Start-up Analysis of a Partial Nitrification Aerobic Granular Sludge System Treating High-Strength Municipal Digestate Supernatant

By

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Abstract

Ammonia pollution is a growing environmental concern affecting wastewater treatment. With rising concerns about energy consumption and increasingly strict discharge regulations, mitigation of energy expenditure and maintenance of high pollutant removal efficiency have fueled advances in biological wastewater treatment. Partial nitrification AGS-SBR technology has shown potential in treatment efficiency and versatility especially under high pollutant loading. However, neither microbial development nor large-scale operation of such systems has yet been tested. This study therefore focused on identification of changes in microbial community over the course of AGS reactor start-up on a laboratory scale under conditions of increased ammonia loading, and the subsequent operation of pilot scale partial nitrification AGS reactors. The study found that ammonia removal remained above 90% for most of laboratory scale operation with efficiency falling at increased ammonia concentrations. Despite the successful inhibition of NOB, AOB enrichment was low despite high ammonia concentration. The second half of this study focused on the operation of pilot scale AGS partial nitrification SBRs. The pilot system was tested with two different start-up strategies on high ammonia centrate and delivered above 80% removal efficiency. It was found that start-up from activated sludge and partial denitrification AGS was more successful and stable than start-up via UASB granular sludge combined with dehydrated AGS granules. HRT was also optimized during this time suggesting an 8hr cycle was sufficient for the maintenance of 80% ammonia removal efficiency. Denitritation was not successfully implemented in the reactors due to recirculation problems but was overall successful in the mitigation of effluent ammonia levels when treating municipal centrate at the pilot scale.

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List of abbreviations

AGS – aerobic granular sludge

ANAMMOX – anaerobic ammonia oxidation

AOB – ammonia oxidizing bacteria

AS – activated sludge

BNR – biological nitrogen removal

COD – chemical oxygen demand

CSTR – continuous stirred tank reactor

DO – dissolved oxygen

EPS -extracellular polymeric substances

FA – free ammonia

GAO – glycogen accumulating organisms

HRT – hydraulic retention time

IFAS – integrated fixed biofilm

MBR – membrane bioreactor

MBBR – moving bed biofilm reactor

MLSS – mixed liquor suspended solids

N - nitrogen

NH_3 - ammonia

NH_4-N – ammonia nitrogen

NO_2^- - nitrite

NO_2-N – nitrite nitrogen

NO_3^- - nitrate

NO_3-N – nitrate nitrogen

NOB – nitrite oxidizing bacteria

PD/A – partial denitrification and ANAMMOX

PN/A – partial nitrification and ANAMMOX

PO_4 - phosphate

PAO – phosphate accumulating organisms

SBR – sequencing batch reactor

SRT – solids retention time

SVI – sludge volume index

SVI_5 – sludge volume index at 5 mins.

SVI_{30} – sludge volume index at 30 mins.

TIN – total inorganic nitrogen

UASB reactor – up-flow anaerobic sludge blanket reactor

VER – volumetric exchange ratio

VSS – volatile suspended solids

WWTP – wastewater treatment plant

1 Introduction

1.1 Background

Ammonia is a common wastewater pollutant across both industrial and municipal wastewater streams. While not formerly well regulated in North America, effluent levels of ammonia have been the subject of increasingly strict regulations. Canada passed legislation banning effluent levels of unionized ammonia above 1.25 mg N/L for all municipal wastewater treatment facilities in 2013 (Environment and Climate Change Canada, 2017). In addition, high ammonia concentrations have been tied to toxicity in fish and other forms of wildlife both independently and as a result of eutrophication (Peng et al., 2004; Li-Long et al., 2013). As such, the removal of ammonia from wastewater has been widely studied, especially in the realm of biological removal. This is due to the relative ease with which microbial degradation may occur. While most innovations have been focused on lower strength wastewater, the main contributors to ammonia discharge come from higher strength wastewaters from industrial sources such as food processing, textile and leather industries, municipal treatment side-streams, and landfill leachate (EPA, 2023; Karri et al., 2018; Zou et al., 2022). Although municipal wastewater is relatively low in ammonia, specifically at the discharge point into the environment, other side-streams, such as biosolids digestate supernatant, contain much higher ammonia concentrations (Morgan & Hamza, 2022).

Aerobic granular sludge (AGS) systems have garnered attention in the field recently due to findings suggesting that this type of sludge has the potential to be used to treat high-strength wastewater both efficiently and at little risk to the biomass itself. Due to the solids retention times (SRT) of these systems, sludge wasting is also lowered, increasing the

attractiveness of AGS for wastewater treatment (Jungles, et al., 2013). Despite this, most AGS processes currently in use are not calibrated for the treatment of high-strength ammonia wastewater. The main reason is the issue of alkalinity loss that occurs as a result of biological ammonia oxidation. When ammonia concentration is high, nitrification becomes more active and large amounts of alkalinity are consumed (Shourjeh et al., 2020). This deficiency can cause dangerously low pH which negatively affects the microbial community, inhibiting nitrification and putting a limit on what is considered biologically treatable wastewater (Zeng, et al., 2008). The only way to combat this is to artificially introduce additional alkalinity into the system that would mitigate pH fluctuations during operation. Artificial addition of alkalinity garners additional costs in both technology and substrate, making this option less attractive to wastewater treatment plants (WWTPs) running on an already constrained budget.

The recovery of alkalinity in the system may be achieved through the second step of the ammonia oxidation pathway, either denitrification or denitritation, which produces alkalinity as a by-product (Zou, 2020; Hu et al., 2011). As such, introducing denitrification or denitritation into the system recovers the alkalinity lost during ammonia oxidation (Metcalf & Eddy, 2014). This has been previously proven to work during the treatment of wastewater with high ammonia concentrations (Zou et al., 2020). It was found that not only may denitritation be established in the presence of high ammonia loading, but that it also sufficiently increased alkalinity in the system while decreasing total inorganic nitrogen (TIN) concentrations in the resulting effluent (Zou et al., 2020). Utilizing partial denitrification instead of traditional nitrification also lowers oxygen and chemical oxygen demand (COD) demand by arresting nitrification in the ammonia oxidation pathway a step earlier than would normally occur (Shourjeh et al., 2020). However, the characterization of partial nitrification

has only been achieved at a laboratory scale level or at low ammonia concentrations which is not suitable for application in wastewaters with high ammonia loading.

There is limited evidence for use of partial nitrification of high-strength wastewater in literature and what has been published is mainly focused on the remediation of landfill leachate. Since high-strength discharge streams are also found in other types of industries, such as municipal side-stream effluent in the form of lagoon supernatant and centrate, it is necessary to determine the functionality of such systems under differing conditions. While microbial community studies do exist in the field of ammonia removal and AGS, analysis of the microbial community in relation to the development of these systems under high ammonia loading has not been done. Since microbial community structure is integral to the wastewater treatment process, it is important to determine the effect of high strength wastewater on the development of the microbial community and how it affects the reactor start-up and granulation process.

1.2 Objectives

The purpose of this study was multifold and conducted in two stages. The first stage consisted of the establishment of a laboratory scale AGS sequencing batch reaction (SBR) which was followed closely during start-up and the simultaneous increase in ammonia loading facilitated by the addition of municipal settling lagoon supernatant. The objective of this stage was three-fold:

1. To follow the development of the microbial community during the reactor start-up period

2. To observe AGS development from AS under a hybrid of lagoon supernatant and synthetic wastewater
3. To monitor reactor performance and compare with the performance of an established reactor run under similar conditions of increased ammonia loading

It was expected that differences between the established and start-up reactor would be present in terms of efficiency and performance. Better performance was expected in the established reactor in both removal efficiency and granulation due to the stability and high biomass found in this reactor compared to the start-up. It was also expected that this reactor would have a more established microbial community higher in ammonia oxidizing bacteria (AOB), phosphorus accumulating organisms (PAO), and other wastewater relevant species as it was run longer under AGS conditions which tend to select for these bacterial types. The results of this study were then used to inform the start-up of the pilot reactors undertaken in the second half of this thesis.

The second stage of this study was focused on the implementation of a pilot scale set-up. To achieve this, a protocol found to be successful at the laboratory scale was implemented in the 20L reactors. To determine the feasibility and optimal function at pilot scale, several different factors were examined for two different purposes:

1. To determine the parameters and technological set-up necessary to achieve successful operation of a pilot system at a minimum optimal hydraulic retention time (HRT)
2. To compare different seed sludge types to determine the most efficient start-up strategy for reactors of this size

For this purpose, two different types of seed sludge were used. It was hypothesized that granular sludge from the up-flow anaerobic sludge blanket (UASB) reactor could be used to accelerate the start-up process by introducing granular structure and thus eliminating the time needed for granulation as planktonic relevant species, such as AOB, would be able to integrate into the granular structures similarly to the formation of biofilm onto a provided substrate. It was also expected that these reactors would be directly scalable from the laboratory to pilot scale.

2 Literature Review

2.1 Environmental impact of ammonia

Ammonia is a pollutant of concern across both industrial and municipal wastewater treatment processes. While ammonia is a commercial commodity mainly used in the production of fertilizer, it can cause much environmental damage when released unintentionally. As such, it has garnered the focus of regulations spanning many countries including the USA, European Union (EU), Russia, and China (Preisner et al., 2020).

2.1.1 Environmental Concerns

The toxicity of unionized ammonia (also known as FA) is well documented, as are the effects of ammonia nitrogen (U.S. Environmental Protection Agency [EPA], 1999). In water ammonia is found in its ionized (NH_4) and unionized (NH_3/FA) forms (EPA, 1999). Both are present in solution and their relative concentrations are based on temperature and pH. FA poses the added risk in that it can evaporate and spread through the air up to several kilometers around the affected body of water (Environment Canada & Health Canada, 2001). FA has been associated with fish kills and toxicity to aquatic life (Camargo & Alonso, 2006). While LC_{50} levels vary between species, some organisms exhibit acute toxicity at concentrations below 0.068–2.0mg NH_3–N/L and chronic toxicity at concentrations of 0.05 mg NH_3–N/L (Camargo & Alonso, 2006; Eddy, 2005). Because of its inhibitory effect on AOB and NOB, high FA levels inhibit nitrification and denitrification resulting in further ammonia accumulation (Camargo & Alonso, 2006).

While NH_4 is not in and of itself toxic to aquatic environments, it is still problematic. This is due to both its ability to convert to FA in the right conditions and its role as a substrate

for nitrification. While complete nitrification processes are extremely useful in the treatment of wastewater, they also produce NO_2^- and NO_3^-, which are both toxic in their own right and contribute to eutrophication and acidification (Camargo & Alonso, 2006). As both NO_2^- and NO_3^- cause toxicity to aquatic life at extremely low concentrations, with amphibians being predominantly affected by NO_3^-, it is recommended that concentrations should be kept at 0.08–0.35 mg NO_2–N/L and 2.9–3.6 mg NO_3–N/L (Camargo & Alonso, 2006).

As nitrification consumes alkalinity, this process has been associated with acidification of aquatic environments. When concentrations of total ammonia are high, nitrification rates go up and more alkalinity is consumed. In addition, ammonia oxidation is associated with the production of NO_x compounds which are emitted to the atmosphere and re-enter aquatic environments as nitric acid. When these two phenomena are combined, acidification occurs (Camargo & Alonso, 2006). This has catastrophic impacts on the diversity of crustaceans and other aquatic animals as well as microorganisms (Camargo & Alonso, 2006).

Eutrophication is perhaps one of the largest problems affecting freshwater bodies today. Due to the algal blooms that occur during eutrophication, dissolved oxygen levels are reduced resulting in the death of aquatic life. Therefore, it is being actively monitored worldwide with much attention being given by both Europe and China (Preisner et al., 2020; Zhang et al., 2018). While increased phosphorus is the main contributing factor of eutrophication, NO_2^- and NO_3^- also play a role in the growth of the algae responsible (Canadian Environmental Protection Act [CEPA], 1999; Folett & Hatfield, 2001).

2.1.2 Regulations

Because of the environmental impacts of ammonia on the environment, the regulation of ammonia concentration in industrial and municipal effluent has been implemented in many countries worldwide. The European Union has been regulating effluent concentrations of TIN since 1998 and only allowing for the discharge of TIN at concentrations below 15mg/L (Urban Waste Water Treatment Directive 98/15/EC [UWWTD], 1998). While the EU does not differentiate between nitrogen types, some European countries such as Germany do (Preisner et al., 2020). While regulations address predominantly municipal wastewater treatment, industrial sources in the USA are also subject to regulations with accepted discharge concentrations ranging between 8-100mg NH_3-N/L depending on the industrial sector (EPA, 2021). While Canada does not have regulations governing the discharge of ammonia from municipal wastewater treatment, the recommended guidelines suggest effluent concentrations of 1.25mg NH_4-N/L or less (Fisheries Act, 2015).

The detrimental effects of ammonia on aquatic environments as well as the resultant regulations put in place have made it necessary to develop ammonia mitigation strategies, especially for ammonia rich wastewaters such as industrial waste streams and side-stream effluents of municipal wastewater treatment plants.

2.2 Ammonia rich wastewater sources

High-strength ammonia wastewater is produced by a plethora of sources, both industrial and municipal. With the growth of urban centres and the subsequent increase in produced wastewater, the volume of high ammonia effluents in municipal waste streams is projected to increase. While mainstream effluent from WWTPs is relatively low in ammonia, this is not

the case with side-stream effluent from municipal biosolids treatment (Ochs et al., 2021). Biosolids in municipal WWTPs are almost entirely comprised of waste sludge from the mainstream process. As this sludge must be treated and dewatered before being discharged into the environment, it is treated first via fermentation and then anaerobic digestion (Metcalf & Eddy, 2014). This is done for the purposes of pollutant removal and elimination of pathogens (Metcalf & Eddy, 2014). The resulting digestate is then dewatered and may be used either in compost or for land application (Metcalf & Eddy, 2014).

There are several dewatering methods used including centrifugation and evaporation lagoons, both of which result in liquid supernatant. This supernatant is called either lagoon supernatant or centrate depending on whether it originated from the evaporation lagoons or centrifugation process. Because the supernatant accumulated from biosolids dewatering is high in both ammonia and phosphorus it may not be released without treatment. It is therefore diverted back into the mainstream treatment pipeline where it comprises approximately 1% of the treated wastewater and 15-40% of the nutrient load (Chandrasekeran, 2007). As such, side-stream wastewater places a significant strain on mainstream wastewater treatment. To alleviate this, alternative measures for the exclusive treatment of side-stream wastewater have been implemented, though improvements to these systems are still being developed (Husband et al., 2010; Rosen et al., 1998).

2.2.1 Lagoon Supernatant

Lagoon supernatant is a product of settling lagoons utilized in the dewatering process of biosolids digestate generated by WWTPs in the sludge treatment process (Zou et al., 2020). After biosolids are separated from wastewater they are diverted and processed via

fermentation and anaerobic digestion (Metcalf & Eddy, 2014). The digestate is then sent to evaporation lagoons for further dewatering. Majority of ammonia found in lagoon supernatant is formed by the breakdown of proteins during digestion, resulting in high ammonia concentrations (OWP, 2009). Because significant ammonia oxidation does not occur in the lagoons, ammonia is not removed, and its concentration therefore increases as water is evaporated. In addition, lagoon supernatant may also be higher in solids if removed when the lagoons are agitated. While lagoon supernatant is responsible for much of the nitrogen loading in mainstream treatment, very limited research has been conducted on side-stream treatment. However, the few studies that have been done show promising results on ammonia removal of this type of side-stream wastewater (Zou et al., 2020).

2.2.2 Centrate

Centrate is another by-product of solids dewatering. While some digested biosolids are sent to settling lagoons for further consolidation, other portions are sent to dewatering facilities which facilitate biosolid consolidation using centrifugation. The resulting supernatant is called centrate. The NH_4-N concentration of centrate may vary and generally ranges between 800-1300mg NH_4-N/L (Metcalf & Eddy, 2014). However, some plants may exceed concentrations of 2,000mg NH_4-N/L (Chandrasekeran, 2007). Unlike lagoon supernatant, centrate is relatively low in suspended solids and VSS, which makes treatment significantly easier. Research on biological treatment on centrate is also limited. While treatment via bacterial systems is possible, most current centrate treatment research is focused on algae (Posadas et al., 2013; Posadas et al., 2017; Ledda et al., 2015; Lu et al., 2018). Ammonia rich centrate is used as feed for algae in energy production, making it feasible

wherever this type of energy generation is being implemented (Posadas et al, 2017; Lu et al., 2018).

2.3 Treatment approaches

The removal of NH_4-N from wastewater has been widely studied with a variety of methods developed. The strategies used for ammonia mitigation may be categorized as physicochemical or biological based on the nature of the process. Physicochemical processes include adsorption and ion exchange, as well as membrane filtration and air stripping (Karri et al., 2018). Biological methods include AGS, biofilm, algae, and ANAMMOX systems (Ronan et al., 2021). While algae have been used extensively in wastewater treatment, the focus of this study was on a bacterial system of removal (Liu, et al., 2017; Posadas et al., 2013). As such, the use of algae in wastewater remediation will not be discussed in detail. Even though highly effective, algae systems have several drawbacks, namely the requirement for influent UV disinfection and intolerance to high-strength wastewater (Ronan et al., 2021).

2.3.1 Physicochemical methods

Many physicochemical methods are currently in use at the industrial scale and are capable of abating even higher ammonia concentrations than those currently treatable via biological approaches (Karri et al., 2018). However, they are also plagued by drawbacks generally associated with technological limitations and high financial requirements (Karri et al., 2018).

The clarity of wastewater is one factor that places limitations on the use of both filtration and adsorption systems, as high solids content causes membrane fouling and inhibition of adsorbent surfaces (Karri et al., 2018). Since higher strength wastewater streams

have suspended solid concentration above 20mg/L they become unsuitable for adsorption (Karri et al., 2018). Other chemical treatments such as ion exchange are highly effective but suffer under elevated financial costs due to the need for expensive reagents (Karri et al., 2018). Processes such as air stripping have issues with efficiency and high energy expenditure, further highlighting the necessity for new technology development.

The drawbacks of physicochemical methods have been instrumental in driving interest towards biological methods of wastewater treatment. This is due to the relatively low cost and high resiliency of biological systems. However, most currently used biological technologies focus on lower strength wastewaters, with limited research addressing the treatment of wastewater with high ammonia loading. High ammonia removal efficiency has mostly been observed in low strength wastewater with removal efficiency dropping once ammonia concentrations exceed 300mg/L (Karri et al., 2018).

As a result, the number of studies focusing on high-strength wastewaters has been increasing. However, peak removal rates found in literature have not been commonly seen to exceed 72%, with most studies ranging below 60% or 50% (Yu et al., 2014; Jenicek et al., 2004). While few studies have shown over 90% ammonia removal rates, these have been associated with the use of partial nitrification SBR systems (Zou et al., 2020; Zhao et al., 2023).

There are several types of biological systems currently in use based on treatment objectives as well as wastewater strength. Biofilm systems are usually found in integrated fixed film activated sludge SBR (IFAS-SBR) or membrane bioreactor (MBR) configurations. MBR systems combine membrane filtration with biological processes and may be used in

tandem with suspended sludge or biofilms. Although MBR systems have been deemed very effective, these reactors are prone to fouling (Ronan et al., 2021). The SBR system has been found to be very versatile and scalable for different wastewater types and volumes and may use AS, IFAS, or AGS biomass configurations. AGS, which can accumulate at incredibly high biomass concentrations exceeding 10g/L, can therefore be enriched to treat increasingly high ammonia concentrations due to the retention of biomass in the system (Song et al., 2013; Wei et al., 2014; Yu et al., 2014). AGS technology is therefore highly attractive for ammonia mitigation strategies.

2.3.2 Traditional Nitrification/Denitrification

Nitrification/denitrification is the most studied pathway for ammonia oxidation in wastewater. This biological process is facilitated by AOB and nitrite oxidizing bacteria (NOB) which convert ammonia into N_2 gas as a final product (Metcalf & Eddy, 2014). The details of this process are described in section 2.4.1. Nitrification/denitrification is relatively common in most biological systems where ammonia is present and has been observed even in activated sludge systems, which are often used to source seed sludge for AGS reactors (Metcalf & Eddy, 2014). Subsequently, it has also been observed in experimental AGS systems treating low ammonia wastewater (Li-Long et al., 2013). While nitrification/denitrification is adequate for use in lower strength wastewater, the inhibition of NOB at high ammonia concentrations does not make this system suitable for use at high ammonia loading rates (Karri et al., 2018).

Because of this, the partial nitrification method, or the nitrite shunt, was suggested as an alternative. This process, which by-passes the second step of nitrification, has been in the research pipeline for high-ammonia wastewater for some time (Peng et al., 2004). However,

most systems, with either full or partial nitrification, examined so far have been more complicated in terms of operation and infrastructure such as continuous stirred tank reactor (CSTR) or multi-reactor SBR systems (Hellinga et al,.1998; Pacek et al., 2016). The most common set-up for SBR treatment is the two-tank system first described several decades ago (Pacek et al., 2016). While relatively effective, using two reactors for the aerobic and anaerobic steps introduces its own set of problems. It increases the chance for malfunction due to installation of additional hardware necessary to connect the two reactors and the need to regulate volume transfer between them.

Single reactor MBR systems have also been developed and shown to successfully treat synthetic centrate (Chandrasekeran et al., 2007). It combines activated sludge with membrane technology where sludge is used to remove pollutants before the feed stream permeates through a membrane and out of the reactor (EPA, 2007). This allows for higher sludge retention without improved settling. Membranes are made in two main configurations: hollow fiber and plate, with hollow fiber membranes being the most common (EPA, 2007). Hollow fiber membranes are installed inside a reactor in bundles (EPA, 2007). The effluent is found on the outside of the fibers with liquid diffusing to the interior of the fiber before being carried out of the reactor (EPA, 2007). However, as only solids are stopped by the membrane alone, it is better to combine the reactor with a technology, such as BNR, to remove dissolved pollutants. Because membranes prevent the washout of sludge and solids, this results in longer SRT which was associated with better nitrification (Chandrasekeran et al., 2007). Improved nitrification was likely a result of the slower growth rate of AOB and NOB, and the longer SRT allowed for better enrichment of these bacteria. Therefore, AOB and NOB frequency in MBRs has been found to be quite high (Wittebole et al., 2008). When nitrification and

subsequent denitrification occur, dissolved pollutants are removed, resulting in an efficient one-step system for wastewater treatment effective at even high ammonia concentrations (Chandrasekeran et al., 2007; Wittebole et al., 2008).

2.3.3 Partial nitrification systems

Partial nitrification is a biological treatment that has been gaining popularity over the past few decades as it reduces both COD and aeration demand by 40% and 25% respectively (Zeng et al., 2009). This has made it an attractive option as countries progressively move toward more stringent energy expenditure regulations (Directive of the European Parliament and of the Council [Directive], 2022). Despite this, literature regarding ammonia removal from high-strength wastewater using partial nitrification is quite limited. Most studies are focusing on laboratory or pilot scale systems using wastewater with relatively low ammonia loading rates. The effects of partial nitrification in the treatment of high-strength wastewater have not been thoroughly researched. However, evidence for NOB inhibition suggests that higher strength wastewaters may be even more conducive to the use of partial nitrification (Wei et al., 2014).

For partial nitrification to become dominant in a wastewater system, the elimination of NOB is integral (Shi et al., 2009). This results in NO_2^- accumulation which is necessary for subsequent denitritation (Peng et al., 2004). As a result, the elimination of heterotrophic nitrification has been the subject of much research due to the resilient nature of NOB (Jenicek et al., 2004; Peng et al., 2004). As AOB and NOB have similar nutritional demands, excepting the energy source, the elimination of one has often been associated with the elimination of the other. Key differences between the sensitivities of these organisms must be considered in the

selective enrichment of AOB. FA, temperature, DO, and pH, concentration are all associated with the inhibition of NOB in wastewater systems (Wei et al., 2014). While FA concentrations have inhibitory effects on both AOB and NOB, NOB have a much higher sensitivity to FA (Li et al., 2022). Inhibitory concentrations for *Nitrosomonas* (AOB) range from 10 to 150 mg/L and from 0.1 to 4.0 mg/L for *Nitrobacter* (NOB) (Yang et al., 2004; Blackburne et al., 2007). Because the FA tolerance of AOB is significantly higher than for NOB, FA concentration may be used as a selection mechanism for AOB enrichment in reactors with high ammonia loading (Sun et al., 2021).

Other methods may be utilized in tandem with FA elevation to limit NOB growth, such as the elevation of temperature to the 20-30ºC range (Wei et al., 2014; Stenstrom & Jansen, 2016). Decreased DO concentration has also been shown to facilitate NOB inhibition, with lower DO concentrations affecting NOB much more than AOB (Blackburne et al., 2008; Ruiz et al., 2003). The relationship between DO and temperature is integral as lowered temperatures have been associated with an increased sensitivity of NOB to lowered DO concentrations (Wei et al., 2014). When NOB inhibition was studied in AGS, it was observed that while FA was effective at NOB inhibition on the surface, DO and pH were more effective at inhibiting NOB deeper in the granule (Kent et al., 2019). For this reason, it was hypothesized that introducing anoxic phases into the operation cycle would aid in the inhibition of NOB. In addition, the oxidation of ammonia was found to increase in reactors with elevated pH between 8.0 and 8.5 with a correlated increase in NO_2^- accumulation (Wei et al., 2014; Qian et al., 2016). This further highlights the necessity of alkalinity maintenance in partial nitrification systems, which may be aided by alkalinity recovery facilitated by the presence of denitritation.

The most commonly used industrial scale partial nitrification system is the SHARON (Single reactor High Ammonia Removal Over Nitrite) process (Hellinga et al., 1998). The SHARON process was the first developed one-reactor system for the purpose of ammonia removal via nitritation (Hellinga et al., 1998). An average NH_4-N removal of 80% was achieved when high-strength wastewater was used. Interestingly, it was found that ammonia removal efficiency dropped at concentrations lower than 250 NH_4-N mg/L (Hellinga et al., 1998). This process was implemented in a CSTR and involved both aerobic and anoxic phases operated at elevated pH levels with temperatures exceeding 35ºC (Hellinga et al., 1998). However, NOB inhibition in this system is dependent on a high ammonia concentration and as such NOB activity becomes an issue when influent ammonia concentration falls (Hellinga et al., 1998). The process also requires sustained high temperatures which result in high energy expenditures when used in colder climates or with lower temperature waste streams such as lagoon supernatant. The SHARON process also does not remove large amounts of NO_2^- or suspended solids, making it impossible to use under current regulations without additional treatment methods (Hellinga et al., 1998). Therefore, it is often combined with other processes such as ANAMMOX (Lackner et al., 2014).

