ is the Arrhenius coefficient, q is the adjusted growth/removal rate, T is the temperature the rate is being adjusted to (in degrees Celsius), and T_0 is the temperature the rate was at originally (in degrees Celsius).

$$q = q_0 \theta^{(T-T_0)} \quad \text{Equation 13}$$

The Arrhenius coefficient, or θ, in Equation 13, that was used during this study was 1.062, which was based on previous findings (Guo et al. 2010, Nifong 2013).

Results and Discussion

Anammox Detection

Anammox activity was first detected in the MBBR with the preliminary biofilm carriers after approximately 1.5 months of operation. This activity was first detected due to the ammonia removal in the MBBR being higher than the amount of removal that could be attributed to assimilation. This anammox activity was confirmed through an activity test 52 days after startup. Previous startup experiments that did not use anammox seed did not detect anammox activity till 84 (Schoepflin et al. 2020) and 110 (Kanders et al. 2014) days after startup, making this anammox growth unexpectedly fast. This could partially be attributed to the high summer temperatures that were present in the mainstream pilot MBBRs, which ranged between 17.4-28.7°C, increasing the growth rate of the anammox.

Following the detection of this anammox activity, activity tests were periodically conducted in the virgin media MBBR every two weeks until anammox activity was detected during a test 86 days after startup due to continuous, anoxic ammonia removal. This initial anammox detection time falls within the expected range based on previous startup experiments (Kanders et al. 2014, Schoepflin et al. 2020). Following the detection of this anammox activity, activity tests in the virgin media MBBR were conducted on a weekly basis.

While anammox activity in both MBBRs continued to increase after it was first detected (Figure 10) anammox growth was likely limited starting in early October (about 107 days after startup) due to several factors, including declining temperatures and limited effluent amounts of phosphorus leaving the MBBRs.

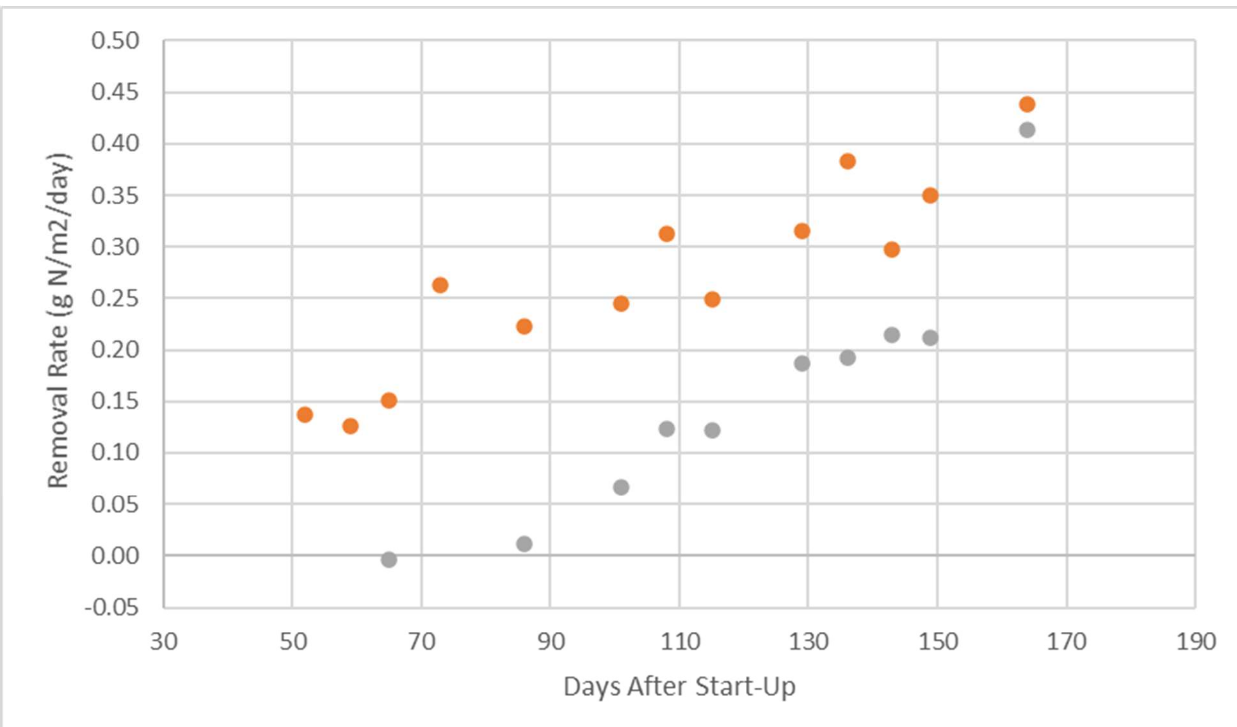

Figure 10. Ammonia removal rates during maximum anammox activity tests adjusted for temperature differences in the preliminary biofilm (orange) and virgin media (grey) MBBRs.

These results indicate that anammox can be grown in mainstream conditions without seed if the proper wastewater conditions are set for anammox activity and growth.

Influent and Effluent Characteristics

Table 2 shows the influent characteristics for both MBBRs throughout the experiment. While the pH fell within the acceptable range for anammox of 6.5-8.0 (Van Hulle et al. 2007), the high influent DO concentrations did result in some additional external carbon demand in the MBBRs to bring the DO down to suitable levels for denitrification and anammox growth. The influent temperature was also relatively high for mainstream conditions due to the startup beginning during the summer months. The average temperature was relatively high for mainstream conditions, which likely sped up anammox growth to a degree. The average AvN ratio of the influent was 0.61 ± 0.30 g/g, which resulted in slightly higher effluent ammonia concentrations in the MBBRs compared to the effluent nitrite concentrations (Table 3), which was caused by full denitrification removing some additional nitrite. TSS and COD concentrations were within the expected range for WWTP secondary clarifier effluent. Influent phosphorus concentrations were low in the influent due to efficient biological phosphorus removal in the full-scale WWTP IFAS tanks. Since both anammox and OHOs require some phosphorus for growth, phosphorus was added to the MBBRs by adding potassium phosphate monobasic into the glycerol stock. This was done to ensure that denitrification and anammox activity were not limited by phosphorus. Based on the average influent nitrite concentrations, a small, but significant, amount of nitrite was also provided for anammox activity in the influent, indicating that a small amount of PdN was likely occurring in the full-scale WWTP IFAS reactors.

Table 2. MBBR influent characteristics throughout the entire startup experiment.

	MBBR Influent
pH	6.68 ± 0.22
DO (mg/L)	3.16 ± 0.39
Temp. (°C)	24.8 ± 3.3
NH3-N (mg/L)	3.13 ± 1.38
NO2-N (mg/L)	1.14 ± 0.32
NO3-N (mg/L)	4.41 ± 1.57
OP (mg/L)	0.08 ± 0.13
sCOD (mg/L)	31.0 ± 5.5
TSS (mg/L)	20.0 ± 7.0

Table 3 shows the effluent characteristics for both the preliminary biofilm MBBR and the virgin media MBBR.

Table 3. Effluent characteristics for both MBBRs throughout the start-up experiment.

	Preliminary Biofilm MBBR	**Virgin Media MBBR**
pH	6.63 ± 0.20	6.64 ± 0.20
DO (mg/L)	0.23 ± 0.32	0.30 ± 0.42
Temp. (°C)	24.6 ± 3.3	24.5 ± 3.3
NH3-N (mg/L)	1.32 ± 0.93	2.01 ± 1.09
NO2-N (mg/L)	1.06 ± 0.60	1.78 ± 1.01
NO3-N (mg/L)	1.27 ± 0.80	1.35 ± 1.10
OP (mg/L)	0.30 ± 0.55	0.32 ± 0.71
sCOD (mg/L)	29.5 ± 5.8	29.9 ± 6.3
TSS (mg/L)	28.4 ± 7.6	28.5 ± 7.0

While the average effluent nitrate concentration was slight over the targeted 1 mg N/L in both MBBRs, the carbon control was still considered effective due to the relatively low nitrate concentrations that were being polished in the MBBRs. Additionally, there were a few days of operation where the carbon feed was blocked, which led to little or no external carbon feed, and thus denitrification, which dramatically affected the average effluent nitrate concentration.

Since both the average influent and effluent sCOD concentrations in the MBBRs were at approximately 30 mg/L, it was assumed that this COD was recalcitrant, or not available to use for denitrification. The similar influent and effluent sCOD concentrations also indicated that neither MBBR was substantially overdosed with glycerol.

Overall, OP addition through the glycerol stock provided enough phosphorus to bring the average effluent OP concentration above the targeted 0.2 mg P/L, which has proved to be a non-limiting effluent phosphorus concentration for denitrification in a MBBR (Peric et al. 2009). There were still some days and time periods during operation, however, where effluent phosphorus concentrations were below the 0.2 mg P/L target due to operating errors, especially between 75 and 120 days after startup.

While the influent and effluent nitrite concentrations appear to be the same, especially in the preliminary biofilm MBBR, all the nitrate that was removed was initially converted to nitrite before either being removed by anammox activity or full denitrification, so some of the nitrate that was converted to nitrite was also used by anammox to oxidize ammonia. Additionally, anammox activity was detected without anammox seed with average effluent ammonia and nitrite concentrations below 2.1 and 1.8 mg N/L, respectively, indicating that large amounts of effluent ammonia and nitrite are not necessary to grow anammox, although it appears to help with encouraging process stability and increasing anammox growth rates.

Partial Denitrification Efficiency

After the PdN carbon control was properly setup around 30 days after startup, the effluent NO_3-N concentration for both MBBRs has held within the range of 0.5 to 1.5 mg/L. Due to the C/N setpoint being too high, however, the carbon control was overdosing external carbon to the MBBRs until approximately 80 days after startup, which led to unsteady and low amounts of PDN in both MBBRs. This overdosing occurred for such a long period of time because the effluent nitrate concentration remained around 1 mg/L, despite the carbon overdosing. The overdosing was only discovered when a lower C/N ratio setpoint was used and the effluent nitrate concentrations remained around 1 mg N/L.

After a controlled nitrate residual was consistently acquired in both MBBRs and external carbon overdosing in the carbon feed program was minimized, the PdN efficiency of the preliminary biofilm media MBBR fluctuated between 23%-83% with an average of 55% ± 15%, while the PDN efficiency of the virgin media MBBR fluctuated between 22%-73% with an average of 53% ± 14% (Figure 11). Therefore, the difference between the partial denitrification efficiencies of the two MBBRs can be considered minimal and likely did not factor much into the difference between the anammox startup times of the two MBBRs. Despite the issues affecting partial denitrification that were faced during the first few weeks after startup, significant amounts of nitrite (1.5-4 mg N/L) were detected in both MBBRs shortly after startup, which helped provide the environment needed for anammox growth.

These PdN efficiencies were lower than many previous PdNA experiments, including Campolong et al. (2019) and Le et al. (2019a), who reported glycerol PdN efficiencies around 90% and above 90%, respectively. It has not been determined why the PdN efficiencies in these two MBBRs were so low, although some potential explanations include low TIN loading rates and the targeted effluent nitrate concentration in the MBBRs that was being too low to support PdN.

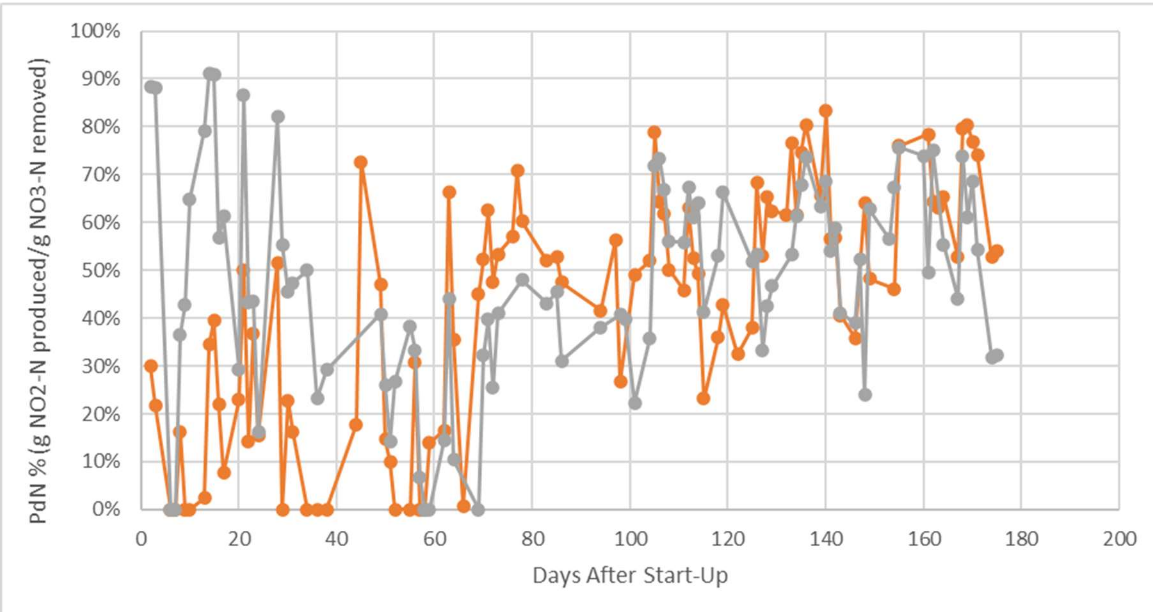

Figure 11. Partial denitrification efficiencies in the preliminary biofilm media (orange) and the virgin media (grey) MBBRs.

Anammox Growth Curves

Anammox growth curves for both the preliminary biofilm and virgin media MBBRs were generated based on the amount of ammonia removal that occurred in the maximum anammox activity tests. The growth curve for the anammox in the preliminary biofilm MBBR was not realistic however, since no maximum anammox activity tests were conducted when anammox activity first began in that MBBR. The growth curve for the anammox in the virgin media MBBR is displayed in Figure 12 below.

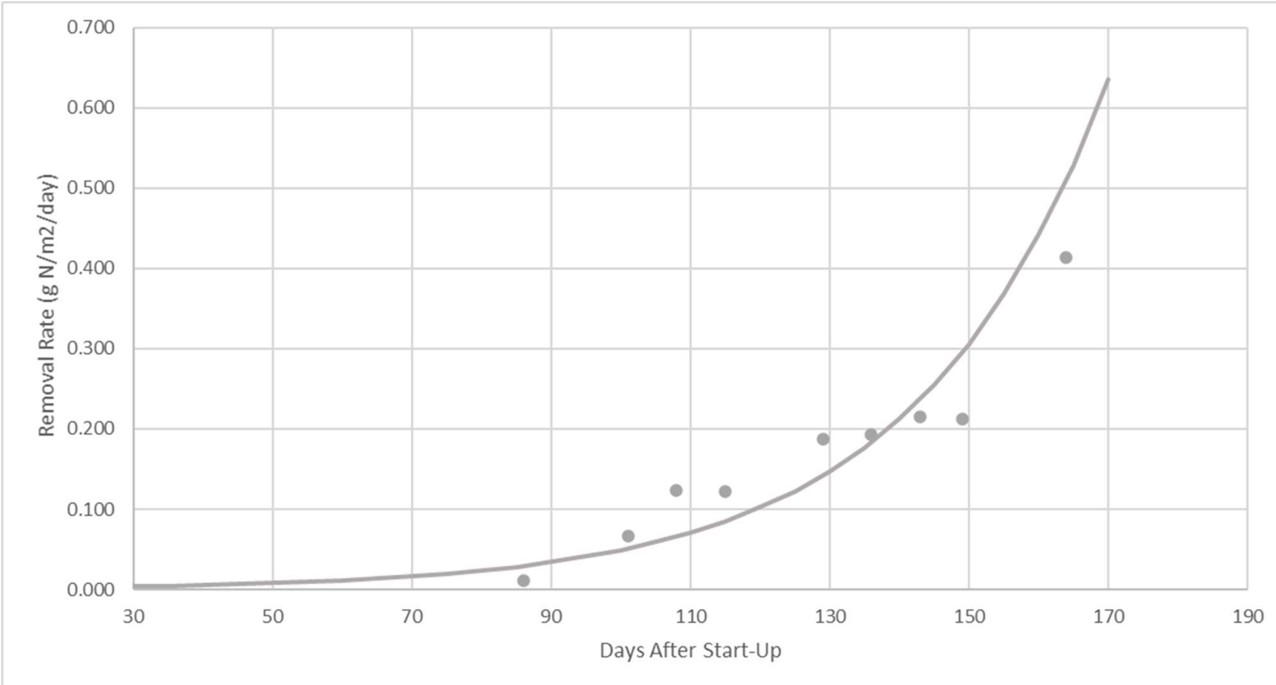

Figure 12. Generated anammox growth curve (grey line) based on maximum ammonia removal rates (grey points) for the virgin media MBBR.

