A STUDY ON THE SIMULTANEOUS NITRIFICATION AND DENITRIFICATION PROCESS OF A MEMBRANE AERATED BIOREACTOR AUGMENTED BY BIOWISH® AQUA

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ABSTRACT

A Study on the Simultaneous Nitrification and Denitrification Process of a Membrane Aerated Bioreactor Augmented by BiOWiSH® Aqua

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Nitrogen pollution is a growing problem that is detrimental to the environment and the economy. Traditional treatment of nitrogen is a multi-stage process, expensive, operationally intensive, and requires large land areas. This research studied the effects of BiOWiSH® Aqua (Aqua), a biological enhancement product, on the simultaneous nitrification and denitrification process in a membrane aerated bioreactor (MABR) to determine if it is a feasible application for wastewater treatment. The MABR used during experimentation was a small-scale batch reactor with a continuous flow of air through a silicone membrane.

The effect of carbon source and concentration on nitrogen removal rates and biomass growth/behavior were determined through a series of laboratory experiments with Aqua and wastewater. With glucose and solely Aqua cultures, average reduction rates in nitrogen concentrations were 1.2 mg-N/L/hour for all C:N ratios investigated. When wastewater was used as the main carbon source, creating a mix of wastewater and Aqua bacteria in the MABR, average reduction rates were 10.9 mg-N/L/hour. A maximum reduction rate of 21.3 mg-N/L/hour occurred at a 2:1 C:N ratio.

This research concluded that pure Aqua cultures are not efficient at removing nitrogen or greatly augment the nitrogen reduction process. MABRs can use the biochemical oxygen demand in wastewater as a useful/viable carbon source. High carbon to nitrogen ratios (C:N ratio of 30:1) did not result in faster nitrogen reduction rates but did experience rapid biofilm growth and death. This shows that high C:N ratios are not an efficient operationally for MABRs due to the excess sludge created. C:N ratios of
approximately 3:1 provided the most consistent nitrogen reduction for both glucose and wastewater. This research concluded that C:N ratios, pH, and oxygen diffusion heavily affect the MABR’s performance. In addition, MABRs can utilize low C:N ratios during treatment, particularly during the treatment of high-strength wastewater.

Keywords: Membrane Aerated Bioreactor, Biological Nutrient Removal, Wastewater
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Chapter 1 – Introduction

Nitrogen is an essential nutrient to the growth of plants and other organisms (Environmental Protection Agency, 2009). However, water quality degrades if excess nitrogen is introduced. This is often an issue in rural areas when a lot of fertilizer is applied to add nitrogen to the soil (approximately 0.45 kg of nitrogen per 1,000 square feet). This balance of agricultural viability to water quality opens the door for public health and environmental crises. Nitrogen comes in multiple forms, but often enters the environment in the form of nitrates and ammonia (Environmental Protection Agency, 2019).

1.1 Nitrogen Pollution

Nitrogen is typically introduced to the environment from fertilizer use or high strength wastewater from agricultural or industrial activities, making it a larger issue in rural areas and in industrial areas (Summers, 2019). However, most domestic wastewater treatment plants will discharge effluent to surface water bodies. Domestic wastewater in urban areas has lower concentrations of nitrogen as the primary contributor is human waste. Overloading the receiving waters with excess nitrogen (nutrient pollution) can cause detrimental effects to the public, environment, and economy.

The most common result of nitrate pollution is eutrophication, the presence of large, algal blooms (often toxic) in surface water bodies. These blooms deplete oxygen availability in the water creating dead zones where little to no aquatic life can be sustained (Environmental Protection Agency, 2019). Direct exposure to toxic algae can result in neurological deficiencies for both fish and humans. Additionally, areas with high nitrate concentrations in their drinking water have high infancy mortality rates due to methemoglobin, colloquially known as blue baby syndrome (Environmental Protection Agency, 2019). Economically, nitrate concentrations cause fishing industries to decline,
wastewater treatment to be more expensive, and can cause a loss of tourism due to aesthetic decreases in public water bodies (Environmental Protection Agency, 2019).