The SHARON process is most commonly implemented in CSTRs. This type of reactor is used relatively frequently in wastewater treatment (Pal, 2017). CSTRs are generally used in AS systems. These reactors operate on a continuous flow basis and rely on sufficient mechanical mixing to lower the concentration of pollutants within (Pal, 2017). This makes them attractive for high-volume flows as no retention time is needed for successful pollutant removal. The CSTR configuration is made up of a treatment tank and a subsequent settling tank (Pal, 2017). The mechanically stirred treatment tank has an influent and effluent port on

opposite sides. As the treated water makes its way from the influent port to the effluent port, biological removal of pollutants takes place. The effluent is then transferred to a settling tank to remove remaining biomass before passing to further treatment or discharge. Sludge accumulated in the settling tank is then returned to the CSTR with the influent (Pal, 2017).

2.3.4 ANAMMOX

ANAMMOX, or anaerobic ammonium oxidation, is a biological process that can degrade ammonia into nitrogen gas (Weralupitiya et al., 2021). This process was first described 20 years ago and has also lent its name to a group of bacteria that facilitate this type of ammonia degradation to N_2 (Podmirseg et al., 2022; Hamasaki et al., 2018). Anammox bacteria are obligately anaerobic and are therefore only found in the center of AGS granules, in biofilms, and anaerobic reactor systems (Weralupitiya et al., 2021). Because anammox bacteria are also very slow growing and prone to nutrient sensitivities, start-up times are extremely long and difficult, placing limitations on the use of this technology in current wastewater processes (Fan et al., 2020). The denitritation ability of ANAMMOX systems does however make up for this and has led to the development of several types of processes for ammonia removal.

ANAMMOX systems are usually implemented in combination with other ammonia mitigating technologies. This is due to the fragility of the ANAMMOX system and the sensitivity of anammox bacteria to nutrient and process changes. These bacteria are immensely sensitive to DO. They are reversibly inhibited by DO concentrations of 2% and above and irreversibly inhibited by DO concentrations exceeding 18% (Weralupitiya et al., 2021). Anammox bacteria also tend to prefer higher temperature systems ranging between 20-

45ºC (Weralupitiya et al., 2021). While ANAMMOX systems have been studied mainly for use in high-strength wastewater, the system appears to be inhibited by high FA concentrations exceeding 1,000mg N/L and elevated NO_2-N concentrations (Weralupitiya et al., 2021). The inhibitory concentration of NO_2-N is quite volatile and dependent on environmental conditions and can range anywhere between 5-280mg NO_2-N/L (Weralupitiya et al., 2021). Consequently, it has been observed that anammox bacteria thrive either in granular or biofilm-based systems since aggregation appears to lend better protection against environmental conditions in the reactor (Weralupitiya et al., 2021). To accommodate these bacteria, ANAMMOX systems are generally used in tandem with other technologies such as partial nitrification or partial denitrification to help ameliorate problems caused by nutrient sensitivities.

Partial nitrification/ANAMMOX (PN/A), or deammonification systems, exploit the denitritation capabilities of ANAMMOX in combination with other nitritation systems (Lackner et al., 2015). In this process, a partial nitrification reactor produces effluent with a high NO_2^- accumulation which is then fed into an ANAMMOX system for denitritation (Deng et al., 2020). An SBR-UASB configuration is one of the more common combinations (Cao et al., 2023; Deng et al., 2020). The ANAMMOX system has also often been combined with SHARON (Lackner et al., 2014). This style of treatment is currently the most common for treating high-strength side-streams in municipal wastewater (Miao et al., 2016).

In recent years, the single-reactor system has become more popular, resulting in modification to more traditional PN/A approaches (Wett, 2007). The CSTR system has remained widely used, however configurations such as the moving bed biofilm ractors

(MBBRs) or MEDIA have also been explored for the treatment of landfill leachate and other wastewaters (Ochs et al., 2021; Sun et al., 2015). Due to the contrasting demands of AOB and anammox bacteria, biofilm systems have been identified as ideal for PN/A (Weralupitiya et al., 2021). In the MBBR, biofilm is grown on carriers which are allowed to move freely through the system via mixing or aeration (Ødegaard, 2006). This reactor works similarly to the IFAS system in that it relies on substrates that are allowed to move freely within the reactor (Ødegaard, 2006). While MBBRs rely only on biofilm biomass, IFAS allows for the return and circulation of AS. Depending on the desired function of the reactor, either may be an asset.

While conventional MBBRs are effective at cultivating a PN/A system, systems such as IFAS configurations have been found to be even better at the simultaneous enrichment of AOB and anammox bacteria (Yang et al., 2020). This is thought to be a result of the limitations on biofilm thickness which dictate the size and composition of a microbial community (Yang et al., 2020). As IFAS SBRs contain suspended sludge and have a larger surface area on which biofilms may attach, the expansion of the microbial community especially among AOB species may be facilitated (Yang et al., 2020). The proliferation of AOB in IFAS SBRs is extremely beneficial as it provides the necessary optimal NO_2-N flow for anammox bacteria (Yang et al., 2020). This makes ANAMMOX attractive for use even in granular systems, which would increase the surface area even more. As such, the possibility of combining ANAMMOX systems with AGS remains an attractive option.

PD/A is a relatively new concept that combines partial nitrification with ANAMMOX and denitrification (Ronan et al., 2021). There are multiple ways to achieve this including

SNAD (simultaneous partial nitrification, anammox, and denitrification) (Ronan et al., 2021). The SNAD process uses the combination of AOB, anammox bacteria, and heterotrophic denitrifiers to remove ammonia from the system at an incredibly high efficiency and reduced cost (Ronan et al., 2021). Therefore, this process could be used to optimize AGS partial denitrification technology.

2.3.1 Sequencing Batch reactor

Current research conducted in our laboratory has previously suggested the ability of SBR systems to remove ammonia from high strength wastewater via biofilm systems. Average removal above 90% and reduced effluent total nitrogen (TN) concentrations have been observed (Zou et al., 2020). To achieve this, an IFAS-SBR was developed (Zou et al., 2020). It was found that implementation of multiple subcycles consisting of aerobic and anoxic phases within the SBR helped with pH maintenance due to the alkalinity recovered via denitritation activity in the anoxic phases of the cycle (Zou et al., 2020).

In addition to pH maintenance, denitritation was also found to be important in the regulation of N_2O gas production (Hu et al., 2010). As N_2O is an extremely potent greenhouse gas with negative effects on the ozone layer, its production during the wastewater treatment process poses a non-negligible environmental risk (Zeng et al., 2003). This is exacerbated in partial nitrification reactors as N_2O emissions tend to be disproportionately correlated to high rates of NO_2^- accumulation (Zou et al., 2022b). It has been observed that 97% of N_2O was released during the aeration phases, especially when NO_2^- concentrations were high (Zou et al., 2022b). It was therefore determined that an increase in the number of subcycles alternating between shortened aerobic and anoxic phases was responsible for a dramatic reduction in N_2O

production (Zou et al., 2022b). Based on these findings, the multi-subcycle strategy was adopted for use in the pilot reactors operated in this study.

The IFAS-SBR is a type of system that operates as a hybrid of a biofilm and AGS system. Biofilm is cultivated on provided structures designed to move in the reactor similar to granules (Metcalf & Eddy, 2014). The structures on which the biofilm can attach may be either sponge or plastic and are allowed to move freely within the reactor along with the suspended sludge. This system therefore retains the benefits of AS in tandem with improved biomass retention (Metcalf & Eddy, 2014).

The bioreactor system tested in this study was the AGS SBR. This type of reactor has been associated with AGS from its first development due to the ideal conditions it creates for its formation (Morgenroth et al., 1997).

In addition, all steps of the process occur in one tank, saving space as well as construction costs and thus reducing the environmental footprint (EPA, 1999b). SBRs follow the same protocol in all set-ups; however, these may be modified based on reactor size and treatment requirements.

The operation of the AGS-SBR is divided into five stages; fill, react, settle, decant, and idle. The fill stage is quite simple and may be conducted via slow or pulse feed. Generally, 40-75% of the total reactor volume is replaced during this stage (Metcalf & Eddy, 2014).

The react stage then begins. This stage may be either aerated or may utilize cyclic aeration and anoxic mixing (Metcalf & Eddy, 2014). At this point, the treatment portion of the cycle occurs. It is responsible for the removal of target pollutants from the wastewater.

Removal of nitrogen through either nitrification/denitrification or nitritation/denitritation as well as COD and phosphorus removal take place during this stage (Dutta & Sarkar, 2015). The react stage of operation is the most versatile and may be configured to almost any set-up necessary with aerobic and anoxic phases being used to facilitate target pollutant removal (Dutta & Sarkar, 2015). This stage takes up most of the operation time and may range in length from hours to days.

Settling is then allowed to occur to separate biomass and solids from the supernatant in preparation for discharge. Settling times may vary depending on the type of reactor. This stage is also used for selection of heavier microbial aggregates, a method integral to the development of AGS (Metcalf & Eddy, 2014).

The decant stage then follows and is generally the shortest of the intervals. Here, the supernatant is removed via a port located at the desired level in the reactor. Effluent discharge, controlled by pump or solenoid valve, may be either slow or rapid. The valve position regulates the VER of the reactor which may range between 40-75% of reactor volume (Metcalf & Eddy, 2014).

The last step is the idle period which may or may not be implemented. This period refers to the time where the reactor is not active and may last anywhere between a few minutes to an hour. The idle period may be used in a multi-tank system to allow for one tank to fill before switching flows to another and/or to allow for changes in capacity due to flow fluctuations such as those caused by seasonal changes or wet weather. The idle phase may also be implemented to balance out timing of the operating cycles.

The key to the effective SBR operation lies in the use of AGS. AGS is formed from AS that has been treated in an aerobic SBR with bubble aeration and shortened settling times. These conditions are conducive to the formation of microbial aggregates that allow for higher biomass and decreased settling times and thus the elimination of secondary settling tanks (Nanchariah & Reddy, 2018). This, combined with the vertical design of the system, allows for both the reduction of facility size and a reduced biological footprint due to increased SRT (EPA, 1999b). As settling of the system improves, greater biomass retention and concentration within the system is achieved, improving the efficiency of nutrient removal, and reducing the need for sludge wasting (Nanchariah & Reddy, 2018).

2.4 Biological ammonia removal pathways

Biological ammonia removal has garnered much attention in the wastewater industry and has therefore been the subject of intensive study. Biological systems are more versatile across wastewater strengths than physicochemical methods and, when done correctly, produce few to no toxic by-products. While biological systems have many benefits, they are also reliant on living organisms that can be temperamental, harder to control, and react both negatively and positively to their environment. It is therefore necessary to have a good understanding of the mechanisms and pathways involved so that the best system may be utilized in each situation.

There are several different biological pathways that have been identified and studied. These include full nitrification/denitrification as well as nitritation/denitritation, also known as partial nitrification or the nitrite shunt. The latter is currently in the research pipeline and includes classical nitritation/denitritation as well as NO_x processing (Paul & Banerjee, 2022).

While NO_x processes may be used in wastewater treatment as well, this review focused on full and partial nitrification, denitrification and denitritation, and ANAMMOX as these pathways were the most relevant to the objectives of this study.

2.4.1 Nitrification/denitrification

There are several steps in the ammonia oxidation pathway, with two different processes, nitrification/denitrification and nitritation/denitritation, occurring under different conditions (Metcalf & Eddy, 2014). While both pathways begin with the same substrate and ultimately end in N_2 and H_2O, differences exist. These must be understood to facilitate the most efficient pathway to N removal.

Nitrification/denitrification is the conventional method for ammonia removal on the industrial scale and is very effective for the treatment of low-strength wastewater (Metcalf & Eddy, 2014). The process occurs via two steps, nitrification and denitrification. In nitrification, ammonia is first broken down into NO_3 via NO_2^- and then to N_2 gas (Fig. 2-1) (Ge et al., 2015). This process is instigated by AOB (Fig. 2-1) (Bellucci & Curtis, 2011). AOB are chemoautotrophic bacteria that utilize CO_2 for a C source and NH_4-N to obtain energy (Metcalf & Eddy, 2014). These organisms are generally classified under *α*-and *β*-*proteobacteria*, with *β-proteobacteria* being the class of interest in the treatment of freshwater wastewater. The genus *Nitrosomonas* as identified with 16S ribosomal RNA sequencing, is the most commonly found genus of AOB (Yu et al., 2020). *α-proteobacteria* are also sometimes found in freshwater treatment but are more common in seawater and related bodies (Metcalf & Eddy, 2014). In the case of AOB populations, *Nitrosomonas* species generally dominate conventional systems (Yao & Peng, 2017). Since AOB share most resources with

NOB, apart from the energy source, the desired bacterial type may be enriched by the manipulation of ammonia and NO_2^- concentrations (Wei et al., 2014b).

Figure 2-1. Nitrification steps associated with its bacterial types. The first step conducted by AOB is also referred to as nitritation.

In the first step of nitrification, the enzymes ammonia monooxygenase and hydroxylamine oxidoreductase facilitate the two-stage breakdown of NH_4^+ to NO_2^-, according to equation 1 (Ge et al., 2015).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

Once NO_2^- begins to accumulate, it is oxidized to NO_3^- by nitrite oxidoreductase as seen in equation 2 (Ge et al., 2015).

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

This second step is performed by NOB (Fig. 2-1) and results in the total ammonia oxidation reaction according to equation 3.

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

The reaction results in the production of NO_3^- which is then metabolized to N_2 during denitrification. Aside from ammonia, nitrification also consumes alkalinity and O_2 (Metcalf & Eddy, 2014). Approximately 4.25g of O_2 and 7.09g of alkalinity as $CaCO_3$ are consumed per g of NH_4-N oxidized to NO_3^- (Metcalf & Eddy, 2014). During this process, NO_3^- and 0.16g of biomass are formed (Metcalf & Eddy, 2014). As NO_2^- is necessary for NOB to thrive, this community begins to appear after sufficient ammonia is metabolized to NO_2^- by AOB (Nowka et al., 2015).

Comammox, an alternate pathway for nitrification was recently identified in the genus *Nitrospira* (Luo et al., 2022). This process has been observed in a large number of nitrification/denitrification wastewater systems but especially in AS where *Nitrospira* make up the largest number of NOB (Daims et al., 2001). Comammox facilitates the oxidation of NH_4-N to NO_3-N in a single step, by-passing the NO_2^- intermediate (Mehrani et al., 2021). Commammox has also been closely associated with the ANAMMOX process. The two bacterial types have a tendency to cooperate, both in reactors and in the wild (Zhu et al., 2023). Because of the high removal rates of this cooperation even in high-strength wastewater, use of a comammox/ANAMMOX system has high potential in the field of wastewater treatment (Zhu et al., 2023).

NO_3^- is still a pollutant that is detrimental to the ecology of water systems. Due to its fertilizing properties, it has been associated with eutrophication and as such, limits on its discharge have been included in most wastewater effluent regulations (Preisner et al., 2020). As such, simple nitrification must be followed by NO_3-N removal by denitrifiers using NO_3-N as an energy source.

Denitrification is the most common biological NO_3-N removal process in wastewater systems due growth strategies of nitrifying bacteria and is the next step after NO_2^- is oxidized into NO_3^-. This process may be either autotrophic or heterotrophic and is performed by a plethora of genera including *Acinetobacter, Flavobacterium, Bacillus, Pseudomonas, Rhizobium,* and *Vibrio* among others (Metcalf & Eddy, 2014). However, while autotrophic reduction has been found to be possible, it is not commonly observed in wastewater treatment.

The first consideration of denitrification is the presence of a carbon source. This may be provided either by an already present constituent of wastewater, but often must be added as many wastewaters are deficient in bioavailable carbon (Metcalf & Eddy, 2014). There are several sources that may be used for this purpose. The most commercially available is waste methanol or glycerin, though acetate or acetic acid may also be used. In the case of this study, carbon was added in the form of acetate. Acetate was chosen as it is the preferred carbon source for nitritation/denitritation. It is important to note that acetate is not generally used as a carbon source for commercial heterotrophic denitrification due to high costs (Metcalf & Eddy, 2014). As such, acetate should be replaced by other types of carbon sources when used in stable industrial scale systems. These may be sourced from other waste streams high in organic carbon.

The process of denitrification constitutes the conversion of NO_3-N into N_2, CO_2, OH^-, and water. The exact nitrate reduction reaction is represented in equation 4.

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \qquad (4)$$

Conversely to nitrification, denitrification produces alkalinity on the scale of one equivalent of alkalinity produced per equivalent of NO_3-N consumed (Metcalf & Eddy, 2014). This equates to 3.57g of alkalinity in $CaCO_3$ produced per g of NO_3-N reduced, leading to the recovery of approximately half of the alkalinity consumed in the nitrification process (Metcalf & Eddy, 2014).

2.4.2 Partial Nitrification

Partial Nitrification is a term used to describe a nitritation/denitritation system. The partial nitrification pathway follows a similar progression to that of nitrification/denitrification.

However, in nitritation, the oxidation of NH_4-N is arrested after the first step performed by AOB (Fig. 2-1). This reaction may be seen in equation 1. The resulting accumulated NO_2^- then serves as a substrate for denitritation.

As nitrification/denitrification is generally the default for aerobic systems, the growth of NOB must be limited for nitritation/denitritation to take place. If NOB are inhibited, the amount of NO_3-N in the system is reduced which instead pushes the system from denitrification to denitritation (Jenicek et al., 2004). As denitritation organisms thrive in anoxic conditions, an anoxic period must be introduced to the system and selection against NOB must be implemented (Jenicek et al., 2004). While this may be done using FA, it has been recently found that enriching AOB through the lowering of DO is more effective at selecting against NOB while leaving AOB unaffected (Zeng et al, 2009). With the introduction of anoxic periods into the operation cycle, it has been observed that denitritation is enriched, while NOB is inhibited (Zeng et al., 2008). This in turn will shift the reactor into the desired type of ammonia oxidation.

Denitritation may occur via heterotrophic denitritation or the ANAMMOX process. While heterotrophic denitritation is known to occur, the mechanism and microbes involved are not well understood. However, it is the biological reduction of NO_2-N according to equation 5 and follows a similar process to that of denitrification.

$$3CH_3COOH + 8NO_2^- \rightarrow 4N_2 + 6CO_2 + 2H_2O + 8OH^- \qquad (5)$$

The main difference between the two is the lowered carbon requirements of denitritation with approximately 67% reduction in acetate demand for denitritation compared to denitrification and improved alkalinity recovery (Metcalf & Eddy, 2014). In addition, denitritation occurs at

conditions of lowered DO, reducing energy costs related to aeration requirements (Le et al., 2020). Additional O_2 is conserved by eliminating the second step of nitrification/denitrification. Production of CO_2 in the nitritation/denitritation process is also lower than that of nitrification/denitrification due to the elimination of the second oxidation step (Metcalf & Eddy, 2014).

2.4.3 ANAMMOX/deammonification

The ANAMMOX process is an ammonia removal pathway which follows a significantly different path to that of nitrification/denitrification. The process is autotrophic and occurs in the anammoxosome of bacteria (Agrawal et al., 2022). This organelle is found mostly in bacteria belonging to the order Planctomycetes and has garnered much attention in the field of wastewater treatment for its efficiency. However, due to the sensitivity of the system and long doubling times of anammox bacteria this system has a more complex start-up than most other conventional processes (Agrawal et al., 2022).

The ANAMMOX process occurs when anammox bacteria metabolize NH_4 via nitrite and hydrazine to N_2 as seen in equation 6 (Agrawal et al., 2022).

$$NH_4^- + NO_2^- \rightarrow N_2 + 2H_2O \qquad (6)$$

The proposed mechanism to achieve the steps in equation 6 is the reduction of NO_2^- to hydroxylamine and then with the aid of NH_4^+ to hydrazine and then N_2 (Metcalf & Eddy, 2014). The steps may be broken down by the enzymes moving the reaction forward (Weralupitiya et al., 2021). First, nitrite is catabolized to nitric oxide via nitrite reductase according to equation 7. This then combines with NH_4^+ and is converted to hydrazine via

hydrazine synthase as seen in equation 8. The hydrazine is then catabolized to N_2 and H^+ with hydrazine dehydrogenase to complete the process according to equation 9 (Fan et al., 2020).

$$NO_2^- + 2H^+ + 1e \rightarrow NO + H_2O \quad (7)$$

$$NO + NH_4 + 2H^+ + 3e \rightarrow N_2H_4 + H_2O \quad (8)$$

$$N_2H_4 \rightarrow N_2 + 4H^+ + 4e \quad (9)$$

Because at least 55% of NO_2^- must be available for the ANAMMOX process to begin, combination with other systems, such as partial nitrification, makes this process more effective (Metcalf & Eddy, 2014).

2.5 AGS for high-strength wastewater treatment

AGS is a unique sludge morphology that is incredibly effective at treating both low- and high-strength wastewater. Because of its higher SRT and increased settling capabilities, it allows for the reduction in sludge wasting as well as the building of more compact systems.

AGS use in wastewater treatment was first described in the 1990s and has been used for various applications since (Morgenroth et al., 1997). The AGS system was traditionally operated as a full nitrification/denitrification system, making it unsuitable for use with high ammonia loading (Karri et al., 2018). As such, most studies have been focused on low strength wastewater treatment. Because granular sludge has higher settling capabilities than AS, settling time and SRT are both improved in the system (Jang et al., 2003). This results in higher biomass and increased treatment potential (Li et al., 2018).

2.5.1 Aerobic granulation process

2.5.1.1 SVI and biomass

Settling and biomass concentration are integral to the operation of an AGS system with SVI tending to be significantly lower in AGS than in conventional sludge (Fu et al., 2010). It is therefore necessary to monitor these parameters through the measurement of MLSS and SVI. SVI is a measure of the settling capabilities of sludge. A good indicator of sludge health and quality, SVI is the ratio of volume to biomass concentration (MLSS). As a result, SVI is a standard measurement utilized by most reactor studies dealing with AGS as it may be used to quantify the health and effectiveness of AGS. Two measurements of SVI are taken, specifically SVI_5 and SVI_{30} corresponding to the values observed at 5- and 30-minute intervals. While SVI values vary depending on the type of sludge for which they are taken, healthy SVI is generally considered to be below 100mL/g and to have an SVI_5/SVI_{30} ratio of 1.0 (Pal, 2017). Due to the relative ease of measurement and the correlation of settleability to reactor health, SVI may be used to gauge the health of an AGS system as well as to help pinpoint any systemic problems, such as the proliferation of filamentous growth before substantial loss of biomass and treatment efficiency becomes irreversible.

2.5.1.2 Granule formation

The AGS system is based on the formation of the aerobic granule. The granule is a microbial aggregate with distinct layers which forms as a result of selective pressures asserted on seed sludge (Nanchariah & Reddy, 2018). The granules are often generated out of AS that is introduced into an SBR and placed under selective pressure which induces microorganisms to form EPS. In this case, the selective pressure is in the form of shear force exerted by fine bubble aeration (Kim et al., 2015). Speeds 1.2cm/s and higher induce microorganisms to begin

the formation of microbial aggregates to protect themselves from the shearing forces (Nanchariah & Reddy, 2018). As the sludge is put under stress, the bacteria begin to produce EPS in the mechanism responsible for biofilm formation (Wang et al., 2016). Biofilms generally form on surfaces in aquatic environments where either toxins, nutrient deficiencies, or shear hydrodynamic forces cause stress to microorganisms. To avoid cell damage, microorganisms use EPS to attach to either surfaces or to each other. The resulting communities are generally more resilient, both to famine and other factors such as toxins or physical damage (Nanchariah & Reddy, 2018),

In the case of the SBR, the biofilm triggers are artificial feast-famine strategies as well as hydrodynamic shear force caused by aeration (Zhang et al., 2016). Since the microbes found in the SBR do not have a reliable surface on which to attach and build the biofilm, they attach instead to each other. This results in the formation of floc which, if given time and the right conditions, then transforms into mature granules (Nanchariah & Reddy, 2018).

Granule formation follows a three-step process beginning with the formation of floc. In this state the sludge is composed of loosely held together cells, with cocci, bacilli, and filamentous types present (Nanchariah & Reddy, 2018). While floc is held together mostly by EPS, the entangling of filamentous bacteria also plays a role in keeping the floc intact. While better than regular AS, this type of sludge still has a lowered settleability. With the presence of selection pressure such as shear force, these flocs further solidify into early aggregates. Early aggregates are more solid than floc, exhibit improved solidity and settleability, yet still contain evidence of filamentous bacteria and lack the defined regions of mature granules (Nanchariah & Reddy, 2018). In cases where filamentous overgrowth is affecting settling and

granulation, COD restriction may be implemented to inhibit the growth of filamentous bacteria. If selection pressure continues to be exerted on the early aggregate sludge, and nutrients for growth are provided, the aggregate will then develop into a mature granule, with distinct aerobic, anoxic, and anaerobic layers (Nanchariah & Reddy, 2018). These mature granules lack filamentous types almost completely, being held together almost exclusively by EPS (Hammiruddin et al., 2019).

While older sludge tends to have larger granules, reactor type and size also affect granule formation. Larger SBRs are known to have lower shear forces, allowing larger granules to develop (Tay et al., 2009). As such, granules may range in size from microscopic up to several cm across. While larger granules have increased mass and therefore a reduced SVI, they also tend to be less dense, and more fragile. Therefore, issues with granule disintegration affect larger granules more frequently (Nanchariah & Reddy, 2018). As large granules are also more mature, they may have lower removal efficiencies.

2.5.1.3 Granule morphology

Granules generally follow the same morphology regardless of shape and size, though size does affect the dominance of certain regions and therefore the microbial communities within the granule (Nanchariah & Reddy, 2018). Each granule layer contains a variety of species which, if necessary, may be enriched to ensure certain functions (Nanchariah & Reddy, 2018). The outside of the granule is aerobic and contains microbes such as AOB and NOB (Nanchariah & Reddy, 2018; Jang et al., 2003) (Fig. 2-2). Because both NOB and AOB occupy the same region, they tend to compete, and as such, selection must be performed to ensure the correct species are enriched in the aerobic zone. As NOB growth rate is

significantly higher than that of AOB, conditions must be adjusted to ensure the enrichment of AOB species in SBRs where the primary objective is ammonia removal (Munz et al., 2011). If this is not successfully achieved, NOB will dominate, reducing ammonia removal efficiency and skewing the reactor towards nitrification/denitrification.

The next layer consists of the anoxic zone (Fig. 2-2). This zone contains PAOs, glycogen accumulation organisms (GAOs), and denitrifiers and denitritation species that complete the conversion of NO_2^- and NO_3^- to N_2 (Nanchariah & Reddy, 2018). The innermost area of the granule is the anaerobic zone, buried at the core of the granule, where oxygen does not penetrate (Fig. 2-2) (Nanchariah & Reddy, 2018). With the exception of anammox bacteria, the core of the granule is not overly relevant to nitrogen removal but has been shown to be the area where phosphorus accumulates (Nanchariah & Reddy, 2018).