The maximum growth rate and doubling time for the virgin media MBBR were calculated to be 0.037 d^{-1} (equivalent to 0.0015 h^{-1}) and 19.0 days, while the initial anammox activity for the curve was determined to be 0.001 g/m^2/day (at 20°C). Equation 14 below shows the full growth curve equation for the anammox in the virgin media MBBR.

$$C = C_0 e^{k\times t} \; where \; k = 0.037 \; \frac{1}{d} \; and \; C_0 = 0.001 \; \frac{g}{m^2 \times day} \qquad \text{Equation 14}$$

This maximum growth rate and doubling time were much slower than values reported in the literature. Anammox in a sequencing batch reactor (SBR) had a doubling time of 11 days and maximum specific growth rate of 0.0027 h^{-1} (Strous et al. 1998), while anammox on gel beads had maximum growth rates of 0.33 d^{-1} and 0.18 d^{-1}, with corresponding doubling times of 2.1 days and 3.9 days (Zhang et al. 2017). A anammox doubling time of 10–12 days was also reported in a full-scale sidestream activated sludge PNA reactor (van der Star et al. 2017). These growth rates from the literature are likely quicker due to the anammox being grown in environments with significantly more nitrite and ammonia present. Considering the relatively low amounts ammonia and nitrite being treated in the PdNA MBBRs, the anammox growth rate demonstrated in this experiment is still significant enough for full-scale mainstream PdNA applications.

Nitrogen Removal

The average amounts of NH_3-N removed per mg of glycerol as COD used in the preliminary biofilm and virgin media MBBRs were 0.044 mg N/mg COD and 0.022 mg N/mg COD, respectively. These values were used to calculate the amount of ammonia removal that could be attributed to assimilation in the MBBRs so the exact amount of ammonia removed by anammox could be determined (Equation 9). Compared to the maximum theoretical assimilation amount of 0.047 mg N/mg COD, which was based on assumed values of 0.087 mg NH_3-N/mg cells as COD and a yield value of 0.54 g cells as COD/mg glycerol as COD, the amount of ammonia assimilated in the preliminary biofilm MBBR is close to the theoretical maximum value, while the amount of ammonia assimilated in the virign media MBBR was not. The amount of ammonia attributed to assimilation in the virgin media MBBR was lower than that of the preliminary biofilm MBBR and the theoretical glycerol amount due to the preliminary biofilm carriers already having pre-established OHOs, which conducted more assimilation in the early days of the experiment and increased the overall average assimilation before anammox detection in that MBBR. While the amount of ammonia removal before anammox activity was detected was consistent in the preliminary biofilm MBBR, the amount of ammonia assimilated in the virgin media MBBR did increase over time, even before anammox activity was detected. During the beginning of the experiment, OHOs had not been established in the virgin media MBBR yet, so less assimilation occurred in that MBBR while OHOs were established, causing the overall average assimilation before anammox detection in that MBBR to be lower.

Both daily *in situ*, or regular operation, removal rates and maximum removal rates that occurred during anammox activity tests were calculated and recorded. The maximum ammonia removal that was accomplished during an anammox activity test in the preliminary biofilm MBBR was 0.413 g/m^2/day when adjusted to 20˚C (Figure 13), while the highest amount of *in situ* ammonia removal, not adjusted for temperature differences, was 0.284 g/m^2/day at 20.4˚C (Figure 13), which is equivalent to 0.227 g/m^2/day at 20˚C. The highest rate of *in situ* TIN removal that was accomplished in the preliminary biofilm carrier MBBR was 0.752 g/m^2/day when adjusted to 20˚C.

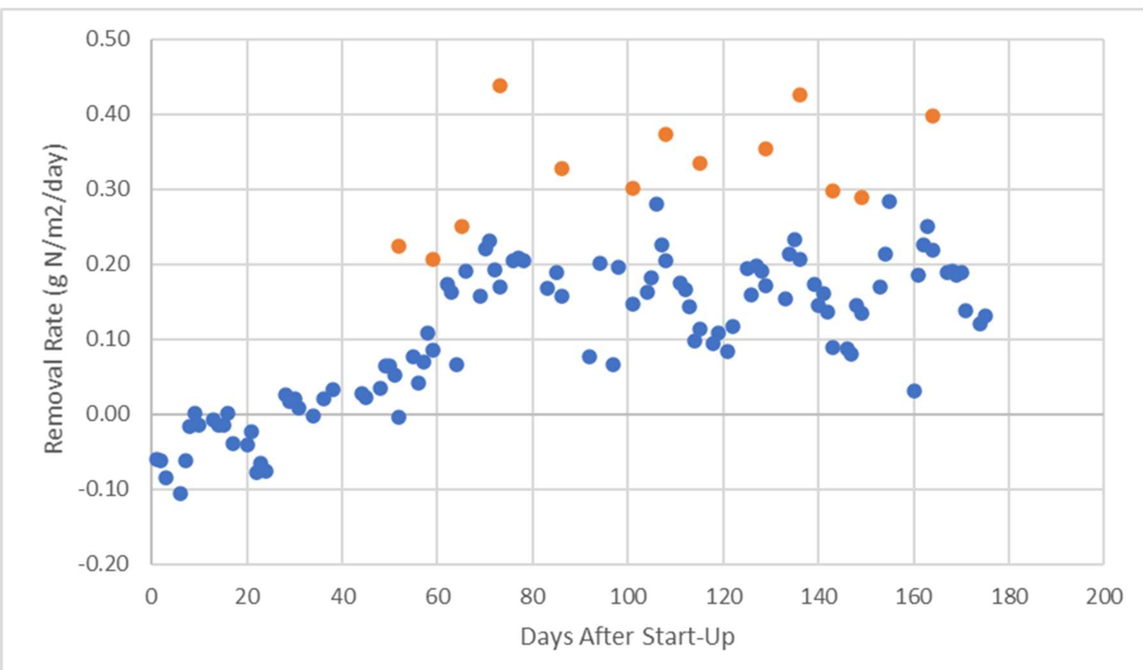

Figure 13. *In situ* (blue) and maximum (orange) anammox ammonia removal rates in the preliminary biofilm MBBR without temperature adjustment.

As seen in Figure **13**, the *in situ* ammonia removal rates in the preliminary biofilm MBBR continued to increase until approximately 80 days after startup, when the rates stopped increasing and began to fluctuate. The removal rate likely stopped increasing due to many factors, including a gradual decrease in temperature, limited effluent phosphorus concentrations, and either limited effluent ammonia or nitrite concentrations due to the AvN control not being finely tuned. Despite these issues though, the average effluent TIN concentration for this MBBR, after the detection of anammox activity, was 3.36 ± 0.94 mg/L (Figure 14).

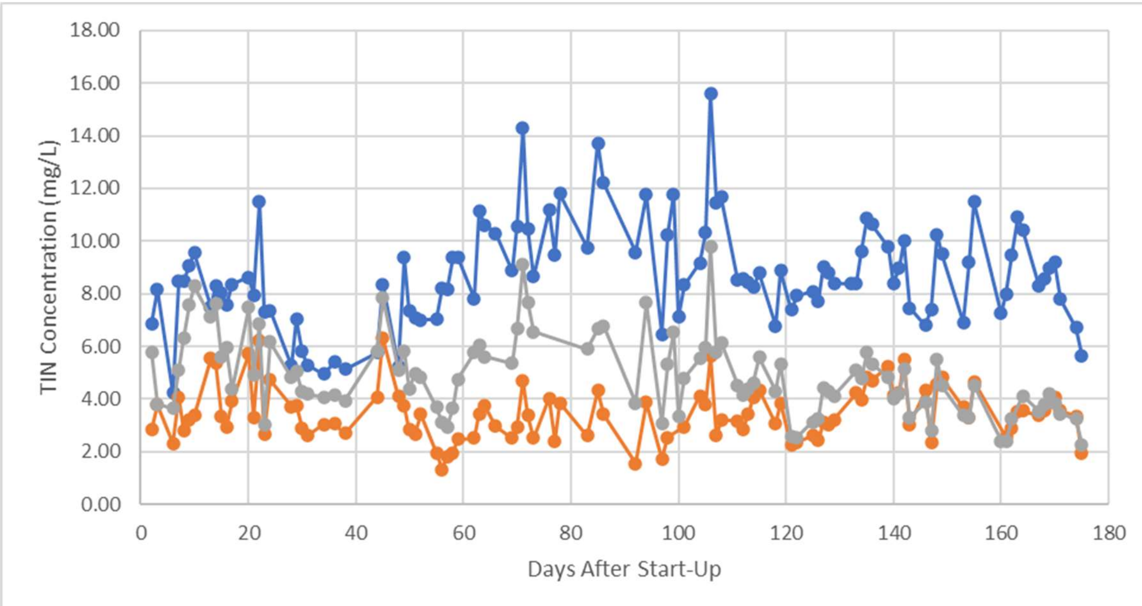

Figure 14. TIN concentrations in the shared MBBR influent (blue) preliminary biofilm MBBR effluent (orange), and virgin media MBBR effluent (grey).

The maximum ammonia removal that was accomplished during an anammox activity test in the virgin media MBBR was 0.390 $g/m^2/day$ when adjusted to 20˚C (Figure 15), while the highest amount of *in situ* ammonia removal, not adjusted for temperature differences, was 0.278 $g/m^2/day$ at 20.4˚C (Figure 15), which is equivalent to 0.271 $g/m^2/day$ at 20˚C. The highest rate of *in situ* TIN removal that was accomplished in the virgin media MBBR was 0.666 $g/m^2/day$ when adjusted to 20˚C, while the average effluent TIN concentration for this MBBR, after the detection of anammox activity, was 4.42 ± 1.39 mg/L (Figure 14).

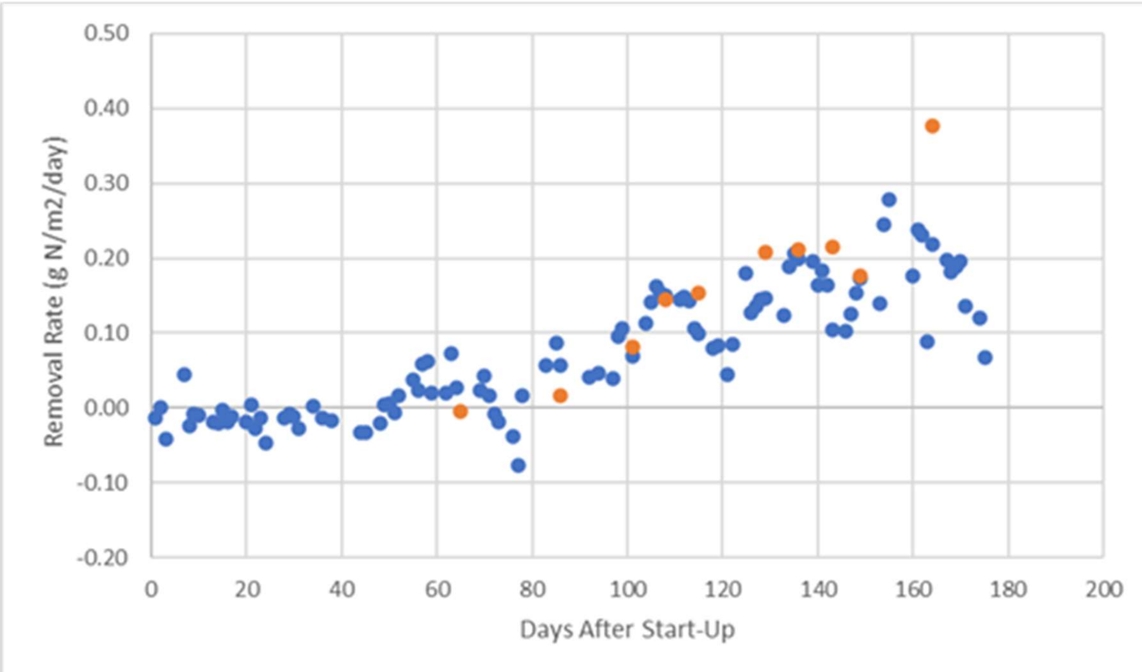

Figure 15. In situ (blue) and maximum (orange) anammox ammonia removal rates in the virgin media MBBR without temperature adjustment.

As shown in Figure **15**, ammonia removal due to anammox activity in the virgin media MBBR began around 80 days after startup and continuously increased till at least 160 days after startup, at which point the ammonia removal rates began to fluctuate like the removal rates in the preliminary biofilm MBBR. Like the preliminary biofilm MBBR removal rates, these rates likely stopped increasing due to multiple factors, including decreasing temperatures, limited effluent phosphorus concentrations, and either limited effluent ammonia or nitrite concentrations due to the AvN control not being finely tuned.

Although both MBBRs reached low effluent TIN concentrations within 180 days after startup, the maximum observed ammonia removal rates in the MBBRs were significantly lower compared to mainstream polishing nitrification rates, which have ranged from 0.7-1.5 g/m^2/day (Hem et al. 1994, Ødegaard 2006) at 15°C, and a typical sidestream PNA ammonia removal rate of 3.5 g/m2/day at 30°C (Klaus et al. 2017), even when the temperature difference and ammonia removal through AOBs are accounted for in the PNA rate. Based on these comparisons, the removal rates in the PdNA MBBRs from this experiment should continue to increase if the properly ratioed amount of ammonia and nitrite are provided and/or the MBBR loading rates are increased.

Biofilm Total Solids

Towards the beginning of the experiment, the preliminary biofilm media had a larger biofilm total solids compared to the virgin media, since a biofilm had already been present on that media when the experiment began (Figure 15). The biofilm on both types of media stayed consistent for the first 2-3 months. About 2-3 months after the pilot was first started, the biofilm on the virgin media began to increase, The biofilm on the media from the preliminary biofilm MBBR, however, remained at a consistent mass until late November (about 5 months after pilot startup), at which point, the biofilm mass began to increase at approximately the same rate as the virgin media. This biofilm growth that occurred on both types of media around 5 months into the experiment was likely due to the noticeable decrease in the pilot influent temperature as the pilot began to operate through the colder, winter months. The biofilm masses likely converged due to similar microbial communities growing within the MBBRs, since they both were treating the same influent and were dosed with the same carbon source.

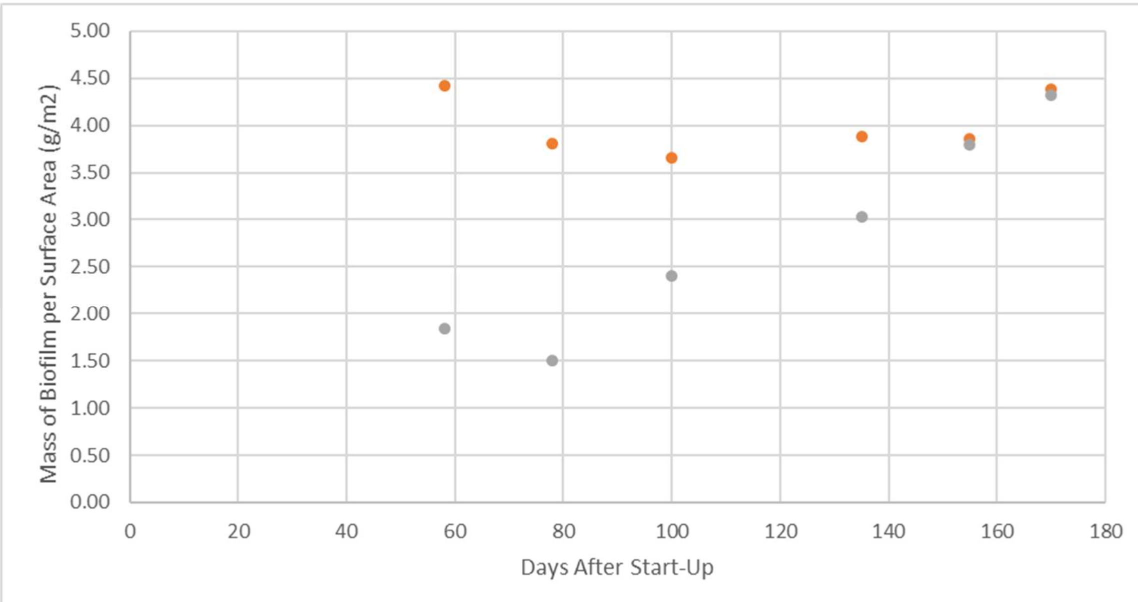

Figure 16. Biofilm mass per media surface area for preliminary biofilm MBBR (orange) and virgin media MBBR (grey).

Conclusions

These results confirmed that starting up a mainstream polishing PdNA MBBR does not require anammox seeding if an environment is established that supports anammox growth. The large amount of anammox biomass/seed that would be required for full-scale mainstream anammox process startup was one of the major barriers preventing the use of these processes, making the confirmation that mainstream anammox startup does not require seed vital for full-scale anammox implementation. Anammox activity was confirmed in the preliminary biofilm and virgin media MBBRs within 52 and 86 days after startup, respectively. These startup times for anammox in the MBBRs were shorter than what has previously been reported, despite having low average effluent ammonia and nitrite concentrations, with ammonia and nitrite effluent concentrations averaging less than 2.1 and 1.8 mg/L, respectively, throughout startup in both MBBRs. This indicates that high effluent ammonia and nitrite concentrations (>10 mg N/L) are not needed for anammox process startups. The relatively high summer mainstream temperatures, which ranged from 17.4-28.7°C, that were present in the MBBRs before anammox activity detection likely helped speed up the anammox growth in the MBBRs, so a startup beginning in colder temperatures would likely not progress as quickly. The study also confirmed that using MBBR carriers with a preliminary biofilm decreases the anammox startup time required by approximately 1 month compared to virgin carriers. TIN removal in both MBBRs could be further increased by increasing the influent nitrogen loading and refining the upstream aeration control to provide a properly balanced amount of NH_3 and NO_x, or AvN, in the MBBRs' influent. Overall, these startup experiments indicate that a full-scale mainstream PdNA MBBR process can be started in under 3 months without anammox seed, provided that the proper conditions and at least a small amount of ammonia and nitrite are present, making full-scale mainstream PdNA processes more accessible for WWTPs to utilize for aeration and external carbon savings.

