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THE EFFECTS OF CARBON CHOICE & NITRATE LOADING ON IFAS PARTIAL
DENITRIFICATION/ANAMMOX PROCESSES

by

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B.A. May 2022, Old Dominion University

Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
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ABSTRACT

THE EFFECTS OF CARBON CHOICE & NITRATE LOADING ON IFAS PARTIAL DENITRIFICATION/ANAMMOX PROCESSES

Lawrence Cornelius
Old Dominion University, 2024
Director: Dr. Gary Schafran

The Hampton Roads Sanitation District's James River Treatment Plant traditionally operated with an A2O configuration and aerobic IFAS. To improve nitrogen removal, eight of the nine treatment trains were reconfigured to a 5-stage process by converting their second anoxic zones to moving media IFAS with WW2 media (World Water Works). This enabled the incorporation of the partial denitrification/anammox (PdNA) process. For effective partial denitrification in the second anoxic zone, a carbon source is required to convert $\text{NO}_3\text{-N}$ (nitrate-nitrogen) to $\text{NO}_2\text{-N}$ (nitrite-nitrogen), which anammox bacteria use. Ethanol was explored as a potential cost-effective alternative carbon source, hypothesized to exhibit a partial denitrification rate between that of methanol and glycerol. A pilot-scale experiment at the James River Treatment Plant was conducted to compare the efficacy of ethanol and methanol as external carbon sources for the PdNA process. Two identical PdNA IFAS reactors were operated in parallel, one fed with ethanol and the other with methanol, under varying COD loading conditions. The methanol-fed reactor consistently showed higher PdN efficiencies, better NH_4 removal, and lower C/TIN values, indicating more efficient carbon utilization, thus establishing methanol as the preferred choice for PdNA. Subsequently, the study examined the resilience of PdNA systems to nitrate loading. The pilot setup was modified to include two reactors, both fed with methanol; one served as a baseline, and the other received supplemental nitrate. These

reactors were operated in parallel and evaluated for removal rates and PdN efficiency. Over time, the nitrate-supplemented reactor developed a thicker biofilm and exhibited increased NO_3 removal, but this came at the cost of reduced ammonia removal. The reactor favored full denitrification over partial denitrification, resulting in lower NO_2 production and thus limited substrate availability for anammox bacteria, leading to lower in-situ NH_4 removal. The results of this study are crucial for designing full-scale PdNA IFAS systems, particularly concerning the choice of external carbon sources and the impact of NO_3 loading on system performance.

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NOMENCLATURE

A20: Anaerobic, Anoxic, Oxic

AOB: Ammonia Oxidizing Bacteria

Aer AOB: Aerobic Ammonia Oxidizing Bacteria

AvN: Ammonia Versus NO_x

BNR: Biological Nutrient Removal

COD: Chemical Oxygen Demand

CSTR: Continuous flow stirred Tank Reactor

D.O: Dissolved Oxygen

GPM: Gallons per minute

IFAS: Integrated Fixed Film Activated Sludge

MIFAS: Moving Media Integrated Fixed Film Activated Sludge

NOB: Nitrite Oxidizing Bacteria

NO_x: Nitrite and Nitrate

PdNA: Partial Denitrification Anammox

PID: Proportional – Integral – Derivative

PNA: Partial Nitrification Anammox

RAS: Return Activated Sludge

SdNR: Specific Denitrification Rate

SWIFT: Sustainable Water Initiative for Tomorrow

TIN: Total Inorganic Nitrogen

W2: World Water Works 2 media

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CHAPTER 1. INTRODUCTION

Exploration of new processes and technologies aims to efficiently treat expanding wastewater quantities while conserving valuable real estate and while improving nitrogen removal performance. Among these methods two of them are gaining significant traction, one of which is partial nitrification with anammox (PNA), and the other is Partial Denitrification with Anammox (PdNA). The traditional processes of nitrification and denitrification, although effective, can be very energy intensive and therefore costly. With increasingly stringent nitrogen limits in wastewater treatment facilities, higher consumption of organic carbon sources and aeration are becoming more of an issue when using traditional nitrification/denitrification processes (Rui 2019). Due to the potential for cost savings and enhanced treatment capacity associated with the integration of anammox bacteria, there is considerable interest in adopting anammox-based processes. These anaerobic ammonia-oxidizing bacteria, known as anammox, exhibit the capability to effectively remove both ammonia and nitrite from waste streams. It does so by utilizing NH_4^+ as an electron donor and nitrite NO_2^- as the acceptor. This results in the oxidation of ammonia NH_4^+ and the reduction of nitrite NO_2^- simultaneously. The product of this reaction is the production of dinitrogen gas N_2 . Partial nitrification with anammox (PNA) is the more ideal approach to shortcut nitrogen removal, however due to its reliance on the out selection of nitrite oxidizing bacteria (NOB), it has proven to be very challenging to implement in full scale mainstream conditions. For this reason, PNA is often used in side stream applications where high temperatures and free ammonia make it easier to out select NOB in favor of ammonia oxidizing bacteria (AOB). On the other hand, the second approach to shortcut nitrogen removal, PdNA, has shown to be very easily implemented in full scale mainstream

applications. This is because PdNA doesn't rely on NOB out selection but instead allows for full nitrification to occur and then shortcuts the denitrification process by attempting to stop a fraction of nitrate NO_3 from fully denitrifying and instead stopping after converting to NO_2 . This way the anammox bacteria can use the NO_2 produced to remove NH_4 from the waste stream. The reduction in supplemental COD requirements for PdNA is approximately 61–65% as compared to full denitrification (McCullough et al., 2022). Beyond this PdNA also offers reductions in aeration requirements being that once anammox is established nitrification of all influent ammonia isn't necessary. Although PdNA is not as cost efficient as PNA due to the requirement of external carbon, the PdNA process has proven to be easier to implement and offer cost savings when compared to traditional nitrification denitrification processes.

Hampton Roads Sanitation District (HSRD) is a wastewater utility company located in southeast Virginia and services 18 cities in the Hampton Roads community. HRSD operates eight large treatment plants across the Tidewater region, including the James River Treatment Plant (JRTP). James River Treatment Plant is a 20 MGD plant that has 9 treatment trains. These treatment trains originally operated in an anerobic, anoxic, aerobic (A20) configuration but now have been upgraded to operate as a 5-stage process with a small second anoxic zone where the PdNA process takes place. The layout for the JRTP treatment train configuration is show below.

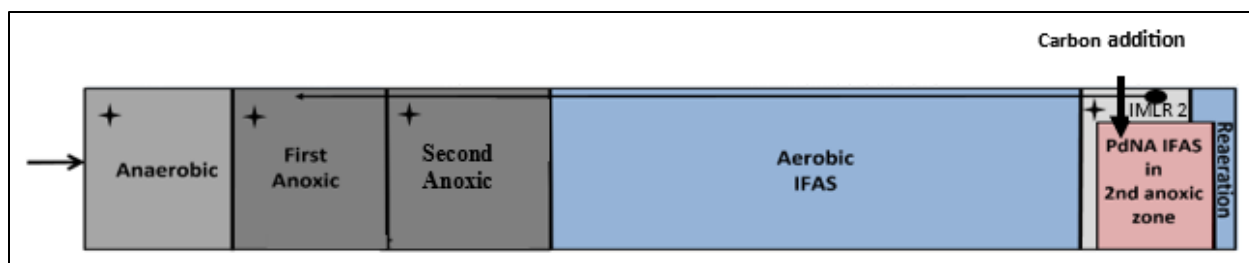


Figure 1: Process flow diagram of HRSD James River Treatment Plant

The successful startup of anammox through PdNA in the second anoxic zone at James River using methanol was a significant milestone (Bachmann, et al. 2024). However, methanol is primarily produced through an energy-intensive process utilizing natural gas and therefore the price of methanol remains interlocked with the fluctuating costs of the fossil fuel market (Bill et al. 2009). Furthermore, due to constraints in methanol storage, the decision was made to transition from feeding methanol to glycerol in the PdNA zone. While glycerol offers effective denitrification, its comparatively higher cost to other carbon sources caused consideration. Beyond this, during piloting, glycerol demonstrated exceptional denitrification capability while also producing an excess of NO_2 (Bachmann, et al. 2024). Nitrite production is beneficial for anammox bacteria but can also presents a challenge for plants adhering to stringent total nitrogen (TN) limits. Furthermore, the elevated levels of NO_2 can have repercussions on the future Sustainable Water Initiative for Tomorrow (SWIFT) facility that will be integrated into the James River Treatment Process. In light of these considerations, HRSD initiated an exploration into alternative external carbon options for the second anoxic zone, aimed at enhancing partial denitrification. After careful deliberation, ethanol emerged as a promising candidate to potentially replace existing external carbon sources, offering substantial cost savings for the facility. Recognizing methanol as the preferred choice for the James River Treatment Plant (JRTP) going forward, a pilot-scale comparison between methanol and ethanol was proposed to

determine the most suitable option for JRTP. During the initial phases of this study, observations made when looking at in-situ removal rates, revealed a significant influence of nitrate loading on the performance of PdNA systems. Consequently, the latter portion of the study was dedicated to investigating the effects of NO_3 loading, determining the thresholds at which system performance might be compromised.

The objectives of this pilot experiment were:

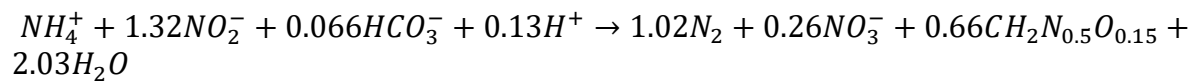
1. Investigate the cost-saving potential of utilizing ethanol as an external carbon source in the PdNA process.
2. Evaluate the limits of nitrate (NO_3) and COD loading for IFAS PdNA.

CHAPTER 2. BACKGROUND OF THE STUDY

2.1 Literature review

2.1.1 Anaerobic Ammonium Oxidation

Anaerobic ammonium oxidizing bacteria, commonly referred to as anammox are a type of bacteria that facilitates the oxidation of ammonia to dinitrogen gas (N₂) under anaerobic conditions. This unique group of bacteria operates through a specialized metabolic process, utilizing ammonia (NH₄⁺) and nitrite (NO₂⁻) as its substrates. By employing (NH₄⁺) as the electron donor and (NO₂⁻) as the electron acceptor, anammox bacteria effectively convert ammonia and nitrite into N₂ gas. The equation for this reaction is below (Kartal et al. 2010).



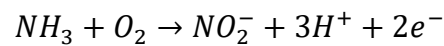
Equation 1

In recent years, the integration of anammox bacteria has gained significant traction within wastewater treatment methodologies. Anammox bacteria, reliant on ammonia as a substrate, alleviate the necessity for complete nitrification of incoming ammonia loads within a system. This unique characteristic allows for a deliberate slippage of ammonia downstream, thereby resulting in substantial cost reductions in aeration which is a primary annual expense for most treatment plants. Moreover, as anammox bacteria also utilize nitrite as a substrate, there is no need to carry out full denitrification of available nitrate. This, in turn, translates to economic

benefits through reduced reliance on external carbon feeds. These microorganisms present a secure and economically efficient means to extract substantial quantities of ammonia from waste streams, rendering them highly appealing to numerous treatment facilities. Implementing anammox bacteria in a system, while highly beneficial, can present challenges due to their slow specific growth rate. With a maximum specific growth rate 0.0027 h^{-1} and a doubling time of 11 days (Strous et al. 1998), these bacteria grow significantly slower than the solids retention time (SRT) of many treatment facilities. The ability to cultivate and retain anammox bacteria for a sufficient period becomes a concern. To address this challenge, the use of mobile biofilm carriers has emerged as one potential solution. Biofilm carriers, available in various shapes, sizes, and materials, have been incorporated into many treatment processes to provide a surface to retain desired microorganisms. Typically, the biofilm carriers often referred to as media, are sufficiently large to prevent washout from the system, providing a surface for the slow-growing anammox bacteria to adhere to and thrive within the facility. James River Treatment plant has implemented anammox bacteria into the mainstream treatment process. Inside of the small second anoxic zone of the 5-stage process, (JRTP) has successfully completed an anammox startup and plans to continue with process intensification upgrades in order to maximize the potential of this zone.

2.1.2 Partial Nitrification Anammox (PNA)

Partial nitrification anammox (PNA) is a process of shortcut nitrogen removal through the partnered use of aerobic ammonia oxidizing bacteria (AOB) and anammox bacteria. Partial nitrification is done by utilizing the AOB to only oxidize ammonia to nitrite which can then be used by anammox to oxidize ammonia anoxically. Below is equation 2 which shows the PN process.



Equation 2

The (PNA) process simplifies wastewater treatment by only requiring the nitrification of a portion of the ammonia in the waste stream. This efficiency stems from the subsequent removal of ammonia by anammox bacteria upon the presence of nitrite. With PNA, solely the initial phase of nitrification, where ammonia is oxidized into nitrite needs to occur, leading cost savings on aeration. Approximately half of the oxygen required for nitrification is consumed during this primary stage, leaving a surplus about 50% aeration capacity. Consequently, the PNA process achieves 50% reduction in aeration requirements compared to traditional full nitrification methods (McCullough et al., 2022). Moreover, the absence of a need for complete nitrification translates into a lack of nitrate production within the system. Consequently, there is about an 90% cost savings on external carbon, a significant advantage over conventional nitrification-denitrification processes. The PNA process is shown in Figure 2 below.

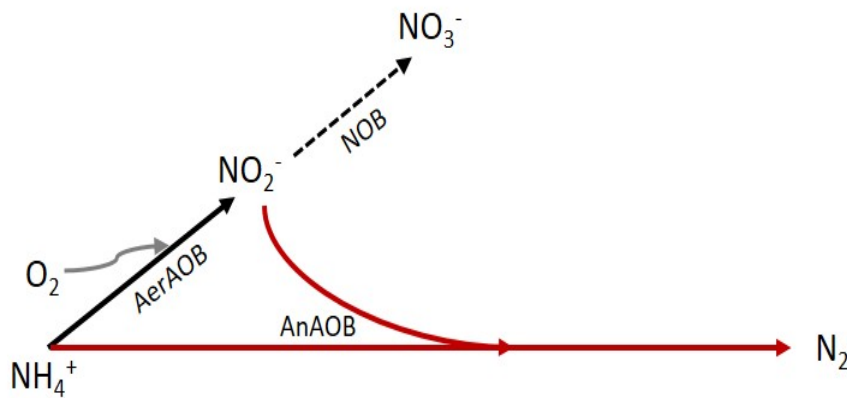


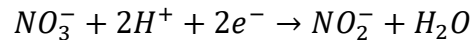
Figure 2: Partial Nitrification\Anammox flow process diagram (Courtesy Hampton Roads Sanitation District).