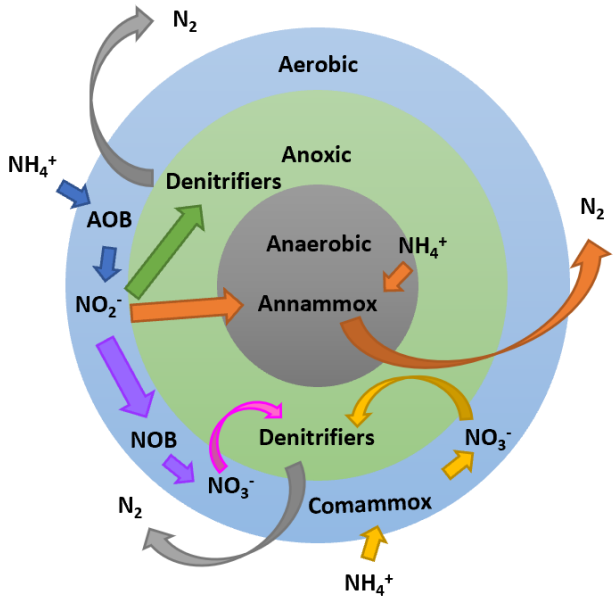

Figure 2-2. Basic structure of an AGS granule with ammonia oxidation processes: nitritation (blue), nitrification (purple), denitritation (green), denitrification (pink), comammox (yellow), and anammox (orange).

2.5.2 Overview of literature

AGS has been shown to be extremely effective at COD removal even at high concentrations (Wei et al., 2012; Sarvajith et al., 2020). When it comes to ammonia removal, the system has been mainly studied using mainstream municipal and synthetic wastewaters which do not tend to have high NH_4-N concentrations. While this has helped to identify the ideal parameters for system operation, it does not address AGS use for side-stream and other high-ammonia effluents. In addition, drawbacks of AGS include both long start-up times and increased energy costs due to aeration requirements (Zhang et al., 2021). It has also been observed that high ammonia concentrations have negative impacts on the granulation process, making it less favorable for high-strength wastewater treatment (Wei et al., 2014).

This is thought to occur due to two different factors: cell hydrophobicity and EPS production. High cell hydrophobicity is integral to the initiation of cell aggregation which is necessary for granule formation (Tay et al., 2009). As high levels of FA lower the hydrophobicity of cells, this can then inhibit the formation of granules (Yang et al., 2004). FA can also inhibit the production of EPS when the C/N ratio is high (Yang, et al., 2004). The findings that high FA concentrations may stop granulation were used to inform the first stage of this study as the effect of high ammonia loading on the microbial community and granulation has not been well researched.

As a result, not much research has been published on the topic of AGS treatment of high-strength wastewater streams. Most studies in this area have reported relatively low removal efficiencies of approximately 50-60% (Yu et al., 2014). However, promising data on AGS combined with ANAMMOX in a PN/A system has been identified, reporting an increase

in ammonia removal efficiency up to 89.6% (Zhao et al., 2023). This confirms the ability of AGS to treat high ammonia wastewaters, especially in conjunction with partial nitrification.

While it has been observed that AGS has the potential to treat high ammonia influents with high removal efficiency, not much research has been published on the subject. Most used activated sludge as inoculum for the reactors (Kim & Seo, 2005; Song et al., 2013; Zhao et al., 2023). Mature granules as seed for the reactors were also used (Yu et al., 2014). It is therefore necessary to determine the possibilities of AGS development from non-granular seed sludge at high ammonia concentrations. The microbial community of reactors with high ammonia loading has also not been well characterized, especially during AGS formation.

In terms of bacterial communities, it was found that AOB such as *Nitrosomonas* were very well enriched, reaching up to anywhere between 4.5%-7% relative frequencies (Kim & Seo, 2006; Morgan & Hamza, 2022; Song et al., 2013). OF the AOB, *Nitrosomonas* species dominate the community with overall relative frequency of 7% (Cao et al., 2023). These bacteria have been observed on the surface of granules in the regions with highest DO penetration (Song et al., 2006). Cultivation of partial nitrification was also successful in high ammonia systems, with the complete elimination of detectable NOB (Song et al., 2013). While FA has been known to inhibit the growth of NOB, it was found that short settling times were also very effective at the reduction of NOB abundance (Kim & Seo, 2005). Lastly, it has been observed that the formation of small granules aid in NO_2^- accumulation (Li et al., 2020). The combination of these factors could then be used to quickly establish purely partial nitrification systems. NOB on the other hand were either not found at all or in incredibly low numbers (Song et al., 2013). Whereas AOB made up of almost 40% of bacteria, only about

1% were NOB (Kim & Seo, 2006). Other than the two studies by Kim and Seo (2006) and Song et al. (2013), no other studies examining the microbial community of high ammonia loading AGS SBRs were found, suggesting a gap in this area of the literature. While *Nitrosomonas* relative frequency in the above studies was still below 10%, this genus was significantly more enriched than what was found in AGS studies exploring lower ammonia loading (Nguyen Quoc et al., 2021). Here, the relative frequency of *Nitrosomonas* was found to be consistently below 3% (Li et al., 2011; Nguyen Quoc et al., 2021). In addition, the relative frequency of NOB was found to be almost 6%, resulting in higher levels of NOB in AGS treating lower strength wastewater (Li et al., 2011).

There is very limited literature on the use of AGS on centrate specifically and no studies found dealing with lagoon supernatant. The studies conducted on the use of AGS for high-strength wastewater treatment focused on either synthetic streams or landfill leachate (Cao et al., 2023; Kim & Seo, 2006; Song et al., 2014; Sun et al., 2015; Yu et al., 2014; Zhao et al., 2023; Zou et al., 2022c). It was observed that increased ammonia concentrations were linked to lowered removal efficiency (Yu et al., 2014). Other studies have observed relatively high removal rates with some reporting over 98% removal efficiency, suggesting that differences in operation, seed, and feed composition likely play a large role in ammonia removal (Zhao et al., 2023). It was found that NO_2^- accumulation was occurring at very high rates in these reactors, making them suitable for partial nitrification (Zhao et al., 2023).

Of all the studies reviewed, only one investigated AGS treatment of real centrate (Morgan & Hamza, 2022). The study was focused on the comparison of real and synthetic centrate with one reactor treating real wastewater and the other two treating synthetic

wastewater. The ammonia removal pathway studied was nitrification/denitrification. The centrate used was also diluted before treatment (Morgan & Hamza, 2022). While MLSS was found to range between 4-7.5g/L, higher biomass was found in the synthetic reactors (Morgan & Hamza, 2022). Ammonia removal efficiency was found to be almost 100% for the real centrate reactor and evidence of partial nitrification and denitritation was observed (Morgan & Hamza, 2022).

While there is good evidence that AGS is a good approach for the treatment of high-strength wastewater, there are still many gaps in the literature. One of these is the absence of research investigating pilot scale reactors. All studies on this subject have only been done on the laboratory scale. Since preliminary data and published results show that partial nitrification AGS can treat high-strength wastewaters, pilot studies working with larger influent volumes are the next step in determining the viability of AGS for industrial use.

3 Laboratory scale start-up and DNA analysis

3.1 Introduction

SBR aerobic granular sludge reactors have been used for the past several decades, both experimentally and commercially. However, the characterization of the microbial community during granule formation has not been very well described. Therefore, the objective of this experiment was to characterize the microbial community of the reactor and any changes that would occur as a result of a gradual increase in ammonia loading during the granulation process. To achieve this, a laboratory scale reactor was set up under the ideal conditions for granulation with the intention of facilitating the environment necessary for formation of AGS. This was done to achieve the following objectives: a) to establish a working AGS reactor capable of removing NH_4-N from the system and to observe the reaction of the reactor to the introduction of high-strength wastewater, b) to characterize changes in the microbial community during this phase, and c) to compare start-up reactor performance (R1) with an established reactor (R2) being run under similar conditions and established on exclusively low loading synthetic feed. This was done to determine the best start-up strategy for a system treating wastewater with high organic loading and whether real wastewater may should be introduced before or after reactor es5tablishment. For this purpose, both performance data in the form of chemical and physiological tests, as well as 16S rRNA sequencing was conducted.

3.2 Methods

3.2.1 Reactor Description

3.2.1.1 R1

A 4L clear plexiglass SBR was operated with a total fill volume of 3.15L and the following dimensions: diameter – 7cm, height – 94.3cm. The port used for effluent was located at 45cm resulting in a 43.2% VER. The target superficial velocity used was 1.6cm/s, which was normalized to 3.7L/min for a reactor of the above dimensions.

This reactor was inoculated with 2L of AS sourced from a large municipal WWTP in Northern Alberta.

4.2.1.2 R2

R2 was a 4L reactor with a 9cm diameter that was engaged in the experiment after full start up had been completed and regular operation was ongoing for several months. Target airflow was calculated to be a superficial velocity of 1.48cm/s. The VER for this reactor was 40%.

3.2.2 Reactor operation

R1 was operated using an aerobic cycle of 4 hours in length with 2.25 hours of aeration, a slow feed spanning 1 hr, a settling time of 30 minutes and a decanting time of 4 minutes. (Table 3-1). Settling time was reduced to 25 minutes after 25 days of operation.

Table 3-1. Operation of R1. 6 cycles were performed every 24hrs, with each cycle lasting 4 hours.

Cycle		Aeration	Feed	Settling	Decanting
1	On	00:00	23:00	2:25	2:55
	Off	2:25	00:00	2:55	2:59
2	On	4:00	3:00	6:25	6:55
	Off	6:25	4:00	6:55	6:59
3	On	8:00	7:00	10:25	10:55
	Off	10:25	8:00	10:55	10:59
4	On	12:00	11:00	14:25	14:55
	Off	14:25	12:00	14:55	14:59
5	On	16:00	15:00	18:25	18:55
	Off	18:25	16:00	18:55	18:59
6	On	20:00	19:00	22:25	22:55
	Off	22:25	20:00	22:55	22:59

R2 was also operated on a basis of an aerobic 4hr cycle, with 2 hrs. 43 minutes of aeration, 10 minutes of settling, 6 minute decanting, and 1 hour feeding per cycle, (Table 3-2). Testing on R2 was begun after the reactor was fully developed and functioning for several months under study for the purpose of PO_4 removal and recovery.

Table 3-2. Operation of R2. 6 4hr. cycles were performed during each 24hr. period.

Cycle		Aeration	Feeding	Settling	Decanting	Idle
1	On	00:00	23:00	2:43	2:53	2:59
	Off	2:43	00:00	2:53	2:59	3:00
2	On	4:00	3:0	6:43	6:53	6:59
	Off	6:43	4:00	6:53	6:59	7:00
3	On	8:00	7:00	10:43	10:53	10:59
	Off	10:43	8:00	10:53	10:59	11:00
4	On	12:00	11:00	14:43	14:53	14:59
	Off	14:43	12:00	14:53	14:59	15:00
5	On	16:00	15:00	18:43	18:53	18:59
	Off	18:43	16:00	18:53	18:59	19:00
6	On	20:00	19:00	22:43	22:53	22:59
	Off	22:43	20:00	22:53	22:59	23:00

3.2.3 Feed

Both reactors were started on and remained on synthetic feed until stable conditions and consistent NH_4-N removal was achieved. For R1, lagoon supernatant sourced from sludge thickening lagoons in Alberta that store and treat effluent of anaerobic digesters treating biosolids from a full-scale municipal WWTP, was gradually added starting with 10% by volume on days 55, 67, 89, and 96 respectively. Concentration was increased by 10-20% increments until 70% supernatant by volume was reached. For R2, concentration was increased in 10% and 20% increments by volume up until 40% concentration on days 4, 17, and 25 respectively.

3.2.3.1 R1

Synthetic feed for R1 was mixed fresh and used up within 48 hours to minimize nutrient and N-species degradation. It was prepared according to the recipe in Table 3-3. For lagoon supernatant supplemented feed, synthetic feed was used as the dilution liquid into which the lagoon supernatant was added.

Table 3-3. Chemical recipe for the synthetic feed of R1. Feed was prepared in 20L batches as needed.

Chemical Name	**g/L**
Sodium acetate (NaAc)	1.875
Sodium propionate (NaPr)	0.417
Ammonium chloride (NH_4Cl)	0.322
Dipotassium phosphate, anhydrous (K_2HPO_4)	0.06
Monopotassium Phosphate, anhydrous (KH_2PO_4)	0.05
Calcium Chloride dihydrate ($CaCl_2 \bullet 2H_2O$)	0.063
Magnesium sulfate heptahydrate ($MgSO_4 \bullet 7H_2O$)	0.025
Iron sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$)	0.02
Micronutrients	1ml/L

Micronutrients were mixed in advance in 5L batches and added as a solution to every 20L batch according to the specifics in Table 3-4. The solution was then stored at 4ºC to inhibit bacterial growth and compound degradation.

Table 3-4. The micronutrient recipe for reactors. The recipe was prepared in 5L batches and refrigerated to prevent contamination and degradation of compounds.

Micronutrients	**g/L**
Boric acid (H_3BO_3)	0.05
Zinc Chloride ($ZnCl_2$)	0.05
Copper(II) Chloride ($CuCl_2$)	0.03
Manganese sulfate monohydrate ($MnSO_4 \bullet H_2O$)	0.05
Ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \bullet 4H_2O$)	0.05
Aluminium chloride ($AlCl_3$)	0.05
Cobalt(II) chloride hexahydrate ($CoCl_2 \bullet 6H_2O$)	0.05
Nickel(II) chloride ($NiCl_2$)	0.05

3.2.3.2 R2

R2 was also initially operated on synthetic feed only. This reactor was fed a different recipe with more limited nutrients as per Table 3-5. This feed was prepared in 20L batches as needed. Lagoon supernatant was also added, beginning at 10% by volume and increasing up to 20% and 40% lagoon supernatant by volume on days 4, 17, and 25 respectively. The diluted supernatant was prepared in the same way as for R1, with chemical constituents as per Table 3-5.

Table 3-5. Synthetic feed recipe for R2 per liter. Feed was prepared in 20L batches.

Chemical Name	**g/L**
Sodium acetate (NaAc)	0.938
Sodium propionate (NaPr)	0.209
Ammonium chloride (NH_4Cl)	0.191
Dipotassium phosphate, anhydrous (K_2HPO_4)	0.03
Monopotassium Phosphate, anhydrous (KH_2PO_4)	0.025
Calcium Chloride dihydrate ($CaCl_2 \bullet 2H_2O$)	0.015
Magnesium sulfate heptahydrate ($MgSO_4 \bullet 7H_2O$)	0.0125
Iron sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$)	0.01
Micronutrients	0.5ml/L

3.2.4 DNA extraction

DNA analysis was performed on R1 and R2. R1 was followed consistently from startup with testing every two weeks or every time there was a change in conditions. R2 had two samples taken for comparison.

DNA was collected during the middle of the cycle from the middle port. 1-2mL of sample were then centrifuged at 4,000rpm and the supernatant discarded. Pellets were then stored at -20ºC and extracted in batches. Extraction was conducted using the DNeasy PowerSoil ® DNA Isolation Kits (QIAGEN, Hilden, Germany). The standard DNA extraction process outlined in the kit was followed. DNA concentration and quality were checked using the NanoDrop™ One (ThermoFisher Waltham, MA), then frozen and stored at -20 °C. The DNA was then sequenced using the Illumina Miseq PE250 platform at Genome Quebec (Montréal, QC, Canada).

3.2.5 Performance testing

After the reactor assumed regular operation, testing for NH_4-N, NO_2-N, NO_3-N, PO_4, MLSS, and SVI was conducted every three days. Influent samples were taken directly from the influent tank. Effluent samples were collected during the discharge period of the cycle using midstream effluent. Samples were then filtered using 0.2µm syringe filters to remove solids before running any chemical tests. To determine ammonia concentration in effluent and influent the HACH Nessler reagent method was used (HACH, Germany). NitriVer ® 2 nitrite reagent, NitriVer ® 3 nitrite reagent, TNT 835 nitrate reagent were used according to the respective procedures provided by the company (HACH, Germany). To analyze the samples DR3900 benchtop spectrophotometer was used (DR3900, HACH, Germany). COD content was analyzed using the Standard Methods (APHA et al., 2022). Sludge characteristics, MLSS and SVI were also measured using the Standard Methods (APHA et al., 2018; APHA, 2020). The Axiovert100-Micro Injection and ImageJ microscope and program were used to analyze sludge morphology and granule size. pH levels were also monitored using an electronic pH meter.

3.2.6 Cycle test

A cycle test for R1 was also performed on day 84 of operation to confirm the behaviour of pollutants within the reactor during the cycle. NH_4-N, NO_2-N, and COD were measured beginning with two 15-minute increments and then switching to 30-minute increments using the same methods as outlined above. NO_3-N was measured every 30 minutes.

3.3 Results and Discussion

R1 and R2 reactors were run for 107 and 43 days respectively. R1 was discontinued after 107 days at a lagoon supernatant concentration of 70%, when NH_4-N removal rates plateaued, and the reactor began to appear more stable. R2 was discontinued after only 43 days at a final lagoon supernatant concentration of 40% due to sludge washout caused by malfunction of the feeding process. Due to the lack of feed, R2's granules disintegrated and washed out, resulting in complete sludge loss in the reactor. Due to this event, further analysis of R2 was not possible.

3.3.1 Sludge morphology

R1 was started initially from AS, to best observe the granulation process. To achieve this, sludge morphology was monitored not only via MLSS and SVI but also via visual examination of sludge both with the microscope and naked eye. Because R2 was past the granulation stage with mature AGS dominant, the progression of granulation in this reactor was not monitored. Granulation was first observed in R1 on day 18 with tiny floc-like granules appearing visible to the naked eye, ranging between approximately 0.5mm and 1mm in size (Fig. 3-1a). Further microscopic analysis confirmed this, finding granules up to 790µm long and 480µm across. Microscopic analysis also confirmed the absence of filamentous growth in the reactor. The granules then continued to develop over a period of 34 days. The resulting change in sludge structure was evident with granules appearing larger and more defined on day 52 than on day 18 (Fig. 3-1b). Granules were found to vary largely by size ranging from approx. 0.5mm up to 5mm in diameter and varying in colour, ranging form tan

to light brown (Fig. 3-1b). This was a marked difference from the fine, relatively uniform granules observed on day 18 (Fig. 3-1a).

Sludge was then examined regularly during SVI and MLSS testing. A second microscopic examination was conducted on day 55 due to settling problems observed during SVI measurements. This confirmed the presence of granules but found evidence of the beginnings of filamentous growth. This, along with the settling issues, was found to resolve once the ammonia concentration was increased via the introduction of lagoon supernatant. This type of settling improvement has been previously observed following decreases in COD loading (Liu et al., 2021). As filamentous growth, which is responsible for bulking, is heterotrophic, a decrease in COD and increase in ammonia loading has a negative effect on growth for this type of microorganism (Rosetti et al., 2005). As such, high ammonia loading has been previously suggested as a remedy for bulking in biological systems.

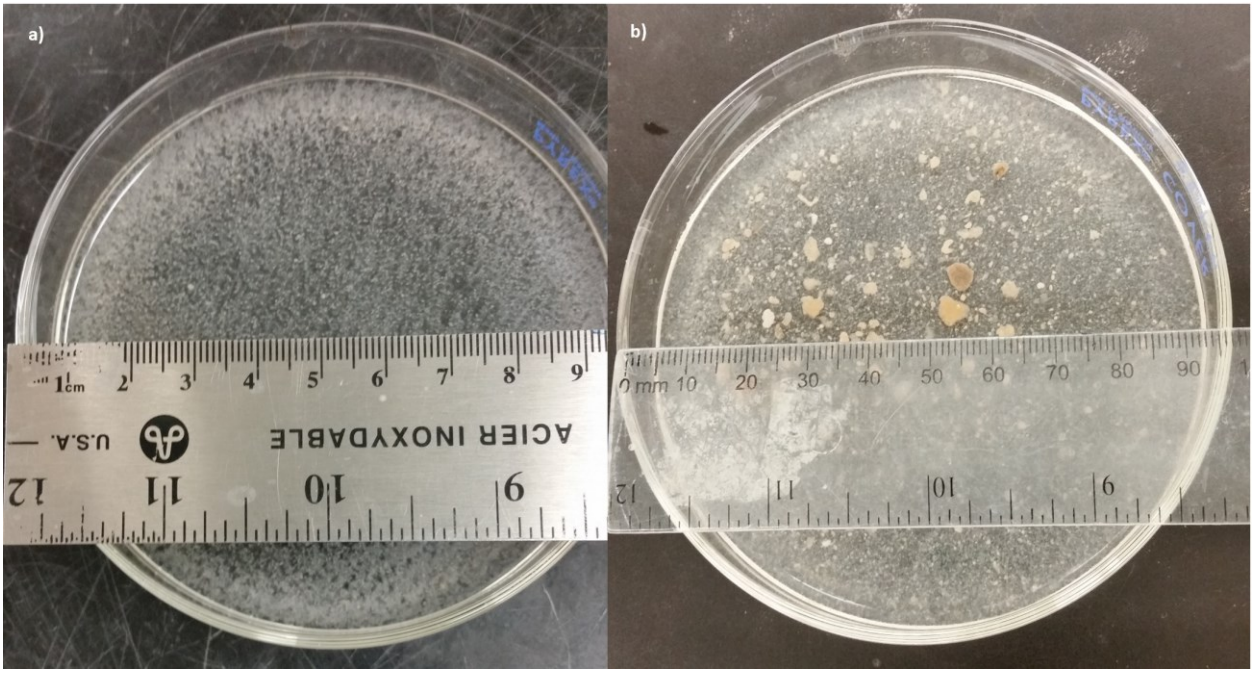

Figure 3-1. Sludge morphology of R1 with a metric ruler for scale showing the various sizes and shapes of the AGS found in the reactor on day 18 of operation (a) versus day 52 (b).

3.3.2 SVI and MLSS

3.3.2.1 R1

SVI and MLSS for R1 remained quite volatile over the period of operation. MLSS was found to decline initially with an increase in both SVI_5 and SVI_{30} during the first 15 days (Fig. 3-2a). This was likely caused by the disturbance to the flocs from the introduction of shear forces caused by aeration. This type of disturbance across settleability and other parameters is often observed after the transfer of seed sludge into an SBR system. In this case, it was likely the result of the disintegration of floc from the shear forces of the bubble aeration. This temporary disintegration of floc would result in an increase in planktonic bacteria which could then be washed out of the reactor. As EPS producing mechanisms were activated, more solid aggregates would begin to form, resulting in a lowered SVI and increased settleability over time. This was evident as MLSS continued to decline but was accompanied by increased settleability as seen in figure 4-2. A rapid increase in biomass was then observed and was accompanied by a drop to the lowest SVI_{30} value observed at 61.9mL/g (Fig. 3-8). By day 34, MLSS had reached 7.4g/L and stayed around this number before beginning to fall dramatically by day 46 (Fig. 3-2). While SVI took some time to be affected, increasing around day 52 (Fig. 3-2). This decrease in MLSS and increase in SVI was correlated with findings of filamentous growth discussed in the previous section which is responsible for reduced settling and the resultant loss of sludge during the discharge phase. The introduction of lagoon supernatant appeared to somewhat remediate the issue, with an immediate rise in MLSS following day 55. SVI decreases followed shortly after, as filamentous growth was reduced. Biomass of the reactor was observed to peak on day 89 at 8.26g/L and SVI_{30} of 92.01mL/g after the introduction of 20% lagoon supernatant feed (Fig. 3-2b). Decrease in SVI values and

increases in MLSS concentration were also found to correlate with increases in lagoon supernatant, likely due to the loss of filamentous growth caused by the decrease in bioavailable COD. The increase in influent concentrations of NH_4-N was not thought to have been the likely cause of the decreases in MLSS as recovery in biomass concentration was observed even after each increase.

The positive impact of increases in ammonia loading may be seen clearly in Figure 3-2a. As NH_4-N was increased over time the average SVI_{30} got progressively lower, balancing out at approximately 100ml/g after an increase to 40% lagoon supernatant. This is representative of a healthy AGS system. While even lower SVI may be achieved, it is generally accepted that anything below 100mL/g is acceptable for AGS systems. As this was achieved by R1, it may be concluded that AGS development was successful in this reactor. While SVI may be used as a measure of granulation, it is also a good early indicator for unwanted microbial growth in the reactor as well. If increasing SVI is accompanied by a decrease in MLSS, it is a good indicator of some sort of problem in the reactor. While not a diagnostic measure in and of itself, it may be effectively used as an early warning system for sludge health in AGS systems.