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Manuscript 2: Nitrogen Removal Capacity and Carbon Demand Requirements of Partial Denitrification/Anammox MBBR and IFAS Processes

Abstract

This study was conducted to investigate the carbon demand and nitrogen removal capabilities of a process with 3 different moving bed biofilm reactor (MBBR) zones, including a partial denitrification/anammox (PdNA) MBBR zone, an anammox (AMX) MBBR zone, and an aerated nitrifying MBBR zone. The PdNA and AMX MBBR zones provided overall TIN removal, while the nitrifying MBBR zone polished any remaining nitrite (NO_2) for the purpose of effluent toxicity and downstream disinfectant demand. The average effluent total inorganic nitrogen (TIN) concentrations for this process throughout operation was 2.81 ± 1.21 mg TIN/L, with an average effluent NO_2 concentration below 0.5 mg N/L at ammonia and nitrite loading rates of 0.055 ± 0.035 and 0.379 ± 0.112 g N/m^2/day for the nitrifying MBBR. The average COD/TIN ratio for the PdNA MBBR was 2.42 ± 0.77 g COD/g TIN. During this experiment, a PdNA integrated fixed film activated sludge (IFAS) reactor was also operated at three different hydraulic retention times and carrier fill fractions, which was done to incrementally increase nitrogen loading to the reactor. The average effluent TIN concentration for the PdNA IFAS reactor was 4.07 ± 1.66 mg/L with an average COD/TIN ratio of 1.08 ± 0.38 g COD/g TIN while the reactor was operated at the original HRT of 29.4 minutes. The average effluent concentration for the PdNA IFAS reactor was 3.30 ± 0.96 mg TIN/L when the reactor's HRT was set to 24.4 minutes, and the average COD/TIN ratio at that time was 2.18 ± 0.99 g COD/g TIN. This experiment concluded that both a mainstream polishing PdNA MBBR and PdNA IFAS reactor can produce low effluent TIN concentrations with relatively low carbon requirements compared to the conventional nitrification/denitrification nitrogen removal process, even with relatively low and unstable PdN efficiencies in both.

Introduction

Integration of anammox into mainstream wastewater treatment technologies and processes has been a focus of researchers in the wastewater treatment industry for over a decade, yet little progress has been made to make anammox implementation into full-scale mainstream processes. Anammox (anaerobic ammonia oxidation) bacteria oxidize ammonia (NH_3) to dinitrogen gas (N_2) in anoxic conditions while using nitrite (NO_2) as an electron acceptor.

PdNA is a biological nutrient removal process that combines partial denitrification with anammox to efficiently remove nitrogen from wastewater. Most notably, this process provides up to 50% aeration and 80% external carbon savings (Strous et al. 1998, Le et al. 2019b, Zhang et al. 2020). While most previously implemented full-scale anammox applications have been PNA processes, the vast majority of these full-scale anammox implementations have been for sidestream treatment. PNA, while providing aeration savings up to 60% and external carbon savings up to 100%, has proven to be difficult to implement as a mainstream treatment process, because PNA requires the outselection of NOB. Sidestream conditions provide the high ammonia (NH_3) concentrations and temperatures that help encourage NOB outselection (Lackner et al. 2014), while mainstream conditions do not. PdNA does not require NOB outselection, potentially making it the most well-suited anammox process for most mainstream conditions.

Maintaining a consistent ammonia to NO_x ratio is critical for optimal PdNA performance, since anammox requires the presence of both ammonia and nitrite. Ammonia versus NO_x (AvN) based aeration control can be used to provide an influent to a PdNA process with the proper amounts of ammonia and NO_x (Regmi et al. 2014, Regmi et al. 2015, Klaus et al. 2020). The optimal AvN ratio setpoint varies between PdNA processes mainly due to differences in partial denitrification (PdN) efficiency.

Efficient nitrogen removal through PdNA requires efficient PdN, meaning that a significant amount of influent nitrate (NO_3) must only be denitrified to nitrite rather than all the way to dinitrogen gas. This is necessary to ensure that an adequate amount of nitrite is produced for the anammox to use to anaerobically oxidize ammonia to dinitrogen gas. Promoting PdN has been done through a variety of methods, which include maintaining a high pH, controlling the hydraulic retention time (HRT), using organic carbon sources, limiting the amount of carbon dosed for every amount of nitrogen removed (or the C/N ratio), and maintaining a nitrate residual in the effluent (Le et al. 2019_b, Ma et al. 2020). Of these methods, using organic carbon sources, limiting the C/N ratio, and maintaining a nitrate residual are the most practical, since maintaining a high pH and controlling HRT in a full-scale plant is not always possible. Organic carbon sources that have supported PdN in previous studies include, but are not limited to, methanol, acetate, glycerol, glucose, ethanol, propionate (van Rijn et al. 1996, Bill et al. 2009, Le et al. 2019a, Campolong et al. 2019). Throughout these studies, glycerol and acetate have demonstrated higher PdN efficiencies compared to the other carbon sources, making them preferable for PdNA processes. Even though several studies have demonstrated that methanol is not inhibitory to anammox, as previously thought, it has consistently produced the lowest PdN efficiencies among these organic carbon sources in studies (Campolong et al. 2019, Le et al. 2019_a), which means a methanol PdNA system would likely provide less nitrite to promote anammox growth and oxidize ammonia with through the anammox pathway.

PdNA processes can be operated in a single-stage or two-stage configuration. A single-stage configuration requires that ordinary heterotrophic organisms (OHOs) and anammox co-exist in a single reactor, Two-stage PdNA separates the OHOs and anammox into two separate tanks so that the partial denitrification occurs in the first tank and the anaerobic ammonia oxidation occurs in the second tank. Separating these two microorganism types is supposed to prevent competition between the two types of organisms for nitrite (Cao et al. 2017, Bahtiar et al. 2020), although previous studies have shown that PdNA can be effectively implemented in a single-stage configuration where anammox must compete with OHOs for nitrite (Le et al. 2019_b). Since single-stage configurations only require one tank, a full-scale implementation of this configuration would also potentially take less space and money compared to two-stage configurations. Another disadvantage of Two-stage configurationsis that they produce a nitrate effluent residual, since anammox produce nitrate in the second reactor. Overall, single-stage configurations appear to be operationally superior to two-stage configurations, since they provide both footprint and financial advantages, do not require additional nitrate residual polishing, and have worked efficiently without separating the OHOs and AMX (Regmi et al. 2015, Le et al. 2019_b, Campolong et al. 2019).

PdNA has been implemented in hybrid granular/suspended growth processes (Le et al. 2019b), deep bed filters (Fofana et al. 2020, Cui et al 2020, Klaus et al. 2020), upflow anaerobic sludge bed reactors (Xu et al. 2020), MBBRs (Campolong et al. 2019), and IFAS (Forney et al. 2020) configurations. All of these are pilot-scale processes except for Klaus et al. (2020), which describes the full-scale implementation of a PdNA deep-bed denitrification filter operating at the Hampton Roads Sanitation District's (HRSD's) York River Wastewater Treatment Plant since 2018.

If PdNA can be implemented using IFAS technology, any WWRF with a second anoxic zone could potentially use that zone for PdNA, which would provide all of the treatment benefits of PdNA at a relatively low capital cost.

The objectives of this study were:

1. Develop a MBBR process with low external carbon demand that can sufficiently polish secondary clarifier effluent to produce low TIN concentrations and minimize effluent NO_2.
2. Investigate the nitrogen removal capabilities of PdNA implemented in a second anoxic zone of a 4 or 5-stage BNR process using IFAS for AMX retention.

Materials and Methods

Pilot Setup

A series of two anoxic MBBRs and one aerobic MBBR were fed effluent from the full-scale IFAS tanks at HRSD's JRTP, which was pumped to the pilot by a Godwin GSP10 submersible pump (Xylem, Rye Brook, NY). Before being fed into the MBBRs, the IFAS effluent flowed through both a 1000-gallon clarifier with a surface overflow rate (SOR) of 260 gallons-per-day/ft2 and a 120-gallon equalization (EQ) tank with an HRT of 30 minutes. The settled solids in the clarifier were drained out with a progressive cavity pump (Seepex, Enon, Ohio) at a rate ranging from 3.5-4 gallons per minute (gpm). The MBBR influent was also chemically dosed with an average of 2.25 mg NO_3-N/L to substitute for any NO_x that was lost due to denitrification occurring in between the end of the JRTP's full-scale IFAS tanks and the pilot MBBRs. The MBBR influent was then pumped from the EQ tank with a progressive cavity pump (Seepex, Enon, Ohio) at a flow rate ranging from 1.7-2.5 gpm. The first anoxic MBBR in the series had a 100-gallon volume, an HRT of 38-60 minutes, and contained preliminary biofilm K3 media (Anoxkaldnes, Lund, Sweden) from the JRTP's IFAS tanks at a 50% fill fraction, or 94.6 m^2 of total media surface area (Figure 17). The media in this MBBR already had established anammox on it during a prior anammox startup study (without seed) in the same MBBR. The second anoxic MBBR in the series had a 40-gallon volume, an HRT of 16-24 minutes, and contained preliminary biofilm K3 media (Anoxkaldnes, Lund, Sweden) from a PdNA MBBR from HRSD's Chesapeake-Elizabeth Treatment Plant (CETP) BNR Pilot at a 50% fill fraction, or 37.9 m^2 of total media surface area. The media in this MBBR also already had established anammox on it during a prior anammox startup experiment in a PdNA MBBR at the CETP BNR Pilot (Schoepflin et al. 2020). The third MBBR in the series was aerobic with a dissolved oxygen (DO) concentration ranging between 4.1-9.4 mg/L and served as a nitrifying polishing zone for nitrite. This MBBR also had a 40-gallon volume, an HRT of 16-24 minutes, and virgin WW1 media (World Water Works, Oklahoma City, Oklahoma) at a 50% fill fraction initially or 49.2 m^2 of total media surface area. The fill fraction in this MBBR was changed to 37.5% and 25% (36.9 m^2 and 24.6 m^2 of total media surface area, respectively) at different times during this study as well though. The media in this MBBR did not have any kind of major biofilm established on it before startup, but it was soaked beforehand to ensure the media was not hydrophobic when placed into the functioning MBBR.

A pilot-scale, anoxic IFAS reactor was also fed effluent from the full-scale JRTP IFAS tanks that was pumped to the pilot with a Godwin GSP10 submersible pump (Xylem, Rye Brook, NY). When the effluent from the full-scale WWRF IFAS aerated zones arrived at the pilot, approximately 4 gpm of flow was redirected to the pilot-scale IFAS reactor with a progressive cavity pump (Seepex, Enon, Ohio). This IFAS pilot influent was chemically dosed with up to 5-7 mg NO_3-N/L as sodium nitrate using a Pro-Series M-2 peristaltic pump (Blue-White Industries, Huntington Beach, CA). 1-2 mg N/L of ammonia was also added to the pilot IFAS influent, in the form of ammonium chloride, towards the end of the experiment with the same pump. The influent was also chemically dosed with up to 2 mg P/L of orthophosphate (OP) at different points throughout the study, also using a Pro-Series M-2 peristaltic pump (Blue-White Industries, Huntington Beach, CA), to determine if phosphorus limited the denitrifying and anammox activity in the reactor. The IFAS reactor initially had a 100-gallon volume and a 25-minute HRT, but the volume and HRT were eventually lowered during the study to 77-gallons and 19 minutes, respectively, to simulate a potential full-scale PdNA IFAS application more accurately and increase the nitrogen loading to the IFAS reactor. The IFAS reactor held a DO range of 0.0-0.1 mg/L and contained WW1 media (World Water Works, Oklahoma City, Oklahoma) that already had anammox established on it during a prior PdNA MBBR anammox start-up experiment (Figure 17). The media fill fraction was initially kept at 38.5%, or 94.6 m^2 of total media surface area, but this fill fraction was eventually increased to 50% when the tank volume was lowered from 100 gallons to 77 gallons 41 days after the experiment had started. The fill fraction was then lowered to a 30.5% fill fraction 85 days after the start of the experiment when media was removed, resulting in a total media surface are of 57.8 m^2, to increase the nitrogen loading rate and help encourage more anammox growth. Additionally, an aerated polishing zone with an HRT of 4 minutes was added approximately two months after the experiment began. This additional tank was used to simulate a potential full-scale aeration zone at the JRTP that could be used for residual OP uptake. This zone did not contain any media and had a DO range of 1.7-5.9 mg/L (with an average of 3.8 mg/L).

Figure 17. Pilot IFAS media (right) versus media from the first pilot MBBR (left).

A distributed control system (DCS) was used for the carbon control program and to control the pilot pump speeds. The NH_3, NO_2, NO_3, and TIN loading rates of both the MBBRs and IFAS reactor were all dependent on the influent coming into the JRTP and the plant's performance. The MBBRs and IFAS reactor were not seeded with any additional anammox during the study. Figure 18 shows the process flow diagram for this pilot configuration.

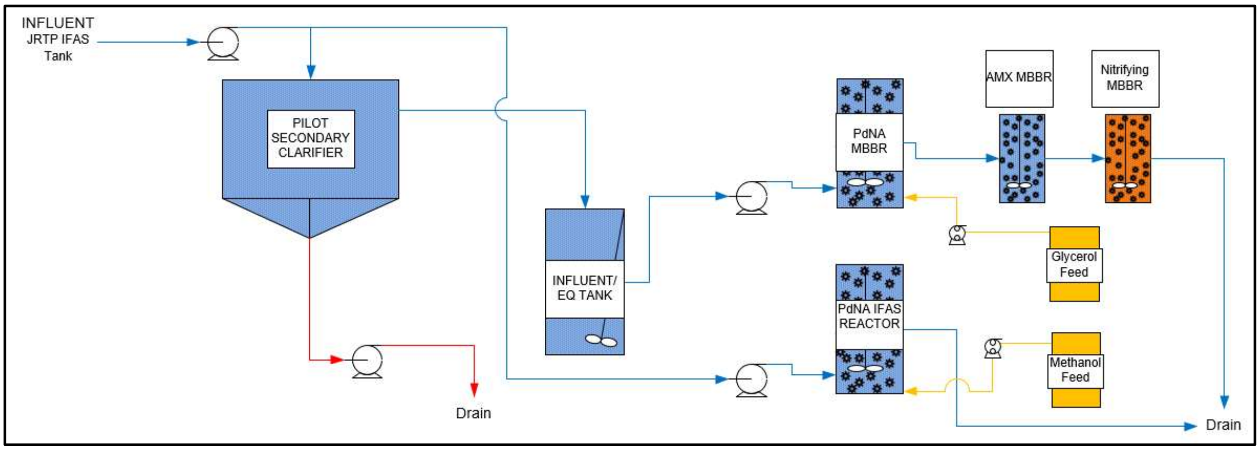

Figure 18. Process flow diagram for PdNA MBBR and IFAS pilot.

During operation of the pilot MBBRs and IFAS reactor, AvN-based aeration control was used in the full-scale JRTP IFAS tanks to produce an influent for the pilot that had a balanced amount of ammonia and NO_x. The controller then adjusts the aeration so that enough ammonia is nitrified to nitrite or nitrate to reach the set ratio of ammonia and NO_x. Throughout pilot operation, the AvN effluent setpoint for the plant's IFAS tanks was adjusted to accurately provide an influent AvN that was suitable for PdNA in the MBBRs. This user-set ratio ranged between 0.8 and 1.0. Since both the pilot MBBRs and IFAS reactor were fed influent from the same full-scale IFAS tank, additional nitrate, as sodium nitrate, was added to the pilot IFAS reactor influent to provide influent with a lower AvN ratio needed for optimal PdNA performance in that reactor.

A picture of the pilot setup at the JRTP is displayed in Figure 19.

