STUDY OF THE EFFECT OF BiOWiSH® AQUA ON SIMULTANEOUS
NITRIFICATION AND DENITRIFICATION IN A
MEMBRANE AERATED BIOREACTOR

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Joelle Arakaki
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COMMITTEE MEMBERSHIP

TITLE: Study of the Effect of BiOWiSH® Aqua on
Simultaneous Nitrification and Denitrification
in a Membrane Aerated Bioreactor

AUTHOR: Joelle Arakaki

DATE SUBMITTED: June 2018

COMMITTEE CHAIR: Nirupam Pal, Ph. D, Professor
Professor of Environmental Engineering

COMMITTEE MEMBER: Rebekah Oulton, Ph. D, Professor
Professor of Environmental Engineering

COMMITTEE MEMBER: Yarrow Nelson, Ph. D, Professor
Professor of Environmental Engineering

COMMITTEE MEMBER: Christopher Kitts, Ph. D, Professor
Director of Cal Poly Center for Applications in
Biotechnology and Associate Dean of
Research

ABSTRACT

Study of the Effect of BiOWiSH[®] Aqua on Simultaneous Nitrification and Denitrification in a Membrane Aerated Bioreactor

Joelle Arakaki

This research entails the investigation of the effects of a bioaugmentation product from BiOWiSH[®] called Aqua, referred to as “Aqua” for the remainder of this paper, on the nitrogen removal rate in a membrane aerated bioreactor (MABR). This research was conducted using a MABR design that consisted of a silicone membrane and continuous flow airline with compressed air. The membrane system was designed to supply oxygen, creating an aerated layer at the membrane-biofilm interface and an anoxic layer at the biofilm-water interface. Laboratory experiments were conducted to compare the nitrogen removal rates of natural bacteria alone to natural bacteria paired with Aqua. However, it was not possible to determine if a difference existed between the nitrogen removal rates of the MABR systems with only natural bacteria versus those with natural bacteria augmented with Aqua. The mean nitrogen removal rate observed when the media in the system reached steady state was 0.39 mg-N/L-hr. with a carbon to nitrogen (C: N) ratio of 12:1. The only increase in the nitrogen removal rate observed was when the C: N ratio was doubled to 24:1 and the nitrogen removal rate increased to 0.56 mg-N/L-hr.

Although it appeared that the Aqua did not have an influence on the nitrogen removal rate in the MABR systems, many other variables still need to be assessed to reach a conclusion. To improve the efficiency of the system more tubing should be added, or the glucose should be removed from the growth media because the maximum O₂ mass transfer rate is only enough O₂ for nitrification. The addition of glucose at 12:1 ratio

increased the O₂ demand in the system to be five times greater than the O₂ supplied from the silicone tubing. This research determined that use of trace minerals, Aqua dosing method, and Aqua dosing concentration were not contributing factors in nitrogen removal from growth media under the conditions of this experiment.

Keywords: Membrane Aerated Bioreactor, Simultaneous Nitrification and
Denitrification, Biological Nutrient Removal

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Chapter 1 INTRODUCTION

Nitrogen is an essential environmental nutrient that plants and other organisms need to grow; however, excess nitrogen becomes detrimental to the environment and human health. Nitrogen often enters the environment in the form of either nitrate or ammonium.

Nitrate is a component of municipal wastewater and is also typically found in fertilizers, so areas with higher agricultural activity typically see more nutrient pollution. This nutrient pollution can cause many detrimental effects on the environment especially when discharging to bodies of water. One example of nutrient pollution is eutrophication or large algal blooms in the water. These algal blooms increase the organic matter in the water that in turn use up the dissolved oxygen during decomposition. This results in oxygen deficiency, a loss of vegetation, and possible aquatic life mortality (Environmental Protection Agency, 2017). High levels of nitrate in drinking water can also have harmful effects on human health. Infants who are exposed to high nitrate levels may become seriously ill with a condition known as blue baby syndrome or methemoglobinemia which causes a shortness of breath, blue-tinted skin and might even cause death (Environmental Protection Agency, 2017).

Similar to nitrate, ammonia may enter the environment through multiple avenues such as the decomposition of organic matter and animal and human waste. Ammonia exists in two forms in water; ammonium (NH_4^+) which is more abundant, or the non-dissociated or unionized ammonia (NH_3) molecule which is more toxic (Environmental Protection Agency, 2017). High levels of ammonia in water can lead to a toxic buildup in internal

tissues and blood that potentially causes death for aquatic life (Environmental Protection Agency, 2017).

Typical influent concentrations of nitrate and ammonia in municipal wastewater are 25 to 35 mg-N/L and 20 to 40 mg-N/L respectively (Environmental Protection Agency, 2017).

The maximum contaminant level (MCL) set by the EPA for nitrate before discharge of treated wastewater is 10 mg-N/L (Environmental Protection Agency, 2017). Currently no MCL exists for ammonia, however, the EPA recognizes that acute exposure at 17 mg-N/L and chronic exposure at 1.9 mg-N/L to ammonia (expressed as Total Ammonia Nitrogen) can be harmful to aquatic life (Environmental Protection Agency, 2017).

The threat of nutrient pollution in the environment emphasizes the importance of biological treatment of wastewater to remove nutrients prior to discharging to nearby bodies of water. Most conventional wastewater treatment plants achieve nutrient removal utilizing the natural nitrogen cycle in which ammonium is nitrified and the nitrate and nitrite is then denitrified to nitrogen gas. This is typically accomplished through systems such as aeration basins with return activated sludge (RAS), or biotowers. For example, the San Luis Obispo Water Resource Recovery Facility (WRRF) currently uses a biotower followed by a set of aeration basins. In this system 30-40% of nitrification is achieved in the biotowers and from there the water is then sent to the aeration basins where nitrification is completed (WRRF Draft, 2014). While this is effective, it does take up a relatively large area or footprint because the water must go from the biotower to a second primary clarifier, then the aeration basins followed by a set of secondary clarifiers (Figure 1-1). While most conventional wastewater treatment facilities do not have two steps of biological treatment like the WRRF, they do still typically require a large

footprint with two different tanks for nitrification and denitrification. With wastewater effluent discharge standards constantly becoming more stringent the industry is always looking for methods to improve biological nutrient removal.

Figure 1-1 Ariel view of the WRRF with the components of the biological treatment outlined in red (GoogleMaps, 2018).

An alternative to conventional wastewater treatment that could improve biological treatment is a membrane aerated bioreactor (MABR). A MABR system uses a membrane to diffuse oxygen into the system and provide a surface for a biofilm to form. The biofilms that develop on the silicone membrane are proven to act as diffusion barriers for the oxygen (Chang and Tseng, 1999). This creates an aerated layer at the surface of the membrane and an anoxic layer at the surface of the biofilm. By combining the aerobic and anoxic zones needed for nitrification and denitrification in one tank the MABR would have a noticeably smaller area or footprint.

An additional factor that could improve biological treatment of wastewater is augmenting the existing bacteria in the system that can achieve nitrification and denitrification through a biological product such as BiOWiSH[®] Aqua, referred to as “Aqua” for the remainder of this paper. Aqua is a blend of bacteria and non-living organic material that helps supplement bacterial growth. It was created to help facilitate biological removal of excess nutrients in wastewater. With less than 1% of active ingredients a majority of the product helps to support bacteria growth. While there have been multiple case studies on the use of Aqua in natural bodies of water, there is still a lot of research to be conducted evaluating the implementation and effectiveness of Aqua in municipal wastewater treatment.

This research aimed to augment a MABR system with Aqua to evaluate if Aqua was capable of increasing nitrogen removal rates. Successful nitrification and denitrification was achieved in previous research using a MABR, however nitrogen removal could be enhanced through bioaugmentation using Aqua. The addition of Aqua will theoretically establish a layer of bacteria to denitrify on top of the layer of nitrifying bacteria that was formed by the natural bacteria existing in the wastewater.

The MABR used in this research was a continuation of the MABR system used by previous graduate student Kirk Waltz. In his design a dead-end configuration was used with compressed air for the MABR. Typically for dead end configurations pure oxygen is used so the membrane system gets a constant supply of O₂. However, compressed air is composed of 78% N₂ gas and the membrane diffuses oxygen at a 2:1 ratio of oxygen to nitrogen. Overtime, this could have resulted in a possible buildup of nitrogen gas in the airline that caused the concentration of oxygen to decrease. This decrease in oxygen

would result in an eventual decrease in nitrification rates which is what was observed previously (Waltz, 2009).

Instead of a dead-end configuration, this MABR design utilizes a continuous flow configuration to allow the excess nitrogen to escape and maintain a consistent oxygen supply. To investigate the feasibility of this system a small scale MABR design was used first. Several screening experiments were conducted using the small scale MABR under the assumption that the Aqua was capable of nitrification. These experiments evaluated the success of a continuous flow configuration for the air line with compressed gas, different Aqua dosing methods, and growth media compositions. These experiments also helped to improve and develop sampling and water quality testing methods.

Once successful nitrogen and ammonium removal was confirmed through the screening experiments, a larger pilot scale MABR was built to further investigate the feasibility of a MABR system in municipal wastewater treatment. The specific objectives of this research included:

1. Confirming nitrogen removal in a continuous flow airline MABR configuration on a lab pilot scale.
2. Comparing nitrogen removal using different Aqua dosing methods.
3. Evaluating the effect of different carbon source concentrations on nitrogen removal.
4. Determining the mechanism of total nitrogen removal in a MABR.
5. Further developing testing and analysis methods to improve understanding of the MABR system.

Chapter 2 LITERATURE REVIEW

This chapter details the previous research done on nutrient removal through biological processes, MABR systems, and Aqua. Previous studies on MABR systems have focused on using alternative configurations such as a dead-end configuration to achieve nitrification opposed to a continuous flow configuration. The research done previously on Aqua is limited but focused mostly on wastewater applications and determining its effectiveness in enhancing nutrient removal in different types of systems.

2.1 Natural Nitrogen Removal Processes

Biological nitrogen removal from municipal wastewater is achieved through the nitrogen cycle. The key steps to nitrogen removal from wastewater are nitrification, denitrification, and assimilation (Figure 2-1). Through these steps, nitrogen is transformed through either biological or non-biological processes, into different chemical forms of nitrogen.

Figure 2-1 Natural nitrogen cycle that can be used to remove ammonium and nitrate from wastewater (Carpenter and Capone, 1983).

2.1.1 Nitrification

Nitrogen in the form of ammonium or ammonia, is converted to nitrate by nitrifying bacteria in the process called nitrification. Some examples of nitrifying bacteria are nitrate oxidizing bacteria or heterotrophic bacteria. Typically, *Nitrosomonas* bacteria are associated with the first step of nitrification where ammonia is converted to nitrite as shown in Equation-1 (Figure 2-1). Other bacteria such as *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, *Nitrosorobrio*, and other autotrophic bacteria are also capable of oxidizing ammonia to nitrite (Tchobanoglous et al., 2004).

The second step of nitrification is completed through nitrite oxidizing bacteria such as *Nitrobacter* (Tchobanoglous et al., 2004). While *Nitrobacter* is the most commonly associated bacteria with nitrite oxidation this process can also be completed by *Nitrospina*, *Nitrococcus*, *Nitroeystis*, and *Nitrospira* (Tchobanoglous et al., 2004). The conversion of nitrite to nitrate is shown in Equation-2 (Tchobanoglous et al., 2004). The growth rate of *Nitrosomonas* is typically slower than *Nitrobacter*. This means that nitrification is usually modeled by the first step of nitrification or the conversion of ammonia to nitrite as the rate limiting step.

Various groups of heterotrophic bacteria and fungi can also conduct nitrification (Tchobanoglous et al., 2004). However, nitrification through heterotrophic bacteria is a slower process. While this type of nitrification can occur in wastewater applications it is not as common as nitrification through autotrophic bacteria.

The main factors that influence nitrification are pH, temperature, dissolved oxygen (DO) concentration, biological oxygen demand/ total kjeldahl nitrogen (BOD₅/TKN), and ammonia and nitrite concentration (Tchobanoglous et al., 2004). These were all important considerations in the MABR design to achieve successful nitrification. For example, the nitrifying bacteria have an optimal pH range of 7 to 8, therefore the pH of the system ideally should be within this range.

2.1.2 Denitrification

In the denitrification process nitrates are reduced to gaseous nitrogen by facultative anaerobes such as bacteria and fungi. These facultative anaerobes break down oxygen

containing compounds such as nitrate. This process is carried out through the reactions shown in Equation-3 and Equation-4 (Figure 2-1).

Heterotrophic and autotrophic bacteria are also capable of denitrification. Heterotrophic bacteria readily metabolize biodegradable substrate under anoxic conditions using nitrate as the electron acceptor. These bacteria would readily use oxygen before nitrate, so oxygen concentrations must be minimized in the bulk water for denitrification to occur.

In contrast, autotrophic bacteria that are capable of denitrification have been divided into three different categories (Zhou, Weili, et al., 2011). The first category is hydrogen-based processes where hydrogen is the electron donor and nitrate is the electron acceptor (Zhou, Weili, et al., 2011). The second is sulfur-based denitrification where either elemental sulfur or thiosulfate are the electron donors with nitrate as the electron acceptor again (Zhou, Weili, et al., 2011). The last category is ANAMMOX, in which anaerobic ammonia oxidization bacteria convert ammonium to nitrogen gas using nitrite as the electron acceptor (Zhou, Weili, et al., 2011).

2.1.3 Nitrogen Assimilation

Another essential step in the nitrogen cycle is nitrogen assimilation. In this step, the ammonia in the system is converted into organic nitrogen. This can result in a steep drop in ammonia or nitrate that might look like nitrification and denitrification. However, what is occurring is that the cells use nitrogen in the system to create new cell material.

Microbial cells are approximately 12.5% nitrogen, so as they reproduce, they are

transforming the nitrogen into organic nitrogen and cell biomass (Tchobanoglous et al., 2004).

2.2 Conventional Wastewater Treatment

The nitrogen cycle is often used to treat wastewater and remove excess nutrients before discharging to the environment. Although nutrient removal can be achieved through air stripping, chemical oxidation by breakpoint chlorination, ion exchange and stream stripping of ammonia, the most common treatment method is through controlled biological processes utilizing the nitrogen cycle (Tchobanoglous et al., 2004). Whichever treatment method is used, the treated effluent must meet EPA standards for nitrogen in the form of nitrate and should be below the recognized chronic exposure level for ammonia prior to discharge.