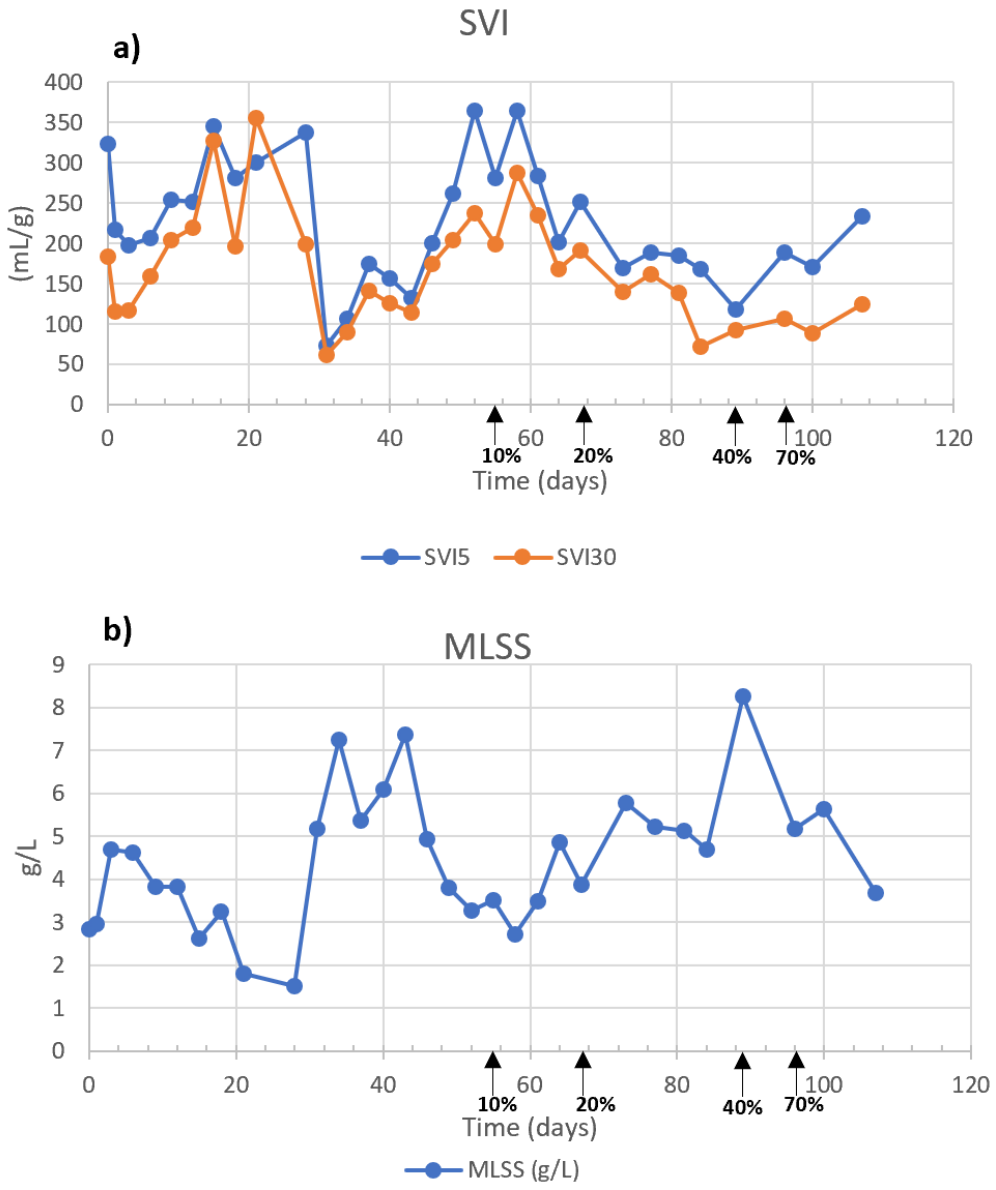

Figure 3-2. MLSS (a) and SVI distribution over the course of operation of R1. Both parameters were measured on average every three days and both SVI_5 and SVI_{30} (b) was recorded.

3.3.2.2 R2

MLSS for R2 was relatively stable, at approximately 5g/L after an initial fall in biomass to 4.17g/L on day 3 of operation (Fig. 3-3b). MLSS remained stable all through the introduction of both 10% and 20% lagoon supernatant concentration increases, only falling

dramatically to 3.3g/L after the introduction of 40% lagoon supernatant on day 25 (Fig. 3-3b). SVI_{30} was observed to be relatively stable as well ranging 70-75mL/g. This is representative of healthy AGS consistent with numbers found in literature (Shi et al., 2011). A dramatic reduction in settleability was observed on day 9, 5 days after the introduction of 10% lagoon supernatant at 170.12mL/g, before falling to 72.16mL/g on day 13 (Fig. 3-3a). At this point, SVI_{30} was observed to remain around 73mL/g until it began rising again on day 32, 7 days after the introduction of 40% lagoon supernatant concentration (Fig. 3-3a). This phenomenon was somewhat similar to what was observed in R1, which showed increases in SVI after introduction of 70% lagoon supernatant (Fig. 3-2). It is possible that this reduction in settleability was tied in part to the increase in ammonia concentration, which has also been observed in other studies (Yang et al., 2004). As higher ammonia concentrations inhibit granulation this would result in looser sludge, leading to reduced settleability which would appear as increased SVI. This would then lead to increased sludge washout and subsequent biomass reduction observed in the reactors. As R2 showed increased sensitivity to increases in ammonia loading, this was likely the reason that SVI increase was observed earlier than in R1. However, while present, the increase in SVI was not overly dramatic, and did not exceed acceptable values for AGS systems. Therefore, it is likely that R2 would have recovered its settling performance had premature termination of operation not occurred.

Despite this, R2 showed much more sensitivity to increases in ammonia loading compared to R1. Where R1 has a relatively upward trend in biomass concentration during the increases in ammonia loading, R2 exhibited the opposite. This was surprising as it is generally accepted that mature AGS tends to be more resilient to changes in environmental conditions.

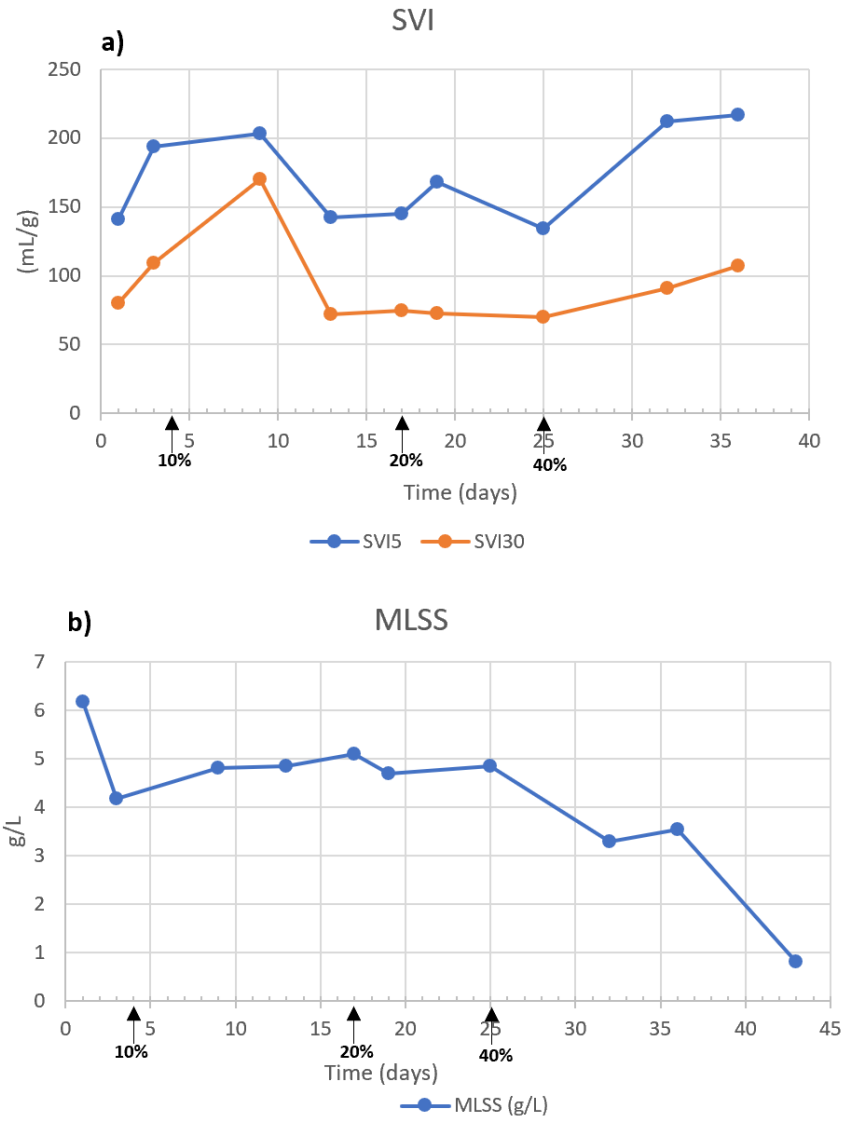

Figure 3-3. MLSS (a) and SVI (b) following the progression of R2 over the course of lagoon supernatant introduction.

3.3.3 NH_4-N Removal

3.3.3.1 R1

In terms of NH_4-N removal, R1 took approximately 34 days to completely stabilize. A decreasing trend in performance was observed up until day 21 of operation after which

performance began to drastically improve (Fig. 3-4). NH_4-N removal then remained relatively stable for the rest of the synthetic feed phase with removal ranging between 90% and 100% (Fig. 3-4). The decrease in performance observed before day 21 was most likely due to the shock experienced by the sludge after being transferred to a radically different environment. As AS, which is usually operated under much lower shear forces, was used for seed, the introduction of the physical SBR environment would likely have a large effect on the microbial community. It was accompanied by a drop in biomass concentration as well (Fig. 3-2). This is a phenomenon that tends to be relatively common during reactor start-up before the microbial community becomes adjusted to new conditions and may be observed over many different measurements (Wei et al., 2014). It is generally a result of selection pressures causing sludge washout, as larger flocs and aggregates are conserved and planktonic bacteria are lost during discharge. This may include any planktonic AOB which would be reflected in the decreasing NH_4-N removal efficiency. As time progresses, however, removal efficiency tends to recover with the healthy establishment of AGS and the subsequent enrichment of AOB within granules. This was seen in R1 in the recovery of NH_4-N removal, an increased MLSS, and reduced SVI.

It was observed that the introduction of increased concentrations of lagoon supernatant had an impact on NH_4-N removal, usually within 4 days of the lagoon supernatant increase (Fig. 3-4). This was most likely due to the delay in growth of AOB organisms which have a relatively slow growth rate (Munz et al., 2011). Because of this, NH_4-N removal efficiency tended to fall after increases in ammonia loading before AOB growth caught up with the increase in available substrate for consumption.

The level of effect that lagoon supernatant had on NH_4-N removal varied depending on the amount increased, with concentrations of 10% and 70% being the most affected (Fig. 3-4). The reason for these two anomalies is likely different. The introduction of 10% lagoon supernatant was the first exposure R1 had to natural wastewater of any kind, after being grown exclusively on synthetic wastewater. In synthetic feed, most nutrients were added to not only simulate wastewater but to make nutrients bioavailable. Other constituents of lagoon supernatant that are non-bioavailable or outright harmful to the microbial community are difficult to simulate and were not added to synthetic feed. This, coupled with a 156% increase in NH_4-N most likely contributed to the reduction in removal efficiency experienced during the first lagoon supernatant addition. The last jump in concentration to 70% was also notable as it was the largest increase in ammonia loading attempted with an increase in NH_4-N of 186% above the previous influent concentration. Therefore, it was likely the cause of this removal efficiency drop as every reactor has a maximum removal capacity. If the maximum capacity of R1 was being reached, a complete removal in 2.25 hours was no longer possible when increased concentrations were introduced. To remedy this, an increase in cycle length could potentially be an immediate solution to this problem, but this option could not be explored due to time constraints. It is also necessary to note that these two concentrations also showed the longest recovery period with the 70% concentration only recovering to 70% NH_4-N removal (Fig. 3-4). This reduction in ammonia removal efficiency was found to be comparable to previous findings, which recorded over 80% removal in high strength wastewater (Zou et al., 2022b). However, this study was conducted in an IFAS-SBR, which had differing sludge morphology due to the addition of a biofilm substrate. In addition, biomass in this study was also higher likely accounting for increased removal efficiency.

Other studies have also developed similar levels of ammonia removal from high strength wastewater (Yu et al., 2014). However, while Yu et al. (2014) utilized slightly higher ammonia concentrations, the MLSS of their reactors was almost 20-fold that of R1. As such, it may be inferred that had biomass concentration been higher, ammonia removal efficiency would have been higher as well, exceeding previously recorded values. This suggests that the implementation of this system was highly successful.

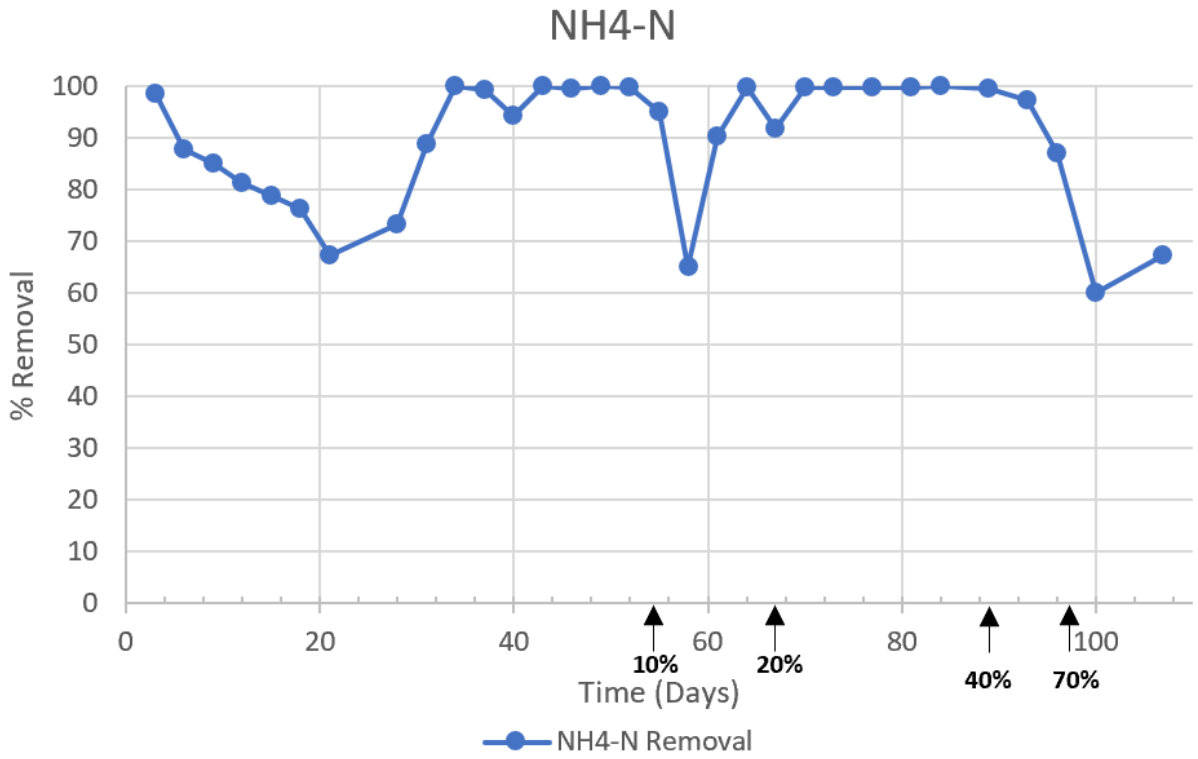

Figure 3-4. NH_4-N removal over the course of R1 operation. Lagoon supernatant addition of 10%, 20%, 40%, and 70% was implemented on days 55, 67, 89, and 96 respectively.

3.3.3.2 R2

Research on R2 began after startup was complete, and initial development of R2 was therefore not recorded. Since the reactor was active for several months before use in this experiment, time was calculated with day 0 being the time of reactor acquisition. As R2 was

initially used for phosphorus recovery research before being acquired for this experiment, the exact date of initial operation was not certain. While some differences in feed did exist between R1 and R2, namely in terms of nutrient concentration, this reactor was chosen for having the closest parameters to R1 out of the reactors available. The full establishment of a reactor with identical conditions to R1 was not possible due to time constraints on this study.

Initial operation resulted in NH_4-N removal rates of 100% with a dip to 84% on day 6 of operation following the introduction of 10% lagoon supernatant (Fig. 3-5). A similar reduction in removal efficiency was observed after the introduction of 20% lagoon supernatant, consistent with the findings in R1 (Fig. 3-4; Fig. 3-5). R2 was found to be more sensitive to changes in ammonia concentration than R1. Three days after the increase of lagoon supernatant concentration to 40%, ammonia removal dropped to approximately 70% efficiency where it stabilized (Fig. 3-5). Recovery above 70% was not observed. Increased sensitivity in R2 was likely caused by the reactor's low removal capacity resulting from minimal NH_4-N loading during initial operation.

Seven days were required for reactor recovery during both periods, suggesting that this reactor was more sensitive to disruption in the form of lagoon supernatant addition as compared to R1. However, differences between the synthetic feed of the two reactors must also be noted. The synthetic feed concentration of all nutrients for R2 was 50% lower than the concentration in synthetic feed for R1. Therefore, it is possible that R2's microbial community was more adapted to a lowered NH_4-N concentration, resulting in a lowered capacity to metabolize high concentrations of the compound. The introduction of 40% lagoon supernatant resulted in a drop in removal efficiency to 68.4% with rates recovering only to 72% and then

remaining stable. This suggests that R2 was significantly less adaptable than R1 which managed to recover to levels above 90% ammonia removal relatively quickly after the introduction of 40% lagoon supernatant. Based on ammonia concentration, this would place R2 at a similar level of performance as other reactors found in common literature (Yu et al., 2014). While it was initially thought R2 would have an advantage in ammonia removal efficiency, this was not case, likely due to its initial low ammonia loading. While this was taken into consideration, R2 was not as adaptable to NH_4-N concentration as was initially thought. This suggests that ammonia exposure during start-up does play a role in the removal efficiency of a reactor, and that start-up conditions play a larger role than sludge morphology in increasing the adaptability of AGS to increased loading.

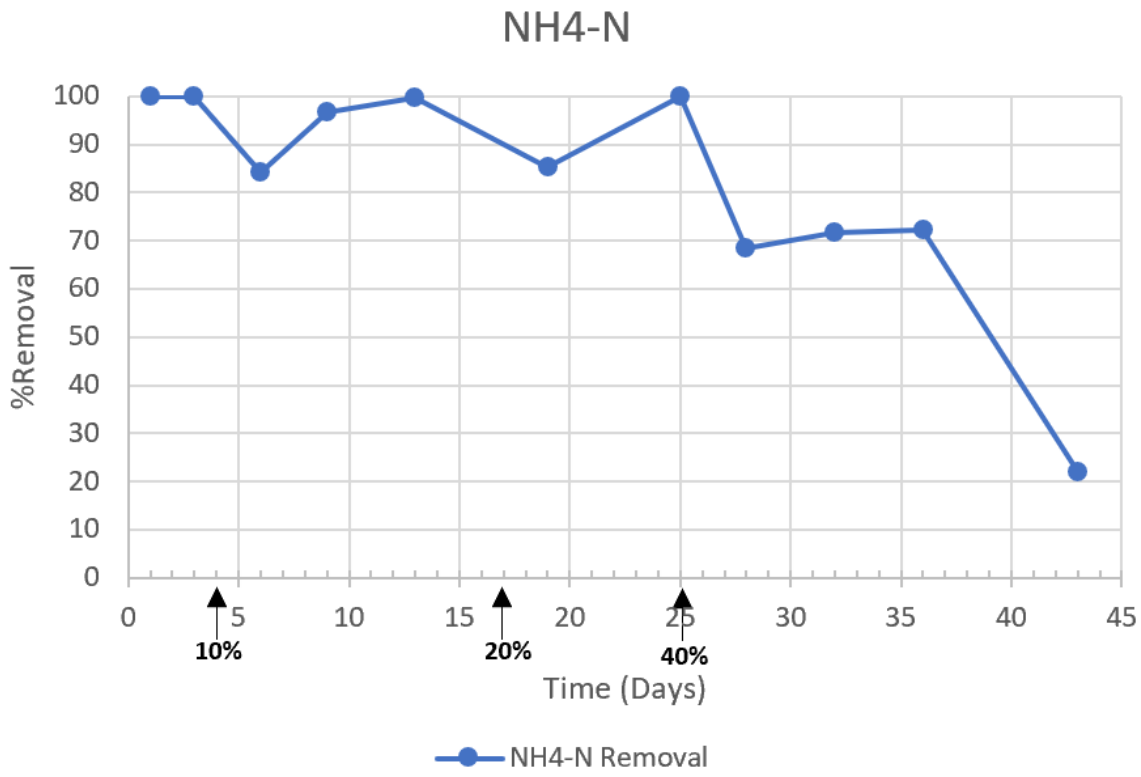

Figure 3-5. NH_4-N removal efficiency over the course of operation of R2. Lagoon supernatant concentration was increased to 10%, 20%, and 40% on days 4, 17, and 25 respectively.

Overall, it was observed that the introduction of lagoon supernatant was instrumental in the temporary disruption of NH_4-N removal efficiency in both R1 and R2. This was likely due to the shock that the system experienced every time this happened. NH_4-N removal in AGS systems is dependent on biological organisms. These in turn are sensitive to their environment and drastic changes are known to cause stress to the overall ecological system, resulting in possible shock to organisms. This is one reason why changes in pollutant loading may cause lowered removal efficiency. The second reason for related to the steep increases in NH_4-N that accompanied the introduction of lagoon supernatant and the subsequent impact on the F/M ratio. The F/M ratio is representative of the amount of substrate a microbial community is capable of metabolizing. Most bacterial systems will grow and aggregate until the ultimate F/M ratio is reached and then plateau. Only after an increase in substrate occurs, such as an increase in ammonia loading, will these organisms begin reproducing. However, due to the slow growth rates of some microorganisms it takes time for this to happen. Before biomass catches up with available substrate, an increase in nutrient wash-out will be observed. However, as R1 showed a lower biomass concentration than is typical for AGS systems, lowered biomass may also have contributed to the lowering of NH_4-N capacity.

While both reactors followed this pattern, R1 showed more adaptability than R2 over the long run, especially at higher NH_4-N concentrations. This suggests that establishment of reactors on purely synthetic feed is not as beneficial as the introduction of industrial wastewaters, during granulation and the start-up period. As a result, it is important to stress the need for higher ammonia concentrations during the start-up period of reactor development to ensure a stable reactor operation.

3.3.4 NO_2-N and NO_3-N

3.3.4.1 R1

NO_2-N and NO_3-N were both measured to monitor the behaviour of nitrification/denitrification and nitritation/denitritation in the reactor. NO_2-N remained relatively stable under 5mg/L up until day 61, after which NO_2-N concentration began rising (Fig. 3-6). The large increase in NO_2-N effluent concentration was correlated with the introduction of lagoon supernatant (Fig. 3-6). Since the concentration of NO_2-N was well below influent ammonia levels, and NO_3-N levels were negligible, this suggested the presence of denitritation activity.

This assumption could be made because the conversion of NH_4-N to NO_2-N is a 1:1 ratio (Metcalf & Eddy, 2014). Therefore, either nitrification or denitritation was occurring during the cycle period. While it was not confirmed which of these processes was responsible for the reduction in NO_2-N over the period of the cycle, sludge lifting was observed multiple times during the settling phase suggesting at least some denitritation activity (Adav et al., 2009). The introduction of lagoon supernatant was found to have catalyzed a large increase in NO_2-N due to the rapid increase in NH_4-N concentration. As the influent concentration of NH_4-N increased, so did AOB activity and thus the concentration of NO_2-N. As a result, the limited anoxic settling period was likely no longer long enough to accommodate the denitritation of all the available NO_2-N. This then resulted in NO_2-N accumulation within the reactor, leading to the exponential increase in effluent concentration observed in figure 3-6.

A small reduction in NO_2-N concentration was observed three days after the introduction of 20% lagoon supernatant and four days after the introduction of 40% lagoon

supernatant (Fig. 3-6). As NH_4-N removal efficiency also dropped during this time, it was likely a direct result of lowered AOB activity.

NO_3-N remained relatively stable at an average concentration of 1.12mg/L. This suggested a low level of NOB in the system. As denitritation activity was an asset in this study, this was considered a good step towards the development of a partial nitrification reactor. Levels of NO_3-N began to increase after the introduction of 20% lagoon supernatant, peaking at 44.4mg/L on day 100, four days after the introduction of 70% lagoon supernatant (Fig. 3-6). This instance was however isolated and may have been related to several factors including the presence of NO_3-N in the feed. It was considered highly unusual as an increase in NH_4-N is generally associated with NOB inhibition (Kim et al., 2015). After this peak, the concentration began to decrease again to a final measured concentration of 10.5mg/L on day 107 (Fig. 3-6). The small concentration of NO_3-N in the reactor is suggestive of either the presence of simultaneous nitrification/denitrification or partial nitrification. However, as nitrification/denitrification systems are generally characterized by low NO_2-N levels, partial nitrification was more likely the dominant system as according to the observed data.

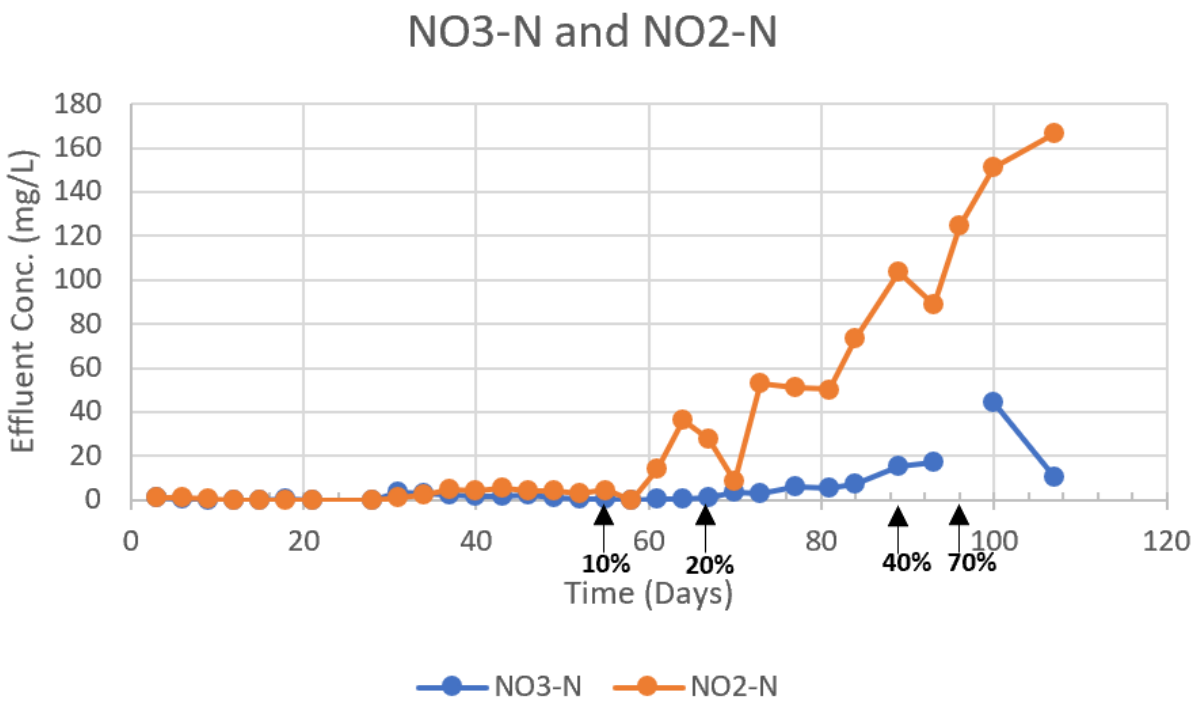

Figure 3-6. NO_3-N and NO_2-N effluent concentrations over the operation of R1.