Implementation of mainstream full scale PNA is difficult primarily because mainstream conditions are not suitable for NOB out selection which is a requirement for successful partial nitrification. During PNA nitrite can be produced through partial nitrification which requires inhibiting NOB growth and activity against AOB (Zhang 2019). Conditions such as high temperatures and high ammonia create an environment where NOB out selection is more feasible. Temperatures higher than 30 °C and pH values between 7.8 and 8.5 have been implemented to inhibit NOB in bioreactors for nitrification (Tao et al., 2012). These conditions are not easily replicated in mainstream treatment processes. As a result of the inability to efficiently create conditions for PNA to take place in many mainstream applications, PNA has largely been used in side stream processes.

2.1.3 Partial Denitrification Anammox (PdNA)

Like PNA, partial denitrification with anammox (PdNA) is a process of shortcut nitrogen removal. PdNA doesn't have as much cost savings associated with it as the PNA process. However, because high concentrations of free ammonia are not guaranteed, NOB in the activated sludge of mainstream processes cannot be inhibited from growing, and therefore nitrite cannot accumulate in high quantities (Fofana et al., 2022). The PdNA process on the other hand, doesn't require such strict accommodations and can result in significant aeration savings and external carbon savings compared to conventional nitrification denitrification processes. The reduction in supplemental COD requirements for PdNA is approximately 61–65% as compared to full denitrification. (McCullough et al., 2022). Although cost savings associated with PdNA are not as appealing as those connected to PNA, the PdNA process has proven to be much easier to implement in full scale applications. The poor efficiency PNA tends to have, results in the unavoidable oxidation of nitrite to nitrate (Izadi et al., 2023). Research on the PdNA process became highly necessary because of the unstable long-term performance of the PNA process.

Partial denitrification works by reducing NO_3 to NO_2 using ordinary heterotrophic organisms (OHO). Below is the reaction for partial denitrification. During this process, the amount external carbon fed controlled so that a large amount of the NO_3 being reduced will stop at NO_2 and not reach full denitrification. The equation for partial denitrification is below.



Equation 3

Through this reduction of NO_3 , NO_2 is produced which acts as substrate for the anammox bacteria. Once NO_2 and NH_4 are available, the anammox bacteria can uptake both and the

product of this reaction is dinitrogen gas N_2 . In the second step of the PdNA process, NH_4 act as an electron donor while the produced NO_2 that acts as an electron acceptor is converted to gaseous N_2 by anammox when in anoxic conditions (Al.-Hazmi et al., 2023). The Figure 3 below depicts the partial denitrification with anammox pathway.

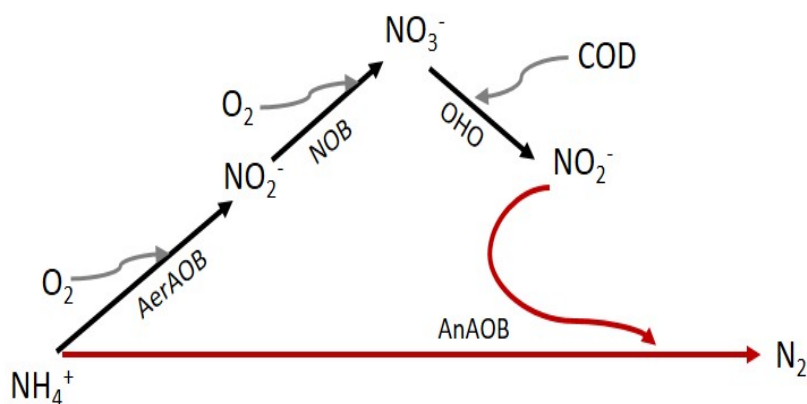


Figure 3: Partial denitrification with anammox process flow diagram (Courtesy Hampton Roads Sanitation District).

PdNA has proven to work in full-scale applications as a result of easier implementation and robust nature of the anammox bacteria. With the combination of partial denitrification accompanied with anammox, high concentrations of NH_4^+ -N and NO_3^- -N can be removed simultaneously from wastewater (Fofana et al., 2022). James River treatment plant is undergoing upgrades to all 9 of its treatment trains for the purposes of successfully implementing this PdNA process. Currently 3 of the 9 treatment trains have media in the second anoxic zone with established anammox activity. Moving forward the rest of the trains will be retrofitted to have this small second anoxic zone filled with media to ensure the PdNA process is successfully completed in every treatment train. Tank 2 of the 9 treatment trains has anammox activity that

contributes 0.3-0.5 g/m²/day of NH₄-N removal. Although this is notable contribution to overall removal rates, HSRD is still looking at ways to further intensify this process in order to meet new permit limits of less than 4 mg/L of total nitrogen (TN) that will be in place 2026.

2.1.4 PdN Efficiency

PdN efficiency measures the extent to which NO₃-N in the system undergoes partial denitrification, converting into NO₂-N without proceeding to full denitrification and producing dinitrogen gas. PdN efficiency can be obtained by calculating the amount of nitrite generated, relative to the amount of nitrate removed in a system (Macmanus et al., 2022).

A relatively high PdN efficiency is preferred for the effectiveness of a PdNA system, as anammox bacteria rely on the presence of NO₂-N to remove ammonia (NH₄⁺) from the system. In the absence of either NH₄ or NO₂, the anammox bacteria cannot effectively contribute to the reduction of Total Inorganic Nitrogen (TIN).

2.1.5 PdNA process control

Control of a variety of components in the PdNA process can ultimately affect the performance of the system. One of the most critical of these is the ratio of ammonia present relative of the amount of nitrite present. Because both NH₄ and NO₂ are substrate for the

anammox bacteria, it is critical that they are both present in stoichiometrically favorable amounts. This way it can be ensured the anammox will remove nitrogen of these species. Anammox bacteria can't metabolize one without the other, therefore keeping them both in relative abundance is ideal. Maintaining this balance is often done by pinpointing a specific ratio of ammonia to NO_x in the system. This ratio of NH₄ to NO_x is referred to as (AvN) (Regmi et al., 2014). Depending on a treatment plant's effluent goals they will set target effluent (AvN) value, and with that control their aeration to ensure the optimal amount of ammonia and NO_x is getting through the system to provide ideal conditions for the anammox bacteria. James River treatment plant utilizes (AvN) to create the best environment for anammox to grow and thrive (Bachmann, et al. 2024). Not only is it beneficial to the anammox but the ability to allow some ammonia to bleed downstream saves on the blower cost as well.

2.1.6 External Carbon Sources for PdNA

The selection of an external carbon source directly influences the efficiency of a system in achieving partial denitrification. Different carbon sources exhibit varying partial denitrification (PdN) capacities, and the extent of their favorable impact depends on the operational practices of the systems as well. A pilot study was conducted at JRTP to investigate if methanol or glycerol would be most optimal for full scale implementation into the PdNA zone. Glycerol proved to have a high affinity to partially denitrify but can be costly. Beyond this the high PdN can be beneficial and detrimental depending on the system it's implemented in. With glycerol, a large amount NO₂ may accumulate, which is a problem if there is not enough AMX activity to uptake the NO₂ (Bachmann, et al. 2024). If NO₂ bleeds out the back of PdNA zone there is a risk of not adhering to TIN permit requirements as well as negatively impacting disinfection processes downstream.

Methanol is a relatively affordable carbon source compared to glycerol and offers effective PdN and has been commonly utilized for full denitrification. The HRSD James River Treatment plant was able to achieve a successful anammox startup in the second anoxic zone of one of its treatment trains using methanol as an external carbon source (Bachmann, et al. 2024). While methanol proves cost-effective, it introduces additional considerations such as the need for appropriate storage facilities, which can entail substantial investments ranging into the millions, depending on volume. This was a primary concern encountered by the JRTP during its deliberations on full-scale methanol implementation. Furthermore, methanol is primarily produced through an energy-intensive process utilizing natural gas and therefore the price of methanol remains interlocked with the fluctuating costs of the fossil fuel market (Bill et al. 2009).

Ethanol has demonstrated its efficacy as a suitable carbon source for facilitating denitrification (Bill et al. 2009). Notably, ethanol remains relatively less explored compared to methanol and glycerol. Ethanol has a carbon to nitrate (C/N) ratio of approximately 5.7 while methanol has a C/N ratio of approximately 4.8. This number represents the amount of carbon needed to remove 1 part of nitrate. The C/N ratio can be directly correlated to cost savings as the more carbon that needs to be dosed equates to more carbon that needs to be purchased. Although ethanol has a higher C/N ratio than methanol it offers faster kinetics than methanol. This attribute could be particularly advantageous for applications requiring partial denitrification. If ethanol offers good kinetics and high PdN, it could be more beneficial to PdNA processes than methanol despite methanol having a lower C/N. In addition, if a facility has a flammable system rated for methanol anyway, it could use ethanol with no modification making it attractive for JRTP specifically.

2.1.7 Moving Media IFAS

The ability to grow and retain slow growing microorganisms for the purposes of increasing efficiency has revolutionized wastewater treatment. Utilization of IFAS biofilm carriers promotes the development of slow growing bacteria such as anammox and prevents wash out of the system, which ultimately increases efficiency (Dias, 2018). In comparison to suspended growth, attached growth technologies like biofilm carriers have proven to be robust and withstand treatment upsets more readily. Biofilm carriers, often referred to as media, have proven to be somewhat hard to implement full scale since they can cause head loss. However, media can be advantageous because the reactor volumes are much smaller than what is required for most systems to implement suspended growth. Attached growth technologies have smaller footprint, and they respond well to system upsets, toxicity spikes, and frequent changes in loading rate and pH (Almomani et al., 2019).

There are various types of moving media, some act relatively similar but others act vastly different. There are several factors that affect moving media performance one of the main being density of the media. Density of the media directly correlates to how well it will mix. High density media will sink faster and require more mixing energy to keep it afloat while low density media could cause stacking closer to the water's surface. Another factor greatly influencing media performance is the geometry of the media piece. The quantity and size of openings in these pieces significantly impact the relative surface area of the media, ultimately dictating where and how effectively microorganisms will colonize the carriers' surfaces. Carriers of different sizes and shapes can influence flow pathways and hydraulic velocities which can impact oxygen and substrate mass transfer along with biofilm dynamics like growth, thickness, and detachment (Dias, 2018). The surface is an extremely important parameter as it will correlate

to the amount of growth that can accumulate on the carrier. Selecting the correct media type per application is imperative as well. This is due to the fact that more surface area doesn't always equate to better activity. For instance, a system with a high chemical oxygen demand (COD) loading may not benefit from media featuring small openings and extensive surface area. The substantial loading within the system could obstruct the small openings, consequently diminishing the anticipated removal efficiency. Instead, a system with high loading may choose a media with a lower rated surface area but larger hole openings. This way the media can handle higher loading conditions without plugging as quickly and still achieve removal requirements.

The available surface area doesn't always equate to surface area that will accumulate growth. The protected surface area refers to the parts of the media that are not as exposed and therefore less susceptible to shearing. The protected surface area is more likely to have biofilm successfully attached without being disturbed as frequently as the biofilm on the exterior edges of a carrier.

Fill fraction of media relative to the amount of water volume in a reactor also influences a system. An abundance of carriers can be beneficial in acquiring more biomass in a tank, however there is a point where too many carriers can also result in worse performance. When too much media is present mixing issues can occur along with the likelihood to see head loss. Beyond the presence of an overabundance of media can cause more shearing amongst the individual pieces as they are colliding more often and thus reducing the total amount of biomass per piece. An increase in fill fraction could increase particle-particle collision and in turn could enhance shearing of the biofilm (Gu et al., 2014). One study done at JRTP showed removal rates of 1 g/m²/day of TIN removal utilizing media (Macmanus 2021).

2.1.8 Effects of nutrient loading and biofilm control

The structure of a biofilm is directly correlated to the performance of the plastic carrier in terms of nutrient removal. A healthy amount of growth on the media pieces is required to see any real in-situ TIN removal rates as a result of the presence of the media. However, a balance must be struck. Obviously if there is no attached growth to the media no removal will be observed, but removal rates can also be greatly reduced by the overabundance of micro-organism on the media pieces. Mass transfer refers to how well nutrients can maneuver to and through an existing biofilm. Mass transfer therefore determines how well the micro-organisms on the plastic media access the surrounding substrate. It has been seen in studies that increase of biofilm thickness can result in stronger mass transfer resistance, meaning increases in thickness are accompanied with decreased microbial activity in the internal biofilm (Cui et al., 2017).

The growth of biofilm is influenced by nutrient loading, with higher nutrient levels typically resulting in a thicker biofilm adhering to the media. This is especially evident in partial denitrification and denitrification applications. As nitrate loading increases (COD) loading must also rise to facilitate the removal of this heightened nitrate. understanding the objectives of a treatment facility is pivotal in determining the optimal type of biofilm growth for the media within a system. In the context of a (PdNA) zone, the presence of denitrifying bacteria is essential. However, it is also noteworthy that anammox bacteria require sufficient coverage on the media for the efficient removal of nitrite and ammonia.

CHAPTER 3. METHODOLOGY

3.1 Pilot Layout

Two reactors with liquid volume of 121L were used for this study. Each reactor was equipped with a mechanical mixer in it that operated at 80 rpm which gave a G-value of approximately 111/sec. This mixer speed was held constant for the duration of the study. A Godwin GSP10 submersible pump (Xylem, Rye Brook, NY) was positioned at the end of an aeration basin within the James River Treatment plant's integrated fixed film activated sludge (IFAS) system. This pump was used to precisely direct a portion of the flow into the pilot plant. As the flow entered the Solids Handling building, home to the pilot plant, it was transferred by a progressive cavity pump (Seepex, Enon, Ohio). Flow was then split and passed through 2 flow meters before reaching two additional Seepex pumps (Seepex, Enon, Ohio) operating in parallel. These pumps were responsible for controlling the flow to the pilot-scale PdNA IFAS reactors, which maintained a steady flow rate of 1.5 gallons per minute (GPM). In Figure 4 is an image of the two-pilot scale PdNA IFAS reactors.



Figure 4: Reactor configuration of PdNA IFAS Pilot

Each of the pilot scale PdNA IFAS reactors had plastic carriers in them to retain anammox. For each phase of the pilot study World Water Works W2 media was used (World Water Works, Oklahoma). World Water Works W2 media was taken from the full scale second anoxic zone and placed in the reactors. Each reactor had a 50% fill fraction, meaning half of the available volume was taken up by the plastic carriers and the other half by mixed liquor. Because the media was taken from the full scale it already had an established annamox population. Because the fill fractions were identical, each reactor had the exact same amount of available surface area for microbial growth. Calculations for this experiment were grounded on the effective surface area of $650 \text{ m}^2/\text{m}^3$ for the W2 media. Below are images of the W2 plastic carriers, one is a virgin piece while the other has a biofilm attached.