In conventional wastewater treatment facilities nitrification and denitrification are achieved separately in aerobic and anoxic stages. Nitrification is typically achieved in activated sludge processes that encourage bacteria to breakdown organic compounds and nutrients by steady mixing and high aeration. This process converts the ammonia in the system to nitrate. The nitrate is then converted to nitrogen gas via denitrification in the anoxic zone.

One process for nitrogen removal was developed by Ludzak and Ettinger, with the anoxic zone first followed by an aerobic zone (Figure 2-2). The nitrates that are formed in the aerated zone are returned to the anoxic zone through the returned activated sludge (RAS). In the anoxic zone the nitrate is oxidized to nitrogen gas that is released to the atmosphere. This process is frequently used to prevent rising sludge in secondary

clarifiers by increasing the recycle ratio of the RAS (Tchobanoglous et al., 2004). The effectiveness of the system is limited because the only source of nitrates to the anoxic zone are from the RAS. For example, if the influent nitrogen concentration is 20 mg/L and the recycle ratio was 1.0 (recycle flowrate divided by influent flowrate) then the effluent nitrate concentration would be around 10 mg-N/L (Water Environment Federation, 2008).

Figure 2-2 Preanoxic treatment train for biological treatment developed by Ludzak and Ettinger (Tchobanoglous et al., 2004).

A Modified Ludzak-Ettinger process is also commonly used to remove nitrogen from wastewater. The internal recycle from the aerobic zone back to the anoxic zone increases the denitrification rate and overall nitrogen removal by increasing the amount of nitrate being sent back to the anoxic tank (Figure 2-3). With an internal recycle of 2 to 4 this process produces effluent with a concentration of 4-7mg-N/L (Tchobanoglous et al., 2004). This process can easily be adapted to existing activated sludge facilities.

Figure 2-3. Preanoxic treatment train with internal recycle known as the Modified Ludzak and Ettinger process (Tchobanoglous et al., 2004).

Another treatment process that might be used in wastewater treatment is a postanoxic design. This single sludge postanoxic process was developed by Wuhrmann to achieve nitrogen removal in an activated-sludge system by having the anoxic zone after the aerobic zone (Figure 2-4). In this process, ammonium is nitrified to nitrate or nitrite in the aerobic zone (Figure 2-4). In this process, ammonium is nitrified to nitrate or nitrite in the aerobic zone. Then in the anoxic zone the nitrate is denitrified to nitrogen gas. This process requires longer hydraulic and sludge retention times to maintain high nitrate removal because the denitrification rate is proportional to the endogenous respiration rate in the mixed liquor (Tchobanoglous et al., 2004). If an additional carbon source such as methanol is added to the system in the anoxic tank then it is possible to achieve effluent nitrogen levels less than 3 mg-N/L (Tchobanoglous et al., 2004). While this is the highest removal of the processes discussed so far, the operating costs will increase from purchasing a carbon source as well as increased costs from controlling the feed of the carbon source (Tchobanoglous et al., 2004).

Figure 2-4. Single sludge postanoxic system treatment train developed by Wuhrmann (Tchobanoglous et al., 2004).

An alternative treatment train used is a multistage process known as the Bardenpho process. This process consists of a series of anoxic and postanoxic tanks to achieve nitrogen removal (Figure 2-5). A 4-stage Bardenpho treatment train can produce effluent nitrogen levels less than 3 mg/L (Tchobanoglous et al., 2004). A benefit of this system is that it is also capable of phosphorous removal. These types of systems require a large footprint and have a retention time ranging from 7.5 hrs. to 20 hrs. to achieve nitrification and denitrification (Water Environment Federation, 2008).

Figure 2-5. Multi stage treatment train with alternating anoxic and aerobic tanks also known as the Bardenpho process (Tchobanoglous et al., 2004).

2.3 Simultaneous Biological Nitrification and Denitrification (SNdN)

An alternative to conventional nitrogen removal methods where nitrification and denitrification occur separately, is SNdN. This type of nitrogen removal has proven to be an effective treatment method producing effluent nitrogen levels as low as 3 mg-N/L (Tchobanoglous et al., 2004).

SNdN can be achieved by both heterotrophic and autotrophic nitrifying bacteria (Tchobanoglous et al., 2004). It was discovered that certain heterotrophic nitrifying bacteria are also capable of denitrifying in aerobic conditions which was beneficial for SNdN systems (Tchobanoglous et al., 2004). The heterotrophic bacteria *Paracoccus pantotropha* have been studied extensively for SNdN. This process requires a carbon substrate such as methanol to supply energy for this process to occur, however, such substrate is typically limited in an aerobic wastewater treatment system (Tchobanoglous et al., 2004). For this reason, growth of heterotrophic nitrifiers is not expected in conventional aerated wastewater treatment systems and might require a different type of bioreactor.

Another type of bacteria that can accomplish SNDN are autotrophic nitrifying bacteria such as *Nitrosomonas europaea* (Tchobanoglous et al., 2004). This type of bacteria can use nitrite to oxidize ammonia with the production of nitrogen gas in the absence of oxygen. If oxygen is present, the bacteria can oxidize the ammonia using oxygen.

The ANAMMOX process can also achieve SNdN, facilitated by bacteria belonging to the order Planctomycetales. If the temperature remains above 20°C, they can oxidize ammonia via the reduction of nitrite under anaerobic conditions. The ammonia oxidation

rate by ANAMMOX bacteria are shown to be 6 to 10 times faster than that for *N. europaea* (Tchobanoglous et al., 2004).

2.3.1 Simultaneous Nitrification and Denitrification (SNdN) Mechanisms

SNdN occurs primarily through two different mechanisms. The first mechanism that SNdN can occur is when regions of low DO or zero DO concentration are present within the basin as a function of the mixing regime (Figure 2-6). In this process the DO is depleted as the mixed liquor travels away from the aerators, creating anoxic zones.

Figure 2-6 Change from the aerobic to anoxic zone in a system with increasing distance away from the aerators described in the first mechanism (Tchobanoglous et al., 2004).

The second mechanism where SNdN can occur is when an activated sludge floc contains aerobic and anoxic zones. In this model the DO and dissolved substrates outside of the floc diffuse into the aerobic zone (Figure 2-7). The size of these individual zones is dependent on the concentration of DO, ammonia, and bCOD in the floc that might hinder the DO from penetrating the floc (Tchobanoglous et al., 2004). The nitrate that is produced during nitrification on the outside of the floc diffuses into the anoxic zones

within the floc where denitrification can occur in the center of the floc. The rate of nitrification in this type of system is not only dependent on the bulk liquid DO concentration but also the BOD that is present.

Figure 2-7. Nitrification and denitrification through the aerobic and anoxic portions of a floc for the second mechanism of S_NdN (Gulshin et al., 2015).

Out of the previously described conventional treatment methods, the most common system that can be reconfigured for SNDN systems are pre-anoxic systems (Tchobanoglous et al., 2004). These are the easiest to retrofit for existing plants. As mentioned, the pre-anoxic system also offers more control of bulking sludge by controlling the recycle ratio (Figure 2-3). Another benefit of this process configuration is that it produces alkalinity in the anoxic zone, before nitrification, raising the pH which is beneficial for nitrifiers that have an optimal pH range of 7 to 8. Although the preanoxic system offers the ability to convert an existing biological treatment system to a nitrogen removal system with relatively short to moderate retention times it still requires a relatively large footprint.

Several essential parameters must be met for SNdN to be successful. In a typical design for a SNDN system the NO_3 is fed to the anoxic portion of the reactor in the form of return activated sludge (RAS) and by pumping mixed liquor from the aerobic zone. The key design parameters for an SNdN system are the anoxic detention time, mixed liquor volatile suspended solids (MLVSS) concentration, internal recycle rate and return activated sludge, influent BOD or biodegradable COD (bCOD) concentration, readily biodegradable COD (rbCOD), pH, and temperature (Tchobanoglous et al., 2004). In a SNdN system the pH should be kept between 6.5 and 8.5.

While this system may have considerable energy savings, it could be limited by the typically larger reactor volume needed for SNdN and the process controls needed to run the system.

2.4 Membrane Aerated Bioreactor

An alternative system that would offer the aerated and anoxic zones needed for SNdN in a single tank is a MABR system. A typical MABR design consists of porous hollow-fiber membranes covered in biofilm that run through the length of the reactor (Figure 2-8).

These hollow-fiber membranes are usually in a dead-end configuration that forces the O_2 through the membrane as the pressure builds. Silicone membranes were ideal for the MABR system because the nitrifying bacteria exhibit a high degree of adhesion to the membrane without clogging the pores (Matsumoto et al., 2007). The hollow fiber membrane serves two major roles: the membrane serves as a carrier to immobilize bacteria and it simultaneously supplies the oxygen to the system (Matsumoto et al., 2007). The oxygen penetrates the membrane into the biofilm that forms on the outer

surface of the membrane. The bacteria in the water consume the oxygen to oxidize the pollutants that are diffusing from the bulk liquid into the biofilm.

Figure 2-8. Typical configuration of a MABR system (Terada et al., 2002).

As the bacteria consume the oxygen that is being diffused the biofilm starts to get thicker, decreasing the amount of oxygen that is diffusing into the bulk water which in turn increases denitrification rates. There is an aerobic zone that is created close to the membrane to support nitrification and as the biofilm gets thicker, an anoxic zone is developed near the biofilm-liquid interface that allows for denitrification (Figure 2-9).

Figure 2-9 Decrease in oxygen concentration further from the membrane and a decrease in substrate concentration further from the biofilm water interface through a biofilm in a MABR (Walter et al., 2005).

A study of MABR systems has shown that DO concentrations at the surface of the silicone membrane were approximately 8.0 mg/L, while at the biofilm liquid interface, the DO was detected at approximately 0.2 mg/L (Matsumoto et al., 2007). As a result, the DO in the bulk water is always approximately 0 mg/L (Terada et al., 2002). This is like the second mechanism described above for SNDN. By having such a low bulk water DO concentration, it is possible to achieve SNDN through a process similar to the one in activated sludge flocs.

The unique structure of the MABR system indicates that it can be used for SNDN in a single bioreactor without pH adjustment, keeping the pH within the ideal range for a SNDN system (Terada et al., 2002). The biofilm structure is much more complicated than a conventional biofilm because two different reactions are occurring under different conditions. Ideally, at the surface of the membrane nitrification is occurring under aerobic

conditions and then at the biofilm liquid interface denitrification is occurring under anoxic conditions. To accomplish SNdN in a single reactor a study published in the Journal of Bioscience and Bioengineering inoculated the MABR in two different stages. The MABR was first seeded with nitrifiers. Once a biofilm was visually confirmed with the nitrifying bacteria, the heterotrophs were added (Terada et al., 2002). This two-step process ensured that the slower growing nitrifiers were not outcompeted by the faster growing heterotrophic bacteria at the aerated membrane surface.

Several important factors that are optimal for SNdN have been identified in previous studies of MABR systems, including membrane surface area, intramembrane pressure, C:N ratio, and temperature. Some studies have shown that the intramembrane pressure does not affect the rate of nitrification if the COD levels are low. However, if COD levels are higher it is necessary to have a higher intermembrane pressure (Hisashi, Satoh, et al, 2004).

Some additional factors that also affected the efficiency of the MABR system were the biofilm thickness and liquid flow velocity. Previous research showed that if the biofilm thickness exceeded 1000 μm then the substrate diffusion was limited, and a decrease in the nitrogen removal rate occurred (Terada et al., 2002). It was also observed that the bulk liquid flow velocity affected the diffusion through the boundary layer, detachment rate, and the maximum biofilm thickness (Terada et al., 2002).

MABR systems have already proven to be successful for nitrification, simultaneous organic carbon removal and nitrification, and the treatment of high strength food processing wastewater. To truly understand the capabilities of this system further

research on MABR systems is currently being done to optimize operation conditions for the system.

2.4.1 Evaluation of Diffusivity of Silicone Membranes

Previous research of silicone membranes has shown that oxygen has a 2:1 higher diffusivity factor compared to nitrogen (Haibing, 2009). The study evaluated gaseous permeability of dimethyl silicone rubber and found that oxygen had a substantially higher permeability. This was confirmed when evaluating peroxide cured silicone tubing from

Cole Parmer that showed that oxygen had a permeability of $6579 \frac{\text{cm}^2}{\text{s} \cdot \text{cm.Hg}} 10^{-11}$

compared to nitrogen with a permeability of $2763 \frac{\text{cm}^2}{\text{s} \cdot \text{cm.Hg}} 10^{-11}$ (Cole-Parmer, 2017).

This high O₂ permeability makes the silicone tubing a good media to use as the aerator in the MABR.

MABR systems are typically run using pure oxygen and do not usually need to consider differences in permeability, however by having a 2:1 diffusivity to nitrogen made it possible to use compressed air as the oxygen source instead of pure oxygen. The mixture of gases does not affect the permeability of other gases in the mixture because the pressure is calculated using their partial pressures (Haibing, 2009). To increase the diffusivity into the water the tubing was looped through the bioreactor to increase turbulence in the air flow paths. If this tubing proves to be an effective aerator with compressed air, it will make upscale of the system more feasible because it is a more affordable option for the than using pure O₂.

2.5 BiOWiSH

To further enhance biological nutrient removal BiOWiSH has created a product, Aqua that was developed for wastewater treatment to facilitate biological removal of nutrients, suspended solids, pathogens, and other contaminants. It is a blend of *Bacillus*, *Pediococcus*, and *Lactobacillus* bacteria that can achieve nitrification and denitrification (Gorsuch et al., n.d.). Aqua consists of less than 1% of active ingredients. Most of the components in Aqua are a carbon source or non-living organic ingredients such as rice brans or soy beans with full fat content to help support the growth of bacteria.

Aqua relies on heterotrophic nitrification by heterotrophic bacteria that use organic compounds as a carbon and energy source. The growth rate of heterotrophic bacteria is higher than the growth rate of typical nitrifiers and some studies have observed that aerobic nitrification and denitrification among certain strains of *Bacillus* (phylum Firmicutes) were at higher rates than previously observed by heterotrophs (Gorsuch et al., n.d.).

The heterotrophic bacteria used in Aqua have several isolated bacteria strains specifically for nitrification and denitrification. Each strain of bacteria in Aqua was tested in DI water reactors and wastewater reactors to determine the nitrification and denitrification rates of each strain (Gorsuch et al., n.d.). This study indicated that aerobic nitrification and denitrification was occurring at high DO concentrations (Table 2-1 and Table 2-2).