3.3.4.2 R2

NO_2-N and NO_3-N were both relatively stable during synthetic feeding, only increasing significantly after the introduction of lagoon supernatant. NO_2-N was found to be no higher than 0.125mg/L over the course of synthetic feed, then increasing up to 19.2mg/L after the introduction of 10% lagoon supernatant (Fig. 3-7). A subsequent increase was observed 2 days after the introduction of 20% lagoon supernatant at 31.4mg/L and falling 1.2mg/L on day 25 (Fig. 3-7). A regular fluctuation in NO_2-N levels was observed where, after the introduction of lagoon supernatant, NO_2-N concentration would fluctuate between 2mg/L or lower and 25mg/L and higher (Fig. 3-7). This occurred in a steady pattern and lacked the exponential increase expected if partial nitrification was taking place. Peak concentration was reached 11 days after the introduction of 40% lagoon supernatant feed at 50.6mg/L, before declining again at day 43 (Fig. 3-4). While during synthetic feeding NH_4-N

concentration was approximately half of that for R, NO_2-N accumulation was quite low suggesting that the nitritation/denitritation was not working properly.

It was also found that NO_2-N concentration was consistently lower than NO_3-N concentration, a notable difference from R1, and was indicative of NOB activity (Metcalf & Eddy, 2014). NO_3-N concentration was stable during the synthetic feed phase, fluctuating between 5mg/L and 7mg/L, suggesting the presence of successful denitrification (Fig. 3-7). After the introduction of lagoon supernatant into the system, NO_3-N concentration increased linearly up until reaching a peak of 141mg/L on day 25 (Fig. 3-7). As NO_3-N was the dominant N species, NOB were likely well enriched in in R2. However, while an increase in NOB was evident, simultaneous nitrification/denitrification was not. This was evidenced by the accumulation of NO_3-N in R2 as NH_4-N levels increased.

After lagoon supernatant concentration was increased to 40%, NO_3-N levels began to fall, following a similar pattern to that seen for NO_2-N (Fig. 3-7). While such decrease may be attributed to the development of denitrifiers in the system, the accompanying fall in removal efficiency of NH_4-N (Fig. 3-5) was in this case the more likely cause.

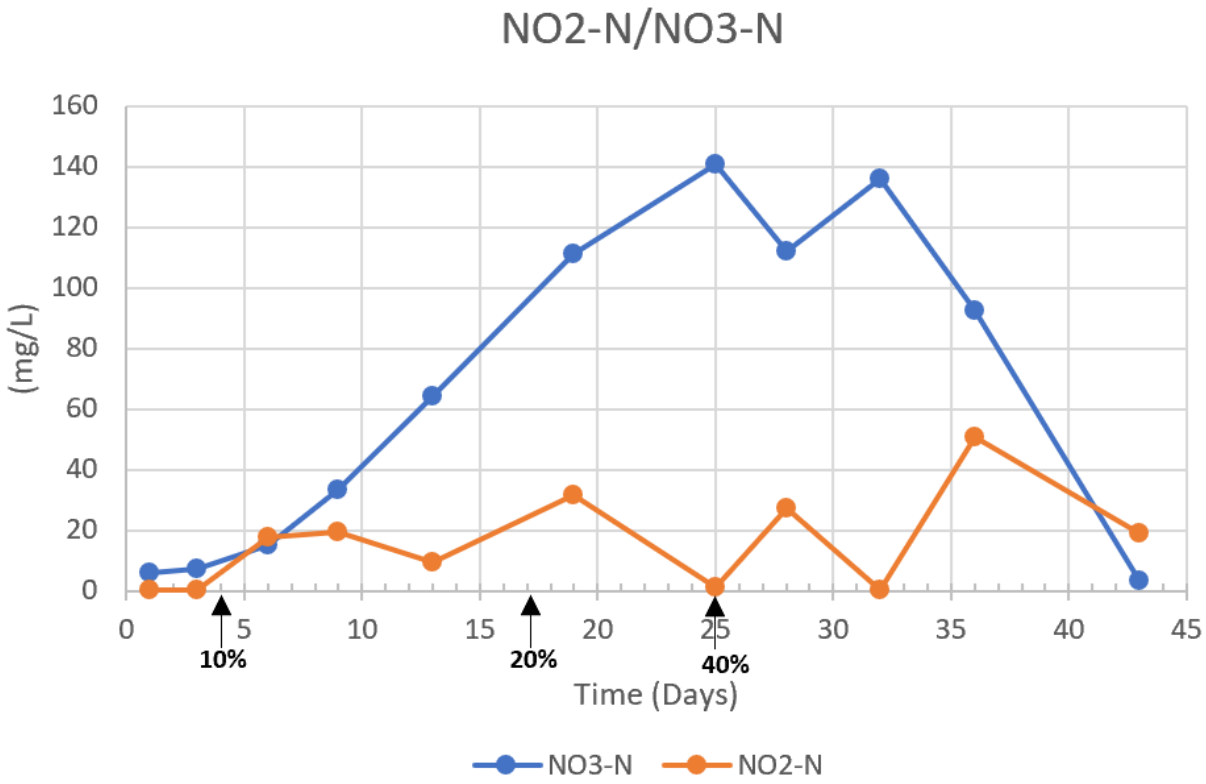

Figure 3-7. NO_2-N and NO_3-N concentration in mg/L duringR2 operation. Lagoon supernatant was introduced on days 4, 17, 25 at concentrations of 10%, 20%, and 40% respectively.

3.3.5 COD

3.3.5.1 R1

COD concentration was monitored since the beginning of start-up with initial removal rates of 97% on day 3, improving up to 100% removal on day 12 (Fig 3-8). A small dip in removal efficiency was seen on day 18, with removal decreasing to 96.9%, but recovering by day 28 (Fig. 3-8). From this day onward, COD removal was found to be between 99 and 100% until the introduction of lagoon supernatant on day 55 (Fig. 3-8). A steady decline in both influent COD and COD removal efficiency was observed with the introduction of lagoon supernatant. This was a direct result of the low C/N ratio of lagoon supernatant. With the

introduction of 20% lagoon supernatant a dip in efficiency to 78.15% was observed, with brief recovery to 83.5% before falling to 31.6% (Fig. 3-8). A small recovery was seen after the introduction of 40% lagoon supernatant before effluent COD concentration began to increase exponentially (Fig. 3-8). COD removal efficiency reached a final value of 17% after the introduction of 70% lagoon supernatant.

Very high COD removal efficiency was observed in R1 during synthetic feed use mainly due to the fact that all COD added was bioavailable. Only NaAc and NaPr were provided as COD sources and could therefore be readily metabolized. When lagoon supernatant was added to the feed, COD composition was changed. As most real wastewater sources have biologically unavailable COD, this would explain the drop in removal efficiency observed after lagoon supernatant addition. Since the analysis method used did not differentiate between total and bioavailable COD, an artificial reduction in removal efficiency would be observed. To further confirm this finding, further testing of COD would be necessary, with a differentiation between bioavailable and non-bioavailable COD.

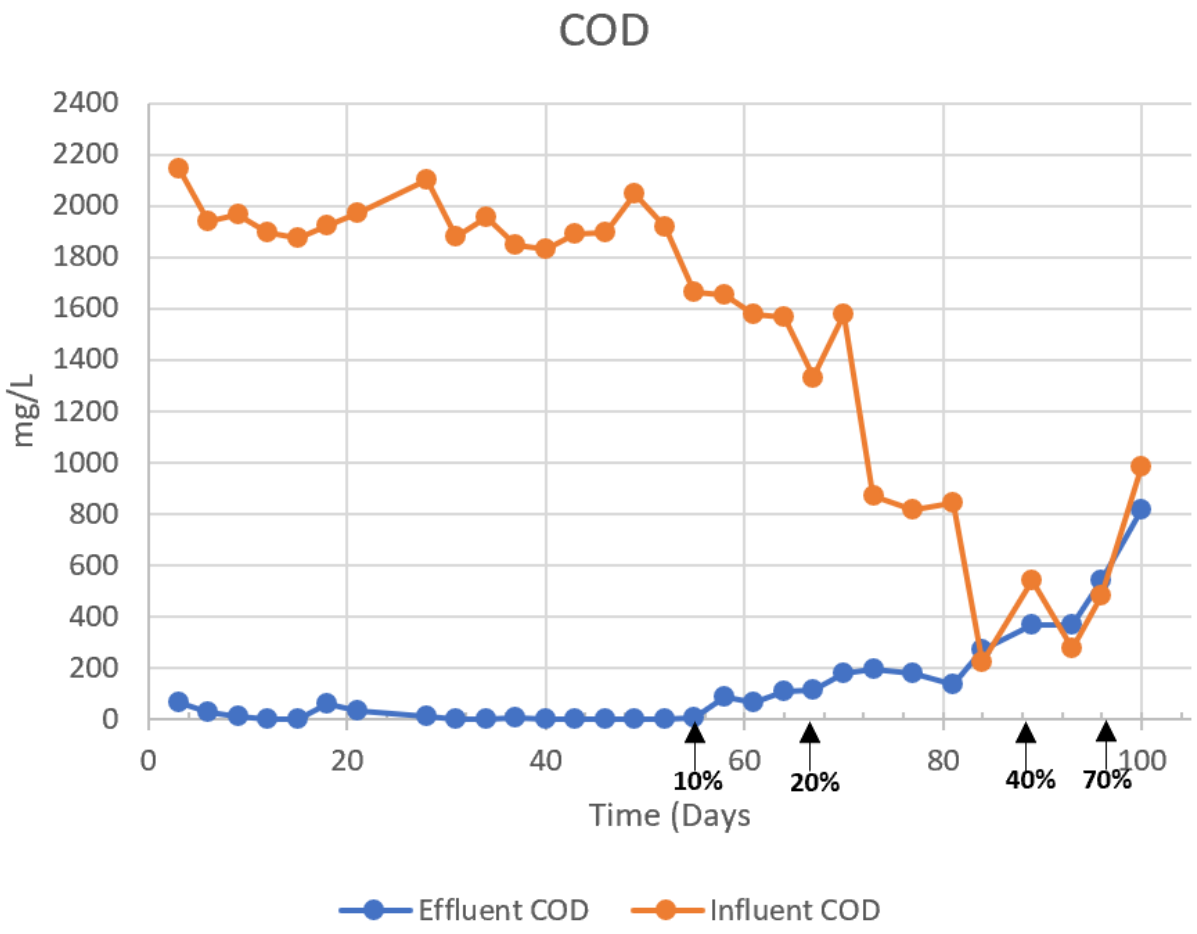

Figure 3-8. COD removal from R1 during operation. The timepoints at which lagoon supernatant concentration was increased were also marked.

3.3.5.2 R2

COD removal for R2 was found to be stable at 100% on synthetic feed, similarly to R1 (Fig. 3-9). The introduction of 10% lagoon supernatant resulted in a slight reduction in removal efficiency before recovering to 100% (Fig. 3-9). For R2, it was found that overall influent COD concentrations were quite volatile over the course of treatment, most likely affecting removal efficiency (Fig. 3-9). As lagoon supernatant was introduced, effluent COD concentrations began to increase in a linear manner, similarly to R1. After this point, removal efficiency began to degrade, reaching a final minimum of 16.4% on day 25 (Fig. 3-9). This

was consistent with what was found in R1 after the introduction of lagoon supernatant. The linear fashion of increase in effluent COD concentration was thought to have been caused by the accumulation of non-bioavailable COD in the reactor. After the introduction of 40% lagoon supernatant the COD removal efficiency began to increase. This was likely due to fact that influent COD concentration was quite volatile while effluent COD concentration was more stable. This would create the impression that COD removal had increased, even though effluent COD concentration was higher than before.

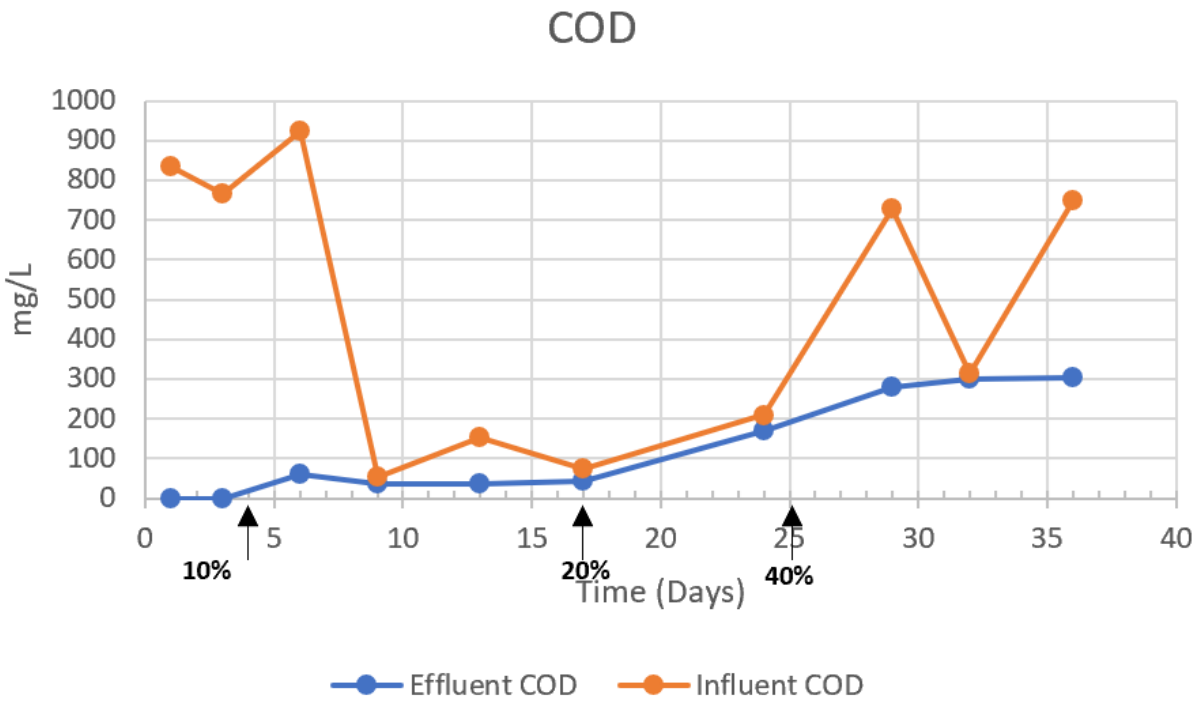

Figure 3-9. COD removal rates of R2 over the course of experimental operation. Lagoon supernatant increases were 10%, 20%, and 40% on days 4, 17, and 25 respectively.

3.3.6 Cycle test

A cycle test was conducted on day 84 of operation when lagoon supernatant was at 20%. It was found that the removal of NH_4-N was relatively linear, reaching 94% removal by

the end point of the aeration phase (Fig. 3-10a). NH_4-N was seen to rapidly increase during feeding up to a maximum concentration of 108mg/L (Fig. 3-10a).

The decrease of NH_4-N is also mirrored in the corresponding rise in NO_2-N concentration (Fig. 3-10b). Notably, an 88% decrease in NO_2-N concentration was observed over the feeding phase before increasing during aeration (Fig. 3-10b). This reduction in concentration would suggest the presence of denitritation. As the hour-long feeding phase was anoxic, and influent facilitated circulation, the feeding phase provided ideal conditions for denitritation. This trend was completely reversed during aeration as NO_2-N began to accumulate, following the conventional pattern observed in a nitritation/denitritation system (Yang et al., 2003).

While NO_3-N concentration was initially low, an increase was observed beginning at 30 minutes into operation with a peak of 8.67mg/L at the 2hr. mark (Fig. 3-10d) This indicated that NOB activity was present but minimal (Fig. 3-10d). The fact that simultaneous nitrification/denitrification was not responsible for this phenomenon was supported by the lack of NO_3-N accumulation during aeration, with a subsequent drop in concentration during anoxic feeding and settling, likely due to present denitrifiers (Davies et al., 1989). As such, majority of NH_4-N removal may be attributed to partial nitrification. After reaching the 2hr. mark, NO_3-N levels began to decrease to a final concentration of 7.5mg/L, suggesting the presence of some, though insignificant, denitrification activity (Fig. 3-10d). COD levels fluctuated but did eventually fall with a final removal of 25%, and a maximum removal of 45.7% (Fig. 3-10c). Although an increase in COD levels was observed after the 1.5hr mark, this phenomenon may have been a result of the conversion of insoluble COD to soluble COD.

As only soluble COD was measured, it was not possible to confirm this theory. However, as the lagoon supernatant used in this study was relatively high in solids, this was likely the case.

Overall, the cycle test demonstrates the effectiveness of the 4hr cycle and the presence of the desired phenomena, namely NH_4-N oxidation during aeration, and denitritation during the anoxic phases. Small amounts of NH_4-N oxidation were also observed during feeding and settling, however, these amounts accounted for less than 10% of the NH_4-N oxidized in each period. This suggests the adequacy of the cycle length for this concentration, especially when total N removal is not required. However, NO_2-N accumulation must also be noted. As a reduction in NO_2-N was evident during the feeding phase, denitritation was likely present. Therefore, enriching denitritation by modifying the cycle to include an anoxic phase would likely aid in the reduction of effluent TIN while simultaneously restoring system alkalinity.

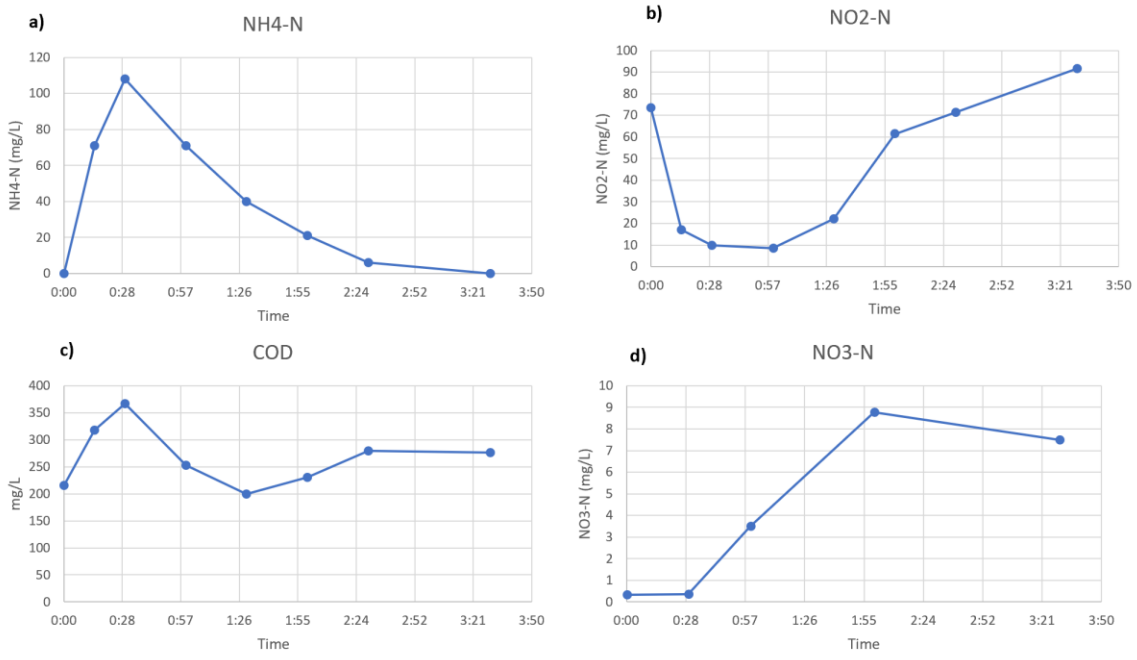

Figure 3-10. Concentration of N species and COD during a single cycle of R1. NH_4-N (a), NO_2-N (b), COD (c), and NO_3-N (d) were measured either by 15-minute or 30-minute intervals.

3.3.7 DNA analysis

3.3.7.1 R1

The microbial community of sludge is responsible for the efficiency observed in a reactor. NA analysis was performed on R1 over the course of its operation. α-Proteobacteria was the most dominant group for majority of operation while the AS used to inoculate R1 was mainly populated by Actinobacteria of the genus *Corynebacterium* (Fig. 3-11). A shift was observed on day 10 of operation with α-Proteobacteria of the genera *Paracoccus* and *Caulobacterales* becoming the dominant types during this time. As *Paracoccus* are a genus responsible for denitrification, these numbers are expected, as denitrification is integral to the NH_4-N removal pathway in AS (Bergast et al., 2010; Dai et al., 2022). The introduction of lagoon supernatant on day 55 was accompanied by the enrichment of Actinobacteria that continued over the course of lagoon supernatant addition. By day 96, the main dominant species had shifted to approximately evenly distributed Actinobacteria and β-proteobacteria. While archaea were also found to be present, all were of the methanogen variety and as such, were not likely instrumental in the biological N removal process.

NOB were confirmed to be present in the form of *Nitrobacter* spp. However, this type was only confirmed at relative frequencies of less than 0.5%, confirming the negligible role in ammonia removal observed across other parameters (Fig. 3-11).

The presence of AOB was also confirmed, with the most abundant genus being *Nitrosomonas*. As expected, enrichment levels were shown to rise over time with the largest increase occurring after the introduction of 20% lagoon supernatant. Relative frequency of *Nitrosomonas* peaked on day 96 at 1.4% coinciding with the introduction of 70% lagoon

supernatant. Interestingly, abundance of all AOB species was found to have fallen on day 89. Among AOB, *Nitrosococcus* species were also present, but were observed at very low levels beginning on day 96. This is likely due to the conditions in the reactor, which was observed to have operated at pH of 7.5 or higher. As *Paracoccus* spp. prefer lower pH environments, this was likely the reason they were not significantly enriched (Fumasoli et al., 2017). Longer testing time would have to be implemented to confirm whether these species were a consistent part of the microbial community and whether they would be enriched given enough time and an increased NH_4-N concentration. Other unidentified members of the family *Nitrosomonadaceae* were also found after day 81 of operation. While they were not identified at the genus level, their pattern of enrichment and classification suggests that they were also AOB (Prosser et al., 2014). Levels of AOB found in R1 were very low compared to literature values as *Nitrosomonas* has usually been observed at a minimum 4% relative frequency in high strength ammonia treatment reactors (Kim & Seo, 2006; Song et al., 2013; Zhao et al., 2023). However, enrichment levels in lower strength wastewater have been observed closer to 1% (Nguyen Quoc et al., 2021) This is congruent with what was found in this study and could therefore explain the lowered ammonia removal efficiency observed at higher NH_4-N concentrations. While very unusual, this may be due to the morphology of the seed sludge used. As no enriched inoculum was added, the AOB population had to be enriched from the lagoon supernatant and AS value, which both tend to be low in AOB. As these organisms are slow growing, it is possible that enrichment would take longer to reach ideal levels.

The presence of filamentous bacteria was also confirmed with 16S analysis determining the presence of various filamentous species from the family *Caldilineaceae* including the genus *Caldilinea*. The filamentous *Caldilineaceae* were quite abundant in the

system comprising approximately 1.3% frequency on day 81, a comparable number to the relative frequency of AOB. As *Caldilineaceae* are known to be relatively ubiquitous in AS WWTP systems, this was quite predictable for a reactor inoculated with AS, such as R1 (Yoon et al., 2010). Filamentous bacteria from the family *Microthixaceae* were also observed on day 68, though at very low levels. This family is generally associated with bulking in biological wastewater systems and as such, a complete absence of these species is preferred (Rosetti et al., 2005). As some bulking was observed in R1, it is possible to suggest that these organisms were a likely cause.

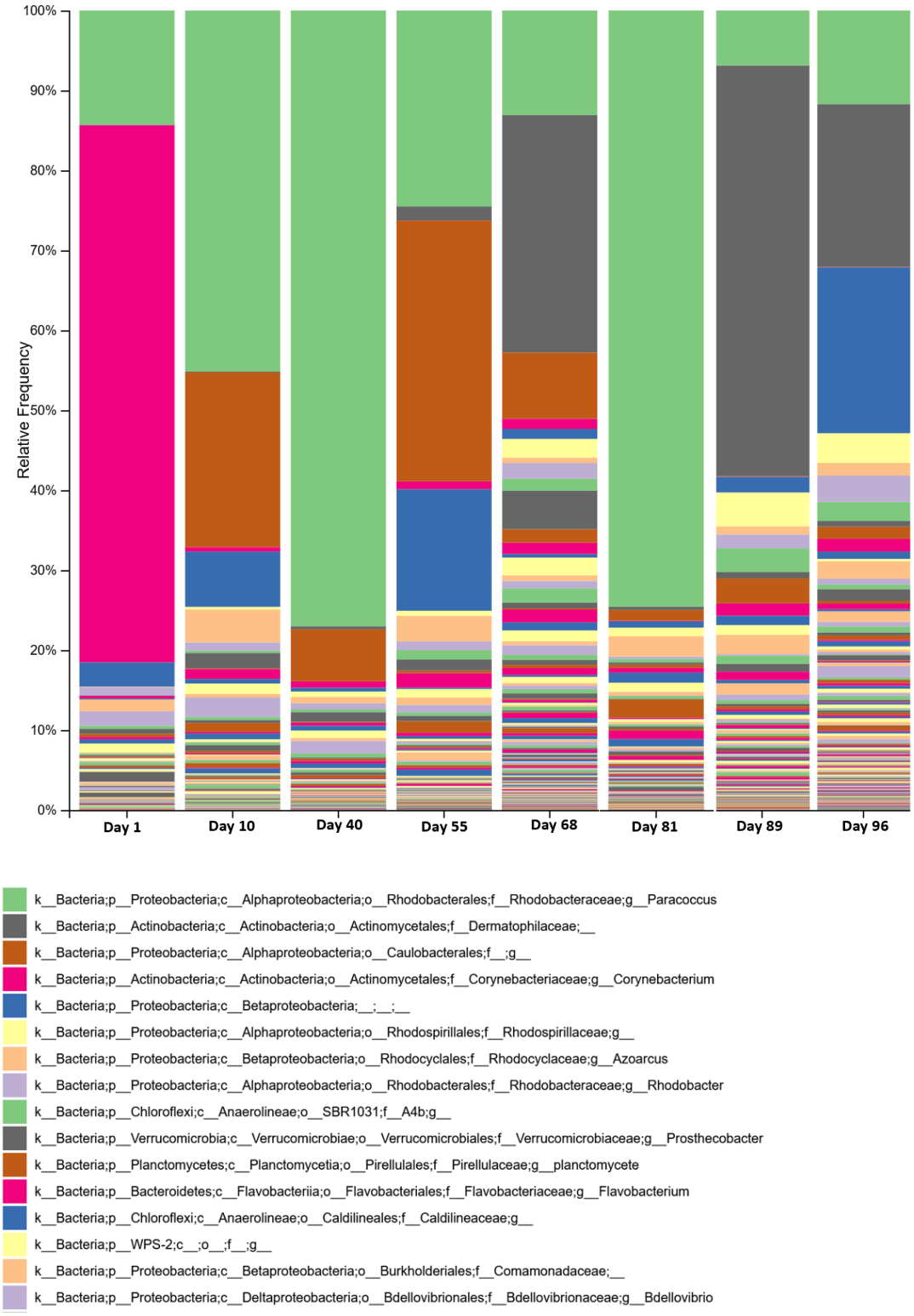

Figure 3-11. Relative frequency of bacterial strains at the genus level during the operation of R1. 16S rRNA sequencing was used to analyze microbial community changes during R1 operation.