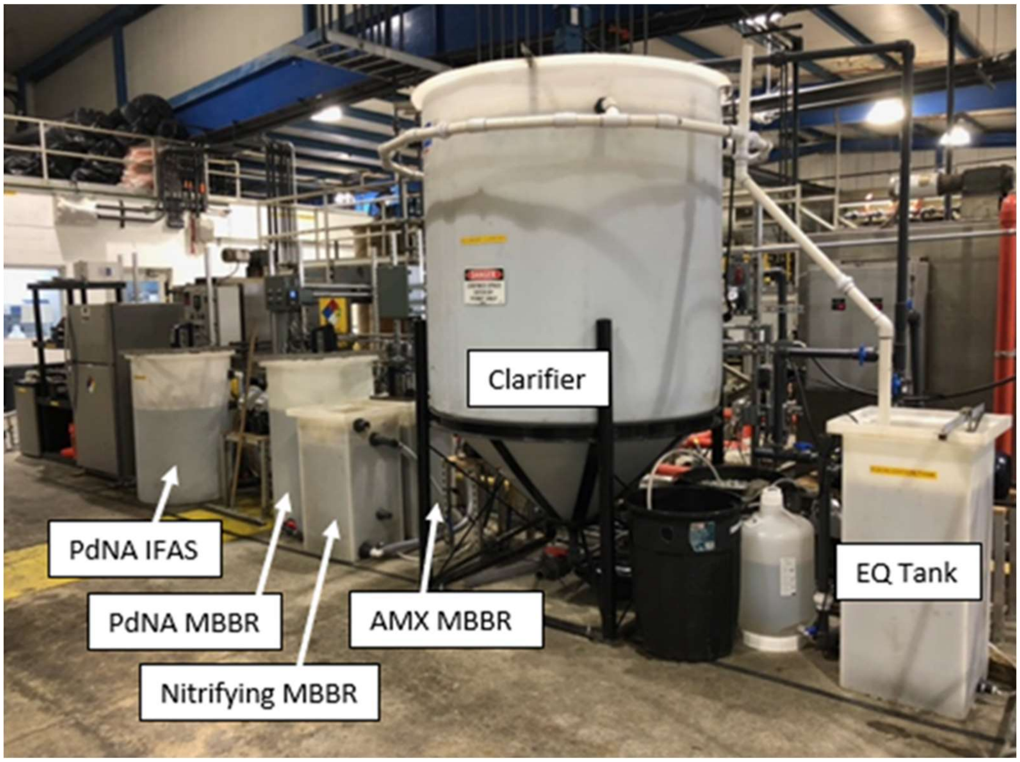

Figure 19. Pilot setup for PdNA MBBR and IFAS nitrogen loading experiment.

Chemical Oxygen Demand (COD) Dosing

The first MBBR was dosed with glycerol using a Pro-Series M-2 peristaltic pump (Blue-White Industries, Huntington Beach, CA) at a rate that was determined by a feedforward control program based on the NO_3-N concentration measured by a Nitratax probe (HACH, Loveland, Colorado) in the EQ tank. The nitrite concentrations in the EQ tank did cause some interference, which was accounted for in the carbon dosing program with a COD/NO_3-N user setpoint, which was adjusted based on nitrate removal performance throughout the experiment. This program also used a nitrate effluent setpoint to ensure a nitrate residual was maintained. The feedforward calculation what was used to determine the volumetric flow rate of the MBBR carbon feed control is displayed in Equation 15.

$$Dosing\ Rate = \frac{(NO_{3in} - NO_{3eff})(Flow\ Rate\ to\ MBBR)(Set\ \ COD/NO_3\ Ratio)}{(Stock\ Solution\ COD\ Concentration)}$$

Equation 15

Glycerol was selected as the carbon source for the MBBR. Potassium phosphate monobasic was also added to the glycerol stock to ensure that the MBBR had an effluent reactive phosphorus concentration of 0.2 mg OP-P/L. The second and third MBBRs did not receive any form of external carbon.

The IFAS reactor was dosed with methanol using a Pro-Series M-2 peristaltic pump (Blue-White Industries, Huntington Beach, CA) at a rate the was determined by a feedback control program that was based on the NO_x-N concentration measured in the IFAS tank. This program used a nitrate effluent setpoint to ensure a nitrate residual was maintained in the reactor.

Nutrient Removal Sampling and Measurements

Five times per week, composite samplers (Teledyne ISCO, Lincoln, Nebraska) collected hourly aliquots over a twenty-four-hour period from the first and second MBBRs and the EQ tank, which served as a representation of the influent.

Grab samples of the influent and effluent of the IFAS reactor were collected five times a week as well. These were collected by first taking an influent sample from a collection port near the inlet to the IFAS reactor, waiting approximately 20 minutes (or 1 HRT), and then collecting an effluent sample from the top of the IFAS reactor. After the aerated polishing zone for the IFAS reactor was added, an additional grab sample was collected from the top of that tank when the OP concentration in the IFAS effluent was found to be higher than 0.1 mg P/L. This sample was taken one aerated zone HRT after the IFAS effluent grab sample was collected.

These composite and grab samples were then run through a 0.45 μm filter. The NH_3, NO_2, NO_3, and OP concentrations in these samples were then measured with HACH tubes (HACH, Loveland, Colorado), and read with a HACH DR-2800 spectrophotometer, to determine the amount of partial denitrification, NH_3 removal, NO_3 removal, and TIN removal in the MBBRs and IFAS reactor. Sulfamic acid was added to samples that had a NO_2 concentration greater than 1.0 mg/L to counter any nitrite interference in the NO_3 and soluble chemical oxygen demand (sCOD) measurements. For these samples, approximately 50 mg of sulfamic acid was dissolved into 5 mL of sample and left to sit for 10 minutes before evaluation. The sCOD concentrations for the MBBR samples were measured to calculate the COD consumption and carbon demand. OP was also measured to validate that the reactors were not limited by phosphorus for microbial assimilation. Lastly, the pH, DO, and temperature of the MBBRs, IFAS reactor, and post-IFAS aerated reactor were also measured when the samples were collected.

Grab samples for the influent and effluent of the third aerated MBBR were also collected five times a week. This was done by first taking an influent sample from the top of the second MBBR, waiting one 15-20 minutes (or one HRT), and then collecting an effluent sample from the top of the third, aerated MBBR. These grab samples were then run through a 0.45 μm filter. The NH_3, NO_2, NO_3, and OP concentrations in these samples were then measured with HACH tubes.

To accurately determine the amount of in situ ammonia removal occurring due to anammox activity, the ammonia removal from heterotrophic organisms had to be considered. This was done by calculating the average amount of total ammonia removal that occurred in an MBBR before anammox activity was detected, which could be entirely attributed to heterotrophic ammonia removal. This average value was then subtracted from the total ammonia removal to determine the amount of ammonia removal from anammox activity.

The partial denitrification efficiency (PdN%), or the amount of nitrate that was only denitrified to nitrite (and not to nitrogen gas), was determined using Equation 16 for both the PdNA MBBR and PdNA IFAS reactor.

$$PDN\% = \frac{NO2out - NO2in + 1.32\ x\ (NH3in - NH3out - NH3assimilate\ \ )}{NO3in - NO3out\ \ \ \ .26\ x\ (NH3in - NH3out - NH3assimi\ \ \ \ \ \ )} \quad \text{Equation 16}$$

This calculation for partial denitrification efficiency accounts for anammox activity by factoring in the amount of NO_2 removed by anammox and NO_3 produced by anammox. These amounts were determined based off the measured NH_3 removal that could be attributed to anammox and the anammox stoichiometry found in the literature. The values used for the amount of NH_3 removed by assimilation were based on the average amount of NH_3 removed per mass of COD dosed in the reactor before anammox activity was detected in it during the prior anammox startup experiment.

The total suspended solids (TSS) concentrations in the three composite samples, from the equalization tank and two MBBRs, were measured on a weekly basis to ensure the concentration of solids in the influent and MBBRs was kept within reasonable limits for a MBBR. The TSS concentrations of the composite samples collected from these three tanks were measured using 2540D in the standard methods.

Media Biofilm Solids Measurements

The mass of the biofilms on the carriers in the PdNA MBBR and IFAS reactor were measured about every three weeks. This process involved collecting samples of media from both reactors, placing the collected media in an oven at approximately 100°C for at least 8 hours, allowing the media to cool, measuring the mass of the media samples, scraping the biofilm off of the media using brushes and by soaking the media in a solution containing 25 g/L of ethylenediaminetetraacetic acid (EDTA), placing the media back into the oven for at least 8 hours, letting the media cool again, and measuring the mass of the media samples without biofilm. The mass of the media without biofilm was then subtracted from the mass of the media with biofilm to calculate the amount of biofilm on the collected media samples. This biofilm mass was then divided by the amount of media samples collected and the surface area of a single piece of media to calculate the mass of biofilm per square meter of media surface area (see Equation 17).

$$\frac{Mass\ of\ Biofilm}{m^2\ of\ Surface\ Area} = \frac{(Mass\ of\ Media + Biofi\ \ \ \ ) - (Mass\ of\ Media)}{(Pieces\ of\ Media)\ x\ (Surface\ Area\ per\ Media\ Piece)} \quad \text{Equation 17}$$

Anammox Activity Testing

The maximum anammox activity in the first MBBR and IFAS reactor were measured through anammox activity tests. The anammox activity in the first MBBR was typically measured through a batch test, while the activity in the IFAS reactor was typically measured through a continuous flow test. The MBBR batch tests were conducted by cutting off the flow to the MBBR, leaving the MBBR to sit for 1 HRT to ensure all available COD was used up by the OHOs, spiking the MBBR with 20 mg NH_3-N/L as ammonium chloride and 25 mg NO_2-N/L as sodium nitrite, and collecting samples to measure the concentrations of NH_3-N, NO_2-N, and NO_3-N in the MBBR over time. These tests typically involved the collection of 5 samples spread out over a minimum of 2 hours. The nutrient concentrations from these samples were measured with HACH tubes and then plotted against the time the samples were taken. A linear slope that best fit the relationship between the different nutrient concentrations and time was derived for each nitrogen species (in mg/L/hour) to determine the current maximum anammox removal rates for NH_3-N, NO_2-N, and TIN and the maximum anammox production rate for NO_3-N at the time of the test.

The pH, COD concentrations, and OP concentrations of the MBBR were measured at the beginning and end of the tests to ensure that the ideal conditions were present for anammox activity. The DO and temperature in the MBBR were also measured throughout the test to ensure the MBBR remained anoxic and to have a proper representation of the average MBBR temperature during the test. These tests were typically conducted on a bi-weekly basis.

Anammox activity was measured using a continuous flow maximum activity test in the PdNA IFAS reactor, rather than through a batch test. These tests were conducted by adding a chemical stock solution containing NH_3, NO_2, and NO_3 to the IFAS influent. For some of these tests the stock solution also contained OP. The stock concentration for each nitrogen species was determined based on a desired influent concentration of 10 mg/L for NH_3-N, NO_2-N, and NO_3-N. The stock's OP concentration was determined based on a desired influent concentration of at least 1 mg P/L when OP was added. After the stock was first added to the influent, stock was continuously added to the IFAS influent for a time period equal to at least 100 minutes (about 5 HRTs) before any samples were collected, in order to ensure the activity in the IFAS reactor had reached steady-state.

Since anammox activity is the only potential explanation for anoxic ammonia removal greater than that which can be attributed to assimilation, the ammonia removal rates (in g/m^2/day) were used as a representation of the amount of anammox present in the MBBRs and IFAS reactor. Due to the effect that temperature is known to have on anammox activity (Dosta et al. 2008) however, these rates had to be adjusted for the changing temperatures in the MBBR. Equation 18 is an adapted form of the Arrhenius equation that can be used to adjust a rate (q_0) at an operational temperature (T_0) to a corresponding rate (q) at a set temperature (T).

$$q = q_0\theta^{(T-T_0)} \qquad \text{Equation 18}$$

The θ variable in Equation 18 is the Arrhenius coefficient, which changes based on the organisms and technologies involved with the activity rate that is being adjusted. The Arrhenius coefficient, or θ-value, that was originally used during this study was 1.062 because of previous findings (Guo et al. 2010. However, during the duration of this experiment, a set of tests were conducted on the media from the first MBBR to determine a coefficient value that was more specifically curtailed for these reactors.

Arrhenius Coefficient Testing

A duplicate set of tests were run to determine an Arrhenius coefficient value for the anammox activity in the first MBBR. Each set of tests involved two tests: a batch maximum anammox activity test at a temperature around 30 degrees Celsius and a batch maximum anammox activity test at the current mainstream operating temperature. The 30-degree test was run by taking approximately 2.4 L of MBBR media and 3.7 L of pilot clarifier effluent and placing them in a small 4 L batch reactor with a mixer. The small batch reactor was then placed in a hot water bath with a pH control system, covered, spiked with approximately 30 mg/L of NO_3-N (to ensure it would not go anaerobic), and left to sit at 30 degrees Celsius for at least 12 hours. Once those 12 hours were over, the reactor was then spiked with 20 mg N/L of ammonia and 25 mg N/L of nitrite. Following the nutrient addition, samples were collected from the batch reactor every 15-30 minutes. The DO, pH, and temperature of the reactor were monitored and recorded at the time each sample was collected. The concentrations of NH_3-N, NO_2-N, and NO_3-N in each sample were measured with HACH tubes and then plotted against the time the samples were taken. A linear slope that best fit the relationship between the different nutrient concentrations and time was derived for each nitrogen species (in mg/L/hour) to determine the current maximum anammox removal rates for NH_3-N, NO_2-N, and TIN and the maximum anammox production rate for NO_3-N at the time of the test. The COD and OP concentrations of the reactor were also measured at the beginning and end of the tests to ensure that the ideal conditions were present for anammox activity. The DO and temperature in the reactor were also measured throughout the test to ensure the reactor remained anoxic and to have a proper representation of the average reactor temperature during the test.

The second test, a batch maximum anammox activity test at the current mainstream operating temperature, was run within 3-4 days before or after the high temperature batch reactor test. This test was conducted by following the process described above in the "Anammox Activity Testing" section. The ammonia removal rates of these two tests were then compared to calculate an Arrhenius coefficient. This was done so using Equation 19, which is an adapted version of the Arrhenius Equation (Equation 18).

$$\theta = \left(\frac{q}{q_0}\right)^{\frac{1}{T-T_0}} \qquad \text{Equation 19}$$

This set of tests was run twice, and the resulting Arrhenius coefficient (θ-values) were averaged and used to adjust anammox activity rates for temperature differences.

Specific Denitrification Rate (SDNR) Batch Tests

SDNR tests were conducted on the effluent from the JRTP's full-scale IFAS reactor, which was also the influent for the pilot IFAS reactor. These tests were run by filling a 5-gallon container with 4.5 gallons of this mixed liquor, submerging a mixer in the container, placing a cover on the top of the mixed liquor, and spiking the mixed liquor with 30 mg NO_3-N/L and a carbon source. When running an endogenous denitrification test, only the nitrate was added. 50 mg/L of COD as methanol was added for a methanol denitrification test, and 150 mg/L of COD as glycerol was added for a glycerol denitrification test. Once the nitrate and COD were given 1-2 minutes to properly mix, mixed liquor samples were collected every 15 minutes over a one-hour period (totaling 5 samples). HACH tubes measuring the NO_2, NO_3, and sCOD concentrations were run for every sample, and the DO and temperature of the mixed liquor were recorded for every sample as well. For the first and last sample, the pH and NH_3 and OP concentrations were also measured.

Once the test was completed, a linear slope was generated for the NO_3, NO_2, and sCOD concentrations over time to calculate a removal or accumulation rate for each in mg N/L/hour. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids

(MLVSS) data was collected using standard TSS and volatile suspended solids (VSS) measurement methods by the JRTP staff. These rates were then converted to mg N/g MLSS/hour and mg N/g MLVSS/hour.

Results and Discussion

IFAS Operational Phases

As mentioned above, the IFAS reactor went through several different phases of operation. These phases differed in terms of the reactor's HRT or the media fill fraction in the reactor. These changes were made over time to make the pilot IFAS reactor more proportional to a full-scale application at the JRTP and to increase the nitrogen loading to the reactor. Table 4 goes into detail on the different phases and the HRT and total media surface area in the IFAS reactor for each one.

Table 4. Changes to the HRT and total media surface area in the IFAS reactor throughout the experiment.

Phase and Days Since IFAS Conversion	HRT (min)	Total Media Surface Area (m^2)	Average NH_3-N Loading Rates ($g/m^2/day$)
Phase 1, Days 0 to 40	29.4	94.6	0.448 ± 0.156
Phase 2, Days 41 to 82	24.4	94.6	0.440 ± 0.174
Phase 3, Days 85 to 141	18.8	57.8	1.21 ± 0.40

Phase 3 was primarily intended to push the nitrogen loading rate to the PdNA IFAS reactor higher to investigate how much nitrogen removal could be provided by this PdNA process. Therefore, this phase was more focused on increasing the nitrogen removal rates rather than maintaining low effluent TIN concentrations for the PdNA IFAS reactor.

Influent Characteristics

Table 5 shows the average influent characteristics for both the MBBRs and the three different IFAS reactor operational phases.

Table 5. Average influent characteristics for the PdNA MBBR and 3 IFAS Phases.

	PdNA MBBR	IFAS Phase 1	IFAS Phase 2	IFAS Phase 3
pH	6.60 ± 0.07	-	-	-
DO (mg/L)	3.87 ± 0.66	-	-	-
Temp. (°C)	16.5 ± 1.98	-	-	-
NH3-N (mg/L)	2.77 ± 0.94	2.10 ± 0.74	1.91 ± 0.77	3.12 ± 1.04
NO2-N (mg/L)	1.50 ± 0.32	1.07 ± 0.16	0.97 ± 0.13	0.94 ± 0.25
NO3-N (mg/L)	3.62 ± 0.96	4.35 ± 1.91	4.93 ± 0.68	5.22 ± 2.05
OP (mg/L)	0.05 ± 0.08	0.09 ± 0.04	0.32 ± 0.83	0.52 ± 0.53
MLSS (mg/L)	-	2.40 ± 0.32	2.51 ± 0.12	2.11 ± 0.18
MLVSS (mg/L)	-	1.03 ± 0.91	1.14 ± 0.95	1.13 ± 0.82
TSS	30.3 ± 8.6	-	-	-

For the MBBRs, the influent pH fell within the acceptable range for anammox of 6.5-8.0 (Van Hulle et al. 2007). The high influent DO concentrations did result in some additional external carbon demand in the MBBRs to bring the DO down to suitable levels for denitrification and anammox growth, but the influent temperature was typical for mainstream conditions. The average AvN ratio of the influent was 0.55 ± 0.19 g/g, which resulted in approximately equal average concentrations of effluent ammonia and nitrite in the PdNA MBBR (Table 6), despite some full denitrification providing additional nitrite removal. TSS and COD concentrations were within the expected range for WWRF secondary clarifier effluent.