Table 2-1 Nitrification rates for the different bacterial isolates in Aqua in DI water reactors versus wastewater reactors (Gorsuch et al., n.d.).

Isolate	DI Water Reactors			Wastewater Reactors		
	Rate (mM h ⁻¹)	DO (ppm) T=0	DO (ppm) T=24	Rate (mM h ⁻¹)	DO (ppm) T=0	DO (ppm) T=24
B.licheniformis (B5)	0.655	6.6	4.2	0.56	7.9	5.8
B.licheniformis (B11)	0.563	7.2	6.3	0.70	6.7	5.8
B.licheniformis (B13)	0.530	8	6.2	0.69	6.2	5.6
B. amyloliquefaciens (B6)	0.146	8.2	4.2	0.11	5.8	4.4
B. amyloliquefaciens (B10)	0.639	7.9	2.4	0.64	5.5	5
B. subtilis (B9)	0.507	6	5.6	0.14	5	4.8
B. subtilis (KLB)	0.141	6.9	4.6	0.01	7	6.5
B. mojavensis (B8)	0.737	6.1	5.4	0.06	6	3.8
B. pumilus (B12)	0.797	6.1	5.3	0.60	5.9	5.6
P. pentosaceus	0.133	8.4	5.6	0.07	7.1	6.8
P. acidilactici	0.639	7.6	6.5	0.13	6.9	5.4
L. plantarum	0.001	7.8	7.6	2.35E-04	6.3	6.4

Table 2-2 Denitrification rates for bacterial isolates in Aqua tested in DI water reactors versus wastewater reactors (Gorsuch et al., n.d.)

Isolate	DI Water Reactors			Wastewater Reactors		
	Rate (mM h ⁻¹)	DO (ppm) T=0	DO (ppm) T=24	Rate (mM h ⁻¹)	DO (ppm) T=0	DO (ppm) T=24
B.subtilis (KLB)	0.010	7.7	6.4	0.009	7.7	6.4
B.mojavensis (B8)	-0.010	8.5	6.8	-0.011	8.5	6.8
B.pumilus (B12)	0.001	7.7	5.8	0.110	7.7	5.8
B. licheniformis (B13)	0.002	7.3	5.4	0.112	7.3	5.4

While these results have not yet been replicated, this study indicates that the bacteria in Aqua should enhance the nitrification and denitrification process.

2.5.1 BiOWiSH Case Study

In a case study for BiOWiSH, a wastewater treatment plant in South Korea approached BiOWiSH about treating their high ammonia leachate that they were receiving from a nearby landfill. The ammonia leachate had high total nitrogen (TN) concentrations between 1000-1200 mg-N/L, and the plant wanted to drop the ammonia to below 50 mg-N/L TN. BiOWiSH's product Aqua was drip dosed into the anoxic tank (Figure 2-10) at the specified daily dosage for the week (BiOWiSH, 2017). In the first week of the 12-week trial the daily dose was 3 kg. In weeks 2 through 10 the daily dose was decreased to 1 kg. Then in weeks 11 and 12 the dose was further decreased to 0.7 kg (BiOWiSH, 2017).

Figure 2-10. Modified treatment train for 12-week trial in South Korean wastewater treatment plant (BiOWiSH, 2017).

For this trial, the total daily flow of $150 \text{ m}^3/\text{d}$ included $130 \text{ m}^3/\text{d}$ of the regular influent and $20 \text{ m}^3/\text{d}$ of the high TN leachate influent. When BiOWiSH was added, a drop in TN of approximately 100 mg-N/L between the influent and effluent water was measured. This result indicated that BiOWiSH allowed the plant to process the high-nitrogen feed with minimal changes to their existing operation.

2.6 Previous Research

Previous work done by Kirk Waltz proved to be inconclusive with a dead-end airline configuration with compressed air. This research included four batch reactor experiments, six continuous flow mineral media experiments, and five continuous flow wastewater experiments. The batch reactor experiments showed positive results indicating nitrification with initial decreases in ammonia with increases in nitrate. The highest initial ammonia removal was 79% and followed first order kinetics (Waltz, 2009). Although there was an 86% decrease in the ammonia concentration in the continuous flow experiments, no increase in nitrate or nitrite was observed (Waltz, 2009). The absence of nitrate or nitrite in the continuous flow experiments suggested that the dead-end configuration with compressed air was not delivering enough oxygen to the system which

could have been inhibiting the nitrification. The MABR design in this study changed to a continuous flow airline to hopefully increase the oxygen delivered to the system.

Some of the major problems identified with the MABR design included control of the airline, consistency of the media composition, and the maximum O₂ pressure that the MABR can handle (Waltz, 2009). One of the main goals for future work identified in Kirk's thesis was to create experimental scenarios that were more controlled. This thesis work focuses on building on the work that was done previously and conducting a study looking specifically at optimizing the bioreactor design and evaluating whether Aqua enhances SNdN in a MABR.

Chapter 3 SYSTEM DESIGN AND INITIAL SCREENING RESULTS

To analyze the feasibility of simultaneous nitrification and denitrification in a MABR, a small-scale bioreactor was first used to conduct screening experiments. When designing a MABR system to work with Aqua, the Aqua dosing methods, concentration of Aqua, the intramembrane pressure, membrane surface area, temperature, nitrogen concentration, and retention time should all be taken into consideration. These screening experiments focused on the following parameters; dosing methods, and concentrations of Aqua, and nitrogen concentration. Because it was not possible to evaluate the effect of all the variables that influence an MABR due to time constraints, those not mentioned were kept constant for the following experiments. The small scale MABR acted as a proof of concept to determine that nitrogen removal was possible in a continuous flow MABR in conjunction with Aqua. Once the removal of nitrate and ammonium were observed, the MABR was scaled to a larger 20 L size to further evaluate the effect of nitrogen concentration, retention time, and dosing methods.

3.1 Small Scale Design

The small scale MABR system used a 1 L. Kontes Cytolift bioreactor (catalog number: 880600). The temperature in the MABR was controlled by a submersible aquarium heater that was placed in the center of the bioreactor.

The media in the reactor was cycled through the MABR with a 0.32 cm. (1/8") inner diameter PVC tubing from Cole Parmer (Item Number: EW-96605-01) that reached the bottom of the MABR. The PVC tubing is not permeable and therefore did not supply any additional oxygen to the system. This ensured that the media entered the system at the bottom and would flow through the entire system to the top to exit the MABR from the

yellow tubing (Figure 3-1). The water was pumped through the system using a Masterflex L/S variable speed modular drive pump (Catalog number: 7553-20, Serial number: 567118).

Figure 3-1 Small scale MABR set up using the Cryolift bioreactor.

The air supply for the MABR was controlled by a series of rotameters, pressure gauges, and valves (Figure 3-2). The compressed air was supplied from Building 13. For the screening experiments the pressure was kept at approximately 15 psi. Initially the air flowrate was controlled using a rotameter with a valve that could be adjusted to restrict the flow and maintain the intramembrane pressure. When more than one airline was needed for multiple MABR systems it was necessary to add a pressure regulator to the airline to control the pressure more precisely than the ball valve that currently controlled the air supply from the building. The compressed air from the building would normally

be around 100 psi, however with the pressure regulator it was possible to maintain the pressure at 15 psi. The final airline configuration for the small-scale system consisted of a regulator, followed by a rotameter, pressure gauge, needle valve and then lastly a final rotameter (Figure 3-2).

Figure 3-2 Diagram of airline configuration for small scale experiments.

As shown, the airline was connected to a silicone tubing that made a U shape in the bioreactor. The surface area of the silicone tubing in the MABR was approximately 0.026 m² (40 in.²). This resulted in a surface area to volume ratio of about 51.6 m²/m³. The surface area to volume ratio remained constant for all the screening experiments.

3.2 Screening Experiment Setup

The screening experiments were conducted with slight variations in both Aqua dosing methods and Aqua concentrations to evaluate how these factors affected nitrogen removal. The two different dosing methods and media compositions used for the screening experiments along with the testing methods are specified below.

3.2.1 BiOWiSH Dosing Methods

For the screening experiments two different methods were used to dose the MABR with Aqua. BiOWiSH specifies that the type of dosing method for the Aqua is typically dependent on the application. Since the MABR system is not a commonly used nitrogen removal technology that is still being researched, two different dosing methods were evaluated and are detailed below.

3.2.1.1 *BIOWISH Aqua Application Method 1*

For the initial experiments conducted the Aqua was not activated prior to being added to the reactor. The dry Aqua was added to the MABR at the specified concentration for each experiment. By not activating the bacteria prior to adding it to the system it could have taken longer to see a drop-in ammonium because the bacteria would still need to go through the lag phase of growth.

One benefit of this dosing method is that it does not limit the type of bacteria that grow in the system in the same way that activating the bacteria first might. Different species of bacteria favor different conditions such as carbon sources, DO concentration, and minerals so inoculating the bacteria prior to adding it to the system could preselect which bacteria grow in the MABR. For example, if the carbon source changed from glucose to sucrose a different species of bacteria might grow at a faster rate. By not activating the bacteria prior to adding it to the MABR it allows the growth of the bacteria that would naturally grow under those conditions.

3.2.1.2 *BIOWISH Aqua Application Method 2*

The second method to dose the Aqua was to activate it prior to adding the Aqua to the system. By inoculating the Aqua this ensured that the bacteria were already either in the

exponential growth or stationary phase. This meant that ideally with an already established population of bacteria the ammonium concentration would start to decrease sooner.

To activate the Aqua for the screening experiments, it was incubated with an aquarium bubbler for about two days prior to adding it to the MABR. This resulted in an inoculum with a high concentration of Aqua that could be diluted and added to growth media for each experiment at the specified Aqua concentration.

3.2.2 Media Composition

To ensure a controlled environment, growth media was used for the screening experiments. Throughout the course of the screening experiments minor changes were made to the mineral media to improve bacteria growth. The main change was in the type of broth that was used to promote the growth of the Aqua for the two different dosing methods.

3.2.2.1 Nitrate Broth Growth Media

The growth media that was used for the initial experiments used a nitrate broth to promote the growth of Aqua. The growth media consisted of minimal minerals with a high concentration of glucose (Table 3-1).

Table 3-1 Nitrate growth media used for initial screening experiments (Holland, 2017).

Chemical	Concentration (mg/L)
Glucose	3000
KH ₂ PO ₄	500
K ₂ HPO ₄	1500
FeCl	2 drops (using 3mL transfer pipet)
MnCl ₂	2.5
Nitrate Broth	50

When preparing the media all the constituents except the ammonium and nitrate were added first. The media was placed on a stir plate until the chemicals were well dissolved. The media was then sterilized using the autoclave. The ammonium and nitrate were added right before the run started to ensure that the NH_4 did not volatilize from the media. For this media the first dosing method was used and the Aqua was added after the autoclaved media had returned to room temperature.

3.2.2.2 Trypticase Soy Broth (TSB) Growth Media

The second growth media used replaced the nitrate broth with TSB broth and was used for the second dosing method. To prepare this media 900mL of growth media was prepared as specified below, excluding the TSB (Table 3-2). 100 mL of the TSB broth was prepared separately from the growth media at the specified concentration (Table 3-2).

Table 3-2 TSB growth media for inoculating Aqua (Holland, 2017).

Chemical	Concentration (mg/L)
Glucose	3000
KH_2PO_4	500
K_2HPO_4	1500
FeCl	.006
MnCl ₂	2.3
BBL Trypticase Soy Broth	30000

The 100 mL of TSB was then added to the 900 mL of the growth media. The growth media was then sterilized using the autoclave. Once the media cooled the Aqua was then added to this inoculum at a higher concentration than what was specified for the bioreactor. This inoculum was then put in an incubator with a bubbler for about two days. Prior to starting the run more growth media was prepared and sterilized in the autoclave

without the TSB in it (Table 3-2). After autoclaving, the desired nitrate and ammonium concentrations were added to the media. Then, before starting the run, a predetermined volume of activated media was added to the MABR to achieve the specified Aqua concentration for the experiment.

3.2.3 Water Sampling, Analyses, and Quality Control

All the screening experiments followed the same sampling methodology to ensure consistency between the experiments. Each sample collected was approximately 15 mL and was collected using a transfer pipette and stored in a glass container. The temperature and pH of each sample was recorded using an Oakton Acorn series pH/temperature meter. To store the samples 96% H₂SO₄ was added to lower the pH below 2 and stop any ammonia from volatilizing from the sample. The sample was then labeled with the reactor letter, date, and time.

All samples were then stored in the refrigerator until they were analyzed for nitrate, nitrite, and ammonium. These are the key components of the nitrogen cycle that are formed and degraded through nitrification and denitrification making them good indicators of how the nitrogen was being removed from the system.

To prepare the samples for analysis they were all double filtered. For all the screening experiments the samples were filtered through 1.2 µm glass fiber filters then through a 0.22 µm nitrocellulose filter. Filtering through the 1.2 µm filters prevented the more turbid samples from clogging the 0.22 µm filters. Filtering the samples through the 0.22 µm filter removed most of the bacteria from the sample to help preserve the nitrate, nitrite, and ammonia concentrations in the water. It was an additional measure to preserve

the samples, ensuring bacteria in the samples were not changing the ionic concentrations over time.

3.2.3.1 Ammonium Analysis

Ammonium analysis was conducted using a TL-2800 automated ammonia analyzer from Timberline Instruments. Once the samples were filtered and acidified the samples were put in 15 mL falcon tubes. Each sample required a minimum of 7 mL of sample in each falcon tube. In this method the sample was mixed with a caustic solution that raised the pH of the sample to at least 11. At this high pH it caused almost all the ammonium ions to convert into dissolved ammonia gas. The caustic and sample mixture then flowed over a tubular membrane where the ammonia gas diffused through the membrane. Within the membrane the ammonia gas was absorbed by a buffer solution with a pH of 6.5. It was then possible to calculate the ammonium concentration in the sample because the concentration is proportional to the change in conductance of the absorbing solution (Timberline instruments, Boulder, CO).

To determine ammonium concentrations a calibration curve was created with known amounts of NH_4Cl . The calibration curve typically ranged from 0 -100 mg-N/L and changed depending on the expected concentration of the sample. Typically, the expected concentration of the samples should be in the middle of the range of the calibration curve. The TL-2800 Data Acquisition software would use this calibration curve to relate the conductance to the concentration of ammonium.