3.3.7.2 R2

R2 had a microbial analysis done at two timepoints, day 4 and day 26 of operation (Fig. 3-12). The initial dominant genus was *Paracoccus*. On day 4, it had a 52% relative frequency, which then fell significantly by day 26 to 11%. This phenomenon was likely caused by the addition of lagoon supernatant. In addition, it was found that while *Nitrobacter* was present on day 26 but was not significantly enriched. This was contradictory to the high levels of NO_3-N in the effluent which suggested high NOB activity (Fig. 3-7). However, other NOB species were also not observed. As such, the fall in abundance of denitrifier species was most likely the cause of the increase in effluent NO_3-N around day 26.

AOB were found to be enriched in R2, though not to the extent of R1. (Fig. 3-12). *Nitrosomonas* was the most common AOB identified in the system with other unidentified *Nitrosomonadaceae* genera present. However, the relative frequencies were far below literature reported levels even for low strength wastewater (Nguyen Quoc et al., 2021;Zhao et al., 2023). *Nitrosococcus* was not present in this reactor. This lower AOB abundance is supportive of the lowered NH_4-N removal found in R2 as compared to R1.

Levels of filamentous bacteria were found to have increased with time in R2 with *Caldilineaceae* genera appearing at a 2.4% frequency on day 4, which was found to be the highest concentration in both R1 and R2. While other genera of the family *Caldilineaceae* were found to decrease over time in R2, the genus *Caldilinea* was found to increase in frequency. This finding was also contrary to the relative frequency of *Microthrixaceae* which, though not very abundant, decreased in frequency. The increase in *Caldilinea* was found to be very interesting as it occurred after the introduction of lagoon supernatant. This is contrary to the common theory that filamentous growth tends to be inhibited by high ammonia

concentrations (Yoon et al., 2010). The reduction in relative frequency of *Microthrixaceae* was also interesting, as *Microthrixaceae* do tend to thrive under higher ammonia concentrations (Rosetti et al., 2005). However, it has also been found these microorganisms are inhibited by aerobic systems which likely occurred here (Gabb et al., 1991). Therefore, as an increase in *Caldilinea* was observed in both R1 and R2, it may be suggested that *Caldilinea* may have been introduced into the system via lagoon supernatant.

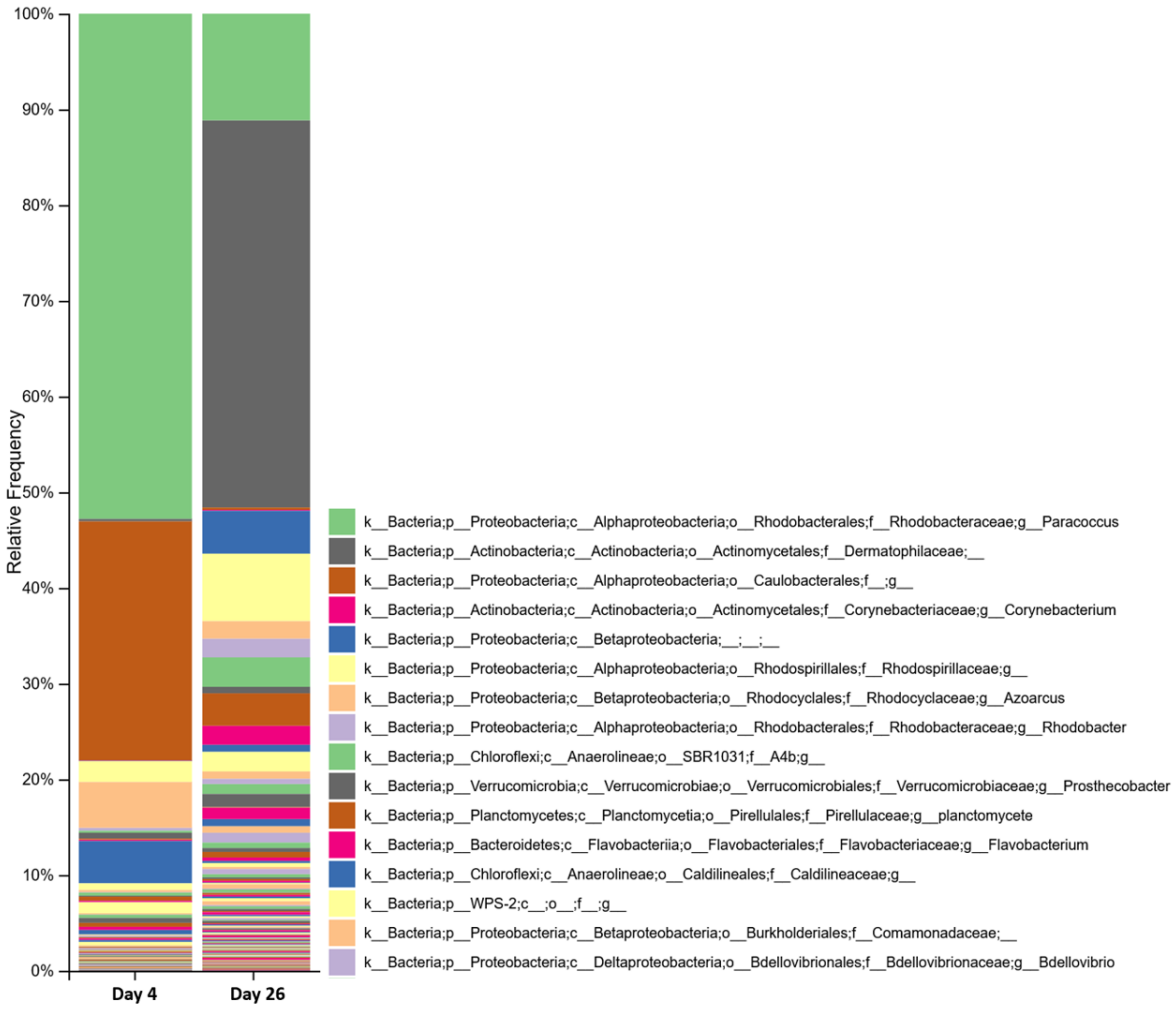

Figure 3-12. Relative distribution of bacteria in R2 over the course of operation. Samples were analyzed using 16S sequencing to determine bacterial community distribution.

3.4 Conclusion

Overall, it was found that R1 had a higher abundance of AOB than R2 as well as better overall performance. R1 was also more resilient to change and increase in ammonia loading compared to R2. The stabilization time of both reactors was however relatively similar in relation to lagoon supernatant concentration increases.

It should be noted that R2 did show more instability in relation to NH_4-N removal in the face of higher nutrient load. This could be due to several factors, most importantly initial feed conditions. While R1 was started for the purpose of NH_4-N removal, R2 was an established AGS reactor with a focus on phosphate removal and recovery. As such, the ammonia loading of its synthetic wastewater was much lower, reducing AOB enrichment. As R2 was not initially primed for the removal of NH_4-N it had a subsequent lowered removal capacity. This reactor also appeared to have a higher level of nitrification that was exacerbated by the introduction of lagoon supernatant. As it operated at relatively low ammonia levels initially, the growth of NOB would have been supported (Yao & Peng, 2017). Since the end purpose of this study was denitritation development, this presence of full nitrification was problematic. It is therefore suggested that the introduction of high-strength wastewater during the granulation process is preferable to the introduction of wastewater after the complete establishment of AGS.

Overall, this stage of the study was quite successful. Based on the collected data, te may be concluded that, for reactors treating increased ammonia loading, high concentrations of ammonia be introduced during the start-up phase as this is correlated to both an increase in AOB abundance and reactor stability over time.

4 Pilot Reactor Establishment and Operation in Calgary, Alberta

4.1 Introduction

For technology to be useful, especially in the environmental field, it must be scalable to industrial levels. Efficient and viable laboratory systems must be subsequently tested on pilot and industrial scales. While industrial AGS SBR reactors have been used in wastewater treatment before, the use of this system for ammonia removal via partial nitrification has not yet been successfully attempted on the pilot or industrial scale. As such, it was the objective of this chapter to test and fine-tune an AGS SBR on a pilot scale. The study was divided into three sub-objectives a) to develop a partial nitrification system with the aid of a combination aerobic/anoxic AGS system, b) to test different seeding strategies for the establishment of an effective and stable pilot reactor, and c) to reduce the HRT to minimum optimal levels. To achieve this, two 20L pilot reactors were established at a large-scale WWTP in central Alberta.

4.2 Methods

4.2.1 Reactor Set Up

Two 20L reactors, PR1 and PR2, were used in this study. The reactors were both 150cm tall with a 15cm diameter (Fig. 4-1). The discharge ports were located at 62cm for PR1 and 43cm for PR2. This resulted in a VER of 39.2% and 56.9% for PR1 and PR2 respectively. A flow rate of 1.6m/s was used for aeration and was normalized to 8L/min for reactors of this size.

PR1 was started first and used to test one set of conditions as well as to troubleshoot initial problems with the system. PR2 was introduced after PR1 was stabilized and one reduction in HRT was achieved.

Figure 4-1. PR1 with added sludge during first stages of operation. The reactor was seeded with AS and nitritation/denitritation AGS from a lab-scale system.

4.2.2 Reactor Operation

PR1 was operated on three HRTs, starting with a total HRT of 24hrs with 8 sub-cycles consisting of an aeration phase of 2hrs, and an anoxic phase of 1hr (Table 4-1). HRT was then reduced to 12hrs with 4 subcycles consisting of 2hr aeration in subcycle 1 and subsequently

alternating 1.5hrs of aeration and 1.5hrs of anoxic mixing (Table 4-2). The last HRT reduction resulted in an 8hr cycle with 3 subcycles with the same configuration as those in the initial 24hr HRT (Table 4-3). A pulse-feeding method was adopted for both reactors with influent feeding times of approx. 10 and 3 minutes for PR1 and PR2 respectively. COD was added through pulse feeding over the first 10 minutes of every anoxic cycle. COD addition was periodically discontinued to address the overgrowth of filamentous bacteria.

PR1 was inoculated with AS from the same large-scale central Alberta WWTP where it was operated, with additional granular sludge from an established lab scale partial nitrification AGS system introduced several days later. PR2 was inoculated using a combination of the lab-scale AGS used in PR1, dehydrated AGS granules from a previous pilot operation, and anaerobic UASB granules obtained from an industry partner.

Table 4-1. First operation schedule for R1. This was used until the cycle test conducted on day 28 of operation.

Subcycle		Aeration	Recirculation	COD addition		Settling	Decanting	Feeding
1	On	12:00	14:00	14:00	On	11:00	11:30	11:50
	Off	13:59	15:00	14:10	Off	11:30	11:35	11:57
2	On	15:00	17:00	17:00				
	Off	16:59	18:00	17:10				
3	On	18:00	20:00	20:00				
	Off	19:59	21:00	20:10				
4	On	21:00	23:00	23:00				
	Off	22:59	0:00	23:10				
5	On	0:00	2:00	2:00				
	Off	1:59	3:00	2:10				
6	On	3:00	5:00	5:00				
	Off	4:59	6:00	5:10				
7	On	6:00	8:00	8:00				
	Off	7:59	9:00	8:10				
8	On	9:00	-	-				
	Off	10:59	-	-				

Table 4-2. Second cycle used for PR1 and PR2. This 12hr cycle was run from day 28 to day 103 for R1 and days 1-59 of operation.

Cycle	Subcycle		Aeration	Recirculation	COD addition		Settling	Decanting	Feeding
1	**1**	On	12:00	14:00	14:00	On	11:00	11:30	11:50
		Off	14:00	15:30	14:10	Off	11:30	11:35	11:57
	2	On	15:30	17:00	17:00				
		Off	17:00	18:30	17:10				
	3	On	18:30	20:00	20:00				
		Off	20:00	21:30	20:10				
	4	On	21:30	-	-				
		Off	23:00	-	-				
2	**1**	On	0:00	2:00	2:00	On	23:00	23:30	23:50
		Off	2:00	3:30	2:10	Off	23:30	23:35	23:57
	2	On	3:30	5:00	5:00				
		Off	5:00	6:30	5:10				
	3	On	6:30	8:00	8:00				
		Off	8:00	9:30	8:10				
	4	On	9:30	-	-				
		Off	11:00	-	-				

Table 4-3. The third operation cycle used. This was used on both reactors from days 103 and 59 for PR1 and PR2 respectively.

Cycle	Subcycle		Aeration	Recirculation	COD addition	
1	**1**	On	12:00	14:00	14:00	
		Off	13:59	15:00	14:10	
	2	On	15:00	17:00	17:00	
		Off	16:59	18:00	17:10	
	3	On	18:00	-	-	
		Off	19:00	-	-	
2	**1**	On	20:00	22:00	22:00	
		Off	21:59	23:00	22:10	
	2	On	23:00	1:00	1:00	
		Off	0:59	2:00	1:10	
	3	On	2:00	-	-	
		Off	3:00	-	-	
3	**1**	On	4:00	6:00	6:00	
		Off	5:59	7:00	6:10	
	2	On	7:00	9:00	9:00	
		Off	8:59	10:00	9:10	
	3	On	10:00	-	-	
		Off	11:00	-	-	
			Settling	**Decanting**	**Feeding PR1**	**Feeding PR2**
1		On	19:00	19:30	11:47	11:57
		Off	19:30	19:35	12:00	12:00
2		On	3:00	3:30	19:47	19:57
		Off	3:30	3:35	20:00	20:00
3		On	11:00	11:30	3:47	3:57
		Off	11:30	11:35	4:00	4:00

4.2.3 Feed

The feed for PR1 and PR2 was the same. Both were fed with centrate from anaerobic digester treating biosolids sourced from a full-scale municipal WWTP in central Alberta. Centrate was taken directly from the centrifuges and stored in large totes before use. For the

first several weeks of operation only partially diluted centrate could be obtained with NH_4-N concentrations ranging from 300-500mg/L. NH_4-N concentration then increased to 800-900mg/L around day 52 and 39 of operation for PR1 and PR2 respectively. Due to foaming issues, the antifoaming agent Flofoam WB45 (Flofoam) from SNF was added right before feeding. This was done to prevent the antifoam from degrading. Antifoaming Agent TRANS48 was also tested. Flofoam was added at the concentration of 0.375ml/L. Alkalinity was added to the feed in the form of $NaHCO_3$ ranging in concentration between 1g/L and 4g/L to aid in the adjustment of pH. The need to supplement alkalinity was decided using effluent pH.

4.2.4 Performance testing

Testing for NH_4-N, NO_2-N, and NO_3-N was conducted according to the methods described in chapter 3 (p. 43) using the DR1900 portable spectrophotometer form Hach (Hach, 2023). COD, MLSS, and SVI were also analyzed according to the methods outlined in Ch. 3 (p. 43). COD and the organic N tests were conducted ever 1-3 days. pH was measured daily using a portable pH meter model ST10 from Ohaus (Ohaus, 2023).

MLSS and SVI testing was conducted every 1-2 weeks. All tests were done on site at the WWTP with the exception of MLSS which was measured using external equipment and laboratory facilities according to the methods described in Chapter 3 (p. 43). AGS formation was analyzed by the naked eye and monitored via SVI and MLSS.

4.2.5 Cycle tests

Several cycle tests were conducted on days 27 and 96 for PR1. Samples were taken at the beginning of feeding, the end of aeration, end of COD addition, end of anoxic periods of

each of the eight subcycles, and from effluent for Cycle test 1. Samples were taken at the beginning of feeding, end of COD addition, and end of anoxic period for all subsequent cycle tests. Cycle tests were conducted for the whole cycle as opposed to separate subcycles.

Samples taken during the subcycle were then allowed to settle, and the clear supernatant was tested for levels of NH_4-N, NO_2-N, NO_3-N, COD, and pH. Excess samples taken were then added back into the reactor during testing to prevent volume loss over the testing period.

4.2.6 Activity Tests

Two activity tests were performed on the sludge from PR1, on days 54 and 117 of operation to determine which processes were present in the reactor. These tests were run to determine the presence NOB, denitritation, and denitrification activity on day 54 and denitritation activity only on day 117.

The sludge was obtained from the reactor and transported to Edmonton, Alberta for testing. It was packed with ice for transportation and tested on the same day.

The sludge was aerated for 1 hour to remove remnant COD and MLSS was taken. A measure of sludge was then taken to ensure an MLSS of 4g/L in each serum bottle, one for nitritation (AOB), nitrification (NOB), and an MLSS of 8g/L for denitritation and denitrification bottles. All bottles were purged with nitrogen gas for 10 minutes before being sealed with rubber plugs and aluminum caps.

NOB tests were conducted in 160ml bottles and contained 30ml of mixed liquor. For the NOB activity test, 200mg/L NO_2-N (as sodium nitrite) and 1400mg $CaCO_3$/L (as sodium bicarbonate) was added and the pH of both bottles was adjusted to a pH of 7.5-7.8. The serum

bottles were then purged with nitrogen gas for 10 minutes. The active agents (NH_4 and NO_2) were added in the form of a standard solution via syringe just before the commencement of the test. The serum bottles were then shaken at 160rpm for 1hr with samples taken every 15 minutes and immediately filtered through 0.45µm syringe filters. The samples were then tested for the presence of NO_3-N for the NOB tests. The NOB activity was calculated by the slope of the linear regression of the curve of NO_3-N over time.

Denitritation and denitrification were measured also over the period of one hour in 120ml Serum bottles containing 80ml of mixed liquor. 2800mg $CaCO_3$/L alkalinity (as sodium bicarbonate) was added to the denitritation bottle and 1400mg$CaCO_3$/L alkalinity (as sodium bicarbonate) was added to the bottles which were then adjusted to a pH of 7.5-7.8. The liquid was then purged with nitrogen gas for 5 minutes and sealed with a rubber stopper and metal cap. 200mg/L NO_2-N and 100mg/L NO_3-N were then injected into the denitritation and denitrification bottles respectively. A carbon source in the form of anhydrous sodium acetate was then injected into the bottles to provide a COD/N ration of 10, for the purpose of facilitating denitritation and denitrification. The samples were then shaken at 180rpm at room temperature with samples at 10 minutes intervals for a total test time of 50 minutes. Slopes of linear regression of NO_2-N and NO_3-N reduction over 15 minutes were used to determine the denitritation and denitrification rates respectively.

4.3 Results and Discussion

The operation of the 20L pilot reactors was only mildly successful by the study's conclusion. This was due to a variety of factors regarding both logistical and technical limitations such as pump failures and malfunctions as well as limited access to laboratory

space. Because of this, it was difficult to determine early signs of dysfunction in the reactor which may have contributed to the problems the reactors experienced. Problems in regard to equipment function were also a factor. As some equipment used was repurposed from laboratory scale this was a somewhat expected disrupting factor. In addition, mixing difficulties experienced were also suspected to be an up-scaling problem and new hardware was obtained to mitigate this. However, Overall, while operation was found to be functional it was found that further testing is needed to fine-tune operation on the larger scale.

4.3.1 Reactor Morphology

PR1 was the first reactor started, consisting of a mix of AS and granules from an established laboratory nitritation/denitritation AGS system. The first issue that had to be addressed was foaming caused by surfactants present in the centrate. The concentration of these surfactants was not possible to determine and was present in varying levels depending on the date, season, and other factors. As such, it was necessary to add antifoaming agents to the centrate before treatment to prevent sludge loss due to foaming. It was determined that silicone based antifoaming agents were effective while oil based antifoaming agents were not. Of the silicone based, the most effective was determined to be Flofoam. While one other silicone antifoam agent was tested, required larger doses to be effective and was thus discontinued.

4.3.1.1 PR1

PR1 was highly effective since the beginning of start-up and began showing granulation early on. During this time, MLSS was also observed to have increased to and then fluctuated around 5g/L (Fig. 4-2a). A large sludge washout occurred on day 20, where PR1

lost approx. 9.5L of sludge due to a mechanical malfunction of the discharge valve (Fig. 4-2). The sludge was recovered and returned to the reactor with no significant effect on either MLSS or SVI. During this initial period, SVI_{30} remained below 100g/L. MLSS began to see a decrease around day 53, with an accompanying increase in SVI (Fig. 4-2b). This likely resulted in the subsequent biomass loss observed (Fig. 4-2a). Higher concentration centrate was also introduced at this time possibly disrupting the system, and inhibiting granulation.

While PR1 had relatively high performance by all other measures, it was not successful in developing denitritation. This was determined to likely have been caused by the inadequate strength of the peristaltic pumps used for recirculation during the anoxic phase. Additionally, the required tubing used for these recirculation pumps had a high rate of breakdown requiring replacement every 3-5 days. While some denitritation was present, as evidenced by activity tests and observed sludge lifting, high effluent NO_2-N concentration and lack of recovered alkalinity suggested a dysfunction in the denitritation system. To attempt to combat this, stronger diaphragm pumps were implemented around day 70. However, the action of the pumps was found to be destructive to AGS granules, resulting in sludge disintegration and subsequent washout (Fig. 4-2). After discovering this, the diaphragm pumps were discontinued, and UASB granular sludge was added to the reactor to increase biomass from the resultant 1.33g/L concentration (Fig. 4-2a).

During this time, PR1 also began to show signs of filamentous growth, as observed in resistance to settling and resulting spike in SVI observed (Fig. 4-2b). As high COD has been observed to impede granulation and settling a COD limitation strategy was implemented and COD addition during the anoxic phase was discontinued (Tay et al., 2003). While this did

result in significantly better settling in the short-term, once COD was reintroduced the settling problems returned relatively quickly, suggesting this to not be a viable long-term solution.

While PR1 was originally relatively stable from a sludge morphology point of view, it was highly sensitive to interventions. This was likely due to the fact that biomass stability was affected by hardware problems experienced, specifically related to the feeding apparatus which was found to be somewhat unreliable. This suggests that diaphragm pumps are not a viable option for larger systems. It is therefore suggested that further research utilize pumps with a higher reliability. The adaptation of peristaltic pumps for recirculation in higher volume reactors is also suggested in order to aid in recirculation without granule disintegration.

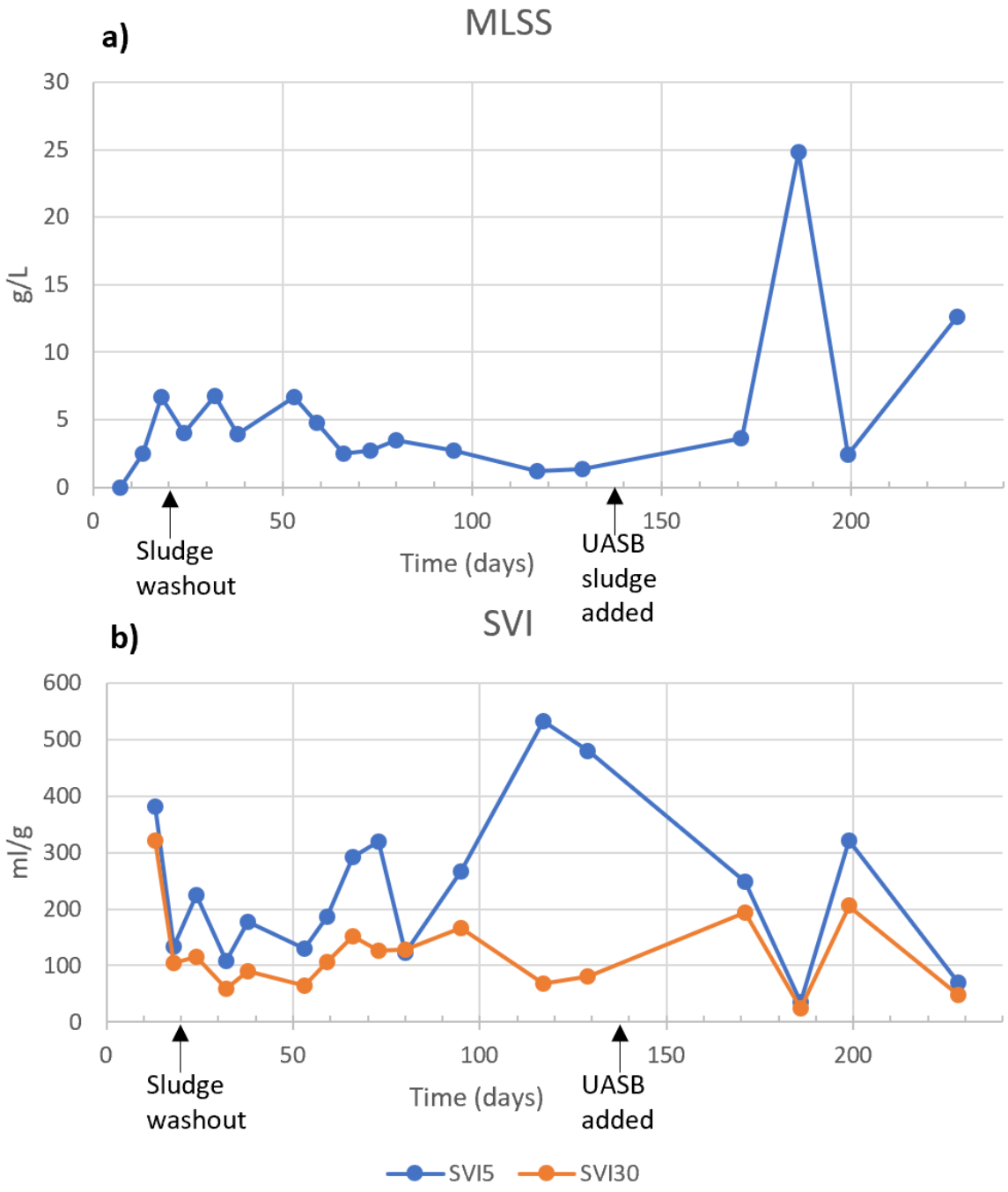

Figure 4-2. MLSS (a) and SVI (b) of PR1 during operation. A large sludge washout on day 20 and the addition of UASB granules on day 138 were suspected of affecting these measures.

4.3.1.2 PR2

PR2 was inoculated with UASB granules obtained from a reactor treating high COD effluent and was run for a shorter time than PR1. It was found that during operation this reactor was much less stable than PR1, in both chemical performance and sludge morphology. While the granular structure of the anaerobic seed was conducive to a low initial SVI and high MLSS, the system showed signs of granule disintegration during the aerobic process that resulted in a drastic reduction in biomass to just 8g/L (Fig. 4-3). The biomass then remained relatively stable at approximately 7.5g/L for about a week (Fig. 4-3a). The next drop is likely to have been caused by the implementation of the diaphragm pump for recirculation as was done in PR1. This resulted in a further drop in MLSS combined with a corresponding increase in SVI (Fig. 4-3). Once the biomass began to drop, pulse recirculation was implemented to attempt to mitigate damage to the granules while retaining recirculation. However, this strategy was found to be only partially effective at preventing granule disintegration (Fig. 4-3a).