Ammonia enters the environment through the decomposition of organic matter and animal or human waste. Ammonia can either be found as ammonium ($\text{NH}_4^+$), which is abundant, or unionized ammonia ($\text{NH}_3$), which is more toxic. High levels of ammonia in water leads to a toxic buildup in the internal tissues, organs, and the blood stream of fish and other wildlife, often resulting in death (Hill, 2014). Ammonia pollution depletes food supplies and can be detrimental to aquatic ecosystems.

1.2 Regulations for Nitrogen

Ammonia concentrations in raw domestic wastewater range from 14 to 40 mg-N/L (Tchobanoglaus & et.al, 2013). Nitrate concentrations can range from 0 to 30 mg-N/L depending on the source. Agricultural runoff or industrial wastewater discharges can contribute to increased nitrate concentrations in the influent (Environmental Protection Agency, 2019). The Clean Water Act allows the Environmental Protection Agency (EPA) to approve state-wide programs for nutrient removal techniques and limits. The EPA set a maximum contaminant limit (MCL) for nitrate of 10 mg-N/L, and most state programs regulate their wastewater effluent to meet the MCL or lower to prevent surface water or groundwater contamination (Environmental Protection Agency, 2009).

There is not a current MCL for ammonium, however, the EPA does recognize that ammonium is toxic to aquatic life and has set an acute exposure limit of 17 mg-N/L and chronic exposure limit of 1.9 mg-N/L (Environmental Protection Agency, 2013). This is measured as total ammonia nitrogen (TAN) to capture both ammonium and ammonia concentrations. Alongside the EPA limits for nitrate and ammonia, there is a push towards sustainable living practices. This often includes water recycling for irrigation and
non-potable uses. As water recycling becomes more common-place, nitrogen limits are likely to become more stringent to prevent contamination of groundwater tables.

1.3 Current Treatment Methods

Biological wastewater treatment has been the main nitrogen removal method for decades. Research on biological treatment has grown drastically to try and meet stricter limits and assuage growing concerns with nutrient pollution. Often, wastewater treatment plants will use a return activated sludge (RAS) system or a biotower to remove organic carbon in the form of biochemical oxygen demand (BOD). If operated at longer solids retention times, these systems can remove nitrogen from the wastewater stream. An example of this is the San Luis Obispo Water Resource and Recovery Facility (SLO WRRF) which utilizes a biotower system and an aeration basin to convert TAN into nitrate. The biotowers themselves remove up to 40% of the TAN, and the additional aeration basin is required to finish the conversion (City of San Luis Obispo, 2015). Many RAS systems and pond systems have anoxic zones, where the main source of oxygen comes from nitrate, allowing for reduction of nitrate into nitrogen gas. However, conventional systems are often operated to focus on BOD and TAN removal. Wastewater treatment plants that aim to remove all nitrogen from the waste stream often need a specific anoxic tank with multiple feed lines to efficiently achieve full nitrogen removal (McCarty, 2018).

Aeration processes require large footprints and due to the need for redundancy, these large systems can require acres of land by themselves. The SLO WRRF’s plant layout is dominated by the biotowers, RAS system, and the secondary clarifiers required (Figure 1-1). While most treatment systems do not use two biological reactors like the SLO WRRF, one aeration basin is still a large undertaking, and those treatment plants will often require additional systems (such as filters) if they want to recycle the effluent. In
wastewater treatment plants that utilize an anoxic tank, this requires double the footprint of a conventional aeration system, while potentially requiring further separation processes as well (McCarty, 2018).

Figure 1-1: Aerial view of the SLO WRRF with the biological treatment systems highlighted in red (Google Maps, 2018).