3.2.3.2 Nitrate Analysis

The nitrate analysis was conducted using a Dionex ICS-1600 Ion Chromatograph. In this test the filtered sample was put into 5 mL Dionex Polyvials and sealed with 0.2 μm

Dionex plain filter caps. The ion chromatography process used in this method occurs in 6 steps (Figure 3-3).

Figure 3-3 The 6 steps in the ion chromatography process (ThermoScientific, 2012).

First, the sample was injected into an eluent stream. The eluent is a 9 mM Na_2CO_3 solution prepared by dissolving 953.9 mg of Na_2CO_3 into 1L of DI water. The eluent and sample then went through the guard that removed any contaminants that could poison the separator column. In the separator column the ions were separated based on the premise that different ions will migrate through the IC column at different rates depending on their interactions with the ion exchange sites (ThermoScientific, 2012). Once the ions left the column, they went through a suppressor that enhanced the detection of the ions simultaneously suppressing the conductivity of the eluent. The conductivity cell then transmitted the signal to the Chromeleon Chromatography Management System that

identified the ions based on the retention time through the separation column. The program then quantified each analyte by integrating the peak area or peak height.

To interpret these results a calibration curve was created using known concentrations of nitrate in a solution prepared using NaNO_3 . The calibration curve would typically consist of points ranging from 0 to 100 mg-N/L. This would change depending on the expected concentrations of the samples that were being analyzed. By putting samples with known concentrations of nitrate, conductivity readings could be related to nitrate concentration.

3.2.3.3 Nitrite Analysis

The same method was used for the nitrite analysis that was used for the nitrate analysis. The main difference in the nitrite sample was that retention time of the nitrite through the separation column is shorter than that for nitrate. The corresponding nitrite concentration for each conductivity reading produced by the ion exchange was determined using a calibration curve created using known concentrations of nitrite. The solution was prepared by adding NaNO_2 at specified concentrations as mg-N/L. Similar to the nitrate calibration curve the range of the nitrite calibration curve would change with the expected concentration of the sample. The nitrite concentrations were typically expected to be much lower than the nitrate concentrations, so the range was typically half of that for nitrate.

3.2.3.4 Quality Assurance/ Quality Control (QA/QC)

The QA/QC methods that were practiced include spikes, splits, and continued calibration verification (CCV). The splits and spikes needed to be within $\pm 15\%$ of the expected value. All CCV values needed to be within $\pm 10\%$ of the expected value. In addition, for

all calibration curves the R^2 values all needed to be ≥ 0.99 . These methods were applied to all the water quality testing that was done.

3.2.3.5 Ammonium Added to System

All nitrate and ammonium were measured as mg-N/L. By having the concentration in mg-N/L it makes the results comparable to EPA standards for wastewater treatment plant discharge that are typically given in mg-N/L.

3.3 Screening Experiments

These experiments looked at assessing the feasibility of using a continuous flow configuration, different Aqua concentrations, different Aqua dosing methods, and different initial ammonia and nitrate concentrations (Table 3-3). The results from the screening experiments were a proof of concept to show that the MABR was capable of degrading ammonium and nitrate using a continuous flow configuration for the airline. For all the experiments the influent flowrate was kept below 5mL/min to avoid scour of the biofilm.

Table 3-3 Brief description of screening experiments that were conducted and results from the small scale MABR system.

Experiment Number	Objective	Observations/Conclusions
1	Confirm diffusivity of oxygen through silicone tubing in continuous flow configuration	Continuous flow configuration is capable of diffusing oxygen.
2	Nitrate and ammonium loss to the atmosphere	No atmospheric losses of ammonia or nitrate for small scale MABR system
3	50 ppm Aqua in growth media with 25 mg-N/L nitrate and ammonium	Fast decrease in nitrate and no decrease in ammonia
4	100 mL of growth media from Experiment-3 with Aqua with 25 mg-N/L nitrate and ammonium	Confirmed a decrease in nitrate and no drop-in ammonia as shown in Experiment-3.
5	25 ppm Aqua in growth media with 100 mg-N/L ammonium and no nitrate	Drop in ammonia within the first 20 hrs. then concentration plateaus at 60 mg-N/L Assumed carbon source was the limiting factor.
6	30 ppm Aqua in growth media with 30 mg-N/L ammonium and no nitrate	Confirmed degradation of ammonia.

3.3.1 Experiment 1: Diffusivity of silicone tubing in a continuous flow configuration

The main objective of the first experiment was to establish that the silicone membrane was diffusing oxygen through the membrane at an intramembrane pressure of 15 psi in a continuous flow configuration. Because the previous work done with the MABR system was conducted using a dead-end airline configuration it was necessary to confirm that the continuous flow configuration would diffuse air through the silicone membrane.

A single reactor was set up with the silicone tubing running through the reactor in a U-shape. The system was checked for any leaks before the first test was conducted. Through

visual observation it was determined that air was being released through the membrane. It was not necessary to take DO measurements of the water because the literature review proved that oxygen has a higher diffusivity than nitrogen through silicone membranes. The MABR in this experiment was filled with deionized (DI) water so no bacterial growth observed.

3.3.2 Experiment 2: Nitrate and ammonia loss to the atmosphere (Trial 1 and 2)

After confirming that the silicone membrane would diffuse oxygen into the reactor a second experiment was conducted to determine if any nitrate or ammonia was being lost to the atmosphere. This experiment acted as a control for the screening experiments because it was not possible to completely seal the top of the MABR and each small scale MABR experiment did not have a control. 1 L of the nitrate broth media was prepared and autoclaved as described previously. The only difference in the reactor setup was that no Aqua was added to the media. This experiment was run over three days with samples taken three times a day at 6-hour intervals starting at approximately 9 AM. Again, no bacterial growth was observed. This experiment was replicated using the same methods.

Both experiments showed that no NH_4 was volatilized, with the ammonium and nitrate concentrations remaining constant (Figure 3-4). The concentration of NH_4 and NO_3 in the water remained relatively constant over the three-day period that samples were taken. This confirmed that any drop in NH_4 in the following experiments would be due to the bacteria or any reactions occurring in the MABR and that the NH_4 was not volatilizing from the system.

Figure 3-4 No degradation of NH_4 , NO_3 , and NO_2 in growth media with no bacteria over the course of approximately 100 hours in Experiment 1.

3.3.3 Experiment 3: Evaluating ammonium and nitrate degradation with 50 ppm

BiOWiSH (Trial 1)

The next step was to determine if the system was capable of nitrogen removal. In this experiment the first BiOWiSH dosing method was used with the nitrate broth media. For this experiment 25 mg-N/L as nitrate and 25 mg-N/L as ammonium were added to the media with 50 ppm of BIOWISH Aqua. The reactor was set up the same as the previous two experiments with the only difference being that the reactor was covered with parafilm to prevent any oxygen in the atmosphere from entering the system. Samples were collected three times a day in 6-hour intervals starting at approximately 9 AM. For the following screening experiments these assumptions were made:

- No oxygen was entering the system from the top of the reactor and all oxygen in the system was only from the silicone tubing

- The water was not mixing and the water flowrate of less than 5 ml/min was low enough to prevent scour of any biofilm.

To determine the success of the system the nitrogen removal rate was calculated by dividing the amount of nitrogen removed by the time it took for the nitrogen removal to occur. This experiment showed that the MABR in conjunction with the Aqua was capable of nitrate removal at a rate of 0.38 mg/L-hr. (Figure 3-5). Small spikes in nitrite were observed that indicate that denitrification could be occurring in the bioreactor. It also appears that nitrogen assimilation was occurring because not all the nitrate that was degrading was showing up as any other form of nitrogen. The slight increase in ammonium over the course of the experiment could be due to the decay of biomass or start of cell lysis as the older bacteria start to die, especially because no solids were removed from the bottom of the MABR.

Figure 3-5 Decrease in NO₃ in the MABR and no drop in NH₄ over 190 hours in Experiment 3.

3.3.4 Experiment 4: Evaluating ammonium and nitrate degradation with established culture of Aqua from Experiment-3 (Trial 2)

This experiment was a replicate of Experiment-3 with the same nitrate and ammonia concentrations. The main difference for this experiment was that 100 mL of the growth media with the bacteria grown in the previous experiment was used to inoculate this MABR. The objective was to enrich the culture by keeping those bacteria that had been removing the nitrate in the previous experiment. Similar to the previous experiment the nitrate broth was used for the growth media. This experiment was run for eight days and samples were collected three times a day at 6-hour intervals starting at 9 AM.

While this experiment showed slower nitrogen removal, the NO₃ still degraded over the course of the experiment. In this experiment small spikes in NO₂ were produced making it

appear again that nitrogen assimilation was occurring as well as denitrification. In this experiment the NO_3 removal rate was around 0.085 mg-N/L-hr. when the nitrogen removal was evaluated over the entire eight days. However, focusing on the initial drop in nitrate from the 50th to 100th hour, the nitrogen removal rate was around 0.15 mg-N/L-hr. (Figure 3-6). Although this was higher than the removal rate over the entire eight days it was still considerably lower than the removal rate that was seen in Experiment-3.

Figure 3-6 Decrease in NO_3 with no production of NO_2 over approximately 270 hours.

The lower removal rate could be because this bioreactor was inoculated using 100 mL of media from the previous experiment. It appears that instead of enriching the bacteria culture it made it so that the bacteria in this MABR were older and might have already entered an endogenous growth stage. If the bacteria population was already decreasing it would justify the decrease in the removal rate. Despite the slower removal rate this experiment still confirmed that the Aqua bacteria were capable of NO_3 removal.

3.3.5 Experiment 5: Evaluating ammonium degradation in the absence of nitrate

To determine if Aqua could decrease the ammonium in the water the ammonium concentration was increased to 100 mg-N/L and no nitrate was added. In this experiment a second bioreactor was made identical to the original one. Both bioreactors were run simultaneously in this run. The objective of only including ammonium in the media was to condition the bacteria that would normally favor nitrate to instead degrade the ammonium. This experiment used the second BiOWiSH application method and TSB broth. By inoculating the bacteria prior to beginning the run it was expected to see improved ammonium and nitrate degradation. This experiment was run for 5 days with sampling three times a day, 6 hours apart starting at approximately 9 AM each day.

While both MABRs received the same amount of ammonium and Aqua MABR 1 appeared to have only a 10-20 mg-N/L drop in ammonium whereas MABR 2 exhibited almost a 40 mg-N/L drop in ammonium (Figure 3-7 and Figure 3-8 respectively). While there was a decrease in ammonium the nitrogen did not appear again in the form of nitrate or nitrite that would have suggested nitrification was occurring.

Figure 3-7 Drop of approximately 15 mg-N/L in ammonium in MABR 1.

Figure 3-8 Drop of approximately 40 mg-N/L in ammonium in MABR 2.

The discrepancy between the decrease in ammonium and increase in nitrate in MABR 2 could be explained through two different mechanisms. One possibility was that the system had a higher concentration of denitrifiers. This could be possible because as mentioned previously the only space for nitrifiers to survive was near the oxygen supply, at the membrane surface. The rest of the system was anoxic which was ideal for denitrifiers. If there was a high population of denitrifiers then the nitrate concentration would not be as high as expected because the nitrate would be denitrified to nitrogen gas. This would be unlikely though because if there was rapid denitrification then it would be unlikely that there would be any nitrite appearing. The other option was that instead of being nitrified the ammonium was being assimilated into biomass. In assimilation there are no intermediate forms of nitrogen going from ammonium to cell mass which would explain why the nitrate or nitrite did not appear.

Around the 50th hour a slight increase in ammonium concentrations indicated that the nitrate was being converted back to ammonium through denitrification and eventually nitrogen gas. It could also be due to cell lysis producing ammonium.

The plateau in the ammonium concentration indicates that nitrification or assimilation stopped. This could be because the nitrifiers and other bacteria in the system no longer had an electron donor source. Glucose was only added in the beginning of the experiment and could have been completely consumed over the initial 24 hrs. In those initial 24 hours the nitrogen removal rate was about 1.875 mg-N/L-hr. in MABR 2. If there was no other energy source after that time for the bacteria, then no more nitrogen removal would occur.

While similar trends can be seen in MABR 1 it was not as defined as in MABR 2. To confirm the results, the experiment was replicated to attempt to achieve complete ammonium removal from the system.

3.3.6 Experiment 6: Evaluating ammonium degradation in the absence of nitrate with continuous glucose feed

Experiment-5 was replicated with a slightly higher concentration of 30 ppm of Aqua using the same dosing methods and media composition. For this experiment 1 g. of glucose was added every 30 hours to promote the growth of bacteria in the system. This experiment also had a lower initial ammonium concentration of 30 mg-N/L. All other parameters and inoculation methods were kept identical to those in Experiment-5.

When preparing the media for this run the NH_4Cl was mistakenly added prior to autoclaving the media. As a result, approximately 20 mg-N/L NH_4 volatilized out of the system. This left the initial concentration around 12 mg-N/L. In this experiment a drop-in ammonium was observed at a rate of 0.58 mg-N/L-hr. (Figure 3-9). This was slower than the removal seen in the previous experiment but confirmed that certain strains of bacteria in Aqua can remove ammonium. While there was one point with a spike in NO_2 , the NO_3 concentration present in the effluent was never as high which makes the point appear to be an outlier because all other NO_2 and NO_3 concentrations were zero. The absence of any other nitrate or nitrite in the system supported the results from Experiment-5 that the main mechanism for nitrogen removal was nitrogen assimilation.

Figure 3-9 Immediate decrease in NH_4 followed by a spike in NO_2 .

3.3.7 Screening Experiment Conclusions

The screening experiments successfully confirmed that it was possible to achieve ammonium and nitrate removal separately in a MABR system in conjunction with Aqua. The success of the nitrogen removal was dependent on the initial concentration of ammonium and nitrate as well as carbon concentration available.

3.4 MABR Design

The large scale MABR was designed using the results of the screening experiments and previous studies done. The MABR was constructed using a 1 m. (40") long 15.24 cm. (6") diameter clear schedule 80 PVC pipe. The bottom of the bioreactor was constructed using a slip fit to female thread connector. To attach the air and water feed lines two 1.09 cm. to 1.27 cm. ($\frac{3}{4}$ " to $\frac{1}{2}$ ") PVC connectors were inserted into holes drilled in the bottom cap of the MABR and sealed with Reactor Seal pipe thread sealant. The air and water

effluent lines were attached to the top of the MABR in a similar method. The only difference was that the top of the MABR was capped with a PVC flex cap (Figure 3-10). To heat the MABR a submersible aquarium heater was used. It was completely submerged at the top of the MABR by running the power cable through a plug with a hole in the middle and sealing any holes with plumber's putty.