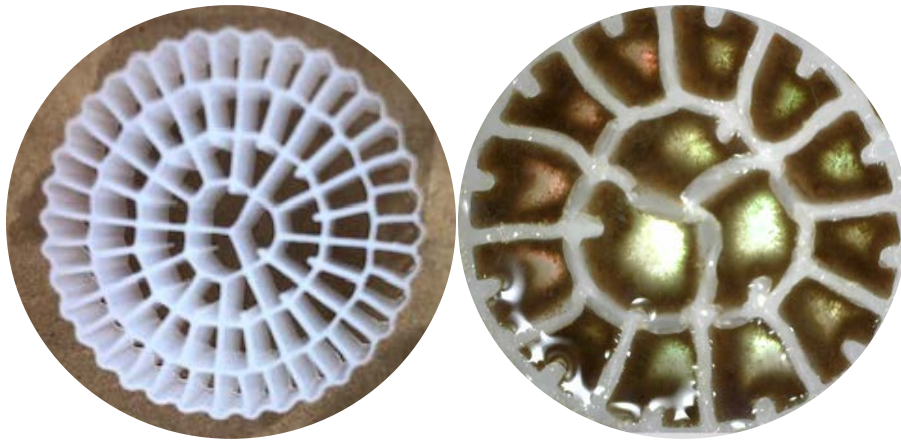


Figure 5: World Water Works W2 Media

Each of these reactors had a liquid volume of 121 a hydraulic retention time (HRT) of 20 minutes per reactor. Effluent drains were equipped with PVC screens designed to allow water to pass through while retaining the media within the reactors. The process flow diagram for the pilot plant is provided in Figure 6.

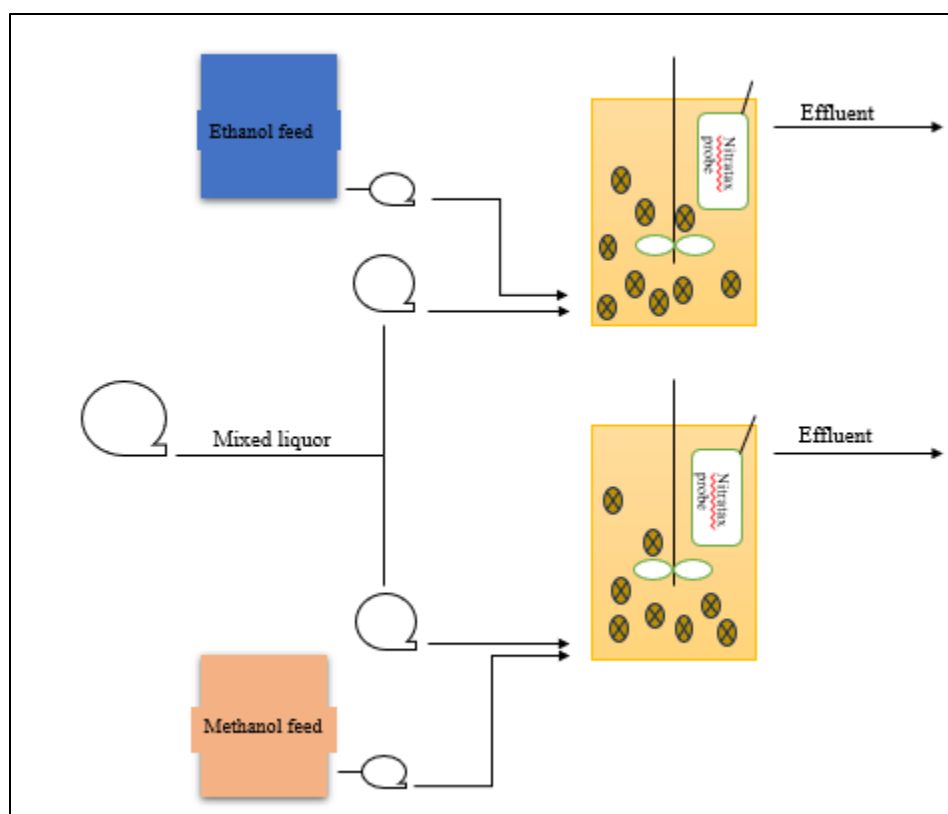


Figure 6: Process flow diagram of PdNA IFAS Pilot

3.1.2 External Carbon Feed System

In order to dose methanol or ethanol to their respective reactors. There was a feedback system with a PID (Proportional – Integral – Derivative) controller in place that utilized a target $\text{NO}_3\text{-N}$ setpoint to determine how carbon should be dosed to the reactor at a given time. The establishment of this target setpoint played a pivotal role in this study, primarily aiding in the regulation of $\text{NO}_3\text{-N}$ residuals within the reactor. Previous research has underscored the significance of nitrate residual as a key factor influencing PdN (McCullough et al., 2022). Throughout the majority of this study, the target effluent $\text{NO}_3\text{-N}$ concentration was maintained at 1.5 mg/L for both reactors, though in some cases the setpoint was not maintained due to the upper bound limitations placed on the COD pump speed.



Figure 7: Carbon feed pumps for pilot IFAS reactors.

Each phase of the experiment implemented a top scale on the carbon feed system that acted as an upper limit for carbon dosing. This ensured that, regardless of the nitrate concentration within the reactors, there existed a maximum amount of (COD) that could be dosed per unit time. At the initiation of phase 1, the carbon feed for both reactors, IFAS #1 with ethanol and IFAS #2 with methanol, was regulated to a dosage limit of 2 g-CODm²/day of external carbon. While the pumps were capable of operating at flow rates below this allowable limit, they were constrained from exceeding it. After operating with this top scale for a 4-week period, the top scale was

increased to allow for 4 g-COD/m²/day of external carbon to mark phase 2. Following this the top scale was increased to 8 g-COD/m²/day at the beginning of phase 3.

3.1.3 Reactor operation for Methanol and Ethanol comparison study

The methanol and ethanol fed reactors operated in parallel and received identical flow rates of 1.5 gpm. Nutrient concentrations in the influent were also the exact same between both reactors in order to ensure an accurate comparison of performance. This study encompassed 3 phases, each operated under a different allowable COD dosage. All three loading phases took place under relatively similar NO₃ loading conditions between 1-1.3 g-N/m²/day. The only distinction between the reactors was the external carbon being fed for PdN. One reactor was fed ethanol while the other was fed methanol. Influent and effluent samples were pulled daily from both reactors and assessed for nitrogen species (NH₄, NO₂, NO₃). After analysis of raw data, concentrations from influent and effluent were used to determine removal rates, PdN efficiency and C/TIN values for both reactors.

3.2 Pilot set-up to observe effects of NO₃ loading.

The pilot design then pivoted to another comparative approach, with one reactor serving as a baseline while its counterpart received additional NO₃-N at a consistent rate to artificially increase loading. This augmented nitrate loading was achieved using a precision Master flex pump. All other equipment such as NO₃ sensors, mechanical mixers and carbon feed pumps remained in the same configuration. Both reactors were also fed methanol during this portion of the study to ensure the only difference between them was the NO₃ they were receiving. Over the ensuing 2-month period, both reactors operated in parallel, allowing for a detailed observation of

how the biofilm and overall microbial activity responded to the increased nitrate loading. In Figure 7 is the pilot configuration during this assessment.



Figure 8: PdNA IFAS pilot set up to assess effects of nitrate loading.

3.3 Daily sampling

A systematic approach was followed for sampling, analysis, and monitoring within the reactors. Once a day, grab samples are collected from each of the pilot scale PdNA IFAS reactors. Samples were collected around mid-day using a syringe and subsequently, these

samples undergo filtration through a 0.45-micron filter to eliminate particulate matter. The collected samples are then subjected to comprehensive analysis to determine the presence of various nitrogen species, including ammonium-nitrogen (NH₄-N), nitrite-nitrogen (NO₂-N), and nitrate-nitrogen (NO₃-N). Additionally, the levels of COD and phosphorus were measured 2-3 times a week. Monitoring of flow rates from the influent pumps into each reactor allowed assurance that the target HRT of the reactors was consistent. Concurrently, environmental parameters such as the temperature, dissolved oxygen levels, and pH of the wastewater was also measured during sample collection. Dissolved Oxygen (DO) and temperature were measured using an InsiteIG Portable Meter (IPM) (Insite, Slidell, LA) with an Insite Portable Dissolved Oxygen Sensor (Insite, Slidell, LA) attached. The pH was measured using a Hach Pocket Pro pH Tester (Hach, Loveland, CO). Nutrient loading to each reactor was assessed on a mass per area basis, enabling a precise evaluation of nutrient inputs and removal rates within the reactors. The collected data was then inputted into an organized spreadsheet for further analysis. This analysis plays a crucial role in providing insights into the performance and efficiency of the PdNA reactors. Based on these findings, necessary adjustments and optimizations were made to the treatment process to ensure the optimal removal of contaminants and the efficient operation of the system. After collection and analysis of concentrations within daily samples, the resulting values were plugged into the equations below to assess loading rates, removal rates, and PdN efficiencies in both reactors.

The equation for loading rate is shown below (Macmanus 2021).

$$\text{Loading rate} = \frac{(\text{Influent Concentration}) * (\text{Flow Rate})}{\text{Total Media Surface Area}}$$

Equation 4

The equation for calculating removal rates is shown below (Macmanus 2021).

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

Equation 5

The equation used to calculate PdN efficiency is shown below. (Macmanus 2021).

$$\text{PdN efficeincy} = \frac{(\text{NO}_2 \text{ out} - \text{NO}_2 \text{ in}) + 1.32 * (\text{NH}_4 \text{ in} - \text{NH}_4 \text{ out})}{(\text{NO}_3 \text{ in} - \text{NO}_3 \text{ out}) + 0.26(\text{NH}_4 \text{ in} - \text{NH}_4 \text{ out})}$$

Equation 6

3.4 Anammox Maximum Activity Test

Maximum anammox activity tests were run every 3-5 weeks to assess anammox activity on the moving media. The test was conducted by first isolating the IFAS reactors. To isolate the reactors the seepex pump (Seepex, Enon, Ohio) that provided mixed liquor was turned off and the blue-white pump that provided external carbon was turned off. The influent valve at the base of the reactors was then closed. Closing the influent valve to the reactors created a batch test environment and ensured no unwanted flow could enter. Once isolated, the reactor was given approximately 10 to 20 minutes to allow all flow above the effluent opening to escape. Dry ammonium chloride and sodium nitrite were measured using an analytical balance. Given that both ammonium chloride and sodium nitrite were in powder form, a small volume of deionized water was added to facilitate dissolution. Once fully dissolved and thoroughly mixed, the chemical solution was gently introduced into the isolated reactor, allowing 3 to 5 minutes for proper dispersion. Subsequently, the initial sample was extracted using a syringe, immediately filtered through a 0.45-micron filter to preserve its concentration from any potential microbial

influence. A systematic sampling protocol was then established for subsequent intervals. This time interval between samples was typically 5 minutes. At the start of the activity test a D.O probe was placed in reactor to monitor dissolved oxygen concentrations throughout the test. The probe also was used to accurately measure temperature during the duration of the test. At the beginning and end of each activity test, pH was taken using a (HACH brand pH meter) which also measured temperature. Samples were collected and analyzed using HACH tubes, focusing on the concentrations of nitrogen species including ammonia, nitrite, and nitrate. Additionally, COD HACH tubes were employed for analysis on the initial and final samples of the test. The equation used to find removal rates from the anammox max activity test is shown in equation 7.

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

Equation 7

3.5 Maximum Specific Denitrification Rate Test

Maximum specific denitrification rate tests were run to assess denitrification rates of specified external carbon sources (Ethanol, methanol, and glycerol). The test was conducted by first collecting 10 Liters mixed liquor from the end of JRTP full scale aerobic zone. The mixed liquor was then placed into a 5-gallon bucket which acted as a batch reactor. This ensured nothing could enter or exit the confines of the existing environment. A small submersible pump was placed in the bucket to provide sufficient mixing through the test. A dissolved oxygen (D.O.) probe was placed in the reactor to ensure minimal oxygen presence and to accurately monitor the temperature throughout the test duration of the test. Following this a foam cover that was cut to the exact dimensions of the surface of the 5-gallon bucket was placed on the surface of the mixed

liquor to ensure minimal D.O intrusion. The foam cover had a small slit in it to allow room for the D.O probe and chemical addition. pH measurements were taken using a HACH brand pH meter at both the beginning and end of each activity test. The pH probe also provided a way to verify the temperature readings from the D.O probe. The bucket was allowed to mix internally until the D.O probe read below 0.05 ppm, this way the absence of oxygen was confirmed before the start of the test. To provide sufficient substrate for the denitrifying bacteria, approximately 15-20 mg/L of Sodium Nitrate ($\text{NO}_3\text{-N}$) along with 200-300 mg/L of COD, via the choice of external carbon for that specific test, was measured and introduced into the reactor. The reactor was then given an additional 3-5 minutes to mix prior to the collection of the first sample. Samples were taken every 15 minutes for the course of an hour using a syringe and filtered through a 0.45-micron filter. Samples were collected and analyzed at 15-minute intervals using HACH tubes to measure nitrite ($\text{NO}_2\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$). Additionally, COD HACH tubes were utilized on the initial and final samples of the test to verify there was a non-limiting carbon.

3.6 Temperature correction

Rates from all activity test and daily samples were temperature corrected to 20 degrees Celsius to allow comparison at a common temperature. The formula used for temperature correction is shown below. The Arrhenius coefficient used for heterotrophs was 1.07. The Arrhenius coefficient used for anammox was 1.09 (Nifong 2013).

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

Equation 8

CHAPTER 4. RESULTS AND DISCUSSION

Unexpectedly, the PdNA reactor that was being fed methanol outperformed the PdNA reactor that was being fed ethanol in both TIN removal and PdN efficiency. This was surprising as the assumption going into this study was that ethanol would show better PdN efficiency due to its faster denitrification kinetics. This comparison was broken into separated phases where performance of each reactor was assessed. Each phase of the pilot study was conducted at a similar nitrate loading but varied COD loading. Phase 1 of this study encompass moderate NO₃ loading accompanied with 2 g/m²/day of COD. Phase 2 of the study was conducted under moderate NO₃ loading conditions and 4 g/m²/day COD. The final phase of this comparison, phase 3, was conducted under moderate NO₃ loading with 8 g/m²/day COD. Phases 1 and 2 of the pilot study occurred during moderately loaded summer conditions, spanning from late May 2023 to early August 2023. Phase 3 of the pilot began in early August 2023 and ended in December 2024 approaching winter conditions. Figure 7 shows the loading rates associated with each phase of the pilot study.