It was interesting to note the smooth curve of both SVI and MLSS, in PR2. This relatively stable and smooth curve found in PR2 was significantly different to what was observed in PR1 (Fig. 4-2). This was initially thought to be a result of inoculation with stable granules which facilitated early good settling, but the subsequent increase in SVI suggests that while UASB granules are effective at producing good settling initially, they are susceptible to disintegration and increased SVI as operation continues. As such, this high settling capability is not sustainable. This was also seen in PR1 after the addition of UASB sludge, further confirming that settling increases from UASB inoculum are temporary. However, due to the logistical constraints of this study, settling and biomass morphology could not be observed as

closely as necessary to make the reliable conclusion that this increase in SVI was caused by seed sludge type alone. As such, it is necessary to observe this phenomenon further, with greater scrutiny on the biomass concentration and SVI.

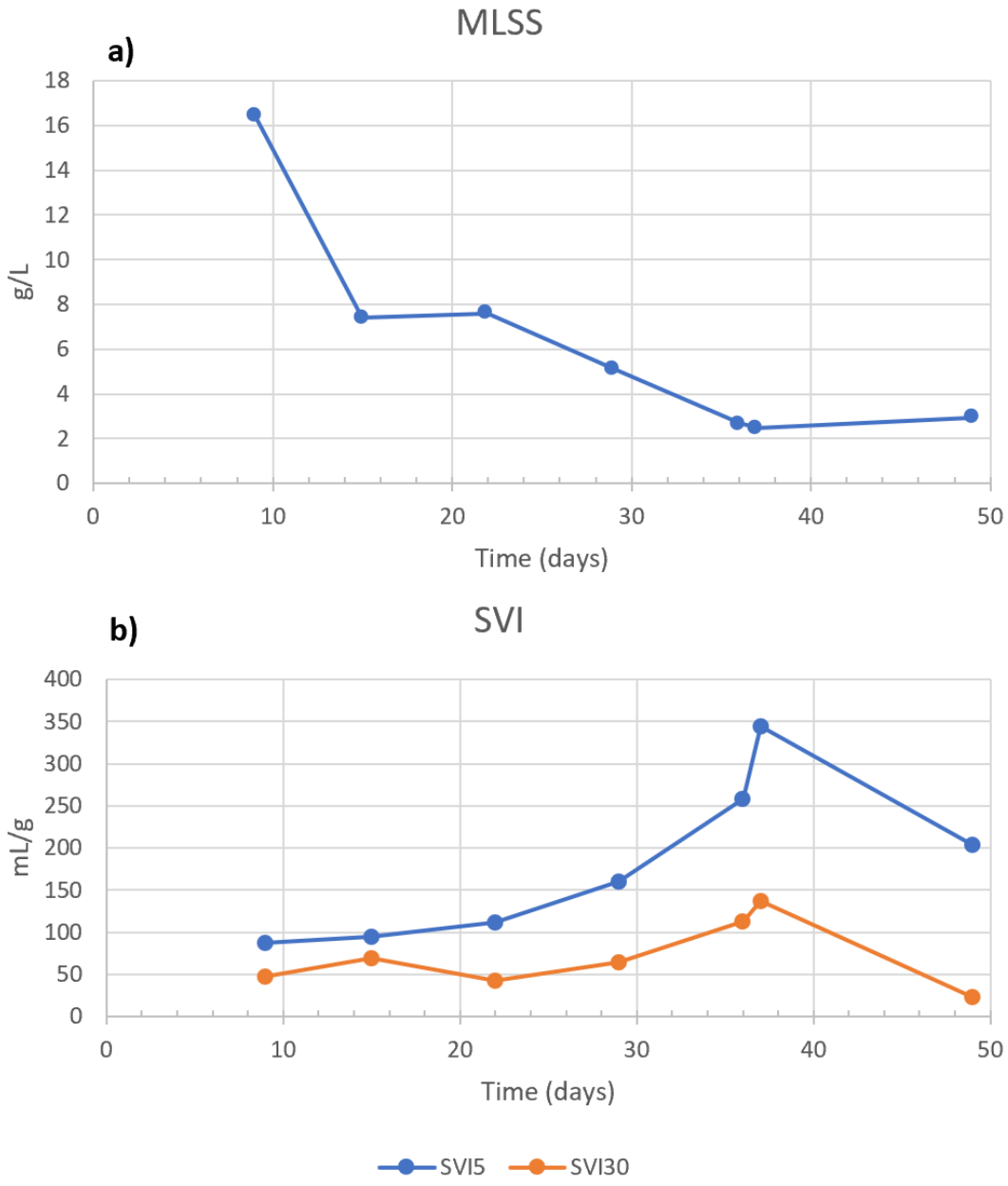

Figure 4-3. MLSS (a) and SVI (b) of PR2 over the course of operation. Both measurements were taken 1-2 weeks apart and plotted over time.

4.3.2 NH_4-N

4.3.2.1 PR1

In terms of NH_4-N removal, PR1 performed quite well, reaching 100% NH_4-N removal in the first 4 days (Fig. 4-4). It was then stable in the 95-100% for the following 40 days. The efficiency then fell slightly hovering at approximately 85-95% with an overall mean removal efficiency of 91.4% (Fig. 4-4).

This drop in reactor efficiency may be attributed to several different factors, though is most likely related at least in part to the change in NH_4-N concentration of the centrate. While initially the reactor was fed with semi-diluted centrate with an NH_4-N concentration of approximately 300-400mg/L NH_4-N, this was switched to undiluted centrate with an NH_4-N concentration ranging between 800 and 900mg/L. Combined with the loss of sludge mentioned in the previous section, this is likely what caused the slight reduction in efficiency over time. As the sludge was having problems, AOB could not develop. In addition, the increase in NH_4-N in the centrate was determined to have been sufficient for the drop in removal efficiency overall.

Despite this small dip, PR1 was considered to have performed incredibly well, especially with the number of disturbances inflicted upon the system. PR1 had a myriad of technical problems, especially regarding feeding and sludge loss, which were not found to affect NH_4-N removal in any significant way. Removal efficiency was also not affected by sludge washout following the sludge disintegration and wasting caused by the installation of the diaphragm pump. While biomass was incredibly low at this point, PR1 still maintained extremely high levels of removal not commonly seen in literature focused on high ammonia

loading (Kim & Seo, 2006; Song et al., 2013). This is indicative of the stability and adaptability of this system even at high pollutant loading conditions. Because of this, it is possible to infer that reactors seeded with AS at the pilot scale are very stable and can maintain removal efficiency in extremely adverse conditions. This in turn would make this type of set-up attractive for use on the industrial scale.

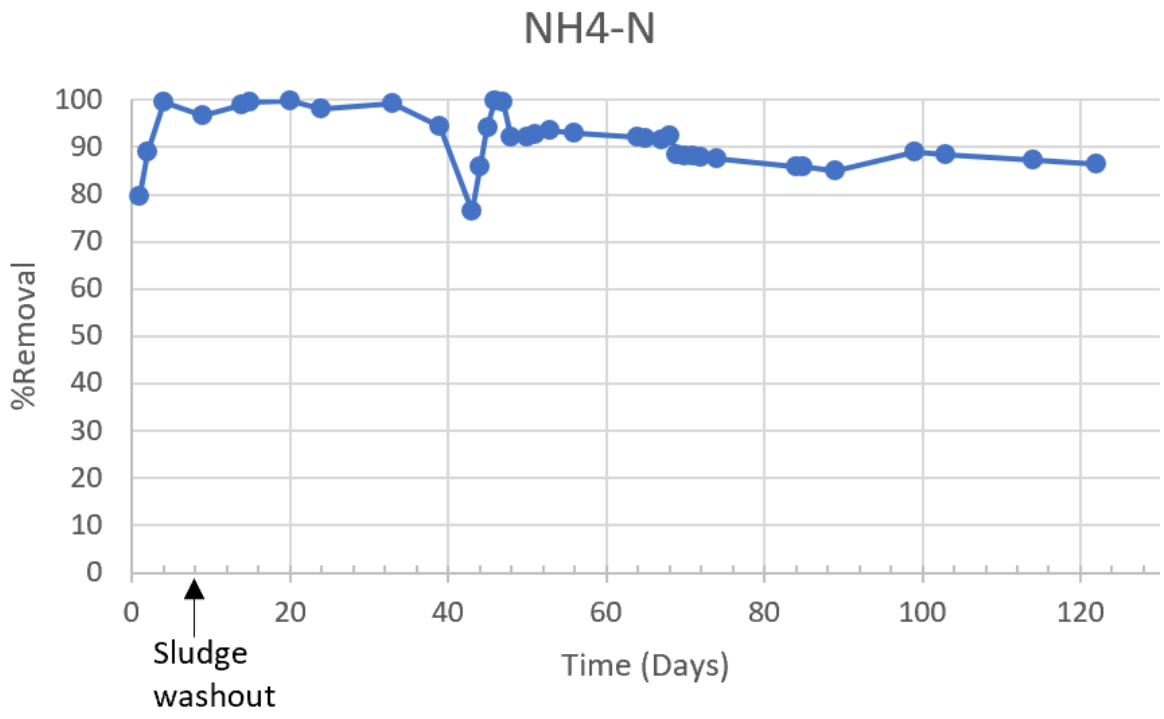

Figure 4-4. NH_4-N removal efficiency of PR1. NH_4-N was measured on a regular basis in the effluent of PR1 and tested for every new batch of centrate used in the feed.

4.3.2.2 PR2

While PR2 was run on the same feed as PR1, the differences in NH_4-N removal efficiency were quite marked. NH_4-N removal was initially high in PR2, but was not stable over time, with a mean removal of 67.4% (Fig. 4-5). This was found to be true over the entire period of operation, with large changes in removal efficiency in short amounts of time taking place (e.g. from 93% on day 13 to 32% on day 21) (Fig. 4-5). PR2 appeared to go through

three distinct waves with NH_4-N rapidly decreasing and then slowly recovering, only for the process to repeat immediately after reaching over 80% removal.

This suggests an instability in the system. While many possibilities exist, this phenomenon was likely caused by disturbances of the PR2 system, which, like PR1, experienced multiple feeding and recirculation issues. However, because the dips in efficiency were not correlated with instances of feeding or discharge failures, this was not likely the cause. Additionally, such disturbances occurred in PR1 as well and the same effects on NH_4-N were not observed, though confirmation via an undisturbed operation run should be done to confirm this was the case. As such, it is suggested that the seed sludge likely had a role to play in the differentiation of NH_4-N removal trends between PR1 and PR2.

The fragility of the seed sludge in PR2 was likely a result of the addition of high volumes of UASB sludge. This was the bulk of the seed stock added to PR2. This sludge was originally cultivated to remediate high COD effluent from industrial processes, specifically dealing with high sugar content. Therefore, the UASB granules used were not only cultivated anaerobically but were also used primarily to facilitate COD removal. As such, AOB were not enriched which would naturally lead to lowered NH_4-N removal rates. The dehydrated AGS granules and nitritation/denitritation sludge added would have then been responsible for the bulk of AOB inoculum. However, as these granules were smaller in the case of the nitritation/denitritation sludge, and lighter in the case of the dehydrated sludge, this would put the AOB containing structures at higher risk of disintegration and washout. As sludge loss caused by technical problems removed the AOB rich constituents, only the anaerobic granules would remain. While the UASB sludge did show some signs of NH_4-N removal, washout of

AOB would cause removal rates to drop. As AOB in the granules were then enriched, removal would increase before being further affected.

As microscopic and DNA analysis were not available due to limited laboratory resources present at the site, the presence of AOB in the UASB sludge could not be confirmed. As such, further tests are recommended to determine the precise morphology of sludge in PR2, and to confirm the source of AOB activity. The ammonia removal performance of PR2 was found to be quite lacking. With a mean removal efficiency of 67.4%, PR2 was comparable to other AGS systems treating high-strength wastewater (Jenicek et al., 2004; Yu et al., 2014). This makes the use of UASB granules counterproductive as using AS for inoculum was found to remedy this problem completely. Therefore, the PR1 system was found to be a better option for NH_4-N removal than PR2.

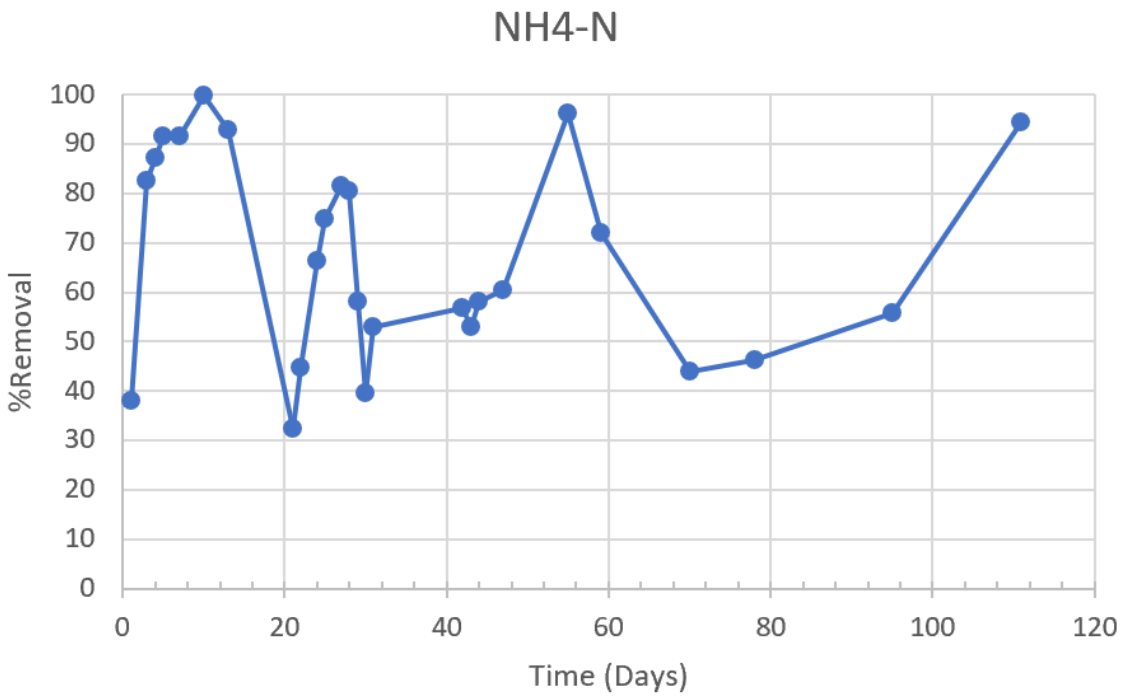

Figure 4-5. NH_4-N removal efficiency of PR2. PR2 was inoculated with nitritation/denitritation and desiccated AGS granules and UASB granules which constituted majority of added biomass.

4.3.3 NO_2-N and NO_3-N

4.3.3.1 PR1

As was expected, PR1 showed relatively high levels of NO_2-N accumulation compared to NH_4-N removal. However, initial concentration of NO_2-N in the effluent was quite low given NH_4-N the concentration. This, combined with the low levels of NO_3-N at this time was indicative of both denitritation and denitrification activity in the reactor. A large spike in NO_2-N concentration was observed around day 24, 4 days after COD addition was turned off to combat settling issues in PR1 (Figure 5-6). As COD is required for denitritation, and the bioavailable concentration of COD in centrate was found to be quite low, this likely caused the increase in NO_2-N accumulation (Gu et al., 2017). The returning of COD back into the system lowered NO_2-N concentration. However, the correspondent rise of NO_3-N effluent concentration could have also influenced this decrease (Fig. 4-6). The next spike in NO_2-N was observed to peak at 1,000mg/L on day 67 of operation. This may have been caused by the introduction of diaphragm pump recirculation (Fig. 4-6). This increasing trend was also observed to begin after the introduction of centrate with higher levels of NH_4-N. If the system was not given time to regenerate the denitritation community to the required levels, this would result in the accumulation of NO_2-N in the reactor and therefore higher effluent concentrations of NO_2-N as were observed.

NO_3-N was found to be low compared to the mean NO_2-N levels, peaking at 204mg/L on day 53 (Fig. 4-6). At this point, NO_3-N was found in the effluent at higher concentrations than NO_2-N, suggesting an increase in NOB during this time. As concentrations of ammonia increased, NOB activity declined as may be seen in Figure 4-6. This is congruent with the

findings in literature which suggest that high ammonia concentrations inhibit the growth of NOB (Sun et al., 2021).

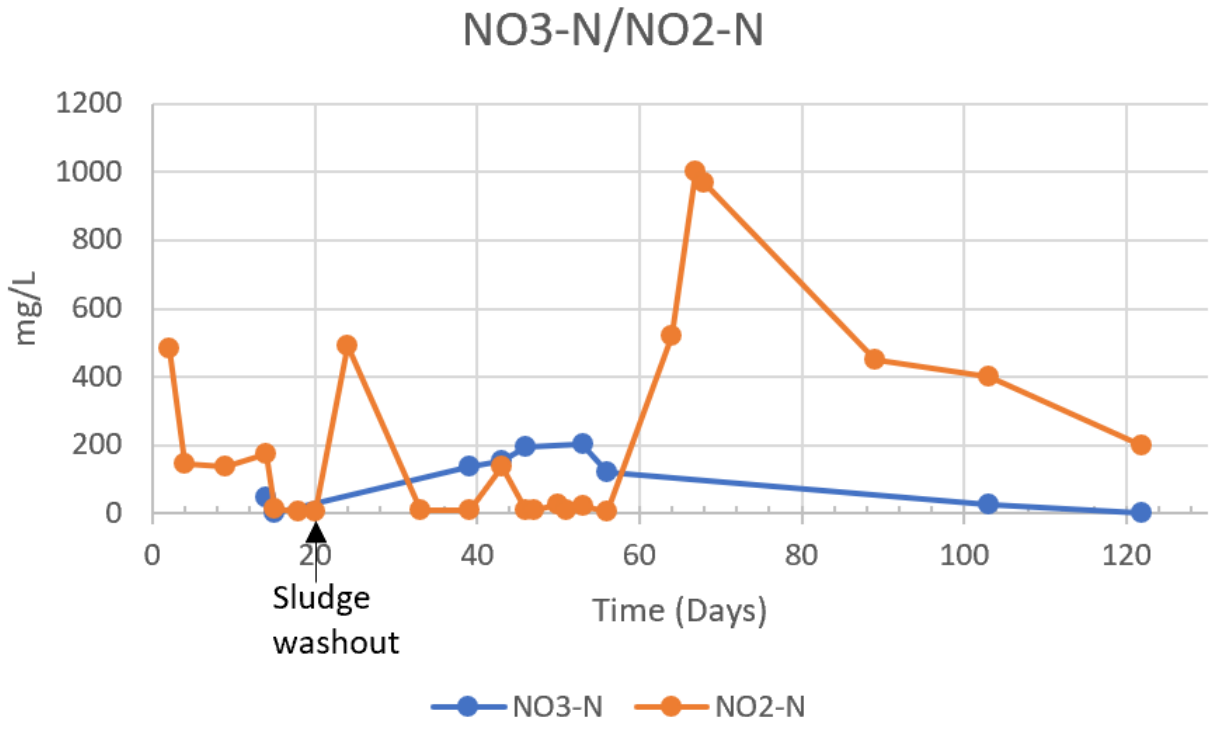

Figure 4-6. NO_2-N and NO_3-N concentrations found in PR1 effluent. The overall higher concentrations of NO_2-N suggest that NOB were not enriched in the system.

4.3.3.2 PR2

NO_2-N concentration in PR2 effluent was quite volatile over the course of operation yet remained well above the NO_3-N concentration observed (Fig. 4-7). NO_3-N concentration stayed relatively stable around 200mg/L over the entire period measured. Unfortunately, measurement of NO_3-N was not possible in the latter days of the cycle due to the lack of testing materials caused by a supply delay and backorder of testing materials. As such, the reason for the rapid decrease in NO_2-N levels observed after day 47 cannot be concretely linked to changes in NOB activity (Fig. 4-7). However, it was observed that this drop in NO_2-N was found to have occurred only one day after the reintroduction of COD into the

recirculation phase. This suggests that the drop could have been caused by the activation of denitritation in the system. However, this could not be confirmed. As such, the main reason for the decrease in NO_2-N was thought to be attributed to the decrease in NH_4-N oxidation observed after day 60 (Fig. 4-5).

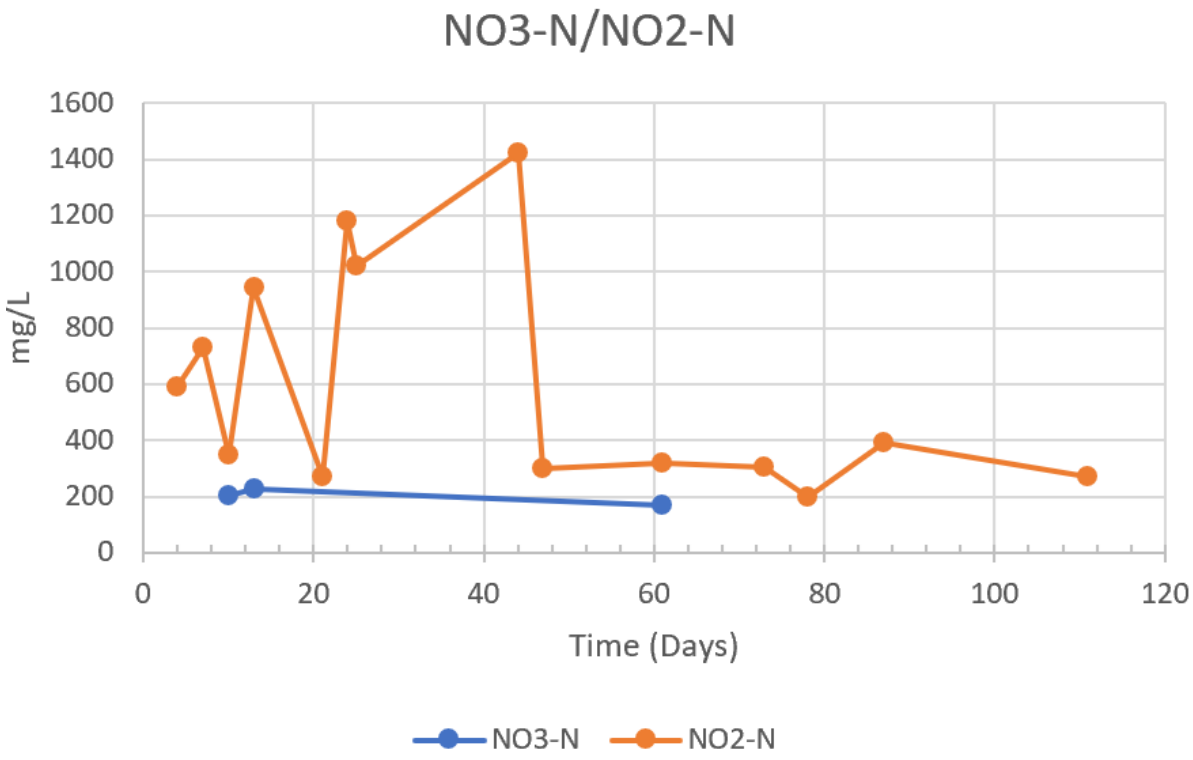

Figure 4-7. NO_2-N and NO_3-N concentrations over the course of operation of PR2. The effluent concentrations were taken and used to monitor the performance of the reactor.

4.3.4 COD

4.3.4.1 PR1

In this experiment, COD was primarily tested to inform optimization of COD addition. As COD is necessary to denitritation, high effluent COD could be used to flag denitritation problems when the appropriate concentrations are added. In the case of PR1, COD was initially added to the reactor, but was eventually discontinued to reduce filamentous growth.

This was done periodically, and COD was returned to the system once settling improved. However, while concentration of the COD stock solution added was kept at relatively stable concentrations, the effluent concentration of COD began to go up substantially starting on day 50 (Fig. 4-8). This was observed immediately following the reintroduction of a consistent COD feeding period. After this point, effluent COD was found to increase rapidly up to a maximum concentration of 1,081mg/L. This was indicative of a lack of heterotrophic organisms, including denitritation facilitating bacteria. This absence of denitritation was further confirmed by a slight decrease in the denitritation activity after prolonged operation of PR1. As these bacterial species were no longer metabolizing COD in conjunction with NO_2-N, this would lead to the accumulation of COD especially in the absence of other heterotrophs (Fig. 4-8). This suggests that while restricting COD may be beneficial to the removal of filamentous growth in the system, it is also detrimental to denitritation organisms and may affect denitritation long after the reinstation of COD into the system. While further study would be necessary to confirm this, these findings should be taken into consideration when attempting to remove suspected filamentous growth from a denitritation system.

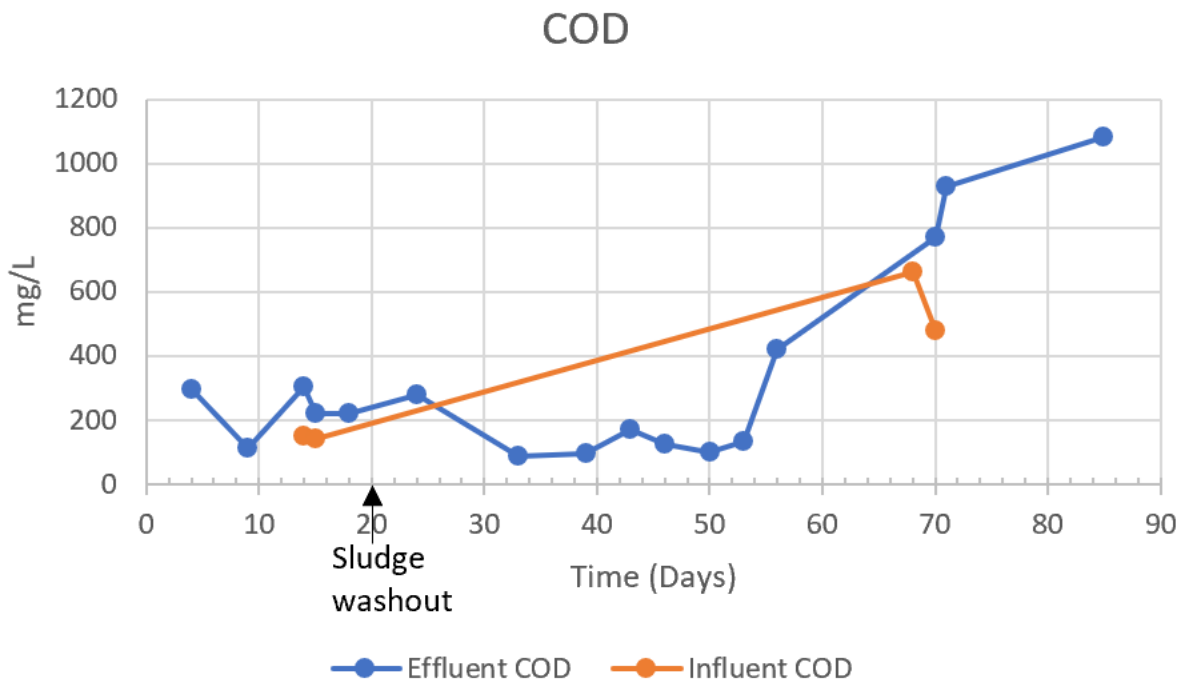

Figure 4-8. Effluent and influent COD concentration of PR1 over time. COD addition was modified according to denitritation needs and to control filamentous growth.