Figure 3-10 MABR cap with the airline on the right, effluent line on the left, and water heater in the middle.

The water was fed through a 0.32 cm (1/8") inner diameter (ID) tube at the bottom of the MABR. The flowrate was controlled using an Ismatec IPC (Model number: 78001-22 and Serial Number: 11441-00045) peristaltic pump. The Ismatec tubing (Tube ID: 3.17) that ran through the pump was connected to 0.32 cm. (1/8") polyethylene tubing that attached to the MABR. The Ismatec tubing had a flowrate range of 0.44 mL/min to 44 mL/min.

The air for the system was fed to the silicone membrane in the reactor from the compressed air supplied from Building 13 (Figure 3-11). The air was controlled with a regulator to ensure the pressure remained constant. The line then went to a rotameter to measure the inflow and then into the silicone membrane in the bioreactor. After the bioreactor the air exited to a pressure gauge to determine the pressure in the membrane. This was followed by a needle valve to control the flowrate because the valves on the rotameters did not work in series to control flowrate. This valve allowed the airflow to be controlled precisely before going to a second rotameter and exiting the system. For all the large scale experiments the intramembrane pressure was kept around 20 psi.

Figure 3-11 Water and air line configuration for large scale MABR.

One important factor in the MABR design was the selection of the silicone membrane. It was important to select a silicone membrane with the right type of coating that allowed for the diffusion of oxygen. A peroxide cured tubing was used because of its higher diffusion coefficient than platinum cured silicone tubing (ColeParmer Masterflex, catalog Number 13-310-108). 4.6 m. (15') of 0.32 cm. ($\frac{1}{8}$ ") ID tubing was used in each of the MABRs. The gas permeability for oxygen is $6579 \frac{\text{cm}^2}{\text{s} \cdot \text{cm.Hg}} 10^{-11}$ which is over two times higher than the permeability of nitrogen gas that is $2763 \frac{\text{cm}^2}{\text{s} \cdot \text{cm.Hg}} 10^{-11}$ for this specific tubing. This was a key component in the design because the higher permeability of oxygen through the tubing allows for the possibility of using air as the source of oxygen for the MABR. The majority of the nitrogen gas in the tubing will continue out the other end through the continuous flow airline.

To keep the influent sterile and avoid contamination polypropylene quick-filling/venting closures were used (Figure 3-12). This helped to slow the contamination of the influent feed because it was not possible to autoclave all the influent media. On one hose barb a 0.45 μm filter was attached to vent the carboy and keep bacteria out. The other hose barb was attached to the influent line going to the peristaltic pump.

Figure 3-12 Polypropylene quick-filling/venting closures used on the influent carboys to pump the influent while keeping bacteria out (“ThermoScientific”).

Chapter 4 SYSTEM IMPLEMENTATION AND TESTING

To evaluate the impact of Aqua on nitrogen removal rates in a MABR system 6 experiments were conducted using the large 20 L MABR design discussed in Chapter 3.4. This chapter will detail differences in the sample collection methods, tests conducted, and media composition from the small-scale experiments as well as the objective and setup of each experiment conducted.

4.1 Water Sampling, Analysis, and Quality Control for Large Scale Experiments

For each experiment, samples were taken twice a day approximately 12 hours apart. For the batch reactor experiment, samples could only be taken through a port at the top of the bioreactor. Approximately 30 mL of water was taken for each sample using a 10 mL pipette. The pH and temperature of the sample was recorded immediately after collecting the sample. To avoid the volatilization of any NH_4 from the sample 96% H_2SO_4 was added to the samples to lower the pH below 2 and were refrigerated until the samples were analyzed.

For the experiments with a continuous influent flow, samples were taken from a sampling tube that branched off from an effluent tube at the top of the bioreactor (Figure 3-10). Similar to the batch reactor the samples were taken twice a day approximately 12 hours apart by opening the valve on the sampling tube. Due to the low flow rate of either 2mL/min or 5 mL/min, H_2SO_4 was added prior to collecting the sample so that the NH_4 would not volatilize in the time that it took to collect the 25 - 30mL of sample necessary for analysis. To measure the pH and temperature, a separate sample was collected with no acid in a 50 mL beaker

Additional QA/AC was done to determine if the sample preparation process influenced the nitrate and nitrite concentrations obtained from the IC. The result of these tests determined that the glass fiber filters that were being used to pre-filter the samples were removing nitrate from the samples. As a result, the pre-filters were switched to 1.22 μm nitrocellulose filters that did not cause any drop-in nitrate concentration. The QA/QC testing also revealed that acidifying the samples with H_2SO_4 reacted with the nitrite at a 2:1 ratio, removing some of the nitrite from the sample. Further details on the effects of the sample preparation can be found in the Appendix (Chapter 8).

4.1.1 Sample Analysis

Similar to the screening experiments, the samples collected were tested for nitrate, nitrite, and ammonia using the same testing methods specified previously. For the large scale experiments an additional test for total nitrogen (TN) was done to estimate the organic nitrogen in the water as well as determine the total nitrogen removed. The concentration of organic nitrogen in the effluent was estimated by subtracting the nitrate, nitrite, and ammonia concentration from the total nitrogen. This would indicate whether the system was accomplishing SNDN or if the nitrogen was being assimilated into biomass.

4.1.1.1 Total Nitrogen Analysis

To test for TN, the high range Total Nitrogen Reagent Set from Hach (Product number 2714100) was used. The test was conducted using the standard procedures provided by Hach. The TN test required that at least 0.5 mL of sample was left unfiltered and acidified to preserve all the nitrogen in the sample. Due to this acidification it was necessary to neutralize the sample before conducting the test by titrating each sample with NaOH. A calibration of the TN test was previously conducted and showed that the

acidification and neutralization of the samples did not affect the concentration if the initial and final volumes of the samples were recorded to account for any dilution that may have occurred.

This method analyzed the nitrogen in the sample using the persulfate digestion method to determine the concentration of total nitrogen. The test converts the ammonia and organic nitrogen to nitrate, leaving the initial nitrate and nitrite unaffected by the digestion.

Following the digestion, the test uses colorimetric determinative methods for TN analysis. To convert the absorbance to concentration one standard calibration curve was used that was created from averaging multiple calibration curves.

4.2 Media Composition

These experiments were conducted with three different types of media. The first media that was used for the initial experiments was a growth media developed by BiOWiSH. It was determined that this media composition was the best for growing the Aqua bacteria. However, after Experiment-3 it was determined that this media did not have enough alkalinity for the acid that was produced from the incomplete breakdown of glucose. This led to a change in media to growth media 2 that was used in a previous study of MABR systems (Chang and Tseng). The media was then modified a third time to try and create a media that could maintain a steady pH in the MABR.

4.2.1 Growth Media 1

This growth media was used in the initial experiments and consisted of the following constituents (Table 4-1). This growth media had a pH around 7.

Table 4-1 Growth media created by BiOWiSH for bacteria inoculation (Gorsuch).

Chemical	Concentration (mg/L)
KH ₂ PO ₄	217.5
K ₂ HPO ₄	85
Na ₂ HPO ₄ 7H ₂ O	630.7
CaCl ₂ 2H ₂ O	27.5
MgSO ₄ 7H ₂ O	22.5
FeCl 6H ₂ O	0.25

The glucose for the media was added at a carbon to nitrogen (C: N) ratio of 12:1. In addition to the constituents listed above, mineral salts were added at a concentration of approximately 1% (Table 4-2). The mineral salts each enhance the bacterial growth in a different way. For example, the Na₂MoO₄ 2H₂O is an essential nutrient for denitrification.

Table 4-2 Mineral salts composition used to enhance the bacteria growth in the MABR (“Trace Mineral Supplement”).

Chemicals	Concentration (g/L)
EDTA	0.500
MgSO ₄ 7H ₂ O	3.000
MnSO ₄ H ₂ O	0.5000
NaCl	1.000
FeSO ₄ 7H ₂ O	0.100
Co(NO ₃) ₂ 6H ₂ O	0.100
CaCl ₂	0.100
Alk(SO ₄) ₂	0.100
H ₃ BO ₃	0.010
Na ₂ MoO ₄ 2H ₂ O	0.010
Na ₂ SeO ₃	0.001
Na ₂ WO ₄ 2H ₂ O	0.010
NiCl ₂ 6H ₂ O	0.020

4.2.2 Growth Media 2

To maintain a pH within the range of 6.5 to 8.5 the growth media was altered slightly by adding NaHCO₃ to act as a buffer for the solution (Table 4-3). In this growth media mineral salts were not added. The pH of the final growth media was around 7.4 which was within the ideal pH for both SNdN of 6.5 to 8.5 and for ANAMMOX from 7 to 7.5.

Table 4-3 Constituents of growth media 2 that was used in the bioreactor (Chang and Tseng).

Chemical	Concentration (mg/L)
NaHCO ₃	500
KH ₂ PO ₄	8.5
K ₂ HPO ₄	21,75
Na ₂ HPO ₄ 7H ₂ O	33.4
CaCl ₂ 2H ₂ O	27.5
MgSO ₄ 7H ₂ O	22.5
FeCl 6H ₂ O	0.25

4.2.3 Growth Media 3

Using growth media 2 the pH was still dropping below 6, so the media was altered again. In this mineral media the concentration of KH₂PO₄ and K₂HPO₄ were increased to act as a buffer in the mineral media. The concentration of each needed in the solution were calculated using the Henderson Hasselbach Equation (Equation -5). In this equation the pKa = 7.2 and the desired pH = 7.4.

$$pH = pKa + \log \frac{A^-}{HA} \dots \dots \dots (5)$$

In addition to the increase in concentration of KH₂PO₄ and K₂HPO₄, the mineral salts from the original growth media described in Chapter 4.2.1 were also added to the media to help promote the growth of Aqua in the MABR (Table 4-4).

Table 4-4 Growth media 3 that had an increase in buffer to maintain pH around 7.4 [23].

Chemical	Concentration (mg/L)
NaHCO ₃	500
KH ₂ PO ₄	526.42
K ₂ HPO ₄	1068
Na ₂ HPO ₄ ·7H ₂ O	33.4
CaCl ₂ ·2H ₂ O	27.5
MgSO ₄ ·7H ₂ O	22.5
FeCl 6H ₂ O	0.25

4.3 Contamination Prevention

For all the large-scale experiments it was not possible to autoclave the media being used or the MABR itself. To prevent contamination in the system from occurring the MABRs, carboys, and tubing were all cleaned with bleach and Alconox to try and prevent contamination. Before starting any run, the MABRs were filled with water and bleach and this solution was cycled through the MABR to ensure that all the attached tubing was cleaned as well. The bleach solution was then drained, and clean water was added. To ensure that no bleach residue remained on the silicone tubing or in the system, the MABR was thoroughly rinsed with water. The carboys were cleaned using the same methods prior to making any new media. As previously mentioned filters were put on the carboy covers to vent the carboys and ideally keep bacteria out.

4.4 Large Scale Experiments

These experiments focused on determining the most important factors that influence SNdN in the MABR system as well as determining the mechanisms for total nitrogen removal. For all the continuous flow experiments in this chapter the initial flowrate in the

system was set to 2 mL/min (hydraulic residence time (HRT) is approximately 7 days).

Once a biofilm was established on the membrane and the Aqua was added, the flowrate was increased to 5 mL/min (HRT is approximately 2.75 days).

Table 4-5 Summary of run times, dosing methods, Aqua concentrations, media used, and objectives of each experiment conducted using 20L MABR system.

Experiment	Run Time (hrs.)	Aqua Dosing Method	Aqua Conc. (ppm)	Media	NO ₃ ⁻ /NH ₄ ⁺ Concentration (mg-N/L)	Objective
1 Batch	490.5	2	50	Growth media 1	35/45 + extra dose of NO ₃ ⁻ periodically	Determine ammonia and nitrogen removal rates in a batch reactor.
2 Continuous Flow	186	2	25	Growth media 1	0/35	Evaluate the difference in nitrogen removal rates with and without Aqua using dosing method 2.
3 Continuous Flow	185	1	45	Growth media 1	0/35	Evaluate the difference in nitrogen removal rates with and without Aqua using dosing method 1.
4 Continuous Flow	113	1	25	Growth media 2	0/35	Evaluate the difference in nitrogen removal rates with and without Aqua using dosing method 1 in growth media 2.
5 Continuous Flow	102.5	1	25	Growth media 2	0/35	Evaluate the influence of doubling glucose on nitrogen removal.
6 Continuous Flow	99.5	1	25	Growth Media 3	0/35	Determine if there is an improvement in nitrogen removal with an increased pH.

4.4.1 Experiment 1: Batch Reactor

The first experiment was set up as a batch reactor. This experiment evaluated the growth rate of the biofilm in the bioreactor and rate of nitrogen removal from the growth media. This information could be used to determine the HRT that was needed for nitrogen removal in this SNdN system. The HRT was a key component in the MABR design because SNdN systems typically need longer retention times. Running the MABR as a batch reactor for approximately 500 hrs. also determined how long the biofilm would stay on the membrane before becoming too thick and sloughing off the membrane.

The MABR was inoculated with 50 ppm Aqua using dosing method 2. Prior to adding the Aqua into the bioreactor 1g. of Aqua was mixed with 150 mL of the growth media 1 and inoculated for approximately two days making an inoculum for the system. To inoculate the MABR the 150mL of inoculum was added to the MABR to achieve a final concentration of 50 ppm in the MABR. Growth media 1 was used in this experiment because it was the media that was found to result in the highest Aqua growth rates from previous research. The initial nitrate and ammonium concentrations in this experiment were 35 and 45 mg-N/L respectively.

4.4.2 Experiment 2: Continuous Flow Configuration Using Aqua Dosing Method 2 in Growth Media 1

In this experiment a second MABR system was used to compare the nitrogen removal rate between a bioreactor inoculated with only RAS (RAS MABR) and one with RAS and Aqua (Aqua MABR) in a continuous flow configuration (Figure 4-1). In this experiment 35 mg-N/L of ammonium was added in the influent feed and no nitrate to imitate concentrations typical of municipal wastewater.

At the start of the experiment 100 mL of RAS from the San Luis Obispo Water Reclamation Facility (WRRF) was used to inoculate each bioreactor. Although the theoretical minimum flowrate to prevent washout of nitrifiers was calculated to be 7 mL/min, the flowrate in this experiment was set to 2 mL/min to ensure that the nitrifiers would be able to establish a biofilm on the membrane without any scour.