Table 1. Description of reactor phases for methanol and ethanol comparison.

Phases	COD loading (top scale)	Actual COD fed (Ethanol)	Actual COD fed (Methanol)	NO ₃ Loading
Phase 1	2 g/m ² /day	1.5 ± 0.32 g/m ² /day	1.3 ± 0.36 g/m ² /day	1.08 ± 0.08 g/m ² /day
Phase 2	4 g/m ² /day	3.3 ± 0.51 g/m ² /day	2.1 ± 0.46 g/m ² /day	1.21 ± 0.07 g/m ² /day
Phase 3	8 g/m ² /day	4 ± 0.71 g/m ² /day	3.0 ± 0.69 g/m ² /day	1.20 ± 0.12 g/m ² /day

4.1 System response to moderate NO₃-N and varying COD loading

Nitrogen species were assessed in both the methanol and ethanol fed reactors daily.

Figure 9 shows results of daily grab samples examined for NO₃ concentrations are shown for both reactors.

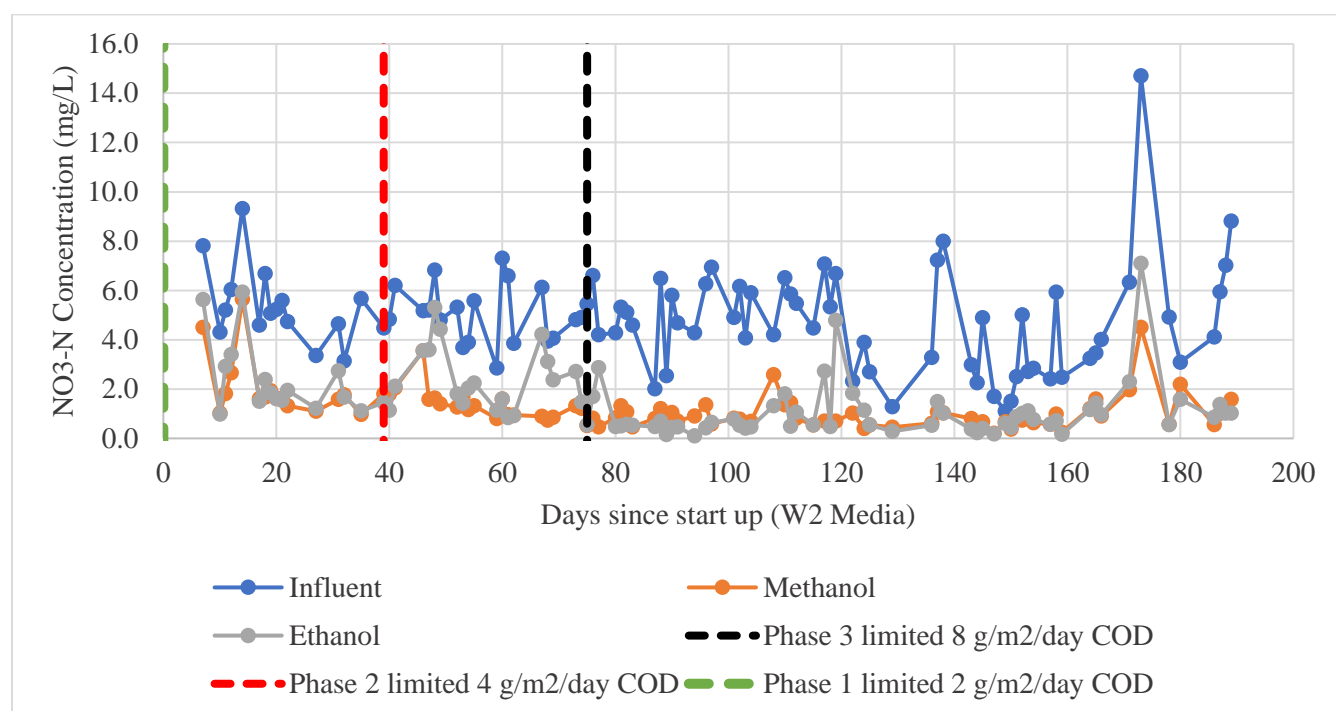


Figure 9: NO₃ grab sample results from methanol and ethanol fed reactors.

Table 2: Nitrate removal rates from all phases of the Methanol and Ethanol comparison.

	NO ₃ removal rate in Ethanol fed reactor (g/m ² /day)	NO ₃ removal rate in Methanol fed reactor (g/m ² /day)
Phase 1	0.45 ± 0.05	0.51 ± 0.06
Phase 2	0.41 ± 0.07	0.50 ± 0.05
Phase 3	0.60 ± 0.08	0.66 ± 0.09

4.1.1 NO₃ trends during Ethanol and Methanol comparison

During phase 1, the ethanol reactor removed 0.45 g/m²/day of NO₃ while the methanol reactor removed 0.51 g/m²/day on average. The ethanol fed reactor was unable to meet the NO₃ setpoint of 1.5 mg/L due to the limited COD feed of 2 g/m²/day. In order to mitigate this, the top scale on the COD feed was increased from 2 to 4 g/m²/day for phase 2.

When top scale on the carbon feed was increased for phase 2, the methanol fed reactor continued to outperform the ethanol fed reactor. On average, NO₃ removal rate from the ethanol fed reactor was about 0.41 g/m²/day. The NO₃ removal in the methanol fed reactor was 0.50 g/m²/day which was identical to what was observed during phase 1.

NO₃ removal rates between both reactors during phase 3 were much closer than in previous phases. The ethanol fed reactor exhibited an average NO₃ removal rate of 0.60 g/m²/day, whereas the methanol-fed reactor demonstrated a rate of 0.66 g/m²/day. This may be attributed, in part, to the higher concentration of (COD) available to both reactors. Being the top scale on the carbon feed was increased it would make sense to see more nitrate removal in this phase as there was more available external carbon to aid in denitrification.

Nitrate removal during all 3 phases was higher in the methanol fed reactor than what was observed in the ethanol fed reactor. On average the in-situ NO₃ removal rate seen in the ethanol reactor was 0.49 g/m²/day while the methanol reactor had an average of 0.54 g/m²/day. Under carbon constraints the methanol reactor was able to remove more nitrate in-situ which can be correlated to its lower theoretical C/N value than ethanol's. In Figure 10 below are the temperature corrected nitrate removal rates combined across all 3 phases for both reactors.

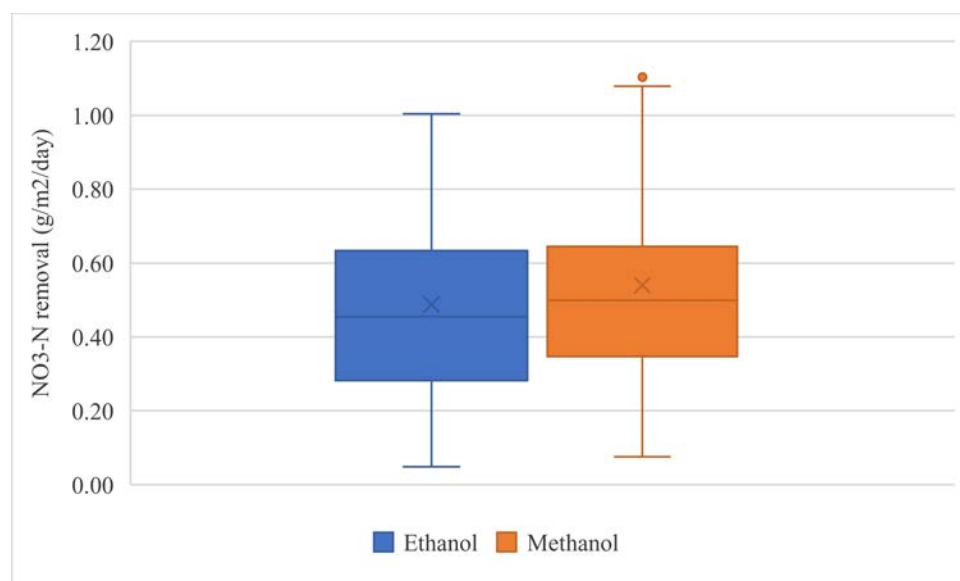


Figure 10: Average $\text{NO}_3\text{-N}$ removal rates in ethanol and methanol fed reactors for the entirety study.

In Figure 11 is the results of daily grab samples examined for NO_2 concentrations in both reactors.

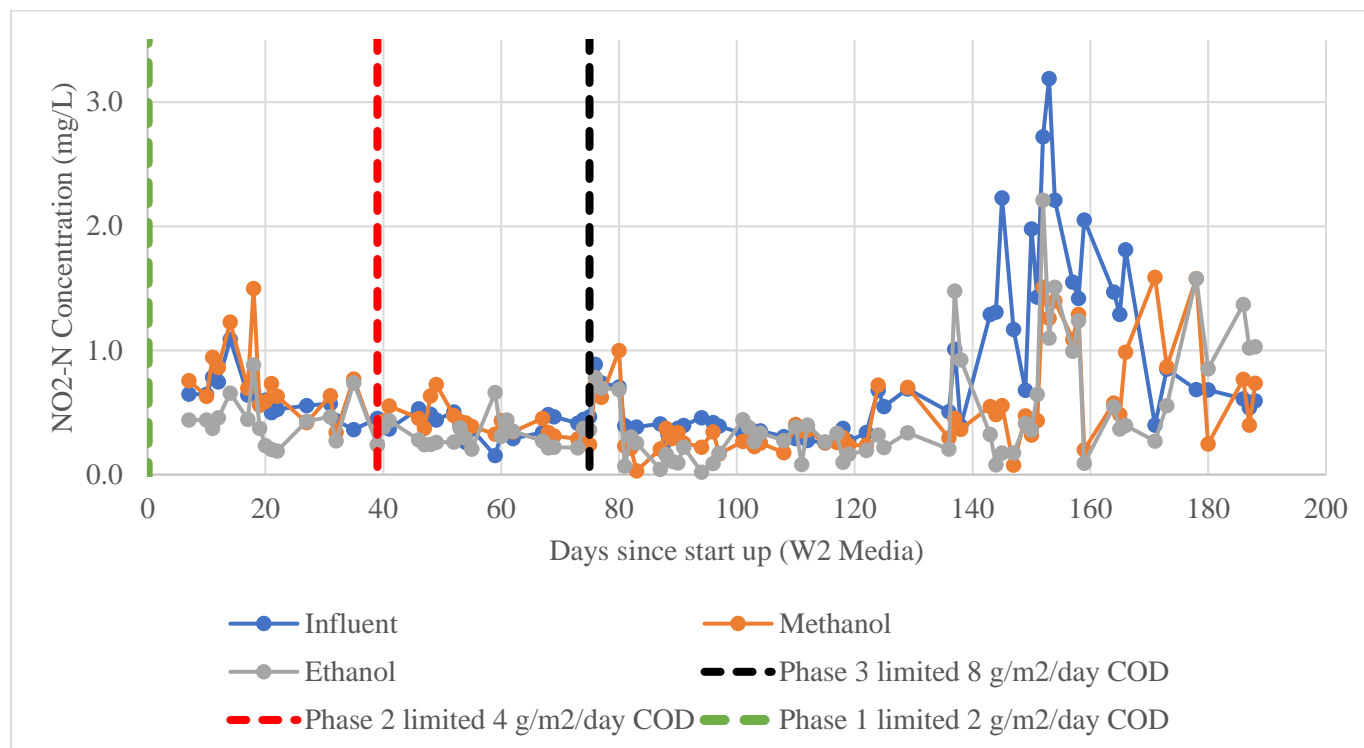


Figure 11: NO_2 grab sample results from methanol and ethanol fed reactors.

4.1.2 NO_2 trends during Ethanol and Methanol comparison

The methanol-fed reactor exhibited a higher accumulation of Nitrite $\text{NO}_2\text{-N}$ compared to the ethanol-fed reactor. Figure 11 illustrates higher NO_2 concentrations in the methanol fed reactor as compared to the ethanol fed reactor. Because these are PdNA reactors the nitrite within them fluctuates, as partial denitrification occurs NO_2 is produced. However, since there is

anammox within the system NO_2 was also up taken. Therefore, simply looking at NO_2 concentrations alone won't tell the full story of how well the reactors are partially denitrifying.

In Figure 12 are the results of daily grab samples examined for NH_4 concentrations in both reactors.

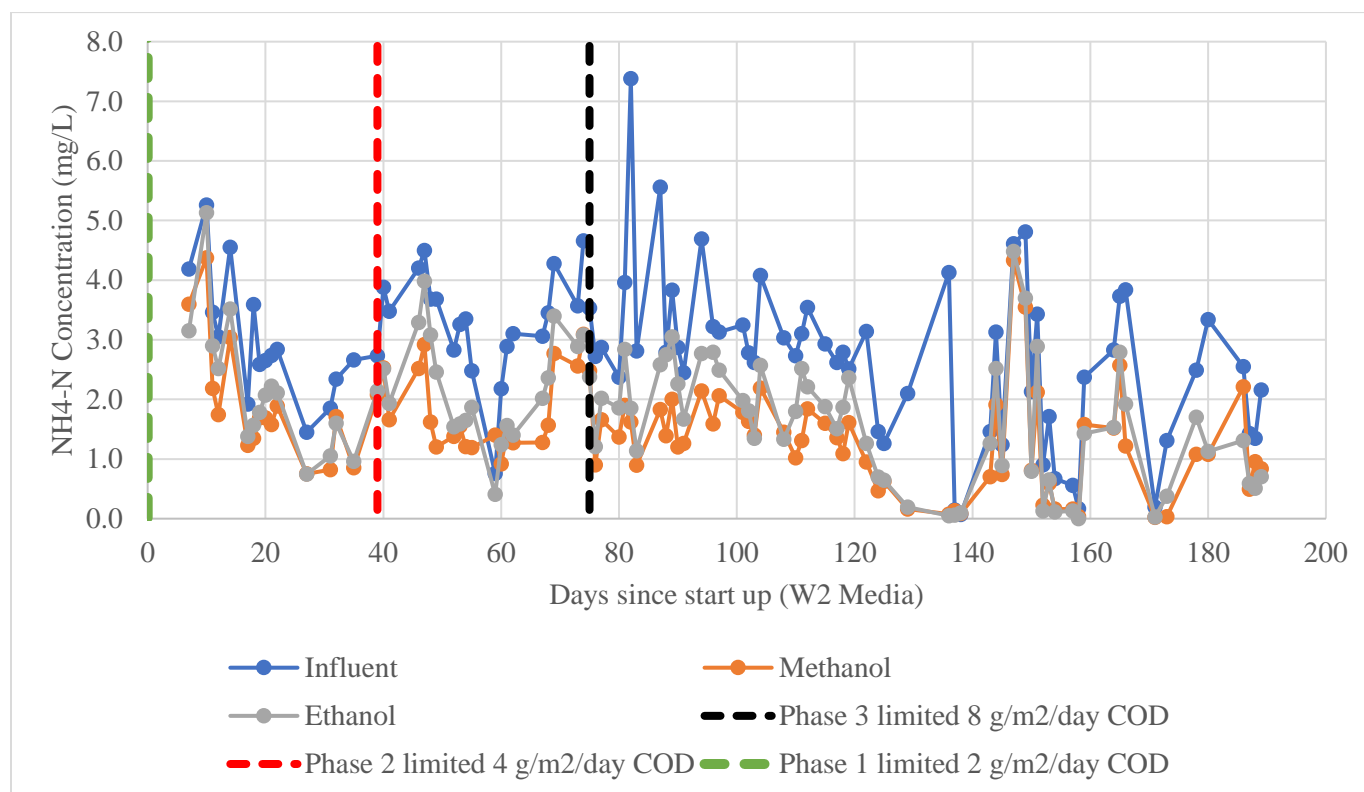


Figure 12: NH_4 grab sample results from methanol and ethanol fed reactors.