The current practice of multiple tank systems puts an undue burden on the municipalities trying to treat their effluent to a high quality, particularly those aiming for recycled water usage (Environmental Protection Agency, 2009). The expenses required for multiple tanks, the maintenance, and the highly trained operators is often too costly to implement for many cities or communities. The large footprint forces wastewater treatment plants to be in remote locations, which can cause the sewer lateral connections to grow in price, on-top of the overall pricing of land. This is especially true for smaller cities that do not have the proper funding to sustain the large infrastructure or the operation and maintenance of these conventional systems.
1.4 Focus of this Research

A potential solution to improve biological treatment is a membrane aerated bioreactor (MABR). MABR systems utilize a membrane to diffuse oxygen throughout the system, with that membrane also providing a growth location for biofilm. The biofilm provides a significant barrier to oxygen diffusion through the bulk water, allowing for complete nitrogen removal within the system (Terada & et.al, 2003). This means that an aerobic zone develops on the biofilm membrane interface, and an anoxic zone develops on the biofilm bulk water interface. This combined system of aerobic and anoxic treatment would lower the land footprint by at least two-fold (General Electric Co's, 2017). MABRs have the potential to lower energy costs by 75% due to the low-pressure requirements and not needing to bubble oxygen through the whole tank (Williams, 2017). These benefits mean MABRs are a potential solution to the concerns surrounding conventional nitrogen removal methods in wastewater treatment.

Another potential way to improve biological treatment is to augment the microbial system in a treatment unit with a commercially available product known as BiOWiSH® Aqua (Aqua). Aqua supplements bacterial growth through a blend of bacterium and non-living organic material. Aqua was designed to facilitate nitrogen removal in biological systems by promoting growth of bacteria known to interact with the nitrogen cycle (BiOWiSH Technologies, 2016). Multiple studies were performed on Aqua’s effects on natural water bodies and proved successful. However, it has not been studied further in domestic wastewater treatment, meaning Aqua’s implementation and effectiveness need to be assessed. If Aqua can augment the nitrogen removal process in biological treatment, it may provide an easier pathway for operators.

Previous research on the augmentation of the MABR with Aqua was performed by Joelle Arakaki. That research focused on a preliminary understanding of Aqua dosing methods,
sampling and testing methods, growth media constituents, the viability of continuous flow configurations for compressed air usage, and briefly touched on the effects of carbon concentrations on nitrogen removal rates (Arakaki, 2018). This research produced testing methods in the form of ion chromatography, TAN analysis with a Timberline, and Hach total nitrogen kits. It proved that an Aqua dosing method of 1g inoculated outside the system is most effective. The continuous flow configuration proved beneficial to the system, proving that compressed air is a viable source of oxygen for the MABR system. However, the research was inconclusive in terms of the effect of carbon concentrations and did not prove conclusive on the actual effectiveness of Aqua as a biological augmentation pathway. The previous research performed a few small-scale experiments but was then scaled up to try and look at implementation on a larger scale. However, there were too many unknowns and variables to make conclusive arguments on the effects of Aqua in domestic wastewater treatment.

1.5 Objectives of this Research

The research discussed in this paper scaled the MABR down to a continuous airflow configuration in a 1 L bioreactor. This scaled down MABR aims to make conclusions about the nitrogen removal pathways within the system. The specific objectives of this research are:

1. Observe the effects of glucose and wastewater as carbon sources at various C:N ratios on the nitrogen removal processes,
2. Understand biofilm growth and the impact it has on nitrogen removal rates,
3. Model nitrogen removal rates to provide a baseline for MABR success,
4. Confirm Aqua’s ability to augment a biological treatment system,
5. Propose MABR design and testing improvements to promote future MABR experimentation.
Chapter 2 - Literature Review

The literature review for this project details industry information on natural nitrogen removal pathways, conventional wastewater treatment, simultaneous nitrification and denitrification, the proposed membrane aerated bioreactor system, and microbial augmentation with BiOWiSH® Aqua. Previous research on membrane aerated bioreactors focused on configurations of air flow and membrane material. Research on BiOWiSH® Aqua is limited but focused on low-level wastewater and surface water treatment efficiency and preliminary strain testing.