Figure 4-1 Continuous flow configuration with one MABR inoculated with RAS (left) and one inoculated with RAS +Aqua (right).

After two days a biofilm was confirmed in the system through visual inspection of the membrane and the Aqua was added to one of the MABRs. To dose the Aqua in this experiment dosing method 2 was used and the Aqua was incubated at a concentration of 1000 ppm in growth media. Growth media 1 was used in the MABR and to inoculate the Aqua. Once the Aqua was added to the MABR the flowrate for both was increased to 5 mL/min. The inoculation of the Aqua and the fact that the Aqua consisted of faster growing bacteria made it so that a long retention time was not needed.

4.4.3 Experiment 3: Continuous Flow Configuration Using Aqua Dosing Method 1 in Growth Media 1

Experiment-3 evaluated if a difference in dosing methods would improve the performance of the Aqua in a continuous flow MABR. The MABR configuration and set up was kept the same as in Experiment-2 with two MABR systems (Figure 4-2). The initial ammonium concentration was kept at approximately 35 mg-N/L.

Figure 4-2 MABR reactors for Experiment-3 with RAS MABR on the left and RAS + Aqua MABR on the right.

The dosing method used in this experiment was a slight variation to dosing method 1 where the Aqua was not inoculated prior to adding it to the MABR. One difference in this method was that the Aqua was slowly fed into the influent line over a period of 11.5 hours. The final concentration of Aqua was 45 ppm. This method slightly diluted the concentration in the bioreactor and increased the flow into the bioreactor with the Aqua.

The suction from the pump was not strong enough to have the line split from the influent carboy before the pump and have a flowrate of 2.5mL/min influent and 2.5 mL/min Aqua, so a separate line was added to the peristaltic pump. The Aqua influent was bled into the system after the pump. This doubled the flowrate in the Aqua MABR to approximately 10 mL/min whereas the flowrate in the RAS MABR remained at 5 mL/min (Figure 4-3). Again, the was not added until the biofilm from the RAS was confirmed.

Figure 4-3 Influent feed line set up to feed Aqua into the MABR slowly over a period of approximately 11.5 hours.

4.4.4 Experiment 4: Continuous Flow Configuration Using Aqua Dosing Method 1 in Growth Media 2

The objective of Experiment-4 was to evaluate how an increase in the pH of the media would affect the nitrogen removal rates. This experiment was done with a control MABR in addition to the RAS and Aqua MABRs to account for the contamination that was occurring in the previous experiments (Figure 4-4). The control MABR was not inoculated with any bacteria. For this experiment growth media 2 was used for all three MABRs and the influent ammonium concentration was approximately 35 mg-N/L. In this experiment the influent line for all three MABRs branched off from one influent container to ensure that they all had the same type of contamination.

Figure 4-4 MABR configuration for Experiment-4; RAS inoculated MABR (left), RAS + Aqua inoculated MABR (Middle), and control (right).

The Aqua in this experiment was not inoculated prior to adding it to the system and it was added all at one time. The Aqua was mixed with DI water then added to the system at a concentration of 25 ppm. Similar to the previous experiments the Aqua was not added to the MABR until a biofilm on the surface of the membrane was confirmed.

4.4.5 Experiment 5: Continuous Flow Configuration Using Dosing Method 1 in Growth Media 2 with 24:1 C to N Ratio

The objective of this experiment was to determine if the ammonium degradation was related to concentration of glucose in the growth media. Similar to the previous experiment the ammonium and Aqua concentrations were kept the same at 35 mg-N/L and 25 ppm respectively and growth media 2 was used. Both the airline and influent lines were kept identical to the setup in Experiment-4 (Figure 4-4). The only factor that changed was that the C: N ratio was increased from 12:1 to 24:1. Both the RAS and Aqua MABRs were first inoculated with 100 mL of RAS from the WRRF. Once a biofilm was established the Aqua was added to the Aqua MABR using dosing method 1. The control MABR was not inoculated with any bacteria. Similar to Experiment 3 the control was not inoculated with any bacteria.

4.4.6 Experiment 6: Continuous Flow Configuration Using Aqua Dosing Method 1 in Growth Media 3

The last experiment focused on increasing the pH in the bioreactor to between 6.5 and 8.5 using growth media 3. Although growth media 2 increased the pH of the solution slightly, it still was not in the idea pH range of 6.5 to 8.5 that nitrifying bacteria typically prefer. As previously mentioned in growth media 2, the NaHCO_3 was the buffer in the solution and the concentration of K_2HPO_4 and KH_2PO_4 was considerably lower than the original

growth media as seen in Chapter 4.2. Although the NaHCO_3 was able to buffer some of the acid that was produced in the MABRs, the pH eventually dropped below 6.

In this experiment all parameters other than the media remained the same as Experiment-4. The intramembrane pressure, the HRTs, the temperature, and the Aqua dosing method and concentration were identical to Experiment-4. The only change was that growth media 3 was used. Again, the control was not inoculated with any bacteria.

Chapter 5 EVALUATION AND DISCUSSION

Each of the large-scale experiments conducted provided valuable insight into the success of a continuous flow MABR system. The experiments helped to further develop the methods to determine if SNdN was possible in the system and if Aqua would increase the nitrogen removal rates. The results of these experiments are discussed in the following chapter. The RAS MABR will be referenced as “R” and the results from the Aqua MABR will be referenced as “A” in the following graphs and tables.

5.1 Experiment 1: Batch Reactor

In Experiment-1 the nitrate removal rate in this experiment was approximately 0.8 mg-N/L-hr. (Figure 5-1). This was greater than the previous nitrogen removal rate seen in the screening experiments of approximately 0.4 mg-N/L-hr.

Figure 5-1 Nitrate and ammonium concentrations in the batch MABR for experiment 1.

When the MABR was dosed with 35 mg-N/L of NO_3 at the 24th and 110th hour, the nitrate depleted so quickly that when the sample was taken a few hours later the concentration had already dropped below 10 mg-N/L (Figure 5-1). The peak after the NO_3 was added around the 110th hour indicated that the drop-in nitrate occurred within the 3 hours between when the NO_3 was added to when the sample was taken.

No degradation of ammonium was observed in the system possibly due to a high initial concentration of nitrate in the media. After the nitrate was completely degraded the ammonium concentration never decreased. The batch reactor was only inoculated with Aqua and no RAS which meant there were no nitrifiers in the system that are typically found in wastewater. This was like what was observed in the small-scale experiment that had nitrate and ammonium in the media that was only inoculated with Aqua. It is possible that the bacteria in Aqua favored nitrate to ammonium and became conditioned to only consume the nitrate.

Although almost all the nitrate disappeared, only a couple very small increases in nitrite were observed as the nitrate decreased. This indicated that there was either rapid denitrification, or the nitrate was being assimilated into organic nitrogen. However, the increase in organic nitrogen was not comparable to the decrease in nitrate (Figure 5-2). There are two possibilities for the decrease in TN from the influent to the effluent; the first is that the organic nitrogen remained in the system in the form of biofilm, or there was another process occurring that was using the nitrate. Although the nitrogen was not being removed through denitrification as expected, this experiment showed that the MABR could still remove the nitrogen from the system in the form of organic nitrogen if

the biomass was filtered from the effluent. There was a 67% removal rate of total nitrogen over the 3-week period that the experiment was run.

Figure 5-2 Total nitrogen removal over the course of 500 hours in the batch reactor.

Over the course of three weeks the ammonium concentration trended upwards, increasing by approximately 5 mg-N/L. This could be because no water was cycling through the system and all the biomass in the system had no way of exiting. As the bacteria got older, cell lysis started to occur and this in turn produced more ammonium. Because there was no ammonium decaying and leaving the system, this would explain why the concentration steadily increased.

Running the experiment for about three weeks, it was possible to observe how the bacteria growth in the system changed over time. Allowing the bacteria to grow for an extended period eventually led to the biofilm becoming very thick. This thick biofilm eventually became too heavy and sloughed off the membrane around the 450th hour (Figure 5-3)

Figure 5-3 Biofilm in the batch reactor was detaching from the membrane because it is too thick.

5.2 Experiment 2: Continuous Flow Using Dosing Method 2 in Growth Media 1

In Experiment-2 the influent ammonium concentration was kept constant for the entire run and no nitrate added to the growth media. The system took until approximately hour 50 to achieve a steady state in the effluent (Figure 5-4). Once the system was at steady state the effluent ammonium concentrations plateaued around 10-15 mg-N/L (Figure 5-4).

Figure 5-4 Ammonium degradation in MABR R and A until the plateau in concentration around 50 hours.

When the system was not at steady state higher concentrations of organic nitrogen were observed than when the system was at steady state (Figure 5-5 and Figure 5-6). This implies that the growth in the bioreactor might have consisted of more suspended solids in the first 50 hours. When the concentration of ammonium in the effluent did reach steady state the biofilm in the system was probably better established and the bacteria growth was mostly on the membrane versus suspended in the media. In the RAS MABR the total nitrogen removal rate increased from around 54% to 63% when the system went from non-steady state to steady state. In the Aqua MABR the total nitrogen removal rate increased from about 46% to 76% once steady state was achieved.

Figure 5-5 Nitrogen removal in the RAS MABR when the MABRs were not at steady state around 42 hours.

Figure 5-6 Nitrogen removal in the RAS MABR when the MABRs were at steady state around 150 hours.

At around the 50th hour the ammonium concentration leveled off at around 10-15 mg/L in the RAS and Aqua MABRs (Figure 5-4). There was also no substantial difference in the nitrogen removal rate observed between the two MABRs. Because this experiment used the second dosing method to add the Aqua to the system, one concern was that the

inoculation process preselected which bacteria grew. Because certain bacteria favor different environments and factors such as DO concentration and temperature the bacteria that grew in the inoculation process could have been different than the bacteria that would have grown naturally in the MABR if it had not been inoculated prior to adding it to the MABR.

5.3 Experiment 3: Continuous Flow Using Dosing Method 1 in Growth Media 1

Using a modified version of method 1 for dosing the Aqua, no substantial difference in the ammonium removal in the RAS and Aqua MABRs was observed from the previous experiment. The rate of the initial drop in the ammonium was almost identical in the initial 50 hours of Experiment-2 and both MABRs had a plateau in ammonium concentration around 10-15 mg-N/L (Figure 5-7). This showed that the dosing method did not affect the nitrogen removal rates in the MABR. The RAS and Aqua MABR systems in this experiment behaved almost identically to each other, also having a similar decrease in pH to approximately 5 that was much lower than the pH that is typically desired for simultaneous nitrification and denitrification.

Figure 5-7 Comparison of the ammonium degradation and pH in the Aqua MABRs between the two different dosing methods.

While a majority of the observed ammonium decrease in this system was likely due to nitrogen assimilation, spikes of nitrite in the effluent of approximately 5 mg-N/L indicate that nitrification was occurring (Figure 5-8). With no other source of nitrogen being added to the system the nitrite was most likely from the oxidation of ammonium. These spikes in nitrite were also observed in the Aqua MABR system. (Figure 5-9).

Figure 5-8 Spikes in nitrite over the course of 180 hours in the RAS MABR.

Another possible explanation of the small peaks of nitrite with less frequent peaks of nitrate is that there was ANAMMOX occurring in the MABR (Equation 6). Typically, if nitrification was occurring there would be more nitrate because the rate limiting step is the conversion of ammonium to nitrite. This means that the conversion of nitrite to nitrate is typically faster, so there would be less nitrite.

While it is not possible to conclude ANAMMOX is occurring without further testing to fully understand the mechanisms that the total nitrogen removal is occurring, it is one possibility that should be investigated in future studies.

The percent removal of the total nitrogen in both the Aqua and RAS MABR systems ranged from 35% to 45% (Figure 5-9 and Figure 5-10 respectively). One difference in the systems was that the concentration of organic nitrogen in the Aqua MABR started to decrease at the end of the run starting at around 140 hrs. This indicates that the Aqua could have been decreasing the suspended solids in the system, however the run would need to be duplicated and run for a longer period to confirm this.

Figure 5-9 Percent of total nitrogen removal in the Aqua MABR shown above each bar with the constituents broken down into ammonium, nitrate, nitrite, and organic nitrogen.

Figure 5-10 Percent of total nitrogen in the RAS MABR shown above each bar with the constituents broken down into ammonium, nitrate, nitrite, and organic nitrogen.

5.4 Experiment 4: Continuous Flow Using Dosing Method 1 in Growth Media 2

The addition of sodium bicarbonate to growth media 2 did help to increase the pH of the growth media in the MABR systems slightly (Figure 5-11). While it did not reach the target pH around 7, this increase in pH resulted in a higher initial nitrogen removal rate in the MABRs. The ammonium concentration dropped to approximately 18 mg-N/L in the first 24 hours with an initial concentration around 45 mg-N/L which is faster than the drop seen in the previous experiments (Figure 5-11). The concentration reached the same steady state plateau at around 50 hours with effluent concentrations around 10-15 mg-N/L, showing that the nitrogen removal rate was only greater in the initial phase of this experiment when there was only RAS in each MABR and that the Aqua did not have an effect.

Figure 5-11 Comparison of ammonium removal to pH between Aqua dosing method 1, Aqua dosing method 2, and growth media 2 in the Aqua MABR.

Despite efforts to keep contamination out of the influent container the influent feed eventually became contaminated with what appears to be bacillus bacteria (Figure 5-12). Bacillus are typically white and will create more filamentous flocs on the membrane surface.

Figure 5-12 Bacteria contamination on the membrane in the RAS MABR causing turbidity.

This type of bacteria typically grows at a faster rate than the nitrifiers and could have outcompeted the nitrifiers in the system. The control MABR was not inoculated with any bacteria, however the control was clearly more turbid after the 4th day than it was initially (Figure 5-13 and Figure 5-14 respectively). The higher organic nitrogen in the effluent could be caused by accelerated cell synthesis because of the longer retention time, higher growth rate, and excess carbon supply.

Figure 5-13 MABRs at $t=0$ hrs.

Figure 5-14 Increased turbidity in MABRs at $t=96$ hrs.