Figure 12 above presents the results of grab samples collected throughout the experiment. It can be observed that concentrations leaving the methanol-fed reactor were typically lower than those observed in the ethanol-fed reactor. In Table 3 ammonia removal rates from all phases of the study.

Table 3: Ammonia removal rates from all phases of the Methanol and Ethanol comparison.

	NH₄ removal rate in Ethanol fed reactor (g/m²/day)	NH₄ removal rate in Methanol fed reactor (g/m²/day)
Phase 1	0.09 ± 0.01	0.16 ± 0.02
Phase 2	0.12 ± 0.01	0.17 ± 0.01
Phase 3	0.17 ± 0.02	0.20 ± 0.03

4.1.3NH₄ trends during Ethanol and Methanol comparison

On average the in-situ NH₄-N temperature corrected removal rates observed from daily grab sample was 0.09 g/m²/day for the ethanol fed reactor and 0.16 g/m²/day in the methanol fed reactor when the carbon feed was limited to 2 g/m²/day. The methanol fed reactor continued to outperform the ethanol fed reactor over the course of phases 2 and 3. At lower COD dosing, the methanol reactor was able to achieve higher removal rates signifying more efficient external carbon use.

Upon comparison of NH₄ removal rates and nitrite trends during phases 2 and 3 of the study, it became apparent that there was consistently more NH₄-N removal in the methanol fed reactor. Regardless of COD feed, the methanol fed reactor achieved higher ammonia removal, suggesting a less efficient partial denitrification in the ethanol reactor. To ensure accuracy, these rates have been temperature-corrected to 20°C, accounting for the varying temperatures experienced throughout the study. Below in Figure 13 are the temperature corrected ammonia removal rates averaged across all 3 phases for both reactors.

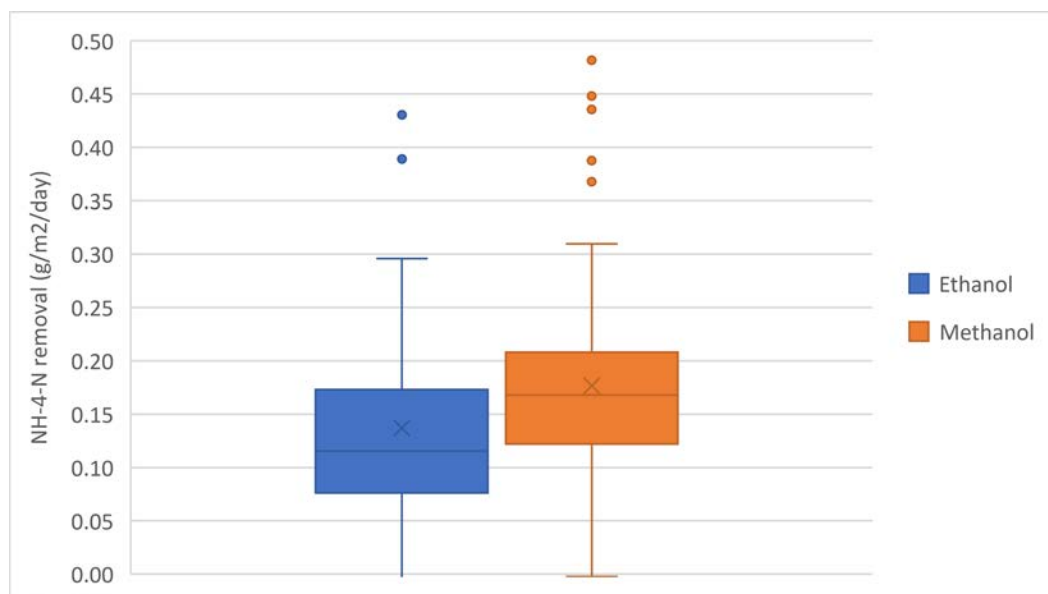


Figure 13: Average $\text{NH}_4\text{-N}$ removal rates in ethanol and methanol fed reactors for entirety of the study.

4.1.4 PdN efficiency for ethanol and methanol fed reactors for each COD loading.

Figure 14 depicts changes in PdN efficiency observed after increasing the top scale on the carbon feed system.

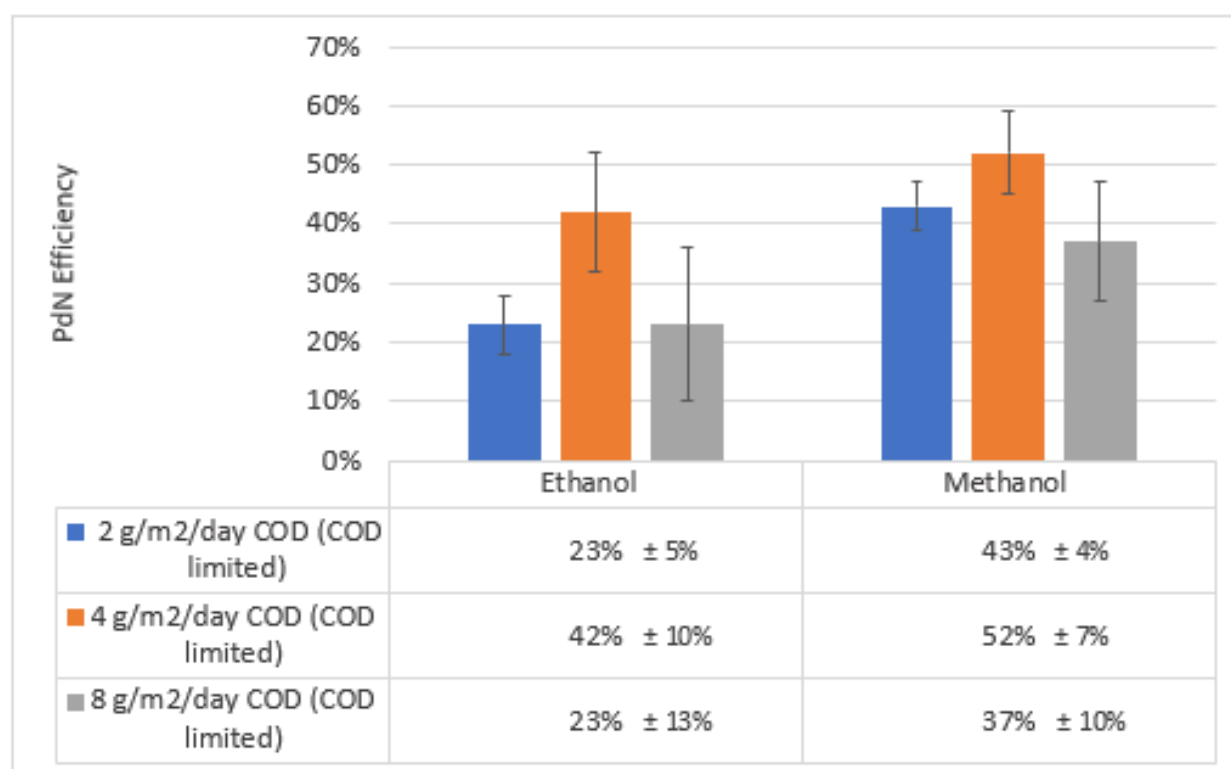


Figure 14: PdN efficiency at each COD loading

PdN efficiency trends during Ethanol and Methanol comparison.

At a COD feed rate of 2 g/m²/day, the methanol fed reactor exhibited nearly double the PdN efficiency compared to the ethanol-fed reactor. Operating under low external carbon dosing, the methanol fed reactor demonstrated over 40% PdN efficiency, a highly advantageous outcome

for potential implementation in a full-scale PdNA system. Both reactors experienced an increase in PdN% when the external carbon feed was raised from 2 to 4 g/m²/day of COD. Although both jumps in efficiency were notable, the ethanol fed reactor took a larger leap when looking at the difference in PdN% before and after the external carbon increase. This ultimately set the stage for the final phase of this experiment which was give each reactor the option to feed higher amounts of external carbon.

Following the promising results observed with ethanol toward the conclusion of phase 2, the question persisted: if the ethanol fed reactor were allowed to operate at even higher COD dosages, could it emerge as a more viable carbon choice for PdNA than methanol? By introducing higher available carbon addition, the aim was to observe another substantial increase in PdN efficiency in the ethanol reactor. As depicted in Table 1, the nitrate loading conditions in phase 3 closely mirrored those of phase 2, with an average TIN loading of 1.8 g/m²/day, approximately 1.1 g/m²/day of which comprised NO₃ loading. The PdN efficiency in both reactors decreased during phase 3. During each of the 3 phases, the methanol reactor demonstrated a greater tendency to avoid complete denitrification compared to the ethanol reactor. However, it seemed during phase 3 that both reactors faced challenges in achieving partial denitrification. Examining the trend in Figure 11, it becomes evident that prior to the introduction of the 8 g/m²/day carbon loading top scale, the methanol reactor consistently accumulated NO₂. Subsequently, when allowed 8 g/m²/day COD, these reactors tended towards full denitrification more frequently than when carbon was restricted to lower dosages.

The ethanol fed reactor operated at an average PdN efficiency 23% and the methanol fed reactor operated at an average PdN efficiency of 37%. This decline in PdN efficiency may be attributed

to the increased availability of external carbon accessible to the reactors. The rise in external carbon from 4 g/m²/day to 8 g/m²/day likely promoted complete denitrification more often than partial denitrification. Consequently, the observed PdN efficiency decreased in both reactors. Despite fluctuations in PdN efficiency within both reactors, the methanol-fed reactor consistently operated at PdN efficiencies significantly higher than those of the ethanol-fed reactor throughout every phase.

C/TIN trends for ethanol and methanol fed reactors for each COD loading are shown below in Figure 15.

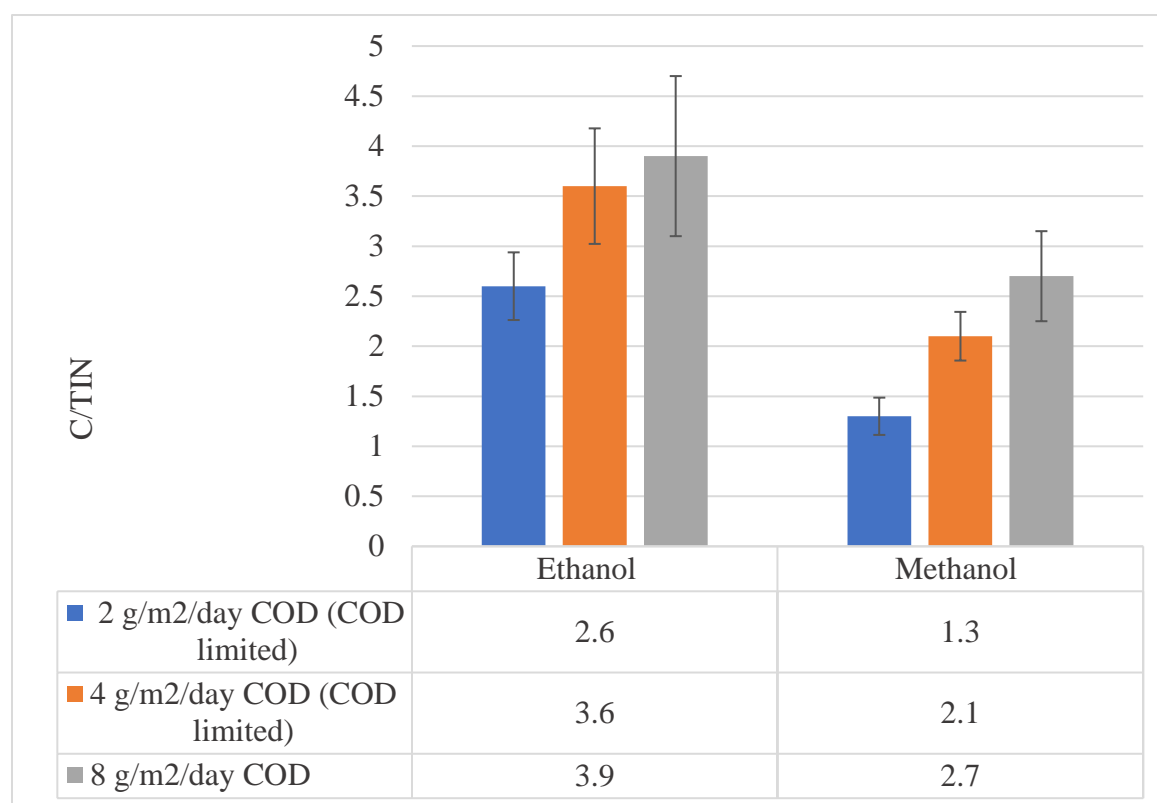


Figure 15: C/TIN averages for both methanol and ethanol reactors during each phase.