For the IFAS reactor, the average AvN ratio was 0.46 ± 0.26 g/g during Phase 1, 0.33 ± 0.13 g/g during Phase 2, and 0.58 ± 0.28 g/g during Phase 3. Phase 2, on average, produced the lowest and most consistent average effluent ammonia concentration of the three phases and still managed to have a low average effluent nitrite concentration (Table 6). The amount of nitrate that was added to the IFAS reactor influent was also increased over time, which can be observed by the increase in the average influent nitrate concentration across the three phases, to push the loading rate higher. Additionally, ammonia was also synthetically added to the pilot IFAS reactor's influent during Phase 3 to further push the nitrogne loading, leading to that phase having the highest average AvN ratio among the three phases. Thus, the PdNA IFAS reactor was receiving and treating more influent TIN than the MBBRs were throughout the study.

Influent phosphorus concentrations were low due to efficient biological phosphorus removal in the full-scale WWRF IFAS tanks. Both OHOs and AMX require some phosphorus for growth. Without adequate amounts of phosphorus being provided, denitrification and anammox activity rates in a PdNA process can both decrease, causing a loss in effluent equality and treatment efficiency. 0.2 mg P/L has proven to be an adequate effluent phosphorus concentration for denitrification in a MBBR (Peric et al. 2009).

Therefore, supplemental phosphorus was added to the PdNA MBBR by adding potassium phosphate monobasic into the MBBR glycerol stock. Phosphorus was also added to the IFAS reactor influent in the form of potassium phosphate monobasic as well during Phases 2 and 3. This was done to ensure that denitrification and anammox activity were not limited by phosphorus, since both anammox and OHOs require some phosphorus for growth. Based on the average influent nitrite concentrations, a small, but significant, amount of nitrite was also provided for anammox activity in the influent, indicating that a small amount of PdN was likely occurring in the full-scale WWRF IFAS reactors.

Effluent Characteristics

Table 6 shows the average effluent characteristics for both the MBBRs and the three different IFAS reactor operational phases.

Table 6. Average effluent characteristics for the PdNA and AMX MBBRs and 3 IFAS Phases.

	PdNA MBBR	**AMX MBBR**	**IFAS Phase 1**	**IFAS Phase 2**	**IFAS Phase 3**
pH	6.58 ± 0.06	6.60 ± 0.06	6.69 ± 0.08	6.62 ± 0.04	6.68 ± 0.07
DO (mg/L)	0.17 ± 0.11	0.01 ± 0.02	0.01 ± 0.03	0.00 ± 0.00	0.01 ± 0.01
Temp. (°C)	16.5 ± 2.0	16.5 ± 1.9	16.4 ± 0.6	14.6 ± 0.8	18.8 ± 1.6

NH3-N (mg/L)	1.26 ± 0.87	0.98 ± 0.81	1.32 ± 0.71	0.97 ± 0.48	2.60 ± 1.06
NO2-N (mg/L)	1.31 ± 0.43	0.88 ± 0.45	0.75 ± 0.36	1.00 ± 0.40	1.18 ± 0.46
NO3-N (mg/L)	1.19 ± 0.64	0.94 ± 0.63	1.99 ± 1.38	1.34 ± 0.62	2.10 ± 1.17
OP (mg/L)	0.39 ± 0.38	0.38 ± 0.40	0.56 ± 1.10	0.56 ± 1.05	0.42 ± 0.61
sCOD (mg/L)	26.1 ± 3.1	25.7 ± 3.3	-	-	-
TSS (mg/L)	35.7 ± 6.3	34.7 ± 7.3	-	-	-

The pH, DO, and temperature for both reactors across all operational phases fell within acceptable limits for denitrification and anammox activity. Non-limiting effluent phosphorus concentrations were also maintained in the PdNA and AMX MBBRs as well, preventing that from limiting anammox activity. Phosphorus was not added to the PdNA IFAS reactor influent during the entire experiment though, so anammox and denitrifier growth could have been limited by low effluent phosphorus concentrations in that reactor at those particular times throughout the experiment.

In the PdNA MBBR, the average effluent nitrate concentration was slightly over the 1 mg N/L targeted by the carbon control program, but the program was still considered to provide effective nitrate removal considering the relatively low influent nitrate concentrations being treated. Occasional clogs in the carbon feed also led to little nitrate removal and a higher average effluent nitrate concentration overall. Approximately 30 mg/L of recalcitrant COD was also observed in the MBBRs based on the influent and effluent sCOD concentrations remaining at approximately 30 mg/L. This also indicates that substantial carbon overdosing did not occur. On average, the effluent TIN concentration from the AMX MBBR was below 3 mg/L.

The methanol feedback control program in the PdNA IFAS reactor, overall, also provided effective nitrate removal. While effluent nitrate concentrations were acceptable during Phase 2, the required amount of nitrate removal was not provided during Phases 1 and 3. During Phase 1, methanol was only dribbled to the IFAS reactor (at about 2.5 g/hour), since methylotrophs had never been established in the reactor before. Therefore, most of the denitrification that occurred during this time was endogenous denitrification, which did not require any external carbon and kept the C/N ratio low for that phase. The endogenous denitrification, however, did not provide enough nitrate removal to consistently reach an effluent concentration of 1 mg/L. For Phase 3 for the PdNA IFAS reactor, the main focus was more on pushing the loading rate to the reactor high in order to investigate how much nitrogen removal could be provided by this process, rather than on maintaining good effluent quality. Therefore, during Phase 3, the influent nitrate concentration was kept around 7 mg/L for several weeks, which was done to increase the loading to the reactor and further strain it. This led to high effluent nitrate concentrations, since the reactor had not handled influent nitrate concentrations that high before and methanol dosing to the reactor was limited by constraints set on the control program.

Nitrogen Removal in PdNA Reactors

Box plots of the effluent TIN concentrations for the PdNA and AMX MBBRs and Phases 1 and 2 for the IFAS reactor are displayed in Figure 20.

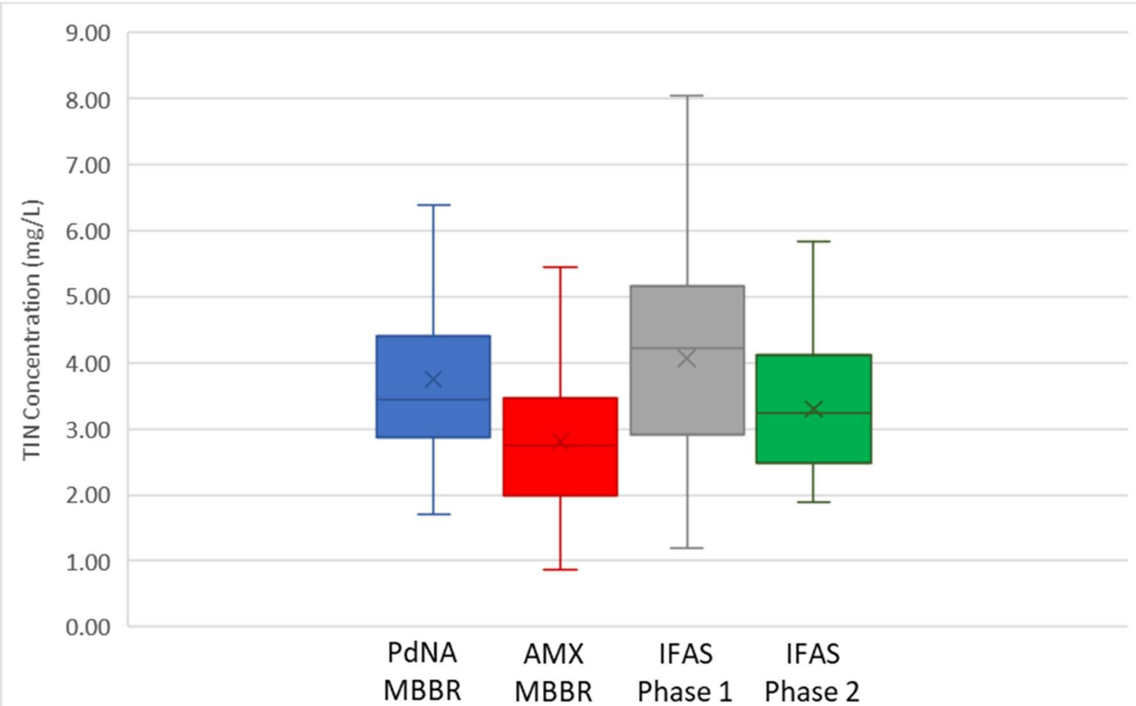

Figure 20. Box plots of effluent TIN concentrations for the PdNA MBBR (blue, n=90), AMX MBBR (red, n=88), IFAS phase 1 (grey, n=23), and IFAS phase 2 (green, n=29).

Both the IFAS reactor and two MBBRs in series managed to produce effluent streams with consistently low effluent TIN concentrations. The average TIN effluent concentrations, amounts of NH_3, NO_2, NO_3, and TIN removed, and removal rates of NH_3, NO_2, NO_3, and TIN for the PdNA and AMX MBBRs and IFAS phases 1 and 2 are included in Table 7. *In situ* amounts and removal rates are those that occurred during regular operation of the pilot MBBRs and IFAS reactor.

Table 7. Average effluent TIN concentrations and nutrient removal rates for the PdNA MBBR, AMX MBBR, and IFAS Phases 1 and 2.

	PdNA MBBR	**AMX MBBR**	**IFAS Phase 1**	**IFAS Phase 2**
Effluent TIN Conc. (mg/L)	3.75 ± 1.25	2.81 ± 1.21	4.07 ± 1.66	3.30 ± 0.96
NH3-N Removed (mg/L)	1.51 ± 0.48	0.27 ± 0.17	0.77 ± 0.30	0.94 ± 0.34
NO2-N Removed (mg/L)	2.89 ± 0.89	0.66 ± 0.18	2.68 ± 0.59	3.57 ± 0.92
NO3-N Removed (mg/L)	2.43 ± 0.98	0.24 ± 0.13	2.36 ± 0.78	3.60 ± 0.76

TIN Removed (mg/L)	4.14 ± 1.23	0.93 ± 0.28	3.45 ± 0.83	4.51 ± 1.05
***In situ* NH3-N Removal Rate ($g/m^2/day$)**	0.171 ± 0.064	0.082 ± 0.062	0.165 ± 0.063	0.217 ± 0.077
***In situ* NO2-N Removal Rate ($g/m^2/day$)**	0.326 ± 0.113	0.188 ± 0.062	0.570 ± 0.108	0.825 ± 0.212
***In situ* NO3-N Removal Rate ($g/m^2/day$)**	0.278 ± 0.131	0.065 ± 0.034	0.501 ± 0.152	0.832 ± 0.176
***In situ* TIN Removal Rate ($g/m^2/day$)**	0.467 ± 0.161	0.270 ± 0.115	0.735 ± 0.159	1.04 ± 0.24

Based on these nitrogen removal rates, the two MBBRs in series provided more TIN removal in terms of mg/L ($p<0.01\%$), but the IFAS reactor had higher removal rates in terms of $g/m^2/day$ due to its lower HRT and total media surface area ($p<0.01\%$).

Figure 21 shows the ammonia and TIN loading rates versus their respective removal rates in both the PdNA MBBR and the IFAS reactor during phases 1 and 2. Overall, the NH_3 and TIN removal rates generally ranged between 25-100% for both reactors, with few exceptions being due to the influent ammonia versus NO_x (AvN) or carbon feed operational issues.

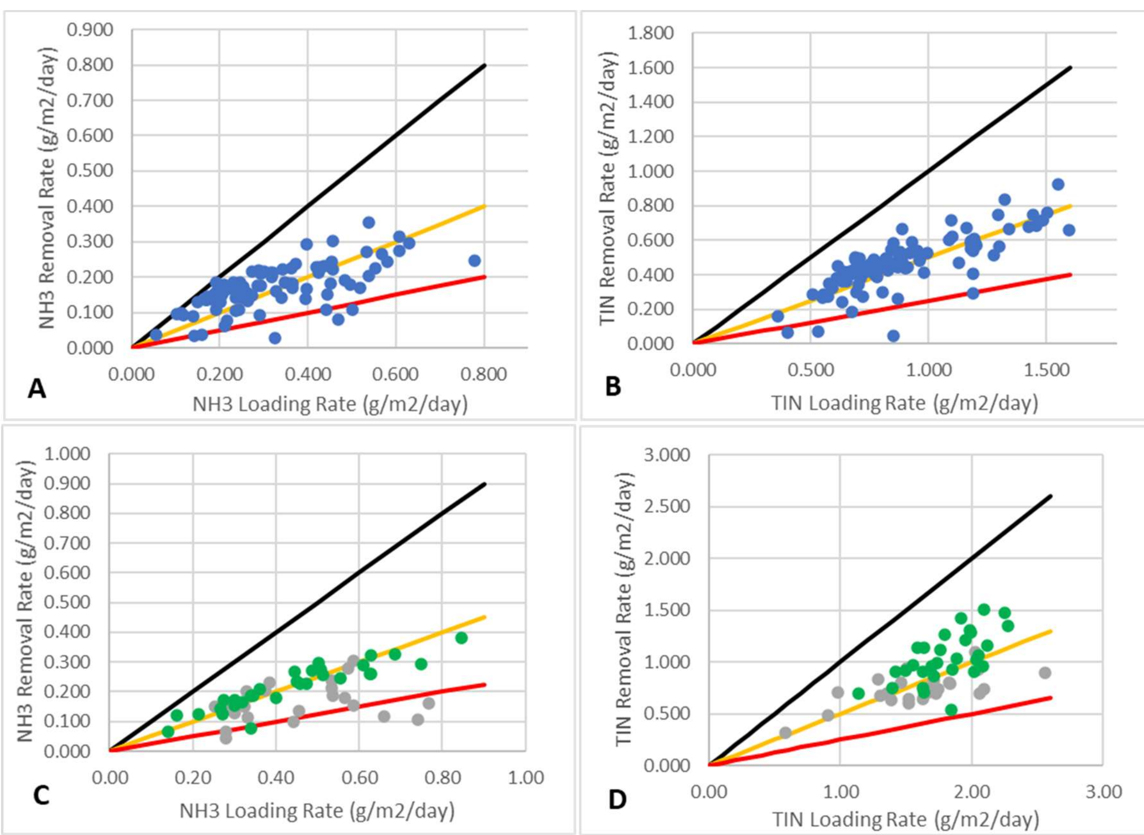

Figure 21. Removal vs. loading rates for NH3 in the PdNA MBBR (A), TIN in the PdNA MBBR (B), NH3 in the IFAS reactor during phase 1 (grey C) and 2 (green C), and TIN in IFAS reactor

during phase 1 (grey D) and 2 (green D) with 100% removal (black), 50% removal (yellow), and 25% removal (red) markers.

The average percent of TIN and NH_3 from the PdNA MBBR were 52.3% ± 12.3% and 58.2% ± 19.2% respectively, while the averages for the PdNA IFAS reactor were 47.5% ± 9.9% and 39.1% ± 15.2% for phase 1 and 57.8% ± 10.2% and 50.8% ± 9.1%. When looking at these ratios of removal rate over loading rate over the entire course of phases 1 and 2, there is no significant difference between the PdNA MBBR and the PdNA IFAS reactor (p=63%).

The PdNA MBBR's treatment performance was generally limited by either low ammonia and nitrite concentrations, although generally, as shown by the average effluent concentrations (Table 6), the amounts of ammonia and nitrite in the PdNA MBBR were both low enough where anammox activity would be slowed significantly by either condition. The amount of anammox activity also did limit treatment performance at certain points throughout the experiment, especially during the winter months when colder temperatures slowed anammox activity and TIN loading rates were generally higher. The AMX MBBR generally provided a small amount of additional polishing for ammonia, nitrite, and nitrate which brought the TIN concentrations down enough, on average, to be below 3 mg/L.