5.5 Experiment 5: Continuous Flow Using Dosing Method 1 in Growth Media 2 with 24:1 C to N Ratio

Increasing the glucose concentration from a 12:1 C: N ratio to a 24:1 ratio resulted in the complete removal of ammonium (Figure 5-15). The increased removal with the increased concentration of glucose indicated that the main mechanism for ammonium removal in the MABR system was nitrogen assimilation. The Control MABR took approximately 24 hrs. longer than both the RAS and Aqua MABRs to reduce the ammonium. The RAS and Aqua MABR both completely reduce the ammonium by the 40th hour, implying that the RAS was increasing the ammonium removal rate. The fact that the RAS and Aqua MABRs completely degraded the ammonium while the previous experiments reached steady state suggested that the limiting factor in the MABR previously was the glucose.

Figure 5-15 100% ammonium removal from the system with doubled glucose concentration.

After observing the increase in ammonium degradation rates with an increased glucose concentration the conversion of glucose to new cells was evaluated to determine if the glucose was in fact the limiting factor. The conversion of glucose to cell mass was modeled assuming a cell composition of $C_5H_7NO_2$ (Equation-7).

Using this equation, the initial 12:1 C: N ratio should have supplied enough glucose to degrade all the ammonia in the system. It appears that the limiting factor in this case was another factor. While it is unclear what the limiting factor was the results clearly indicated that the increase in glucose impacted the ammonia removal rate.

The increase in glucose concentration and oxygen demand could also explain the disappearance of any nitrate or nitrite in this experiment. If the oxygen needed to convert the glucose and ammonia to cell mass was much higher than the oxygen that is supplied

to the MABR, the entire system would become anoxic. This would inhibit nitrification explaining why there was no nitrate or nitrite in the effluent.

In this experiment the pH dropped lower than the previous experiment even though growth media 2 was used again. This was most likely due to excess glucose in the system that completely consumed the oxygen. This incomplete degradation of the glucose could have produced lactic acid that lowered the pH in the system.

Through visual inspection it appears that all three MABRs in this experiment were also contaminated (Figure 5-16). Like the previous experiment all three experiments had white bacteria growth. The control MABR looked just as turbid as the RAS or Aqua MABR systems even though the control was not inoculated with any bacteria.

Figure 5-16 High turbidity indicating contamination in all three of the MABRs on the Day-5 of the experiment.

5.6 Experiment 6: Continuous Flow Using Dosing Method 1 in Growth Media 3

Instead of increasing the NaHCO_3 concentration to help buffer the system, the K_2HPO_4 and KH_2PO_4 concentrations were increased in Experiment-6. This helped to maintain the

pH above 6 creating an environment that was more typical for nitrifiers. Although more nitrite was produced indicating that nitrification could be occurring no spikes in nitrate appeared. In addition, the ammonium degradation showed a similar trend that was observed in the previous experiments using the 12:1 C: N ratio, however in this experiment it appears that the ammonia reached a steady state around the 30th hour which was sooner than what was observed previously (Figure 5-17). The plateau in ammonia concentration was also slightly lower, closer to the range of 5-10 mg-N/L. While it appears the increase in pH may have slightly improved the nitrogen removal rates, the experiment would need to be replicated to confirm these results.

Figure 5-17 Decrease in ammonium concentration with the change in the pH over the course of 100 hours.

The MABRs also had similar contamination to the previous experiments in the MABR as well as the influent feed. To confirm that the ammonium removal was not occurring in

the influent, a sample was taken from the influent carboy. The sample confirmed that the ammonium concentration entering the MABR was at 35 mg-N/L and that the degradation was occurring in the systems.

5.7 Limiting Factors

The contamination and lack of DO and total organic carbon (TOC) data for the large-scale experiments make complete analysis of the success of the MABR system difficult.

After evaluating the ammonium removal from all three experiments done with a control MABR it appears that the ammonium removal occurring is primarily from the bacteria that is contaminating the system. The ammonium removal rates in the control were consistently as high as the removal rates in the RAS and Aqua MABR systems for all three experiments. Better contamination control is necessary to make any definitive conclusions on the success of the MABR in conjunction with Aqua.

5.7.1 Estimating Theoretical DO Concentrations

When evaluating the ammonium removal in each of the experiments conducted it was not possible to record the DO in the water because the biofilm acted as a diffusion barrier and without a micro DO probe it was not possible to measure the DO at the membrane surface. This means that if the DO of the bulk water was measured it would not be representative of the oxygen that was being supplied to the system.

To determine if the oxygen was the limiting substrate in nitrogen removal in the MABR systems the theoretical O₂ transfer rate was calculated. Knowing the intramembrane pressure, and the permeability, surface area, and thickness of the silicone membrane it was possible to calculate the volume of oxygen and nitrogen that was diffusing through

the membrane (Equation-8). It was assumed that the concentration of oxygen in the compressed air flowing through the silicone membrane was 21%.

$$V = \frac{P \cdot A \cdot t \cdot (p_2 - p_0)}{\delta} \dots \dots \dots (8)$$

Where V= volume of air diffused, P=membrane permeability, A = membrane surface area, t=time, P₀= 0 atm., P₂= 0.21 atm, and δ= membrane thickness.

The oxygen mass transfer rate in the water for all the experiments was 1.75 mg O₂/m².

This meant that in each bioreactor there was a total of approximately 138 mg of O₂ diffusing into the system. Knowing the maximum possible oxygen mass transfer rate in the water made it possible to predict bacteria growth rates as well as gain a better understanding of the MABR and by what mechanism total nitrogen removal was occurring.

The oxygen demand for cell synthesis of heterotrophic bacteria using glucose as its carbon source, was predicted for the system (Equation-7). This mechanism in the MABR most closely modeled nitrogen assimilation that was occurring in the MABR and the main cause for the decrease in ammonium concentration. With a 12:1 C: N ratio it was determined that to fully degrade all the glucose in the water approximately 500 mg O₂/L was needed. This suggests that the oxygen was being completely consumed. However, the complete removal of ammonium from the increase in glucose seen in Experiment-5 suggests there is some other mechanism for NH₄ removal such as assimilation, but O₂ may have been a limiting factor for nitrification.

The oxygen needed to degrade all the ammonium in the system through nitrification was also calculated (Equation-9). The maximum amount of oxygen that was needed was approximately 138.46 mg O₂/L. From these calculations it appears that if there was no

glucose in the system then the oxygen would not have been a limiting factor in nitrification, however this would again need to be confirmed by measuring the DO.

5.7.2 Growth and Removal Rates

Another factor that was assessed was the growth rate of nitrifiers and heterotrophic bacteria such as *Bacillus* causing the contamination in all the experiments. Because the systems were continuous flow systems the ammonium concentration in the effluent feed was assumed to be representative of the system and was used to calculate the theoretical growth rate of *Nitrosomonas*. It was not possible to take grab samples from the MABR and using the influent substrate concentrations would have been an overestimate of the growth rate. However, when evaluating the heterotroph growth rate, the influent glucose concentration was used because the effluent was not tested for TOC. While it is an over estimate, it is useful in evaluating the general difference between the autotrophic nitrifying bacteria growth rate and the heterotrophic growth rate of the *Bacillus* bacteria contaminating the system.

To calculate the growth rate of *Nitrosomonas* in the MABR systems Equation-10 was used (Nutrient Control Design Manual, 2010). In this equation it was assumed that $K_N = 0.74 \text{ mgVSS/L.bsCOD}$ and $K_0 = 0.5 \text{ g/m}^3$ (Tchobanoglous et al., 2004). Term A in the equation represented the maximum growth rate possible and was temperature dependent. Term B accounted for the effect of ammonium concentration on the growth rate and term C accounted for the influence of dissolved oxygen on the bacteria growth. To determine the maximum potential growth rate, it was assumed that the system was not oxygen

limited. These calculations determined that the mean maximum potential growth rate of *Nitrosomonas* in the MABRs was 0.74 g VSS/g VSS-d.

$$\mu = 0.47e^{0.098(T-15)} * \left[\frac{N}{K_N+N} \right] * \left[\frac{DO}{K_0+DO} \right] \dots\dots\dots (10)$$

The growth rate for the heterotrophic bacteria was calculated using Equation-11 and typical kinetic coefficients for heterotrophic bacteria (Table 5-1.) (Tchobanoglous et al., 2004). This equation does not take into consideration the effect that oxygen has on the growth rate because heterotrophic bacteria rapidly use up the oxygen in the system. The potential growth rate of the heterotrophs with a 12:1 C: N ratio was 1.83 g VSS/g VSS d which is more than double the estimated growth rate for *Nitrosomonas*. This high potential growth rate was due to the high glucose concentration in the MABRs. As expected when the C: N ratio was doubled to 24:1 the maximum potential growth rate doubled to 3.75 g VSS/g VSS d.

$$\mu = Y \left[\frac{K*S}{K_s+S} \right] - K_d \dots\dots\dots (11)$$

Table 5-1 Typical kinetic coefficients for heterotrophic bacteria (Tchobanoglous et al., 2004).

Coefficient	Unit	Typical Value
K_s	g. bCOD/m ³	40
Y	g. VSS/ g.bCOD	0.4
K_d	g. VSS/g.VSS d	0.10
K	g. bsCOD/gVSS-d	5

Although the heterotrophic growth rates were an overestimation because the influent glucose concentration was used, it still indicated that the *Nitrosomonas* were likely outcompeted in the system. With such a high carbon source for the aerobic heterotrophs they likely grew much faster until reaching a steady state that resulted in the plateau in ammonium concentration around 10-15 mg-N/L in the experiments with a 12:1 C: N ratio.

While the plateau in the ammonium concentration and theoretical calculations suggested that the oxygen supply may have been limiting nitrification, the increase in the nitrogen removal rate when the air supply was kept the same and the glucose concentration increased indicated nitrogen assimilation was dominating the system. The average nitrogen removal rate for the experiments that had a 12:1 C: N ratio at steady state was 0.39 mg-N/L-hr. with a standard deviation of 0.05. The removal rate then increased to 0.56 mg-N/L-hr. with a standard deviation of 0.01 when the C: N ratio increased to 24:1. The relationship between nitrogen removal rates and C: N ratios should be explored further through varying ratios as well as conducting TOC testing and taking DO

measurements. This would give a better understanding of the amount of glucose being consumed and what the limiting factors in the MABRs are.

5.7.3 Hydraulic Retention Time

The plateau in ammonium concentration could also be due to the change in HRT after the Aqua was added to the system. As mentioned previously the HRT was kept at approximately 7 days for the initial 40-50 hours when the MABR was inoculated with RAS. This was to avoid wash out of nitrifiers and to allow time for a biofilm to form on the membrane surface. This was also the period before all the MABRs reached a steady state. Almost immediately after the increase in flowrate the ammonium concentration plateaus between 10-15 ppm (Figure 5-18).

Figure 5-18 Comparing ammonium removal for Aqua MABR system with varying Aqua dosing methods and growth medias.

This plateau was also aligned with the plateau in pH for all the experiments. Initially it appeared that the drop in pH was the cause of the plateau in the ammonium concentration, however Experiment-4 and Experiment-6 showed that the increase in pH still resulted in a plateau in the ammonium removal.

Keeping the HRT at 7 days should not influence the pH. The pH drops initially in the first 20-30 hours of each experiment but, the pH equilibrates prior to the increase in flowrate (Figure 5-19). This suggests that the HRT does not affect the pH.

Figure 5-19 pH in the RAS MABRs for each continuous flow experiment with a 12:1 C: N ratio with the change in HRT noted with a red marker.

Chapter 6 CONCLUSION

While MABR systems have proved to be successful in previous studies using pure oxygen to aerate the systems, further work can be done to make the system more feasible for upscale by replacing the pure oxygen with compressed air. The MABR design used in this research showed promising results that indicated that it could possibly be used in the future for SNdN with further improvements. However, simultaneous nitrification and denitrification was not observed in these experiments. While denitrification was observed under some conditions, nitrification was not. The primary mechanism of NH_4 removal appears to have been assimilation. Nitrification may have been limited by O_2 availability because heterotrophic consumption of glucose in the growth media likely outcompeted nitrifiers for O_2 . Theoretical calculations of the O_2 mass transfer rate and O_2 required for nitrogen assimilation and nitrification indicated that without glucose in the system there was sufficient O_2 in the system for nitrification to occur.

Ammonium degradation was observed in all the experiments with a continuous flow that had ammonium and no nitrate in the media. For the experiments with a 12:1 C: N ratio the nitrogen removal rate was a mean of 0.39 mg-N/L-hr. with a standard deviation of 0.05. This mean included the RAS, Aqua, and the Control MABRs. The relatively similar nitrogen removal rates indicated that contamination had a large influence on the results. Changes made to the growth media, Aqua dosing method, and Aqua concentration had little effect on the nitrogen removal rate between experiments. The experiments would need to be replicated with an improved method to control contamination in the future. The only factors that caused the nitrogen removal rate to change was the increase in glucose concentration to a 24:1 C: N ratio, the addition of nitrate to the media, and

possibly the pH. The increased glucose concentration resulted in a higher mean nitrogen removal rate of 0.56 mg-N/L hr. with a standard deviation of 0.01 between the RAS, Aqua, and Control MABRs. All three MABRs reached a 100% ammonium removal by approximately 50 hours. The similarities in the nitrogen removal rate between the RAS and Aqua MABR to the Control again indicate that the contamination was a large factor. With the addition of nitrate to the growth media in the batch reactor no ammonium degradation was observed, however the nitrate was degraded at a rate of 0.8 mg-N/L-hr. The last factor that could be influencing the nitrogen removal was the increase in pH. It appeared that the system reached a steady state sooner than the previous experiments and the effluent ammonium concentrations were approximately 5 mg-N/L lower. These results imply that the composition of the media has a large influence on removal rates. While it is not possible to confirm the mechanisms by which the nitrogen is leaving the system, the highest total nitrogen removal percentage was 76% in the continuous flow Aqua MABR using dosing method 2 in growth media 1. For the two other experiments that tested for total nitrogen, the total nitrogen removal ranged between 35-45%. To determine if the nitrogen is being nitrified or assimilated further testing is needed. Through the course of both the small and large-scale experiments valuable insight was gained that providing more direction for the design and methods to achieve SNdN in a MABR system using a continuous flow airline. From the experiments conducted it was not possible to conclude whether the Aqua had any effect on the removal of nitrogen in conjunction with a MABR. The results from this study indicated that by adding Aqua to a system with an established population of nitrifiers it could possibly improve denitrification, however further research is needed.