4.1.5 C/TIN efficiency trends during Ethanol and Methanol comparison

At the start of phase 1, the maximum allowable external carbon that could be fed to each reactor was 2 g/m²/day. During this COD loading period the ethanol reactor operated at a C/TIN ratio of 2.6, while the methanol reactor operated at a C/TIN of 1.3. After assessing removal rates and PdN% at this loading rate, it quickly became apparent that the more cost-effective carbon source when operating in this range is methanol. After running at this capacity for a roughly a month and assessing daily in-situ removal rates, the top scale was raised so that both reactors could receive up to 4 g/m²/day of external carbon which marked the beginning of phase 2. Following this adjustment, there was a noticeable shift in both reactors' performance concerning the amount of carbon dosed in relation to the total inorganic nitrogen removed. The ethanol reactor, on average, operated at a C/TIN ratio of 3.6, while the methanol-fed reactor operated at a C/TIN ratio of 2.1. It could be observed that with more available external carbon available, there was less efficient use of this carbon between both reactors. This trend continued with each increase in the COD top scale. Phase 3 held the highest C/TIN ratios for both reactors. However, the methanol fed reactor, under all 3 loading conditions, exhibited lower operating C/TIN values on average while also removing larger amounts of nitrogen. This proved methanol to be more cost effective and operationally viable.

4.2 Biofilm resulting from each loading phase.

4.2.1 Biofilms in ethanol fed reactor during each phase.

Biofilms on the plastic carriers in the media became noticeably thicker when comparing phase 3 to phase 2 of the study. Specifically, the ethanol-fed reactor received an average of 1.1 g/m²/day of NO₃ loading and 3.3 g/m²/day of COD in the form of ethanol at the end of phase 2.

During phase 3 where the top scale was raised to allow 8 g/m²/day COD to feed which seems to have contributed to higher biomass on the carriers. Below in Figure 16, on the left, is an image showing the biofilm associated with the ethanol-fed reactor during phase 2. On the right is the biofilm associated with the ethanol-fed reactor during the period where the top scale was set to 8 g/m²/day of COD. The biofilm in phase 3 appears slightly thicker than that of phase 2.

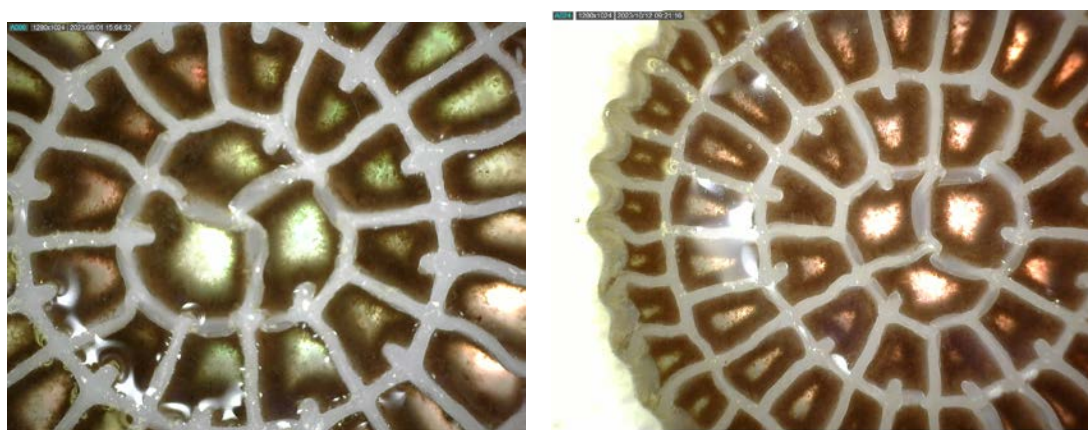


Figure 16: Media in ethanol fed reactor.

4.2.2 Biofilms in methanol fed reactor during each phase.

The methanol fed reactor showed a similar trend to that of the ethanol fed reactor. Biofilm in both reactors became noticeably thicker by the end of phase 3. The methanol fed reactor received an average of 1.1 g/m²/day of NO₃ loading and 2.1 g/m²/day of COD as methanol at the end of phase 2. Like the ethanol fed reactor, the methanol fed reactor received almost identical NO₃ loading during phase 3 as it saw in phase 2. However, during phase 3 the methanol fed reactor dosed an average of 2.8 g/m²/day of COD as methanol during this phase.

The biofilm observed during phases 2 was relatively thinner than that of phase 3. In Figure 17 on the left is a biofilm image from phase 2 and on the right is an image from phase 3.

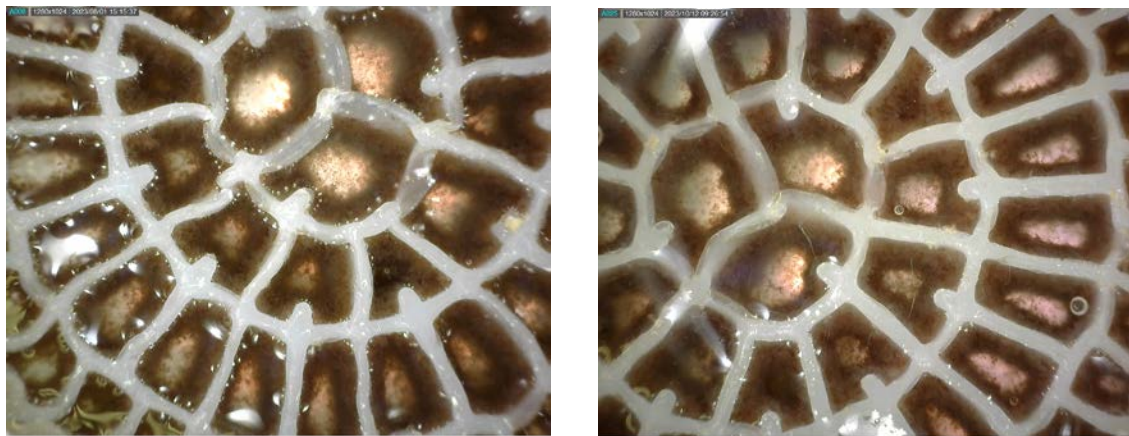


Figure 17: Media in methanol fed reactor.

4.3 Steady anammox activity on World Water Works W2 media

Throughout the methanol and ethanol pilot study, numerous tests were conducted to assess the anammox activity. The consistency of anammox activity across both reactors facilitated a more precise comparison of removal rates between them. This stable anammox activity enabled a more effective utilization of daily data to analyze the impact of the external carbon sources, methanol, and ethanol, and evaluate their respective effectiveness. Below is a time series depicting the maximum anammox activity measured in both reactors over the course of the study.

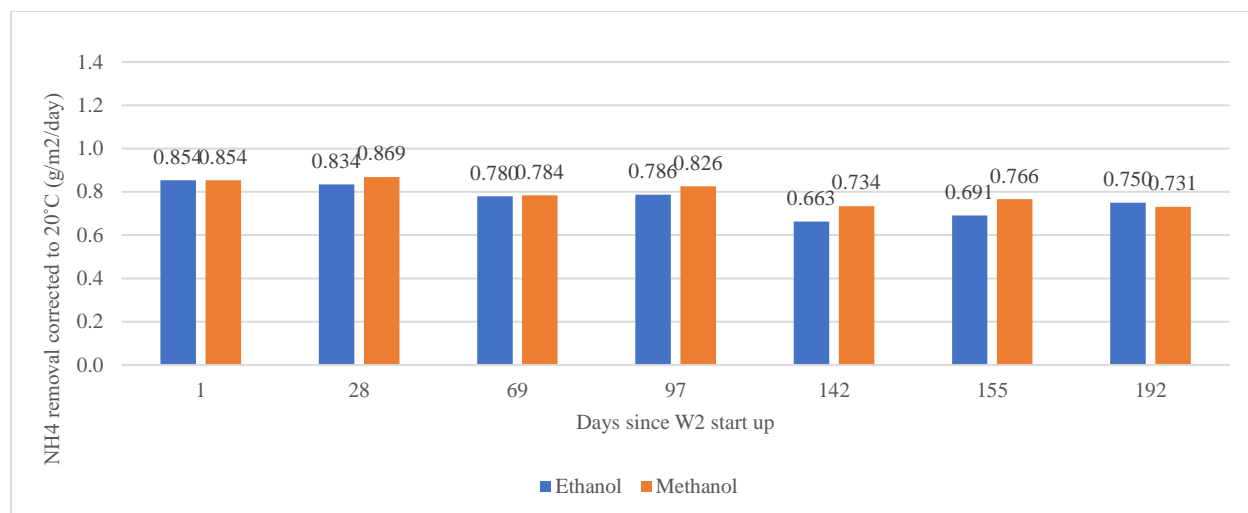


Figure 18: Anammox activity test for duration of methanol vs ethanol pilot study

4.4 Results of Maximum Specific Denitrification Rate Testing

Following the collection and analysis of results of the comparison of ethanol and methanol, maximum specific denitrification test was run to see how these rates compared to what was observed from these reactors in-situ. Maximum SdNR test were not only ran on ethanol and methanol, but glycerol as well since this was a carbon choice that was previously piloted and implemented full scale. In Table 4 below are temperature corrected denitrification rates observed from each of the 3 carbon sources.

Table 4: Temperature corrected denitrification rates observed from each of the 3 carbon sources.

External Carbon	Specific denitrification rate	PdN %
Ethanol	4.8 mg/L/hour	-15%
Methanol	3.1 mg/L/hour	5%
Glycerol	6 mg/L/hour	31%

Running a specific denitrification rate test using ethanol yielded a NO_3 removal rate of 4.8 mg/L/hour. No nitrite accumulated during this test but instead was removed. As a result, the PdN efficiency observed was -15%. Ethanol was more inclined to do full denitrification rather than partial, which is a trend that was observed during the comparison of ethanol and methanol fed reactors. During this test NO_2 was unable to accumulate at a faster rate than it was being turned to N_2 . As a result, the ethanol SdNR test yielded the lowest PdN efficiency of the 3 carbon sources tested. This lines up with ethanol fed PdNA IFAS reactors inability to partially denitrify at an efficiency higher than that of the methanol fed reactor.

Running an SDNR test using 300 mg/L of COD as methanol the observed NO_3 removal rate was 3.12 mg/L/hr. The PdN efficiency observed in this test was 5%. Nitrite accumulated over the time span of the test. This was indicative that a portion of the nitrate being removed was converting nitrite and remaining without fully denitrifying into dinitrogen gas N_2 .

When the specific max specific denitrification rate test was ran using 300 mg/L COD as glycerol, the NO_3 removal rate observed was 6 mg/L/hour. This rate was faster than the rates seen in both ethanol and methanol maximum SDNR test. More nitrite accumulated in this test then both other carbon sources tested which was exactly what was expected based on previous pilot work comparing methanal and glycerol. The PdN percent seen in this test was 31% which was well above the 5% observed in the methanol test and the -15% observed in the Ethanol SDNR test.

CHAPTER 5. EFFECTS OF NO₃-N LOADING ON AN IFAS PdNA SYSTEM

5.1 Introduction of Nitrate loading experiment

After an extensive 6-month evaluation comparing ethanol and methanol as potential external carbon sources for the PdNA, it was conclusively determined that methanol stood as the more suitable choice. However, despite its viability, the threshold at which the methanol fed PdNA (IFAS) system might reach its operational limits remained unknown.

After careful review of the daily In-situ removal rates of both reactors, it was evident that loading conditions also influenced partial denitrification and ultimately NH₄-N removal rates. Thus, the pilot study transitioned from a carbon source comparison to a deliberate investigation into the upper bounds of nitrate loading capacity.

During the comparison of ethanol and methanol each reactor received different carbon source. For the next part of the study both reactors received methanol as the external carbon source while maintaining identical Hydraulic Retention Times (HRT) and NO₃-N effluent targets. Additionally, the media within both reactors was again exchanged for the plastic media directly from the JRTP full-scale second anoxic zone, ensuring a uniform anammox activity starting point. For this portion of the study, one reactor operated as a baseline while the other was fed supplemental NO₃ to see how this would ultimately effect PdN and ammonia removal. Samples were pulled daily from both reactors and analyzed for nitrogen species. In Figure 19 and Figure 20 below are results of grab samples measuring NO₃ concentrations from both reactors.

5.2 NO₃ trends in the nitrate supplemented IFAS #1 reactor and the baseline IFAS #2 reactor.

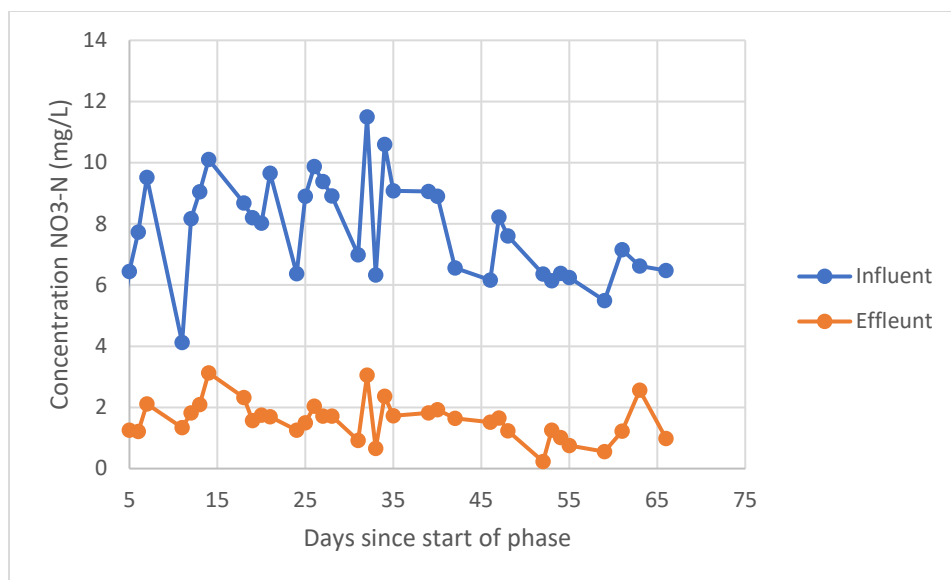


Figure 19: NO₃ grab sample results from the NO₃-N loaded reactor.