The PdNA IFAS reactor's treatment performance was generally limited by a variety of different factors throughout the three phases. During Phase 1, partial denitrification and nitrate removal were limited due to the amount of methanol dosed to the reactor, while during Phase 3, the nitrogen loading rate was increased drastically to push the reactor's nitrogen removal capabilities. These factors led to high average effluent nitrate concentrations in the reactor during those phases (Table 6), which also significantly raised the average effluent TIN concentrations during those phases as well (Table 7). This lack of partial denitrification also led to the general limitation on anammox activity in Phases 1 and 3 being the nitrite concentration as well, since not enough nitrite was being produced through PdN for the anammox to use to continue removing the additional ammonia left (Table 6). Based on the average effluent TIN concentrations, the PdNA IFAS reactor did provide its most effective nitrogen removal during Phase 2, which was likely due to the methanol dosing program being properly set up. This allowed the methanol dosing control program to provide adequate amounts of external carbon that supported partial denitrification, which brought the effluent nitrate concentrations downand provided more nitrite for anammox activity. During this phase, both the average effluent ammonia and nitrite concentrations were both low enough where anammox activity would be slowed significantly by either condition. The amount of anammox activity was not considered to be a limiting factor on performance in the PdNA IFAS reactor due to the removal rates not reaching the levels seen in continuous flow maximum anammox activity tests conducted in the same reactor (Figure 26).

Effect of Influent AvN on Effluent TIN

Maintaining the optimal AvN ratio is extremely important to obtain the maximum amount of nitrogen removal possible from anammox activity. If the anammox run out of either NH_3 or NO_2 to remove, then activity will stop, leaving whatever is left of the other nitrogen species to remain in the effluent. Therefore, finding the maximum AvN of an AMX process where the desired effluent TIN concentrations are produced is important for effective and reliable process operation. For the PdNA MBBR, an influent AvN ratio of 0.6 or below was found to produce significantly lower effluent TIN ($p<0.01\%$) and ammonia ($p<0.01\%$) concentrations compared to an influent AvN ratio greater than 0.6 (Figure 22).

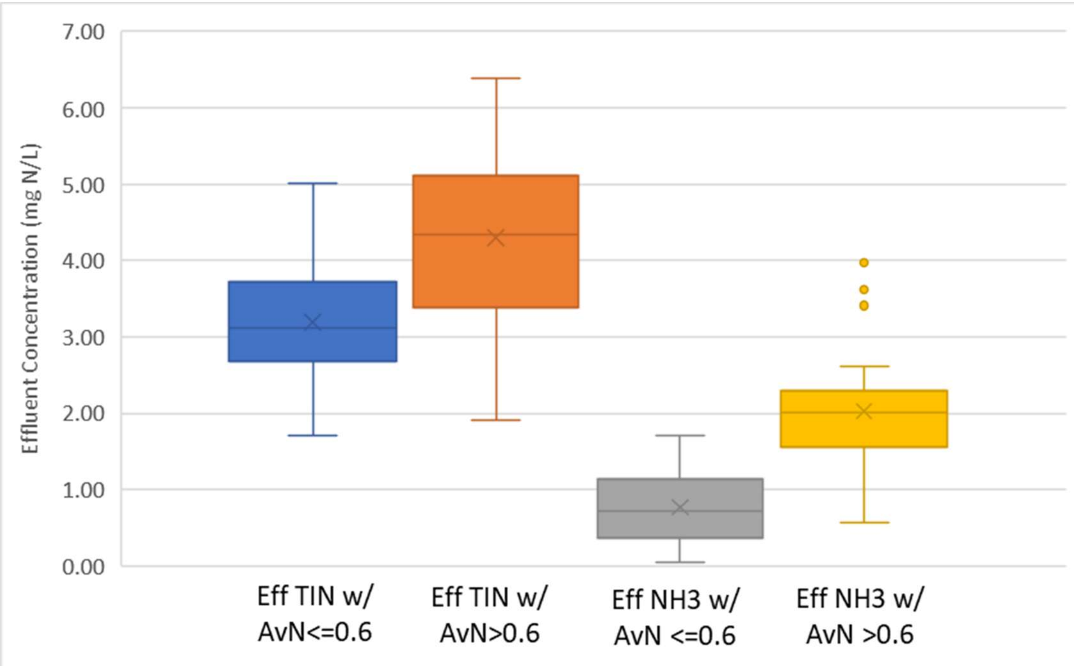

Figure 22. PdNA MBBR effluent TIN concentrations with an influent AvN below or equal 0.6 (blue, n=54) and above 0.6 (orange, n=30) and PdNA MBBR NH_3 effluent concentrations with an influent AvN ratio below or equal to 0.6 (grey, n=54) and above 0.6 (yellow, n=30).

The same ratio was also found to produce significantly lower effluent TIN ($p<0.01\%$) and ammonia ($p<0.01\%$) concentrations in the AMX MBBR as well (Figure 23).

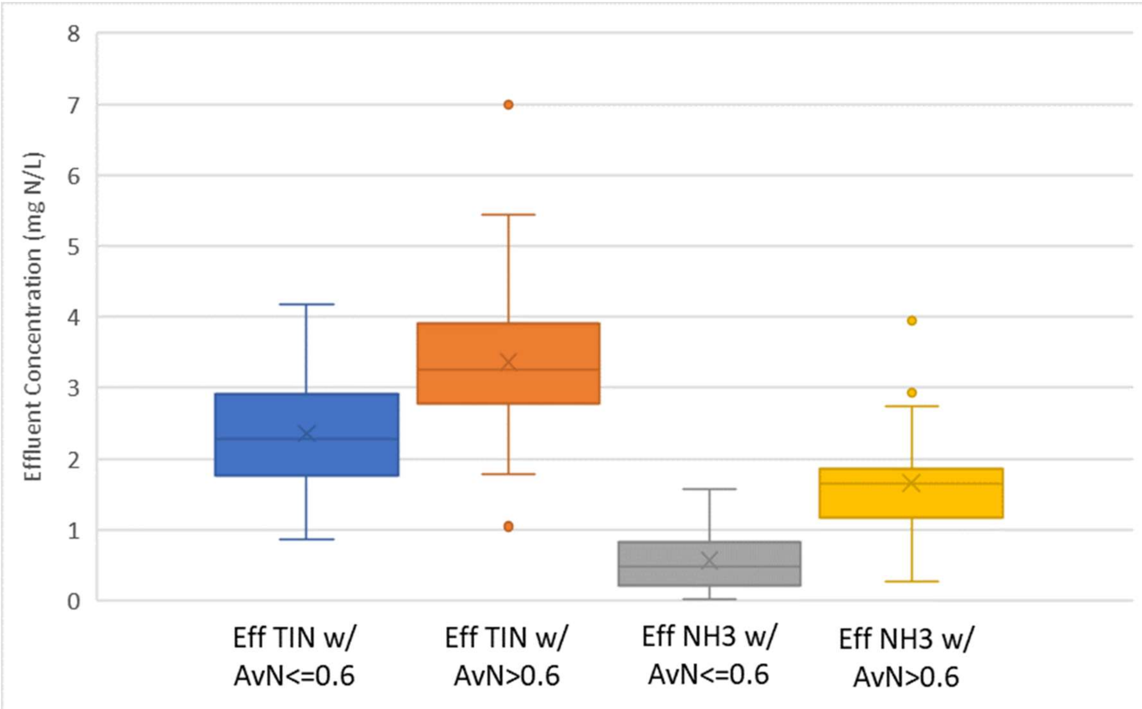

Figure 23. AMX MBBR effluent TIN concentrations with an influent AvN below or equal 0.6 (blue, n=56) and above 0.6 (orange, n=30) and PdNA MBBR NH3 effluent concentrations with an influent AvN ratio below or equal to 0.6 (grey, n=56) and above 0.6 (yellow, n=30).

For the PdNA IFAS reactor during phases 1 and 2, a maximum AvN ratio was much harder to determine because of the uncontrolled endogenous denitrification occurring in the activated sludge. An influent AvN ratio of 0.4 appeared to produce the clearest difference between the effluent TIN (p=5.9%) and ammonia(p<0.01%) concentrations from the PdNA IFAS reactor (Figure 24).

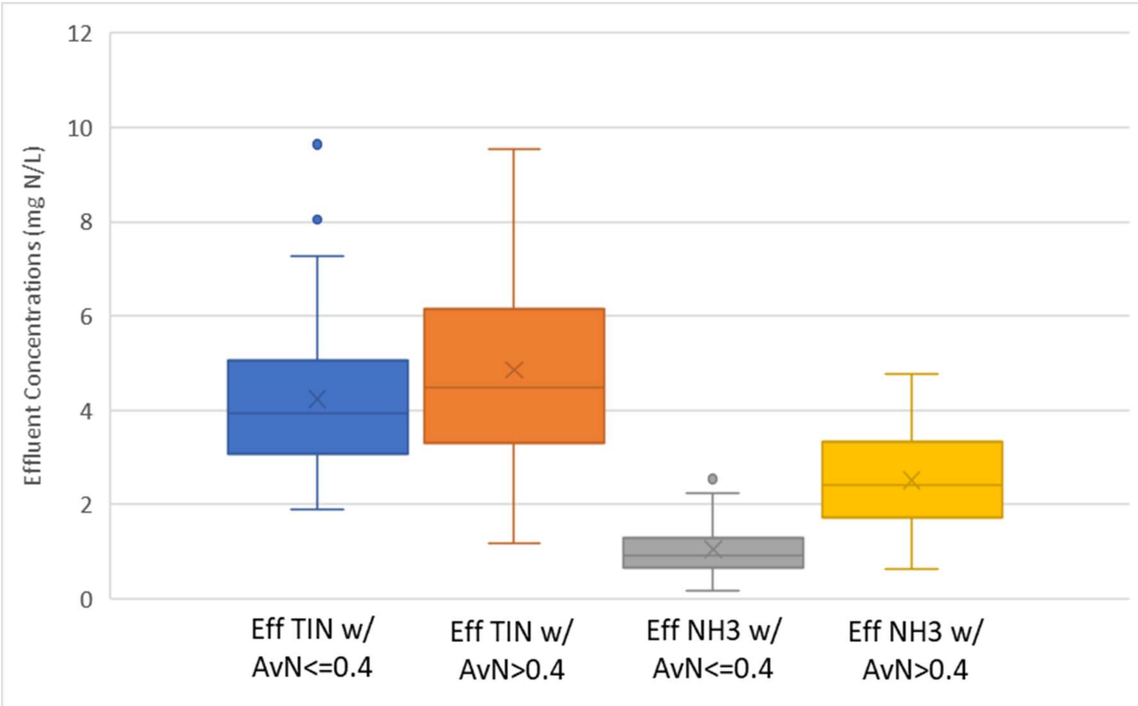

Figure 24. PdNA IFAS effluent TIN concentrations with an influent AvN below or equal 0.4 (blue, n=49) and above 0.4 (orange, n=43) and PdNA IFAS NH3 effluent concentrations with an influent AvN ratio below or equal to 0.4 (grey, n=49) and above 0.4 (yellow, n=43).

Based on these analyses, maintaining an influent AvN ratio below 0.6 for the PdNA MBBR and below 0.4 for the PdNA IFAS reactor will help produce lower effluent TIN and ammonia concentrations for these specific PdNA processes.

Carbon Requirements of PdNA Reactors

Figure 25 is a box plot of the ratios for COD dosed over TIN removed and COD dosed over NO_3-N removed. These parameters are typically used to describe the carbon demand of a biological process.

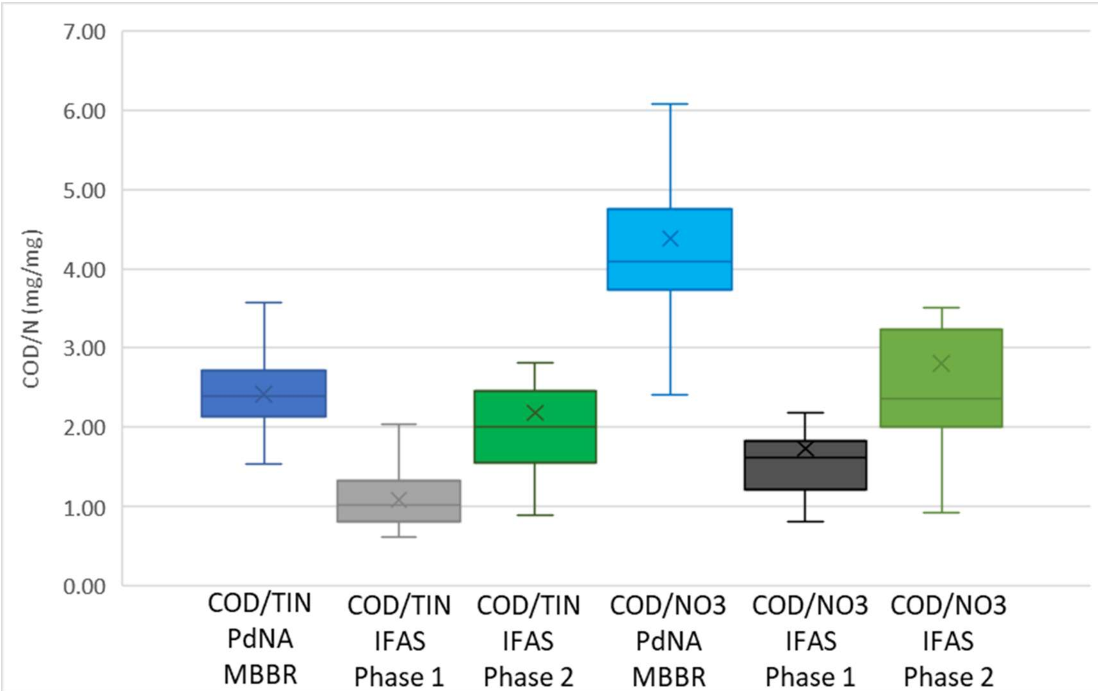

Figure 25. Box plot of COD/TIN ratios for the PdNA MBBR (blue, n=90), IFAS phase 1 (light grey, n=23), and IFAS phase 2 (green, n=29) and COD/NO3-N ratios for the PdNA MBBR (light blue, n=90), IFAS phase 1 (dark grey, n=23), and IFAS phase 2 (lime green, n=29).

The average amount of COD dosed per gram of TIN removed in the PdNA MBBR was 2.42 ± 0.77 g COD/g TIN, which is significantly lower ($p<0.01\%$) compared to the stochiometric C/N ratio for glycerol in a full denitrification process at 7.16 g COD/g NO_3, assuming a yield of 0.54 g/g. Based on these COD/TIN ratios, this process provided 66.2% of average carbon savings compared to the conventional nitrification/denitrification nitrogen removal process. The average amount of grams of COD dosed per gram of NO_3-N removed in the PdNA MBBR was 4.38 ± 1.72 g COD/g N.

The average amount of COD dosed per gram of TIN removed in the PdNA IFAS reactor during Phase 1 was 1.08 ± 0.38 g COD/g TIN, and the same ratio was 2.18 ± 0.99 g COD/g TIN during Phase 2. Both C/N ratio averages are also significantly lower (both $p<0.01\%$) compared to the stochiometric C/N ratio for methanol in a full denitrification process, which is 4.8 g COD/g NO_3 assuming a yield of 0.40 g/g. Based on these COD/TIN ratios, this process provided 77.5% and 54.6% of average carbon savings during Phases 1 and 2, respectively, compared to the conventional nitrification/denitrification nitrogen removal process. The average amount of grams of COD dosed per gram of NO_3-N removed in the PdNA IFAS reactor was 1.73 ± 1.08 g COD/g N during Phase 1 and 2.81 ± 1.79 during Phase 2.

As TIN and NO_3 loading were both slowly increased to the IFAS reactor throughout Phases 1 and 2, the C/N ratio began to increase as well. This increase in demand of carbon per mass of TIN removed was related to how much of the total denitrification occurring in the reactor was endogenous. During most of Phase 1, almost all the denitrification that occurred was due to endogenous denitrification, which meant that the amount of carbon dosed was very low, resulting in a low C/N ratio. As the experiment continued into Phase 2, the control program was allowed to dose more methanol, which produced lower effluent nitrate concentrations and resulted in a higher C/N ratio.

Determined Arrhenius Coefficient

Duplicate sets of tests were run to accurately determine the Arrhenius coefficient for the PdNA MBBR. Each set of tests included a maximum anammox activity test in the pilot PdNA MBBR at the current operating temperature and a maximum activity test on a defined volume of media from the PdNA MBBR at a temperature around 30°C. These two tests were typically conducted 3-4 days apart. The determined ammonia removal rates from these two tests were then plugged in to Equation 19 to calculate an Arrhenius coefficient. Table 8 summarizes the results from these tests.

Table 8. Arrhenius coefficient test results.

High Temp. Test Date	Test 1 – 2/10/2021	Test 2 – 5/5/2021
Operating Temp. Ammonia Removal Rate	0.290 g/m2/day	0.405 g/m2/day
Operating Temperature	14.9°C	20.2°C
High Temp. Ammonia Removal Rate	0.696 g/m2/day	0.805 g/m2/day
High Temperature	29.5°C	29.3°C
Calculated Arrhenius Coefficient	1.062	1.079

For Test 1, the operating temperature and its corresponding ammonia removal rate were based on two tests, one which occurred 5 days before the high temperature test and one which occurred 2 days after the high temperature test. The temperature and ammonia removal from both tests were averaged together, and the averages are the reported values in Table 8. Only one operating temperature maximum anammox activity test was conducted within 7 days before or after the second high temperature test (Test 2), so the average temperature and ammonia removal rate from that one test are the reported values in the table.