4.3.4.2 PR2

Even though PR2 was predominantly seeded with COD digesting sludge, this reactor did not exhibit high levels of COD removal as might have been expected. This was likely a result of the die-off of the anaerobic COD removing organisms populating the UASB granules after the introduction of aeration. The effluent COD for PR2 fluctuated dramatically but stayed for the most part in the 700-900mg/L range (Fig. 4-9). Effluent COD was significantly higher than influent COD found in centrate which reached a peak at 630mg/L (Fig. 4-9). As COD was not being consumed during the cycle, denitritation was likely not occurring. This pattern was found to be present for most of operation until COD addition to the system was discontinued at which point effluent concentrations reached 0mg/L (Fig. 4-9). This suggests that while the system was not capable of dealing with the high levels of supplemental COD, it was capable of removing small amounts. Based on the COD effluent concentrations and the

activity test performed on day 73, it was not likely that denitritation was responsible for COD reduction during the latter period of operation but rather heterotrophic growth.

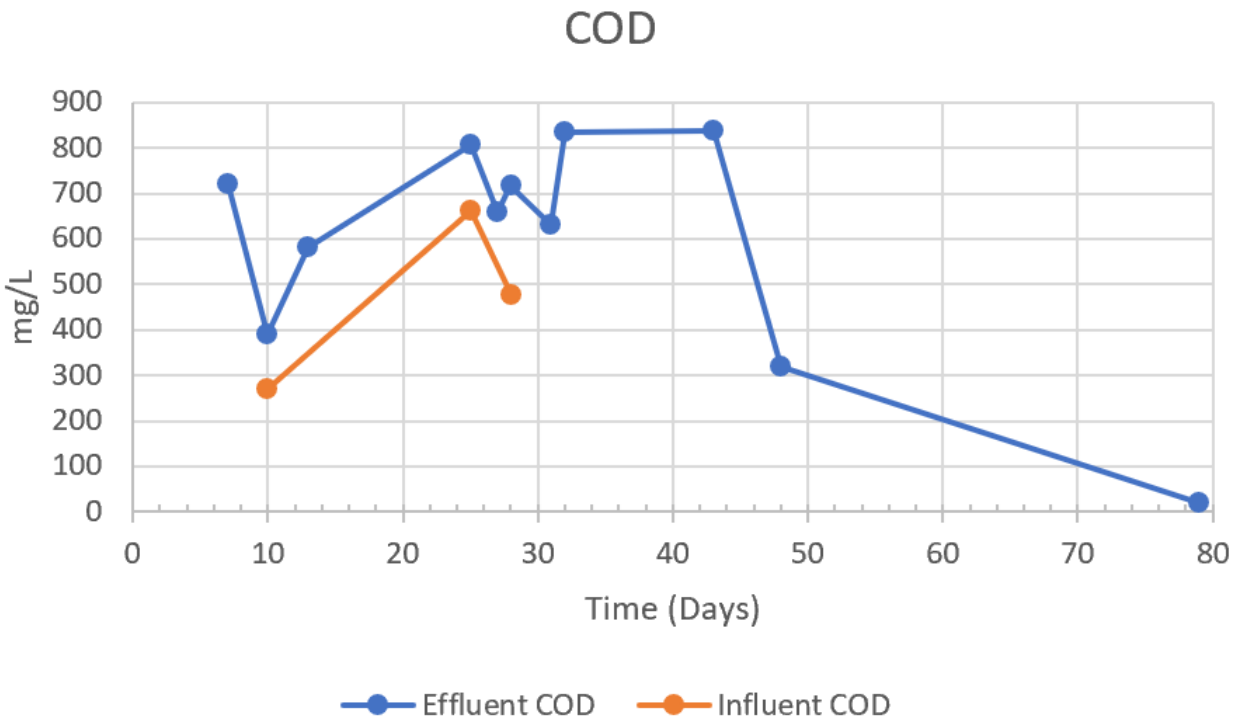

Figure 4-9. COD concentration of influent and effluent of PR2. COD was followed for the first 79 days of operation before being discontinued to facilitate filamentous growth removal.

4.3.5 Cycle Tests

One of the objectives of this study was to find the lowest optimal HRT necessary to facilitate NH_4-N removal from wastewater. To achieve this, two cycle tests were conducted on PR1 and HRT was then reduced as needed.

The first cycle test was conducted on day 26 when PR1 was still operating on a 24hr HRT. It was found that NH_4-N was depleted completely at the end of subcycle 4 (Fig. 4-10a). NO_2-N accumulation did not follow the pattern of NH_4-N removal instead exhibiting a drastic rise and subsequent decline which was accelerated substantially after all NH_4-N was depleted (Fig. 4-10a). This pattern suggested that while denitritation was occurring, it was at very low

rates. COD removal followed a similar pattern and evened out at around 150mg/L during the 7th subcycle (Fig. 4-10b). As such, all added and some centrate COD was removed. Since the main objective of this study was NH_4-N removal and HRT reduction, cycle time was reduced as a result of this cycle test and an HRT of 12hr was adopted. Despite reducing the HRT by 50%, NH_4-N removal was not affected (Fig. 4-2).

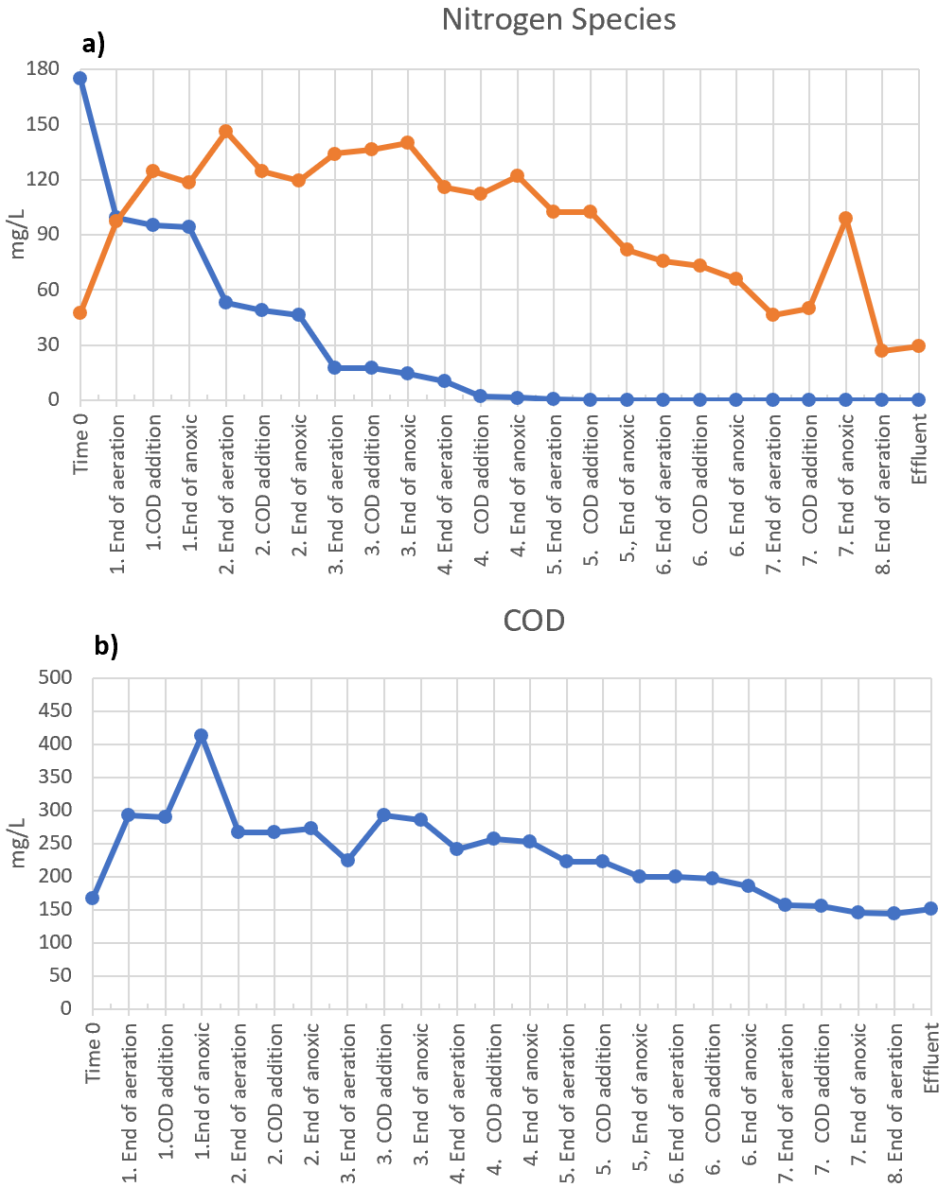

Figure 4-10. Results of the cycle test run on PR1 on day 26 of operation. The cycle test measured NH_4-N. NO_2-N, and COD levels to determine optimal HRT.

The second cycle test was conducted on day 96 of operation. This cycle found a 66% removal of NH_4-N by the end of the second subcycle (Fig. 4-11). Unlike in the previous test, NO_2-N did not show any removal with accumulation occurring instead (Fig. 4-11). However, this was expected as COD was restricted during this time.

It must also be noted that, over the course of the cycle, NO_3-N concentrations were negligible at best reaching a peak of 0.33mg/L in the effluent (Fig. 4-11). As no significant NO_3-N was produced, it is possible to determine that nitrification/denitrification did not play a significant role in N removal in PR1.

As NH_4-N removal was above 66%, the HRT was subsequently reduced to 8hr on day 103. Despite PR1 being accustomed to an extended HRT, it exhibited a swift recovery with removal rates returning to 87% within 10 days (Fig. 4-2). An 8hr cycle was then adopted for the rest of PR1 operation. While it is possible that an even shorter HRT may be possible for the removal of NH_4-N, time constraints on the length of this study did not allow for further examination of this objective. As such, further research would be necessary to determine if shorter HRT is possible and whether 8hr is the optimal operation time for this type of system.

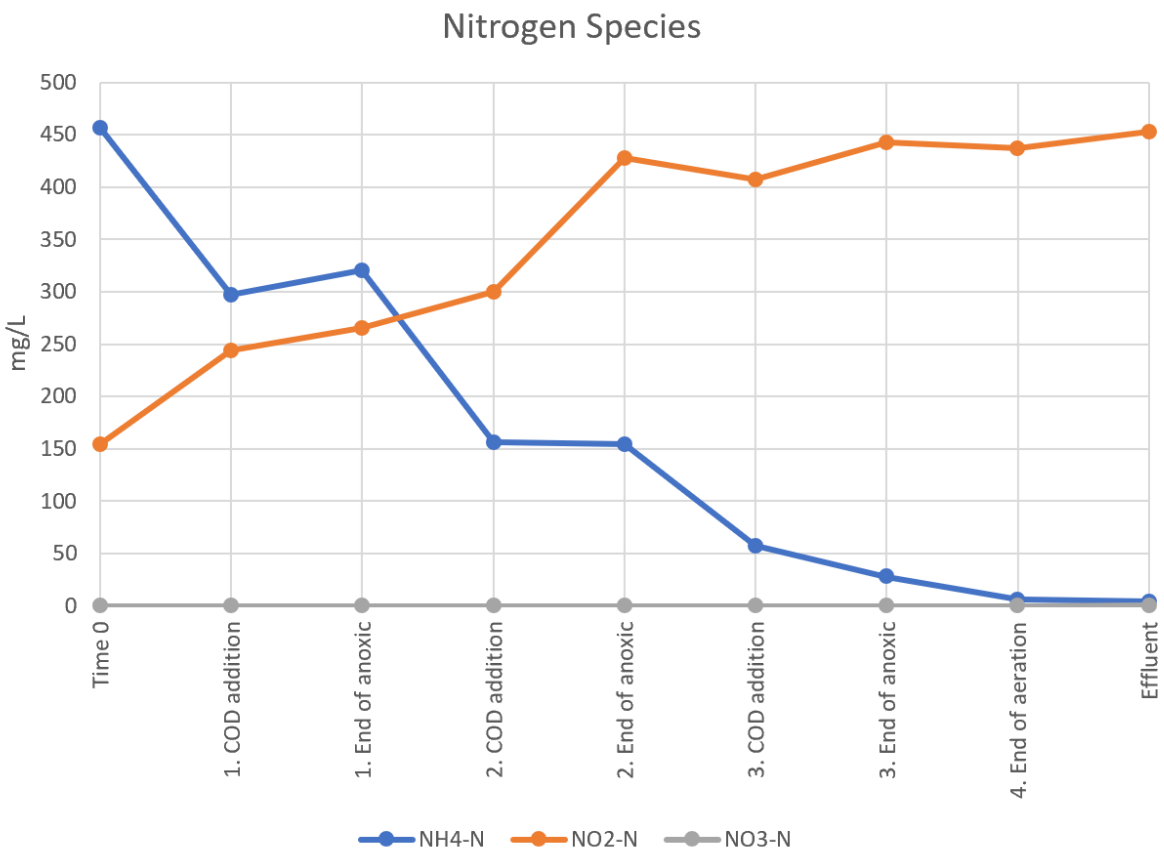

Figure 4-11. Concentrations of NH_4-N, NO_2-N, and NO_3-N during the 12hr HRT. Samples were taken at the end of each phase in all subcycles to observe the performance of PR1.

4.3.6 Activity Tests

The first activity test was conducted on PR1 only on day 54 of operation. This test examined the reactor's capability to metabolize and remove both NO_2-N and NO_3-N as well as NOB activity in the system. It was found that, while NOB activity was present, it was not very high with a peak NO_2-N oxidation of 17%, a final NO_3-N concentration of 28.5mg/L, and an oxidation rate of $0.1161 mg \bullet L^{-1} \bullet min^{-1}$ (Fig. 4-11a). It was interesting to note that NO_3-N concentration began to decrease after the 30-minute mark before beginning to rise again (Fig. 4-11a). This was likely an indication of the presence of simultaneous nitrification/denitrification occurring in the system, resulting in the fluctuation of NO_3-N

concentration. Denitrification was also found to be present in PR1 at this time, following a linear model of removal with a removal rate of 1.16mg●L^{-1}●min^{-1} (Fig. 4-12b). Denitritation was also found to be present with a rate of 1.341.16mg●L^{-1}●min^{-1}, thus confirming what was found in the previous performance and cycle tests (Fig. 4-4; Fig. 4-10a; Fig. 4-12c). As such, it was concluded that denitritation enrichment was possible for PR1.

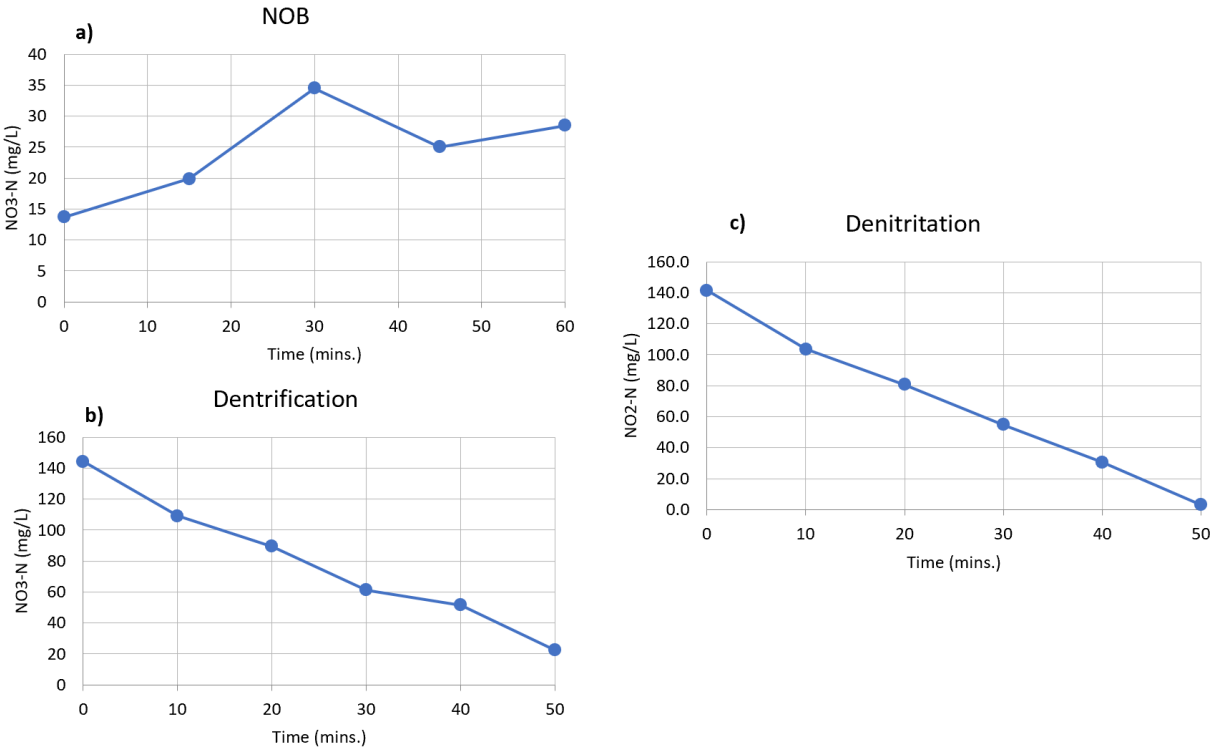

Figure 4-12. Results of the activity test conducted on day 54 of operation. Duplicate tests were conducted for NOB (a), denitrification (b) and denitritation (c) activity.

The second activity test was conducted on both reactors on days 117 and 73 for PR1 and PR2 respectively. This test focused on denitritation activity only. Here, PR1 showed a significant decrease in denitritation activity, compared to the previous activity test conducted on day 54. Even so, some removal of NO_2-N was still present, suggesting denitritation activity in the system (Fig. 4-12).

For PR2, it was found that denitritation activity was not present at all (Fig. 4-12). This is congruent with all other measures of performance taken for this reactor. While some nitritation/denitritation sludge was added to PR2 initially, this activity test showed that any denitritation activity that was present initially had been removed by day 73 of operation, which was congruent with the observations on all other measures. As such, it is possible to conclude that UASB sludge is not a viable inoculant for nitritation/denitritation systems.

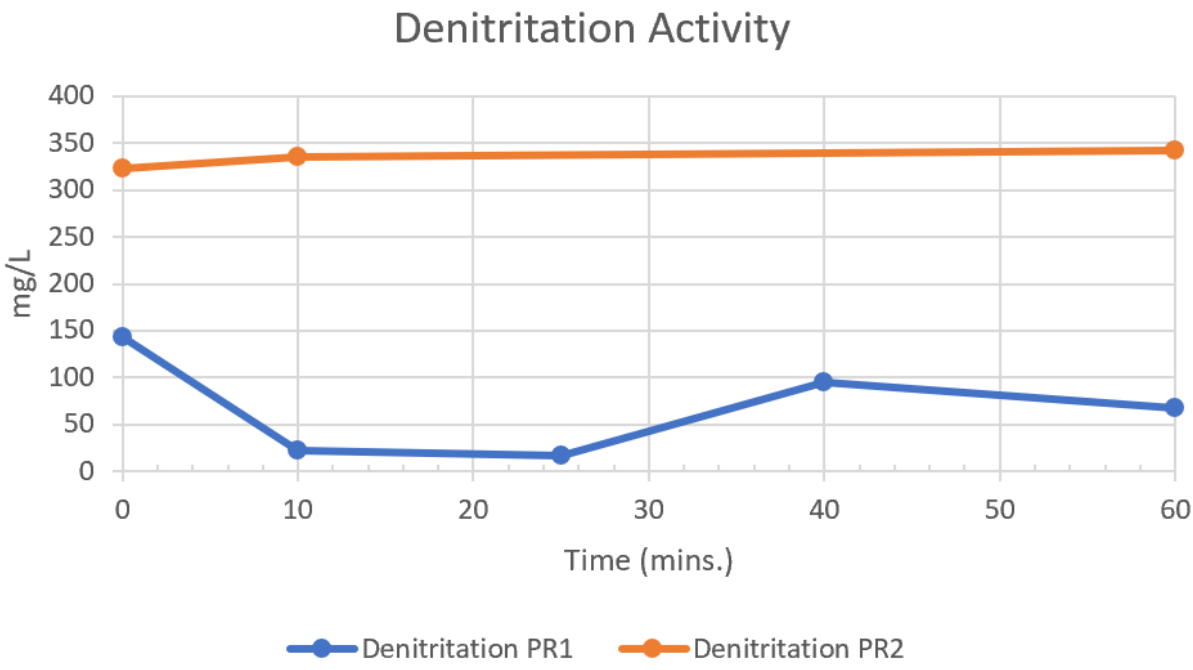

Figure 4-13. Denitritation activity of PR1 and PR2 on days 117 and 73 respectively. Samples were taken at 10, 25, 40, and 60 minutes and analyzed for NO_2-N concentration (mg/L).

4.4 Conclusion

This study of partial nitrification on a pilot scale experienced multiple problems in both technological and logistical senses. The limitations of conducting fieldwork was at least partially responsible for difficulties in operation. Limitations in testing frequency of some parameters due to logistics inhibited the examination of certain operational aspects in greater

detail. Issues with technology related to scale-up, such as insufficiencies in pump strength, were also experienced. However, the analysis of these two reactors was instrumental to the identification of challenges in the scale-up process. These findings provide a foundation instrumental to the set-up of future systems. It was determined that, while UASB granular sludge does increase initial settling capacity and biomass, it was also significantly less stable and less efficient in NH_4-N removal in the long-term compared to the AS-inoculated system. While AS did take more time to granulate, it allowed for an overall more stable and robust system and NH_4-N removal model compared to UASB sludge. Unlike the UASB system, the AS seeded reactor also showed signs of denitritation, resulting in the potential for a total nitrogen removal system using this method. Therefore, the AS model was found to be the better approach for the implementation of pilot partial denitrification SBR systems.

5 Conclusion and future directions

The presence of ammonia in municipal side-stream and industrial effluents is a growing concern worldwide. Because of this, it is important to develop new, better methods to mitigate discharge of this pollutant. AGS technology has been found to be reliable and efficient on the laboratory scale at reducing both ammonia levels in wastewater and overall operation costs. This study examined the use of AGS sludge and the partial nitrification pathway to remove ammonia from side-stream wastewater. While it was found that an AGS-SBR partial nitrification system is capable of ammonia removal from high-strength wastewater at both the laboratory and pilot scale, this study was a mixed success. While the characterization of microbial community development from AS was quite successful, the pilot experiments were less so.

5.1 Laboratory scale start-up and DNA analysis

It was determined that introduction of real wastewater during the granulation process was effective if introduced in gradual increments with no real advantage seen when transitioning an established reactor. It was also determined that it is more beneficial to begin the introduction of lagoon supernatant during the start-up process. As wastewater treatment could be commenced sooner a reduction in start-up time and resources would be achieved, reducing financial burden.

The examined bioreactor had a lower biomass than is generally found in AGS SBR systems. As such, maximization of sludge retention during the granulation process is something that should be researched further. To confirm the reliability of the data collected in this study, this system should also be tested on other feed types, such as industrial effluents

from meat production and petrochemical manufacturing to further explore the impact of feed on microbial community. It was also found in both laboratory reactors that the abundance of AOB was relatively low compared to values found in literature. While this is likely due to the initial low concentration of NH_4-N and would therefore correct with time, further research should be conducted to confirm if this is truly the case.

While it was found that reactors started earlier on high ammonia feed were more stable over time, there were several uncontrolled variables present in this study. Even though both reactors were fed the same proportionally, the feed added to R2 was more dilute, possibly affecting results. This reactor was lost due to technical difficulties relatively early on as well. Due to this, better normalized study of the differences between established and start-up reactors should be conducted to ensure differences are due to ammonia concentration and no other variables present in this study.

5.2 Pilot Reactor Establishment and Operation in Calgary, Alberta

The information on start-up obtained in the laboratory was used to advise the pilot scale reactor set-up. The results of the pilot experiment were unfortunately not successful in establishing a long-term functioning system but did highlight the necessary parameters for a system of this size. This study demonstrated that the N removal capacity of seed sludge was more important than the presence of granules for long-term reactor stability. While UASB granular sludge did perform better than AS on settling and biomass metrics early on, it was not stable over time and did not provide the same levels of pollutant removal on any parameter tested. On the other hand, AS sludge performed well on pollutant removal metrics, including denitritation, as well as exhibiting more stable biomass and settling over time.

PR1also showed signs of granulation relatively early on, before the introduction of diaphragm pumps. Therefore, using AS as seed for this system was demonstrated to lead to longer-term success.

Minimum HRT is a required factor for all successful wastewater treatment processes. It was found that an 8hr HRT was sufficient to degrade NH_4-N completely in the described conditions, however, time constraints on the duration of these experiments and relative degradation of the reactors did not allow for the testing of even shorter HRT. However, due to the positive response to lowered HRT observed in PR1, it is likely that even lower HRT could be achieved with further study, resulting in even more attractive cycle times if given the ideal conditions for AGS growth.

In terms of technological observations, it was found that pump strength was problematic during operation. As good circulation during the anoxic phase is necessary for denitritation, proper strength pumps and large enough tubing must be used. The peristaltic pumps used in this experiment were either too weak to provide adequate mixing, or caused rapid degradation of tubing which is not feasible for long-term operation. Diaphragm pumps were also tested, but while they provided optimal recirculation conditions, they also caused granule disintegration, resulting in almost complete sludge loss in PR1. While stronger peristaltic pumps appropriate for this reactor size are available, their use was not feasible due to high costs. As such, other options, such as mechanical mixing, which has been used on the laboratory scale, need to be explored to maximize denitritation activity in a reactor of this size (Guo et al., 2016).

Overall, this study was successful in not only identifying areas of concern in pilot SBR operation, but also provided insight into the start-up process on both the lab-scale and pilot level. While more research is needed before this type of AGS SBR nitritation/denitritation system may be used on a commercial level, this set-up shows much promise in the treatment of high-strength ammonia wastewater.

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