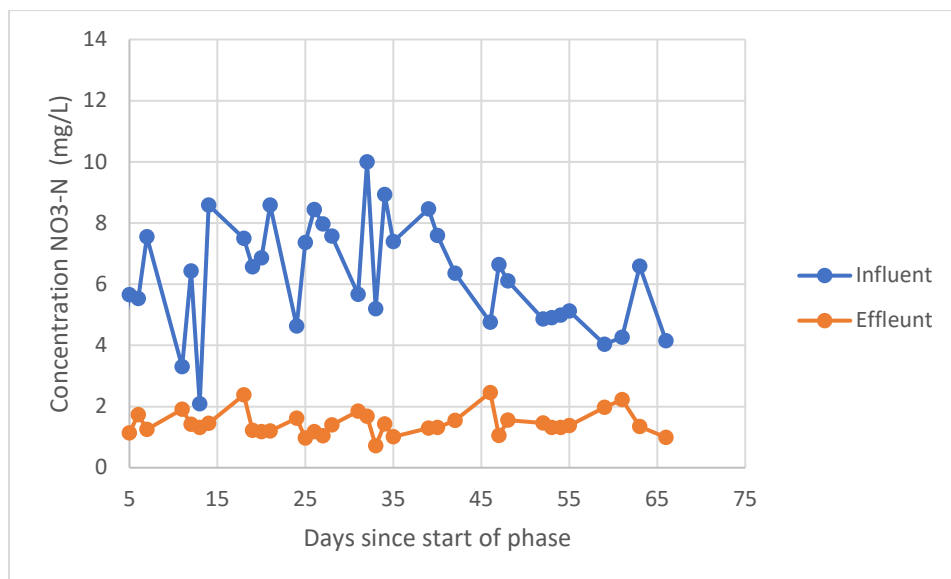


Figure 20: NO₃ grab sample results from the baseline reactor.

IFAS #1 demonstrated higher in-situ NO₃-N removal rates than the baseline reactor, IFAS #2. This observation was expected, given its higher NO₃-N input compared to the baseline reactor. The substantial biofilm development on the plastic carriers within the nitrate-loaded reactor consisted predominantly of denitrifying bacteria. The greater population of these

denitrifying organisms attached to the plastic carriers logically corresponds to the observed trend of increased $\text{NO}_3\text{-N}$ removal rates. The 24 below shows a comparison of the in-situ $\text{NO}_3\text{-N}$ removal rates observed from each reactor during this comparison.

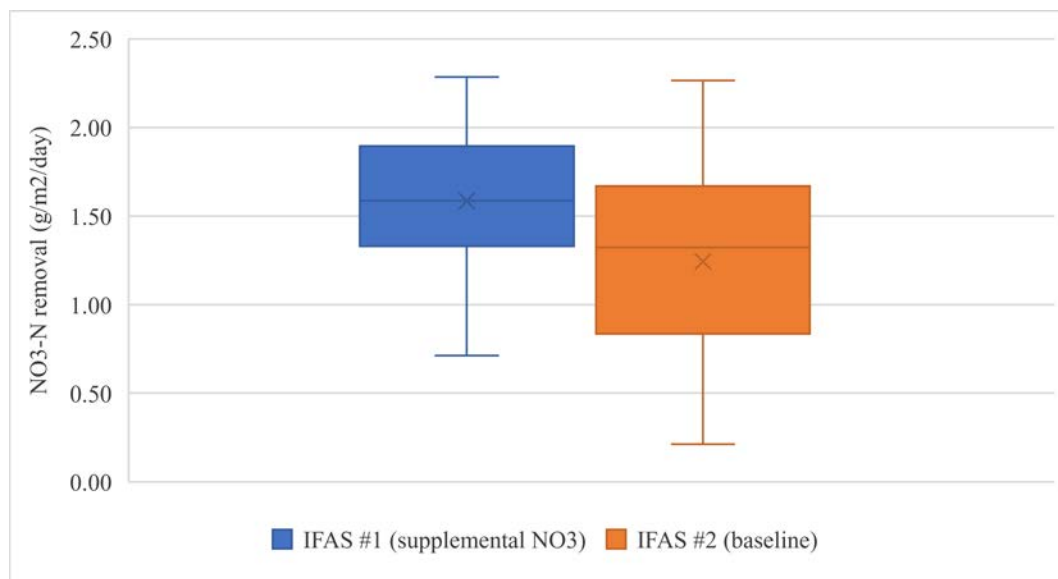


Figure 21: $\text{NO}_3\text{-N}$ removal rates in the nitrate loaded reactor and the baseline reactor, temperature corrected to 20°C .

5.3 NO₂ trends in the nitrate loaded IFAS #1 reactor and the baseline IFAS #2 reactor.

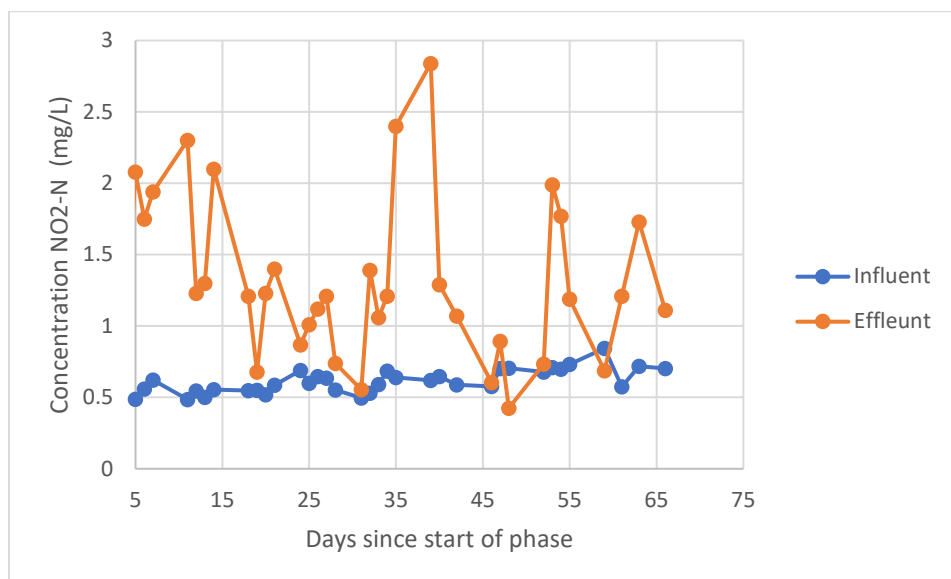


Figure 22: NO₂ grab sample results from the NO₃-N loaded reactor.

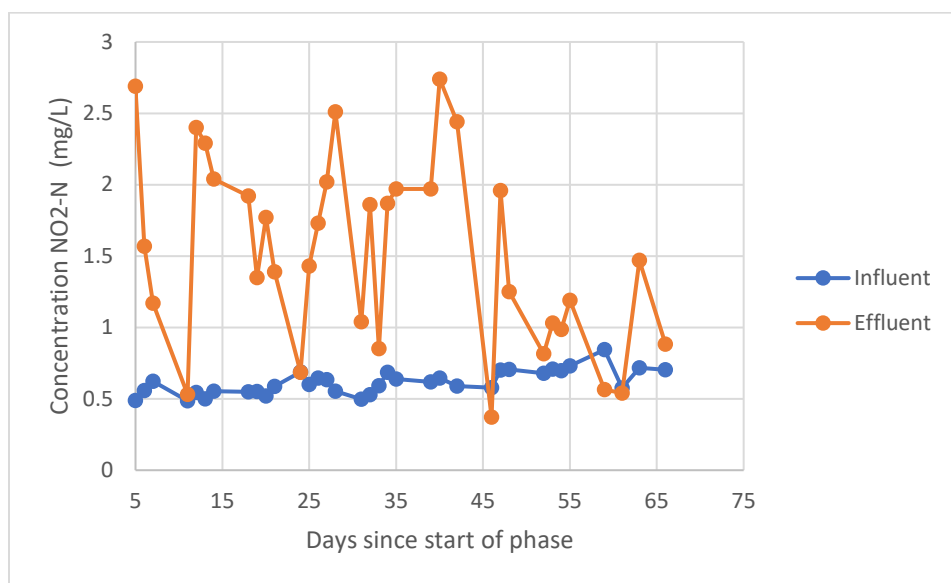


Figure 23: NO₂ grab sample results from the baseline reactor.

When looking at Figure 22 and Figure 23, it can be seen higher NO_2 concentrations were observed in the baseline reactor on average than in the nitrate loaded reactor. Average NO_2 concentrations were 1.2 mg/L in the nitrate loaded reactor and 1.5 mg/L in the baseline reactor. Because both reactors were receiving the same external carbon this observation points better PdN within the baseline reactor. The presence of more NO_3 requires more external to be fed, this increase in external carbon pushed the reactor to do full denitrification more often than partial.

5.3 NH_4 trends in the nitrate loaded IFAS #1 reactor and the baseline IFAS #2 reactor.

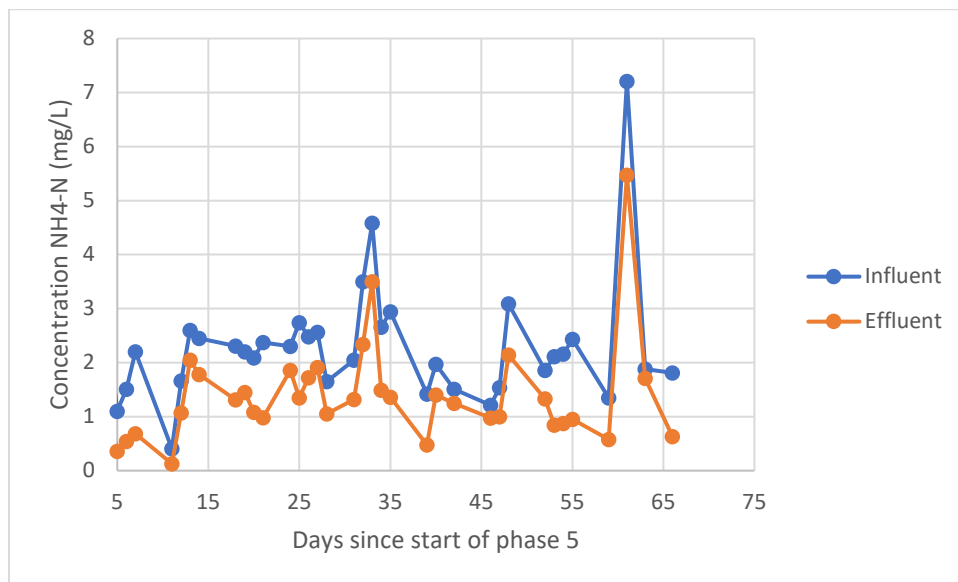


Figure 24: NH_4 grab sample results from the $\text{NO}_3\text{-N}$ loaded reactor.

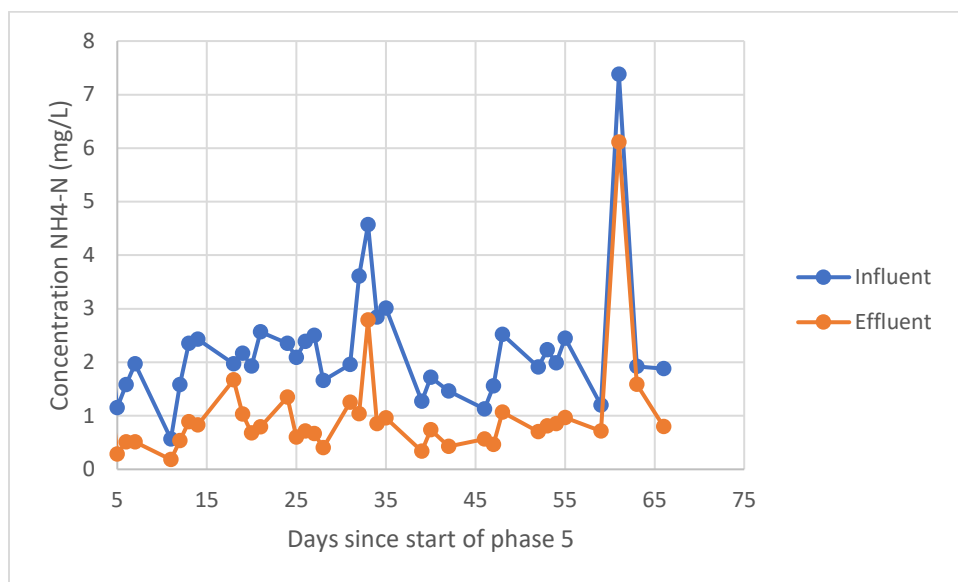


Figure 25: NH_4 grab sample results from the baseline reactor.

In this phase of the study, greater $\text{NH}_4\text{-N}$ removal was observed in the baseline reactor compared to the reactor receiving supplemental $\text{NO}_3\text{-N}$. Despite the baseline reactor having a

thinner biofilm and consequently a smaller microbial population attached to the plastic media carriers, it exhibited higher in-situ ammonia removal. This is mainly attributed to the lower PdN occurring within the nitrate supplemented reactor. Referring back to Figure 22 and Figure 23, it can be seen that NO_2 concentrations in the nitrate supplemented reactor were lower than that of the baseline reactor. Lower PdN resulted in less available substrate and ultimately lower in-situ NH_4 removal rates. Mass transfer could also play a role as the loaded reactor would certainly gain more biomass as the phase over the course of this comparison, which would ultimately hinder anammox from removing larger quantities of ammonia.

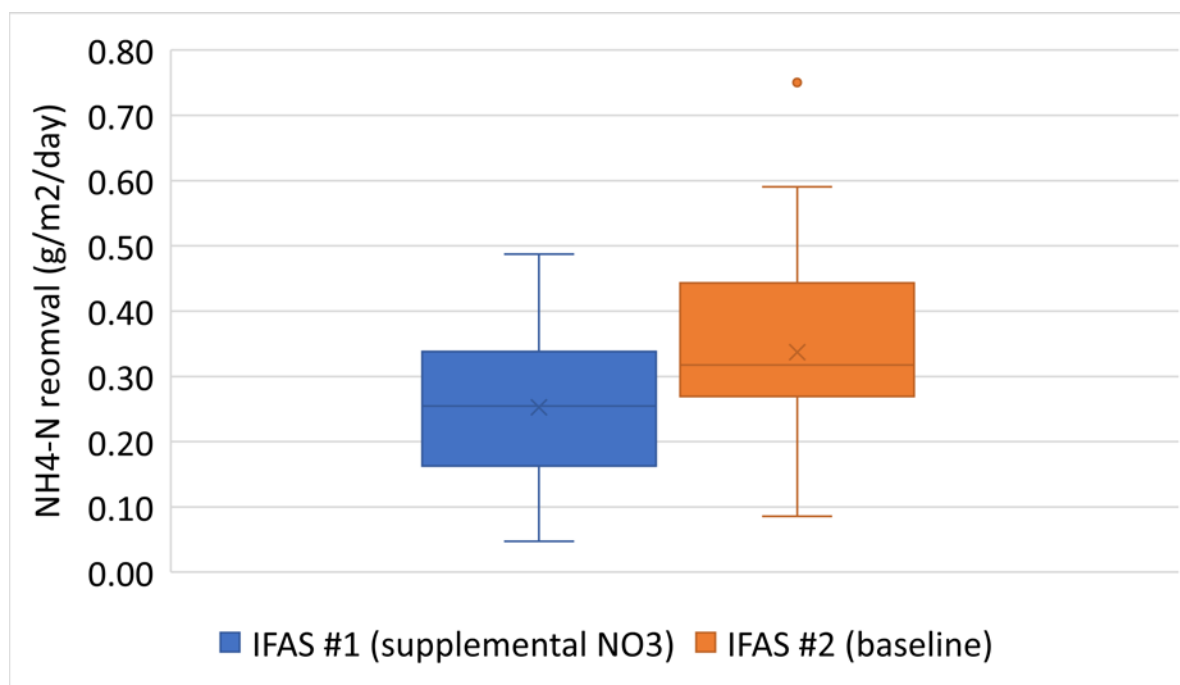


Figure 26: $\text{NH}_4\text{-N}$ removal rates in the nitrate loaded reactor and the baseline reactor, temperature corrected to 20°C.