Several studies, with a variety of different wastewater treatment technologies, have calculated an Arrhenius coefficient for anammox at a variety of temperature ranges, which range from 1.05 to 1.17 (Strous, Kuenen, and Jetten 1999, Dalsgaard and Thamdrup 2002, Rysgaard et al. 2004, Dosta et al. 2008, Isaka 2008, Guo et al. 2010, Nifong 2013). Some studies also concluded that different Arrhenius coefficients were needed for different temperature ranges (Guo et al. 2010, Lotti et al. 2015).

The average of the two calculated Arrhenius coefficients from the two sets of tests run during this study was 1.070. Arrhenius coefficients determined at similar temperature ranges (15-30°C) during earlier studies ranged from 1.062 to 1.10 (Dalsgaard and Thamdrup 2002, Nifong 2013, Guo et al. 2010). The Arrhenius coefficient calculated during these experiments falls well within this range, which helped its validity and made it appear to be reasonable. This value was used in the Arrhenius equation to factor in temperature adjustments for the maximum anammox ammonia removal rates (Figure 26) that were measured during anammox activity tests. Since temperature is known to have a major effect on anammox activity, this allowed for all the maximum anammox ammonia removal rates to be compared evenly so they could properly be used as an indicator of anammox growth.

Anammox Growth in PdNA Reactors

Maximum anammox activity tests were conducted consistently in both the PdNA MBBR and IFAS reactors. The ammonia removal rates from these tests, adjusted for temperature differences, are graphed over time in Figure 26.

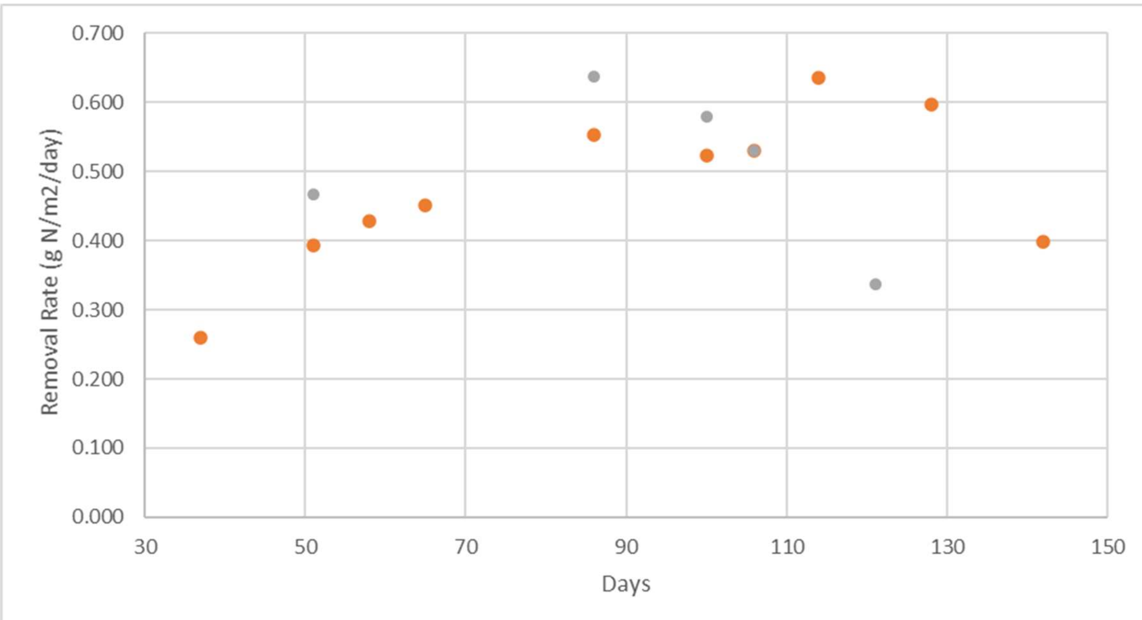

Figure 26. Ammonia removal rates, adjusted to 20°C, from maximum anammox activity tests in the PdNA MBBR (orange) and IFAS reactor (grey).

In the PdNA MBBR, the ammonia removal rate continuously increased until day 114, at which point the removal rate appeared to remain around 0.6 g/m2/day before decreasing to 0.399 g/m2/day on day 142.

In the PdNA IFAS reactor, the ammonia removal rate also increased until day 86, at which point it slowly began to decrease. This decrease appears to correspond with an increase in the amount of nitrate that was being added to the influent, which led to an increased amount of methanol being dosed to the reactor. During this period, it was also noticed that the anammox activity in the daily grab samples was also decreasing. While a recent studies have shown that methanol is not inhibitory to anammox, as previously thought (Le et al. 2019a, Campolong et al.2019), it is possible that severely overdosing methanol to the reactor, which was done at this time, did inhibit the anammox and caused this decrease in anammox ammonia removal.

SDNR Batch Test Results

Glycerol was dosed to the full-scale IFAS tanks at the JRTP for 27 days. The affect this had on the endogenous, glycerol, and methanol specific denitrification rates was observed with SDNR batch tests. The nitrate removal rates from these tests are displayed in Figure 27.

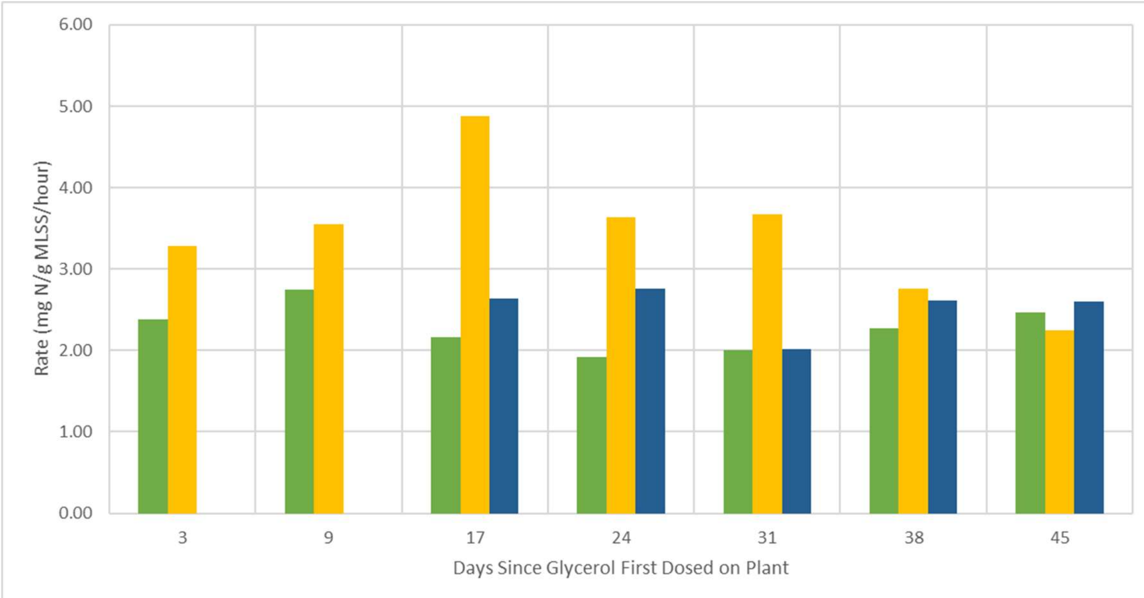

Figure 27. Nitrate removal rates from endogenous (green), glycerol (yellow), and methanol (blue) SDNR batch tests.

After glycerol started to get dosed to the main plant, the glycerol removal rate slowly increased and stabilized around 3.65 mg N/g MLSS/hour. The glycerol feed to the plant was stopped on day 27, at which point the glycerol removal rate decreased in the following two tests. Overall, the endogenous and methanol removal rates appear to remain relatively consistent throughout this time, with the average endogenous nitrate removal rate being 2.28 ± 0.28 mg N/g MLSS/hour and the average methanol removal rate being 2.53 ± 0.29 mg N/g MLSS/hour. The average glycerol removal rate was 3.43 ± 0.83 mg N/g MLSS/hour. The corresponding nitrite accumulation rates from the same SDNR tests are displayed in Figure 28.

The endogenous SDNR tests displayed high rates of denitrification, especially when considering that typical endogenous rates are from 0.2-0.8 mg N/g MLSS/hour (Kujawa and Klapwijk 1999). That makes the average endogenous nitrate removal rate from these tests over double that which is typically seen. While it is not known exactly how these high amounts of endogenous denitrification occur, Vocks et al. (2005, n.d.) also displayed large amounts of endogenous denitrification in a post-denitrification setting with rates of 2.2 mg N/g MLVSS/hour and 1-4 mg N/g MLVSS/hour, respectively. Those studies concluded that some currently unknown form of internally stored carbon, that was uniquely present or usable by the microbial community in activated sludge used for enhanced biological phosphorus removal, was being used for this large amount of endogenous denitrification in a post-denitrification setting (Vocks et al. 2005, n.d.). This has major implications for a PdNA IFAS reactor since the presence of the activated sludge in an IFAS reactor allows for this endogenous denitrification to be taken advantage of to lower the process's carbon demand even more.

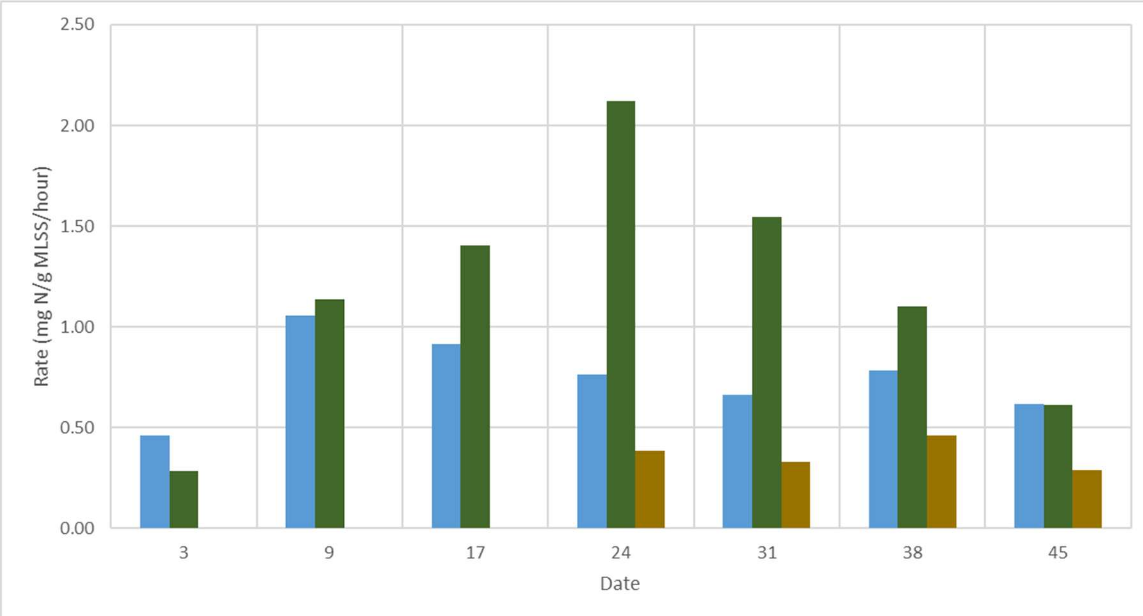

Figure 28. Nitrite accumulation rates from endogenous (light blue), glycerol (dark green), and methanol (brown) SDNR batch tests.

The glycerol nitrite accumulation rate also increased while glycerol was dosed to the main plant but did not appear to reach a point where it began to stabilize. This increase in nitrite accumulation led to an increase in PdN efficiency in the glycerol tests as well. Once glycerol was no longer being dosed to the main plant on day 27, the glycerol nitrite accumulation rate began to drop. The average glycerol NO2 accumulation rate was 1.19 ± 0.58 mg N/g MLSS/hour. Again, the endogenous and methanol rates stayed relatively consistent, with an average endogenous NO2 accumulation rate of 0.72 ± 0.25 mg N/g MLSS/hour and an average methanol NO2 accumulation rate of 0.33 ± 0.10 mg N/g MLSS/hour. Since the average methanol accumulation rate was lower compared to the endogenous rate, the addition of methanol significantly decreased the PdN efficiency of the activated sludge when compared to the endogenous rates (p=0.43%). Based on the average endogenous nitrite accumulation and nitrate removal rates, the average PdN efficiency for the endogenous denitrification in the pilot PdNA IFAS reactor was 32.1% ± 10.5%. These nitrite accumulation rates also make the use of this endogenous denitrification in a PdNA IFAS reactor promising, since a significant amount of PdN and nitrite accumulation is occurring without the use of external organic carbon sources.

Partial Denitrification Efficiency

The partial denitrification efficiencies in both the PdNA MBBR and PdNA IFAS reactor were both unstable throughout operation (Figure 29).

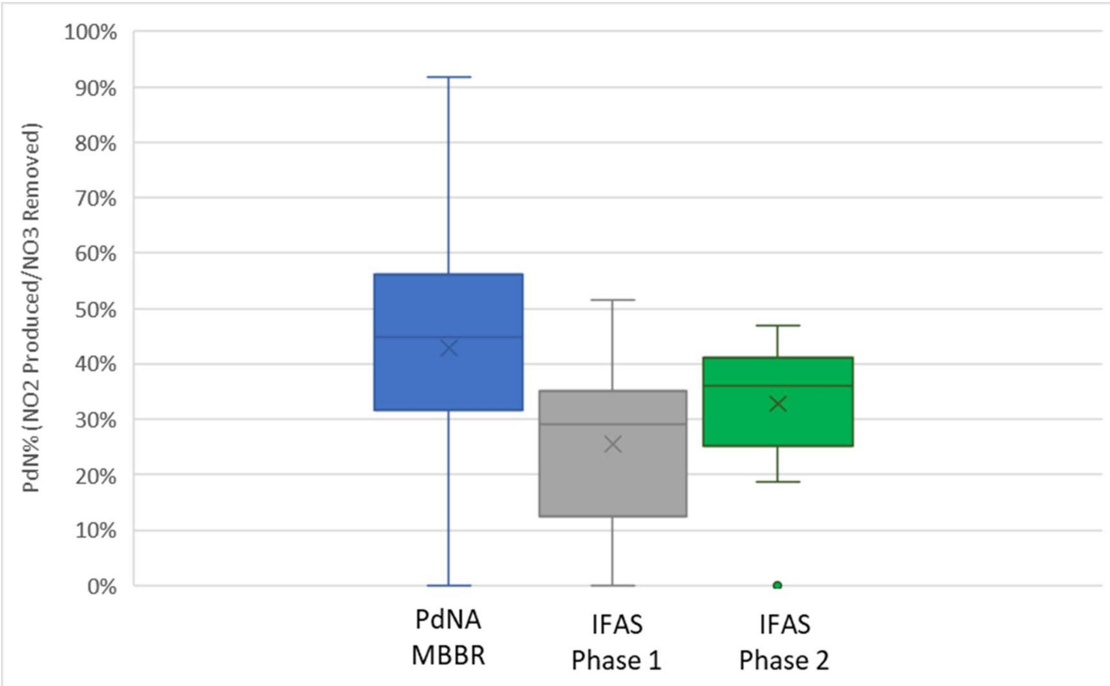

Figure 29. Box plots of the PdN efficiency during operation in the PdNA MBBR (blue, n=90), PdNA IFAS reactor duing phase 1 (grey, n=23), and PdNA IFAS reactor during phase 2 (green, n=27).

The instability of the partial denitrification in both the PdNA MBBR and IFAS reactor is believed to be related to the amount of anammox activity that was occurring in the reactor due to a noticeable relationship between the percentage of TIN removed by anammox and the PdN efficiency. Therefore, any operational factor that would prevent anammox activity from occurring, including running out of nitrite, ammonia, and/or phosphorus, low pH, and/or a high DO, led to the loss of the nitrite sink that the anammox nitrogen removal pathway served as. With the anammox unable to use the nitrite, the nitrite would sit in the reactor until OHOs reduced it to dinitrogen gas through the full denitrification nitrogen removal pathway.

The average PdN efficiency in the PdNA MBBR was 42.9% ± 20.0% throughout operation. The average PdN efficiency in the PdNA IFAS reactor was 25.5% ± 15.2% during phase 1 and 32.8% ± 10.7% during phase 2. When comparing the PdN efficiency of the PdNA MBBR and IFAS reactor during phases 1 and 2 together, the PdNA MBBR had a higher PdN efficiency (p=0.53%).

The lower PdN efficiency in the PdNA IFAS reactor compared to the PdN efficiency in the PdNA MBBR was likely due to two factors. The first was the use of methanol in the IFAS reactor, which has previously demonstrated consistently lower PdN% compared to glycerol in past experiments (Campolong et al. 2019, Le et al. 2019a). The second factor was the relatively low amount of partial denitrification that was provided by the endogenous denitrificaiton, which, based on the SDNR tests, had an avarege PdN efficiency of 32.1% ± 10.5%. The IFAS reactor likley had a lower PdN efficiency during Phase 1 compared to Phase 2 because almost all of the denitrification that occurred during Phase 1 was endogenous, which supported less partial denitrification compared to methanol-driven denitrification.