6.1 Proposed Design Improvements

One main improvement that can be made to the design of the MABR system is the addition of more silicone tubing in the system. As discussed previously the concentration of oxygen needed to achieve nitrogen assimilation and nitrification in the system is much higher than the theoretical O_2 supplied to the system. This could have been a limiting factor in the success of nitrogen removal. In addition, the silicone membrane was rated to be used at a maximum pressure of 20 psi that limited the air pressure in the system and oxygen diffusion. This means that the only way to increase the amount of oxygen in the system would be to either increase the surface area of membrane in the MABR or to change the type of tubing used to one with a higher-pressure rating to increase the intramembrane pressure. This would theoretically result in a higher volume of oxygen diffusing through the membrane.

To gain a better understanding of the effectiveness of the continuous flow configuration for the airline it would be beneficial to take DO readings using a micro DO probe at the membrane surface and biofilm surface. These readings could then be compared to previous literature that evaluated the DO concentration using pure oxygen. In previous studies the DO at the membrane surface was 8 mg/L and was approximately 0 mg/L at the biofilm surface. Due to the design of the MABR it was not possible to take DO readings and only the theoretical maximum DO concentrations were available.

Another improvement to the system would be improving the airline configuration. Currently the airline consists of one regulator from the wall and the line then branches to each of the MABRs. The airline could be controlled better if the airline split into three lines initially and then each line had a regulator. This would ensure that each line is

getting uniform airflow because each line would have uniform resistance. Because air is a fluid and will take the path of least resistance, if one of the needle valves on the airlines was closed more than another, one of the lines was most likely receiving more air flow. The intramembrane pressures of all three MABRs remained within 1-2 psi of each other and did not appear to have a considerable difference in airflow but, this new configuration would ensure that the airflow was uniform. The main improvement with the airline should be to increase the surface area in the MABR but, having more control over the airflow would decrease the number of unknown variables in the system.

6.2 Experimental Methods Improvement

Through the course of the six large scale experiments several new findings had altered the approach to evaluating the original objective of determining if Aqua improved the nitrogen removal rates in a MABR. The experiments conducted helped to further develop methods to continue the research in the future. The testing can be improved through varied media composition, dosing methods, experimental run time, hydraulic retention time, sampling methods, and water analysis.

6.2.1 Growth Media Composition

The first variable that greatly influenced the success of the MABR system was the growth media composition that was used. The initial media composition was one that proved to be successful in previous research with Aqua. However, those experiments were conducted on a much smaller scale and did not have the same problems, particularly with the pH that were exhibited with the larger 20 L system. Throughout the six large scale experiments the pH was a constant problem in the system. Even with slight adjustments

to the media to add more buffer the pH was still not within the ideal range of 6.5-8.5 for SNdN.

For future studies the initial carbon concentration should be greatly reduced. Not only would this help to raise the pH, but it would also help to control the contamination. As previously discussed the glucose uses a large concentration of oxygen through nitrogen assimilation and any glucose that is not fully degraded results in the production of acid in the MABR causing the system to go sour. This was creating two problems; it was depleting the oxygen while lowering the pH and contributing to the contamination problems in the system. The glucose was supplying the aerobic heterotrophic bacteria with a surplus of organic material that resulted in contamination. Because the heterotrophs typically have a higher growth rate they immediately took over the system. To give the nitrifying bacteria a chance to establish a biofilm on the membrane surface where the highest oxygen concentration is, the initial glucose concentration should be much lower or omitted completely at the start of the experiment. If the aerobic heterotrophs do not have a high concentration of organic matter the growth rate will be much lower and the nitrifiers will be the first ones to use the oxygen that is supplied to the system.

To prove that this method would help to establish a healthy population of nitrifiers in the MABR the effluent should be sampled each day until clear signs of nitrification are occurring. This would include the appearance of nitrate and some nitrite in the system when the ammonium concentration was decreasing. Once this is established then the Aqua could be added to one of the MABR systems and glucose could be added in the media. This would ensure that the nitrifiers did not get washed out or outcompeted in the

system and that they were closest to the oxygen source. Visually inspecting the membrane for biofilm formation proved to be an ineffective method to determine if the nitrifiers had been established in the system.

Another variation in the media that could be considered would be to remove the sodium bicarbonate from the system and instead only use the K_2HPO_4 and KH_2PO_4 as a buffer.

The bicarbonate is a part of the ANAMMOX reaction (Equation 6). This means that if the media is relying on the sodium bicarbonate to buffer the system and the bacteria that can potentially achieve ANAMMOX are consuming the carbonate, the pH in the system will drop below the ideal range for SNdN. To first establish a media that can support SNdN in the system the carbonate should not be included. However, if nitrification is successful it would be interesting to add more carbonate to the system later and evaluate if Aqua is capable of ANAMMOX.

6.2.2 Aqua Dosing Methods

The similarities in the nitrogen removal rates between the two different Aqua dosing methods used in Experiment-2 and Experiment-3 confirmed the findings in previous research that the method used for dosing the Aqua did not make a difference in the effectiveness of the product. The ammonium removal methods were almost identical between the two experiments. For future studies using Aqua it is suggested that it is not inoculated prior to being added. This is most likely the method that would be used in wastewater treatment and would be most representative of the effectiveness of the product.

6.2.3 Experimental Run Time

When the continuous flow experiments are conducted they should be run for longer periods of time. Due to the slower growth rate of the nitrifiers it will take a longer time to establish a population of nitrifiers in the MABR. To ensure that they do not get outcompeted, this step should not be rushed and closely monitored to ensure that nitrification is occurring. Once the Aqua is added it would be useful to run the experiment until the biofilm sloughed off the membrane to assess if it is possible for the system to reestablish the nitrifier population without assistance. In the batch reactor it appeared that the biofilm was starting to slough off the membrane around the 450th hour. The longer run time would improve the understanding of when and if the biofilm will reach a steady state and make the analysis of the total nitrogen test more useful. However, if the biofilm thickness is constantly changing then the total nitrogen test will not be able to give an estimate of the organic nitrogen in the system because of the unaccounted-for biomass on the membrane. It is recommended that the small-scale experiments are run for 4 weeks to gain a better understanding of the bacterial growth, and steady state in the MABR.

6.2.4 Hydraulic Retention Time (HRT)

Another important factor that could have been influencing the nitrogen removal rate was the hydraulic retention time. As mentioned previously the plateau in the ammonium concentration was consistently occurring after the change in hydraulic retention time. The flowrate should be kept at 2 mL/min for the entire experiment to evaluate the effect on the nitrogen removal rate. This will give a clear indication if the HRT is one of the main factors that are influencing the nitrogen removal rate in the experiments.

6.2.5 Sampling Methods

Improving the sampling methods would also be beneficial in future studies. Although the current method is effective at taking samples from the system without disturbing the MABR or introducing more air into the system it can sometimes take long to collect the sample which introduces the possibility of contamination from the sampling tubes as well as making the temperature readings not representative of the temperature in the MABR. This could be accomplished by adding another port to the top of the MABR so that samples can be pipetted from the MABR using sterile transfer pipettes. This would also provide a way to measure the DO in the MABR. Although it might not be possible to get a micro DO probe, it could be beneficial to know the DO of the bulk water and if the biofilm is acting as a diffusion barrier for the oxygen.

In addition to sampling the effluent, the influent should be tested periodically to ensure that the ammonium degradation that was occurring was in the MABR, and not due to the contamination. This could be done by adding a T from the influent line to a ball valve that could be opened to collect samples and confirm influent concentrations.

6.2.6 Sample Analysis

To gain a better understanding of the processes occurring in the MABRs two new tests could be conducted to provide more insight. The first test is for TOC. Measuring TOC would provide more information about the growth rate of the heterotrophic bacteria in the system. In Experiment-5 that doubled the glucose it would have been beneficial to be able to compare the effluent carbon concentration compared to the experiments that had a 12:1 C: N ratio. The theoretical calculations show that the glucose in the system should have been enough to remove all the ammonium, however that was not the case. With

double the glucose all the ammonium was removed, however it was not possible to determine if all the glucose had been removed as well without conducting the TOC test.

The second test that would be useful to confirm SNdN in the MABR would be one to estimate the amount of biomass on the membrane. The TN tests that were conducted suggested some oxidation of nitrogen from the system, however without knowing the concentration of biomass in the system it was not possible to estimate the organic nitrogen concentration. Although it is a good indicator that the total nitrogen in the effluent is lower and that the system can remove nitrogen it is unknown whether the drop in TN is oxidized nitrogen or if it is still in the form of organic nitrogen on the membrane surface.

6.3 Future Work

One concern with this research is that a study that was conducted simultaneously with this study revealed that Aqua was not capable of nitrification (Kalvass, 2018). This altered the interpretation of the small-scale experiments and changed how the Aqua was applied to the system. Although the experiments conducted were able to improve the methods for testing the system it is suggested that the system is scaled back to the smaller scale MABR systems first to confirm SNdN. If the small-scale systems are capable of SNdN using the new MABR inoculation methods developed in this research, then the large scale MABR system should be revisited. It will also be important moving forward with the research to first prove that the system is capable of SNdN without Aqua using the continuous flow airline. Once the system is successful then the Aqua can be added to ensure that the only variable that is changing in the system is the addition of the Aqua.

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Chapter 8 Appendix A

The sample preparation for the large-scale experiment differed slightly from the screening experiment procedures after conducting quality assurance testing. There was some concern that the sample preparation and storage methods for the Ion Chromatograph used for nitrate and nitrite testing may have been altering the ion concentrations. Further evaluation of the methods showed that the glass fiber filters were removing nitrate from the samples, and the use of H_2SO_4 to acidify samples was altering the nitrite and nitrate concentrations (Figure 8-1)

To assess the effects of the sample preparation the effects of the acidification and filtration were assessed separately. Samples A1 and A2 were both acidified with H_2SO_4 dropwise till the pH dropped below 2. Samples F1 were only filtered through the 1.2 μm glass filter and samples F2 were filtered through both the 1.2 μm glass fiber filter and the 0.22 μm nitrocellulose filter. These samples were all done in triplicate and were compared the control that had no acid or filtering.

Looking at the F1 samples there is a clear drop in nitrate. The error bars on these are larger than the other experiments, but this was because one of the F1 samples was accidentally filtered through two 1.2 μm filters causing the concentration to drop further. This confirmed that the glass fiber filters were removing nitrate from the samples. Looking at samples F2 there was no further drop in nitrate or nitrite during this step indicating that the nitrocellulose filters did not remove any nitrate or nitrite (Figure 8-1).

Figure 8-1 Change in concentration of 50 ppm stock solution through various stages of sample preparation for IC analysis.

Once this decrease in nitrate was observed from the glass fiber filters the prefilters were immediately changed to nitrocellulose filters. All the samples after T9 of the MABR batch reactor run were filtered with only nitrocellulose filters. This should have stopped the adsorption of nitrate during the filtering process.

Another discrepancy in these data was the 5 mg-N/L decreases in the nitrite for all the samples that had H_2SO_4 added to them. This decrease in concentration is only seen for the nitrite but not for the nitrate (Figure 8-1). This drop could have been caused by the chemical reaction between the NaNO_2 and H_2SO_4 . The NaNO_2 reacts at a 2:1 mole ratio and one of the NO_2 ions forms NO gas and bubbles out of the sample as shown in Equation-12. The acid used to acidify the samples had a concentration of 96% which is what caused the substantial drop in nitrite. The nitrate did not see similar losses because

the NaNO_3 reacts with the sulfuric acid at a 1:1 ratio and does not result in any loss of NO_3 as shown in Equation-13.

To further evaluate the effect of acidifying the samples on nitrite concentrations a second methods calibration test was conducted. In this test two different acids at different concentrations were used to acidify a standard with 13 ppm of NO_3 and 7 ppm of NO_2 . Sample A1 was 98% H_2SO_4 , sample A2 was 2 M H_2SO_4 , A3 was 0.1 M H_2SO_4 , and A4 was 12.1 M HCl . For each type of acid, the acidification was repeated three times. A control was also run to confirm the initial concentration of nitrate and nitrite.

These samples were acidified using the same methods that are used when taking samples. To do this the acid was added drop wise to the sample until the pH dropped below 2. As the acid became more dilute the amount of acid needed to drop the pH increased. In this case the pH of the samples being acidified with the 0.1M H_2SO_4 (A3) did not drop below 2. Due to the increase in volume needed to acidify the sample this could have been diluting the concentration of nitrate and nitrite in addition to the reaction occurring with the acid.

The samples that were acidified with more concentrated sulfuric acid had a decrease in nitrite concentrations and a slight rise in nitrate concentrations. When the weaker sulfuric acid, A3 was used less nitrite was loss and the nitrate did not increase as much as samples

A1, and A2 (Figure 8-2). This experiment also indicated that using hydrochloric acid (A4) still reduced the concentration of nitrite in the water.

Figure 8-2 Effect of different types and concentrations of acid on nitrate and nitrite concentration.

To determine the extent of the effect of acid on the nitrite and nitrate concentration another experiment was conducted using known volumes of acid. This time instead of using the pH as the indicator for how much acid to add, a known volume of acid was added and the pH of the sample at that volume was recorded. In this experiment A1 was 0.05 mL H₂SO₄ (96%), A2 was 0.1 mL of H₂SO₄ (96%), A3 was 0.2 mL H₂SO₄ (96%), and A4 was 0.1mL HCl [12.1N]. This experiment confirmed what was observed in the previous experiment. The addition of H₂SO₄ cause the nitrite concentration to decrease, and the nitrate concentration to increase (Figure 8-3).

Figure 8-3 Effect of acid on nitrate and nitrite using known volumes of acid.

Sample A1 had the least amount of H_2SO_4 added to the sample and had the lowest drop in nitrite in the sample. Sample A2 and A3 that had higher nitrite removal and higher amounts of acid. Through doing a t-test between samples A2 and A3 it determined that these were not statistically different and that the change in concentration in acid did not make a difference. Another similarity between the samples was that sample A1 through A3 all saw an approximate increase in nitrate by about 15 ppm. This increase in nitrate which cannot be explained by the chemical reaction with the H_2SO_4 indicates that there may be another factor influencing the concentration or that the acidification may be altering the retention time through the separation column in the IC in some way.

The apparent decrease in nitrite concentration and increase in nitrate concentration is an important factor to consider when evaluating the results from the IC. In most cases

almost all the nitrite is removed from the sample which could drastically skew the results. It could also cause the nitrate concentration to appear higher than it is.

Moving forward in the experiment it was important to keep these effects from filtering and acidifying the samples in mind. Since the samples are acidified with a transfer pipet and the exact volume of acid is unknown it would be difficult to do a calibration of exactly how much nitrite is being removed for the tests.