5.4 Biofilm from nitrate loaded and baseline reactors.

The biofilm present on the plastic carriers within the nitrate-loaded reactor IFAS #1 exhibited more thickness compared to that observed on the plastic carriers in the baseline reactor IFAS #2. While not reaching identical thickness, the attributes of this biofilm bear resemblance to those observed in the methanol fed reactor during phase #1 of this study. The nitrate-loaded reactor started to demonstrate diminished $\text{NH}_4\text{-N}$ removal efficiency with the growth of the biofilm. A visual representation of the plastic carriers and their associated biofilm in IFAS #1 is provided in Figure 27 below.

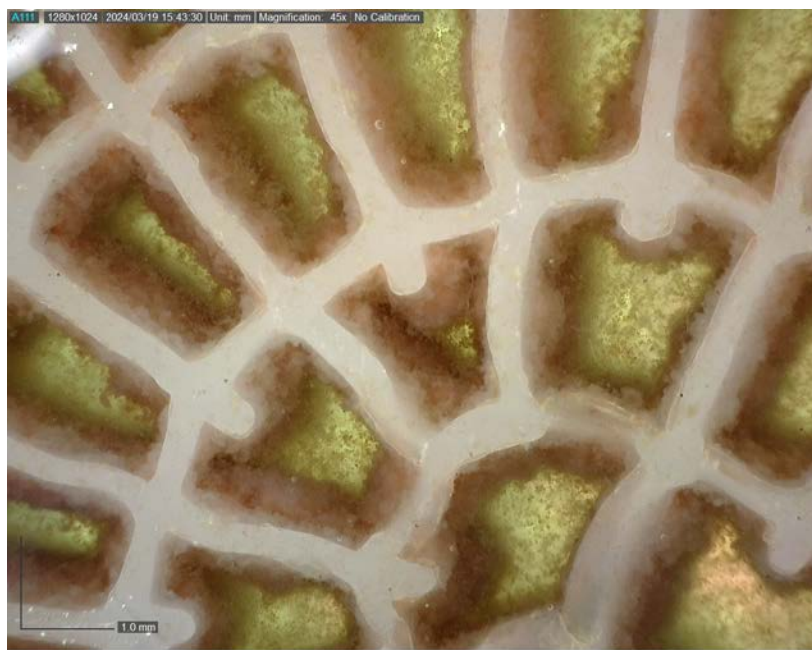


Figure 27: Media in the IFAS #1 nitrate loaded reactor.

The biofilm observed on the media in the IFAS #2 baseline reactor appeared thinner compared to the biofilm in the $\text{NO}_3\text{-N}$ loaded reactor, IFAS #1. This thinner biofilm likely facilitated improved mass transfer across the surface area of the plastic carrier. Consequently, this plays a significant role in the greater in-situ $\text{NH}_4\text{-N}$ removal observed in the baseline

reactor, in contrast to the reactor receiving supplemental $\text{NO}_3\text{-N}$. The biofilm associated with IFAS #2 is depicted in Figure 28 below.

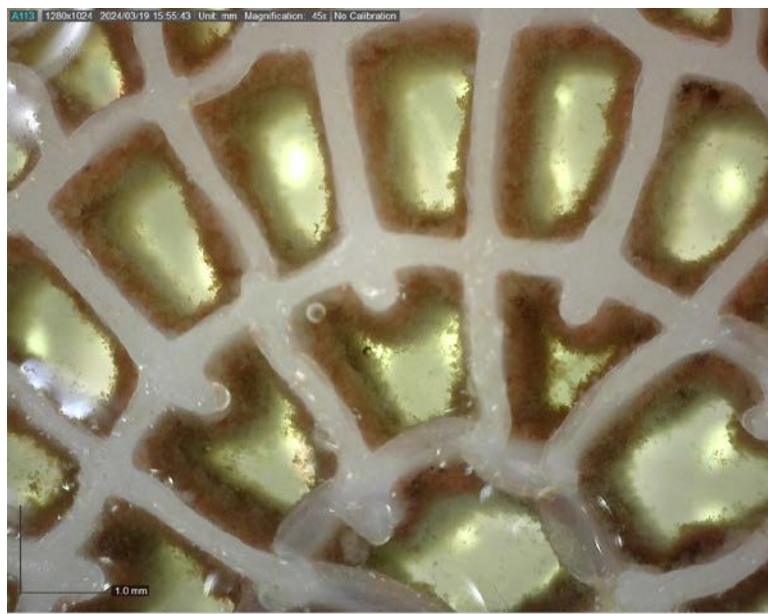


Figure 28: Media in the IFAS #2 baseline reactor.

5.5 PdN Efficiency during comparison of the nitrate loaded reactor and the baseline reactor.

The Partial Denitrification (PdN) efficiency serves as a metric depicting the effectiveness of a system in achieving partial denitrification. In Partial Denitrification with Anammox (PdNA) systems, the ability to generate and maintain $\text{NO}_2\text{-N}$ is crucial for the subsequent uptake of $\text{NH}_4\text{-N}$ by anammox bacteria. Throughout Phase 5, the IFAS #1 reactor, which was loaded with $\text{NO}_3\text{-N}$, exhibited lower PdN efficiency compared to the IFAS #2, the baseline reactor. Particularly on days when the influent $\text{NO}_2\text{-N}$ load exceeded $2 \text{ g/m}^2\text{/day}$, the reactor displayed a reduced PdN percentage. Over the course of Phase 5, IFAS #1 demonstrated a PdN efficiency of $32\% \pm 17$,

whereas IFAS #2 exhibited a PdN efficiency of $46\% \pm 12$. This discrepancy was noteworthy, especially considering that both reactors received the exact same external carbon source (methanol) to achieve treatment objectives. Below are the PdN efficiencies recorded from both reactors during phase 5.

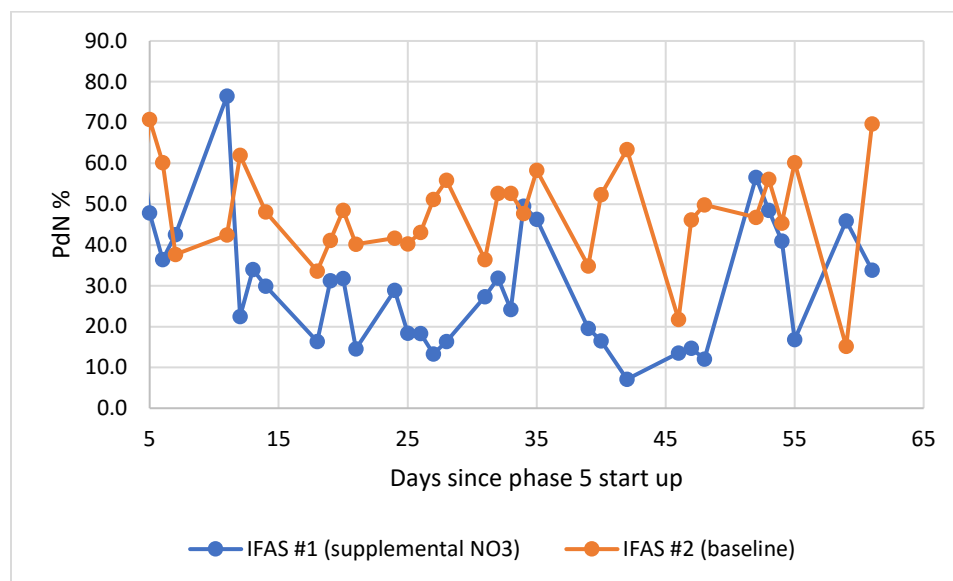


Figure 29: PdN efficiency trend of IFAS #1 nitrate loaded reactor and IFAS #2 baseline reactor.

5.6 C/N ratio during comparison of the nitrate loaded reactor and the baseline reactor.

During the initial two weeks of Phase 5, both reactors maintained a relatively consistent C/N ratio. C/N, referring to the ratio of external carbon dosed (in this case, methanol) to the amount of nitrate removed, remained stable during this period. However, a notable shift in this pattern emerged thereafter. The IFAS #1 reactor, which received supplemental nitrate, required increased methanol dosages to achieve the same level of nitrate removal as IFAS 2. On average, the nitrate-loaded reactor operated at a C/N ratio of 4.7 ± 2.3 , while the IFAS #2 baseline reactor operated at a C/N ratio of 2.6 ± 1.6 . This was a trend that was seen previously in the study, where

more external carbon is available the higher the C/N the reactor will operate at. According to literature and prior studies, the theoretical C/N ratio for methanol is established at 4.8. This signifies that operating at or above this value ensures complete denitrification. However, the objective of a Partial Denitrification with Anammox (PdNA) reactor is not full denitrification, but rather achieving partial denitrification. Therefore, operating below this theoretical C/N ratio is crucial to facilitate nitrite accumulation within the reactor. Below is the C/N of both reactors.

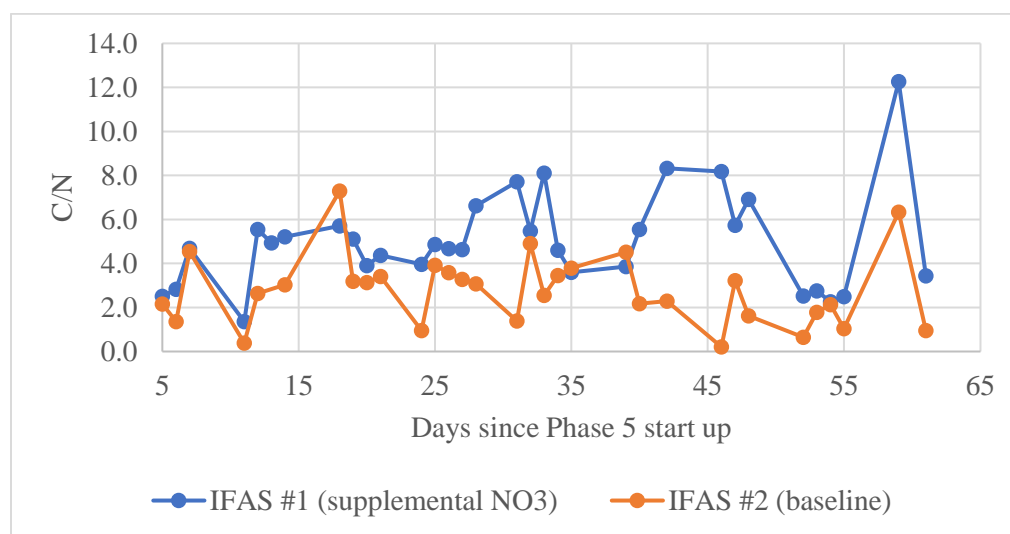


Figure 30: C/N trend of IFAS #1 nitrate loaded reactor and IFAS #2 baseline reactor.

CHAPTER 6. CONCLUSION

The comparison of methanol and ethanol yielded surprising results. The ethanol fed PdNA reactor showed lower removal rates and PdN during high and moderate COD loading conditions. The methanol reactor outperforms the ethanol fed reactor in terms of TIN removal, PdN efficiency, and operated at a lower C/TIN which directly correlates to cost savings on external carbon. The higher PdN efficiency observed in the methanol-fed reactor provided an increased availability of substrate, $\text{NO}_2\text{-N}$, for uptake by the anammox bacteria, thereby facilitating enhanced $\text{NH}_4\text{-N}$ removal. This positive correlation between PdN efficiency and $\text{NH}_4\text{-N}$ removal was evident across all the phases of the methanol and ethanol pilot study.

NO_3 loading also plays a crucial role in reactor performance, particularly when considering in-situ removal rates. As nitrate load increases, so too does the COD being dosed to remove it, which can cause biofilms to thicken with denitrifying bacteria. While $\text{NO}_3\text{-N}$ removal is beneficial, it's not worth sacrificing in-situ ammonia removal in a Partial Denitrification and Ammonia (PdNA) system. Managing biofilm characteristics should align with treatment goals. If left unchecked, an overgrowth of the microbial population on the media within the system could have adverse effects, ultimately leading to a degradation in effluent quality due to poor removal of ammonia or nitrite.

In terms of performance comparison, the IFAS #1 reactor, loaded with supplemental NO_3 , demonstrated notably higher ($\text{NO}_3\text{-N}$) removal rates when compared to the baseline IFAS #2. Although nitrate removal was superior to that of the baseline reactor, the $\text{NO}_3\text{-N}$ loaded

reactor also completed full denitrification more often than the baseline reactor. Nitrate removal is crucial in wastewater treatment processes, yet systems utilizing Partial Denitrification and Ammonia (PdNA) rely on efficient partial denitrification to provide substrate for anammox bacteria. Therefore, if full denitrification occurs much more often than partial denitrification, in-situ ammonia removal rates may suffer as the anammox bacteria lack sufficient substrate for optimal removal. Conversely, IFAS #2, operating at a lower loading level, exhibited superior removal of ammonia $\text{NH}_4\text{-N}$ in contrast to IFAS #1. Additionally, the baseline reactor displayed better partial denitrification (PdN) efficiency which was evident when looking at $\text{NH}_4\text{-N}$ removal rates between the 2 reactors. The baseline reactor, IFAS #2 also operated at a low C/N ratio, indicative of its more efficient utilization of carbon in the removal of Nitrate ($\text{NO}_3\text{-N}$) when compared to the reactor that was receiving higher loading. It was observed that nitrate loading can ultimately cause PdNA systems to do full denitrification turning NO_3 into dinitrogen gas rather than stopping at NO_2 to provide substrate for anammox bacteria. For the configuration utilized in this study it seemed as though $2 \text{ g/m}^2/\text{day}$ of NO_3 loading was the capacity of the system before defaulting back to complete denitrification. This study directly correlates to design parameters concerning full scale PdNA implementation not only at James River Treatment plant, but any facility looking to incorporate a PdNA system.

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