When compared to many previous PdNA experiments, the PdN efficiencies in the PdNA MBBR and PdNA IFAS reactor were notably lower. Campolong et al. (2019) and Le et al. (2019a) reported glycerol PdN efficiencies around 90% and above 90%, respectively. The cause of these lower PdN efficiencies was not definitively determined, but some potential reasons could be related to low TIN loading rates or the targeted effluent nitrate concentrations being too low to support high PdN efficiencies. The main conclusion that was made from the PdN efficiencies in this experiment, however, was that these PdNA processes still provided efficient nitrogen removal and maintained low effluent TIN concentrations with relatively low and unstable PdN efficiencies.

Media Biofilm Solids

The biofilm solids on the media increased in mass on both the PdNA MBBR carriers and the PdNA IFAS carriers for the first 60 days after the IFAS reactor was started up from a MBBR (Figure 30). The biofilms likely grew during this time due to the cold reactor operational temperatures that were there and the growth of more anammox on the carriers.

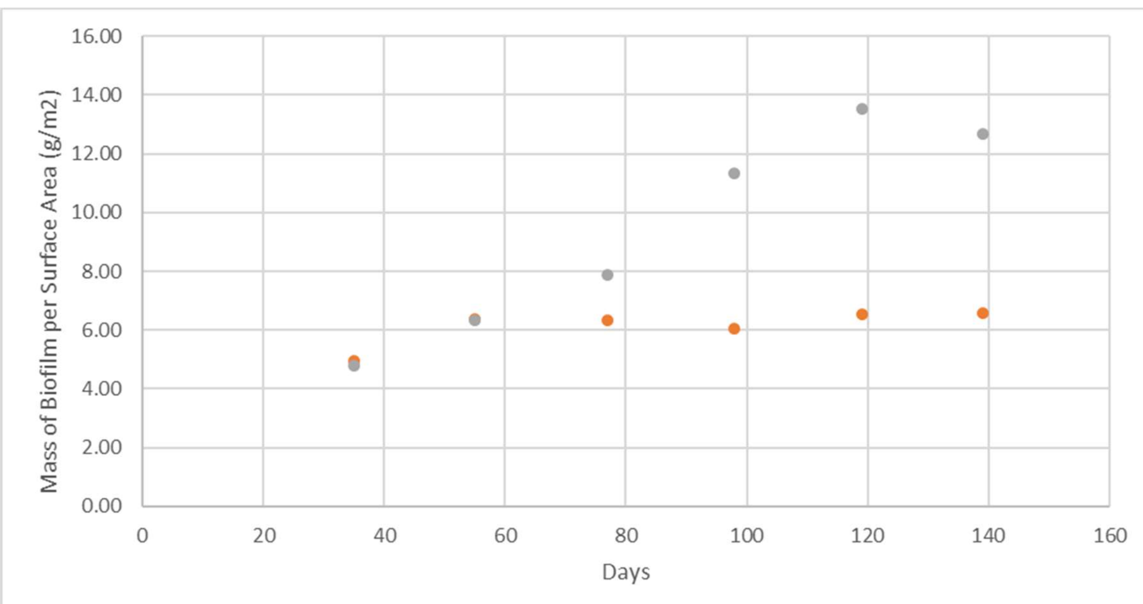

Figure 30. Biofilm mass per media surface area for PdNA MBBR (orange) and IFAS carriers (grey).

Beyond that point, the biofilm on the IFAS carriers continues to increase in mass per surface area, while the biofilm on the PdNA MBBR carriers continued to remain around 6.0 g/m2 for the remainder of the study. While the biofilm on the PdNA MBBR carriers did not appear to grow after the 60-day mark, the biofilm on the IFAS carriers likely continued to increase in size because the nitrogen loading rate to that reactor was continuously increased throughout the whole experiment.

MBBR Nitrite Nitrification Limits

In this MBBR process, the purpose of the final, aerated MBBR zone was to polish any remaining nitrite left in the effluent from the AMX MBBR. The primary purposes for trying to maintain low effluent nitrite concentrations in a WWRF process are limiting effluent toxicity and downstream disinfectant demand. The amount of nitrite that was oxidized in the nitrifying MBBR was typically dependent on the pilot's influent characteristics and the treatment effectiveness of the PdNA MBBR and AMX MBBR. Samples were collected on many days where the influent of the nitrifying MBBR already had a low concentration of NO2-N in it (<0.7 mg N/L), so little nitrite was available be oxidized to nitrate by NOB on those occasions. With the objective of this study being to see how low the effluent nitrite concentration could be maintained however, due to the concern over nitrite ozone demand in the water reuse facility, the major concern was not the nitrite oxidation rate but rather the nitrite effluent concentration. Figure 31 shows the sampled influent and effluent NO2-N concentrations from the nitrifying MBBR throughout operation.

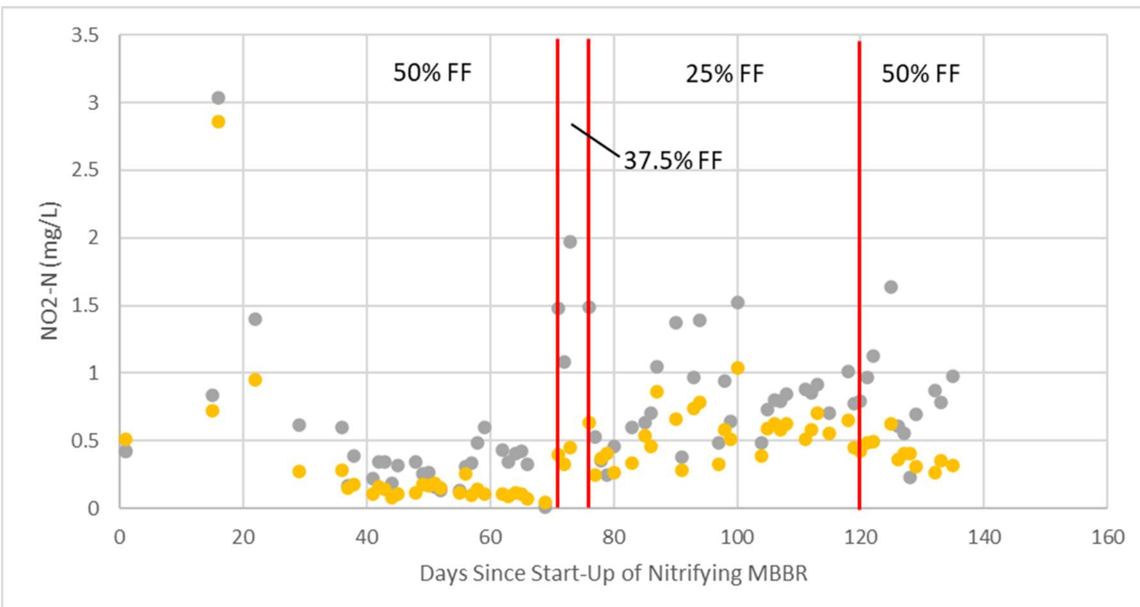

Figure 31. Influent (grey) and effluent (yellow) nitrite concentrations for the aerated nitrifying MBBR.

The media fill fraction was changed three different times throughout operation to determine which fill fraction was necessary to maintain a low effluent nitrite concentration. These different fill fractions, along with the average ammonia loading rate and effluent nitrite concentrations, are listed in Table 9.

Table 9. Influent Loading Rates and Effluent Nitrite Concentrations for the Nitrifying MBBR at Different Fill Fractions.

Days After Startup	Fill Fraction (%)	Average NH3-N Loading Rate (g/m2/day)	Average NO2-N Loading Rate (g/m2/day)	Average Effluent NO2-N Concentration (mg/L)
0-70	50.0	0.170 ± 0.149	0.090 ± 0.105	0.151 ± 0.078

71-73	37.5	0.055 ± 0.035	0.379 ± 0.112	0.392 ± 0.058
76-119	25.0	0.624 ± 0.457	0.403 ± 0.187	0.546 ± 0.187
120-135	50.0	0.383 ± 0.290	0.238 ± 0.101	0.403 ± 0.101

The first 22 days after start-up, nitrification rates were limited due to a lack of NOB. More NOB were grown during this time though, and after day 22, the average NO2-N concentration at a 50% media fill fraction was 0.151 ± 0.078 mg/L. With this fill fraction providing a very low nitrite rate, the fill fraction was decreased to 37.5% on day 71, which produced an average NO2-N effluent concentration of 0.392 ± 0.058 mg/L over three days of sampling. This low average effluent nitrite concentration was reached at this fill fraction even with high nitrite influent concentrations to the nitrifying MBBR (ranging from 1.08 to 1.97 mg N/L). On day 76, the fill fraction was decreased again to 25% to continue to increase the nitrite loading. Nitrite effluent concentrations at this fill fraction varied greatly, with the average concentration being 0.546 ± 0.187 mg/L. The final fill fraction modification was made on day 120 when it was increased to 50% again by adding the same media that was previously taken out back into the MBBR. Since half of the media did not have any kind of biofilm or NOB on it, an increase in the nitrification rate was no seen immediately. The average NO2-N effluent concentration during this time was 0.403 ± 0.101 mg/L. Based on these results, the maximum ammonia and nitrite loading rates that should be used to design a post-PdNA nitrite polishing MBBR are 0.055 g/m2/day and 0.379 g/m2/day, respectively, to maintain a target nitrite effluent concentration of less than 0.5 mg N/L.

Aerated Polishing Tank Phosphorus Uptake

An issue that was noticed with the PdNA IFAS reactor were unexplained spikes of orthophosphate in the effluent. To deal with these spikes of OP, an aerated activated sludge tank was added to serve as an OP polishing step for the IFAS reactor. It is typical for full-scale processes to have a small reaeration zone after a second anoxic zone, which was simulated by this aerated tank. Grab samples were taken from this tank when high OP concentrations (>0.2 mg/L) in the IFAS reactor effluent were detected. The recorded influent and effluent OP concentrations for this aerated polishing tank are displayed in Figure 32.

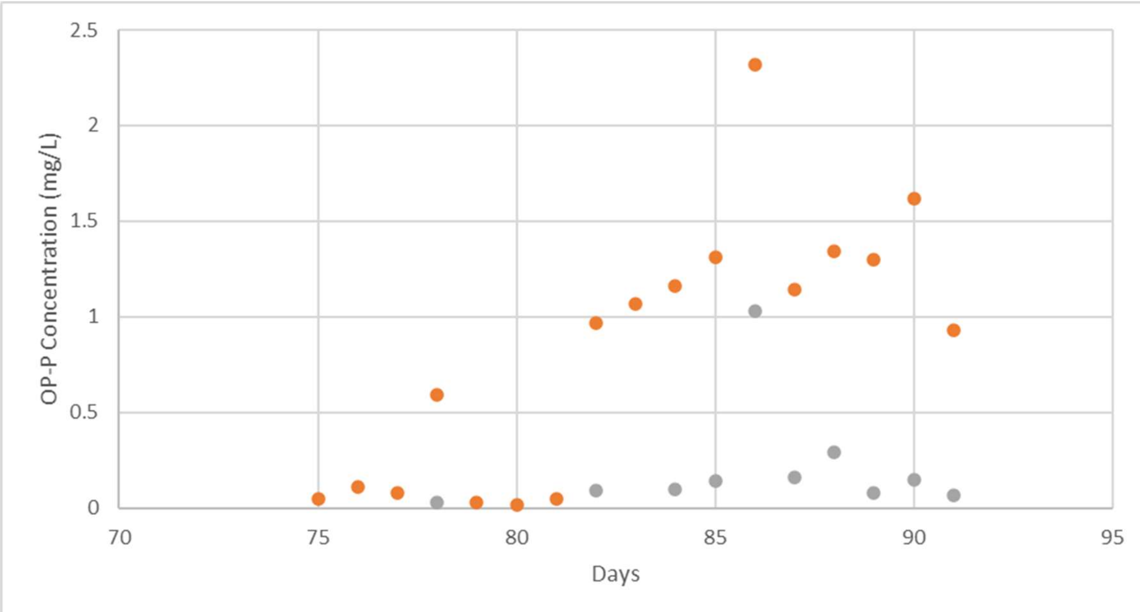

Figure 32. Influent (orange) and effluent (grey) OP concentrations for the aerated activated sludge polishing tank.

Even with only a 4-minute HRT, the aerated polishing tank provided up to 1.47 mg/L of OP uptake, and consistently brough OP influent concentrations above 1 mg/L to concentrations less than 0.2 mg/L. This indicates that a small, aerated zone with a short HRT could be used to deal with these spikes of orthophosphate in the PdNA IFAS reactor's effluent.

Conclusions

Low effluent TIN concentrations (< 3 mg/L) were consistently maintained in both a mainstream polishing PdNA MBBR and a PdNA IFAS reactor, even with variable PdN efficiencies. These low TIN concentrations were reached with COD/TIN ratios of 2.42 ± 0.77 g COD/g TIN for the MBBR, 1.08 ± 0.38 g COD/g TIN for the IFAS reactor during Phase 1, and 2.18 ± 0.99 g COD/g TIN for the IFAS reactor during Phase 2. Based on these COD/TIN ratios, these processes provided 66.2%, 77.5%, and 54.6% of average carbon savings, respectively, compared to the conventional nitrification/denitrification nitrogen removal process. Implementing PdNA in an IFAS reactor provided even more carbon savings due to internally stored carbon, potentially even eliminating the need for external carbon addition. The maximum anammox in the PdNA IFAS reactor, when adjusted to 20°C, increased from 0.467 $g/m^2/day$ at 51 days after startup to 0.638 $g/m^2/day$ at 86 days after startup, indicating anammox growth occurred in the PdNA IFAS reactor. The pilot nitrification MBBR showed that it was possible to consistently maintain an effluent NO_2-N concentration of 0.5 mg/L or below with average ammonia and nitrite loading rates of 0.055 ± 0.035 and 0.379 ± 0.112 g $N/m^2/day$. Overall, performance in the PdNA MBBR and PdNA IFAS reactors were similar in terms of TIN removal and external carbon savings, but PdNA implementation in an IFAS reactor can take advantage of influent carbon and can be implemented in any process with a second anoxic zone with minimal capital costs.

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Summary and Engineering Significance

This research produced valuable insight into anammox process design, mainstream anammox startup duration, and mainstream anammox startup optimization methods through a startup experiment and operation of pilot-scale PdNA processes. First, the startup experiment demonstrated that detectable anammox activity could be obtained in less than three months in a mainstream polishing PdNA MBBR with either virgin or preliminary biofilm carriers. While still longer than the startup times for most BNR processes, this startup time is much lower than initially expected and feasible for full-scale implementation, if planned properly. It is also important to note that, contrary to numerous other reports in the literature of establishing anammox, this work was done without exogenous addition of nitrite, which is relevant to full-scale conditions. The success of the PdNA MBBR startups appears to indicate that, if ammonia, nitrite, and a biofilm-based technology are present in an anoxic environment, anammox growth will occur. They two key components of an anammox startup, based on this research, are providing the anammox bacteria plenty of surface area to grow on and producing a significant amount of nitrite consistently. Additionally, the preliminary biofilm MBBR in this experiment having detectable anammox activity in it over one month before the virgin media MBBR confirmed that anammox were able to grow quicker and more easily on/in the preestablished biofilm compared to virgin media.

The operation of the PdNA MBBR, AMX MBBR, and PdNA IFAS reactor demonstrated that these processes could remove enough nitrogen from secondary clarifier effluent to reach very low TIN effluent limits. This was also done with low amounts of external carbon being added and inconsistent PdN efficiencies in both the PdNA MBBR and PdNA IFAS reactor. Days with better PdN efficiency though still tended to be produce effluent with lower concentrations of TIN. Based on the maximum anammox ammonia removal rates, anammox growth did occur in both the PdNA MBBR and PdNA IFAS reactor, indicating anammox growth could continue in both and that a PdNA startup in an IFAS reactor is potentially feasible. For these processes, it was determined that an influent AvN ratio below 0.6 for the PdNA MBBR and below 0.4 for the PdNA IFAS reactor generally produced lower effluent TIN concentrations, although these ratios can deviate between different reactors and locations. An average Arrhenius coefficient of 1.070 was calculated for the anammox activity in the PdNA MBBR, which allowed for anammox growth to be tracked via maximum anammox ammonia removal, even with significant changes in operating temperatures. Sufficient nitrite polishing (<0.5 mg N/L) of the effluent from the AMX MBBR was also acquired in a 40-gallon aerated MBBR at media fill fractions of 50% and 37.5%, or 49.2 and 36.9 m^2 of total surface area, respectively.

These two research projects showed that mainstream AMX implementation through PdNA is realistic and should be considered at WWTPs who are interested in process treatment intensification or increasing process efficiency, particularly when it comes to aeration, external carbon addition, and solids production. With how quickly anammox activity was detected in the two pilot-scale PdNA MBBRs, it appears that biomass inoculation and/or carrier modifications are not needed to have a reasonable process startup time, making PdNA appear much more feasible to many WWTPs. The use of PdNA in an IFAS reactor also has major implications for any WWTP with a second anoxic zone, since such a zone could be converted to a PdNA zone through the addition of carriers and controlled external carbon. Doing so could provide treatment intensification and efficiency without building any new tanks, taking up more space, or spending a lot of money. This research has demonstrated that PdNA can be implemented into full-scale processes much more quickly and cheaply than what was anticipated before, which will potentially make PdNA more accessible and common in WWTPs throughout the world.