2.1 The Nitrogen Cycle

Municipal wastewater treatment achieves biological nitrogen removal through the nitrogen cycle. The nitrogen cycle (Figure 2-1) shows the multiple pathways for nitrogen conversion through various biological and non-biological processes (Tchobanoglaus & et.al, 2013). The main stages utilized in wastewater treatment plants are nitrification, denitrification, and assimilation.

Assimilation, nitrification, and denitrification are the typical stages utilized in wastewater treatment because they are easier to achieve through known treatment techniques. Varying aeration and a high large mixed liquor suspended solids (MLSS) can result in any of these stages occurring. These techniques are also used to remove carbonaceous biochemical oxygen demand (CBOD₅), making it an easy transition to nitrogen removal.
2.1.1 Nitrification

Nitrification is the process of oxidizing a reduced nitrogen compound, primarily TAN, to nitrite, and sequentially, nitrate. Two known groups of autotrophic nitrifying bacteria perform nitrification in two-steps. In Step 1, (Equation 1), ammonia is converted to nitrite through an aerobic, oxidation reaction. Step 2, (Equation 2), converts nitrite to nitrate through another, subsequent, oxidation reaction.

Figure 2-1: Typical pathways for natural nitrogen removal for TAN and nitrate.
\[ \text{NH}_3 + \text{O}_2 \rightarrow \text{NO}_2^- + 3 \text{H}^+ + 2 \text{e}^- \]  \hspace{1cm} (1)

\[ \text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2 \text{H}^+ + 2 \text{e}^- \]  \hspace{1cm} (2)

Step 1 involves ammonia-oxidizing bacteria (AOBs) such as *Nitrosomonas*. While *Nitrosomonas* is associated as the primary genus for this step, *Nitrosococcus* and *Nitrosospira* have shown to be capable of this step as well. Step 2 involves nitrite-oxidizing bacteria (NOBs), with the main genus being *Nitrobacter*. *Nitrospina*, *Nitrococcus*, and *Nitrospira* are also capable of performing step 2 of nitrification.

Heterotrophic bacteria and fungi can contribute to nitrification. However, these two groups of microorganisms perform nitrification at a much slower rate than autotrophic organisms and are often considered a negligible contributor to the overall process in wastewater treatment (Environmental Protection Agency, 2009).

Nitrification requires free molecular oxygen to occur. Along with requiring oxygen, nitrifying bacteria are temperature sensitive and operate best at a temperature of 20 °C, meaning it is more difficult to maintain consistent nitrogen removal during winter seasons. Nitrification will occur best at a pH between 7.2 and 8.0. During the summer, if the wastewater treatment plant is not operated ideally, algal blooms can cause pH to increase, reducing nitrogen removal. Additionally, due to the slow growing rate of nitrifying bacteria, the solids retention time needs to be approximately double that of a carbon removal system to ensure they are not out competed (Tchobanoglaus & et.al, 2013). For a wastewater treatment plant to achieve nitrification, it needs to be operated with these constraints in mind.

2.1.2 Denitrification

Denitrification is the reduction of nitrate to nitrogen gas through bacteria known as facultative anaerobes. Denitrification can only occur once dissolved oxygen
concentrations have been depleted, forcing the facultative bacteria to consume nitrate as the electron source. This is known as an anoxic condition where dissolved oxygen concentrations are less than 0.5 mg/L. Preferably the dissolved oxygen concentrations are as close to 0 mg/L as possible (Environmental Protection Agency, 2009).

Denitrification is a one step process (Equation 3). As seen in Equation 3, denitrification does require a carbon source to occur, and methanol (CH$_3$OH) is being used as a placeholder.

$$6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 5 \text{CO}_2 + 7 \text{H}_2\text{O} + 6 \text{OH}^- \quad \text{.................................................. (3)}$$

While facultative anaerobes are considered the main denitrifiers, heterotrophic and autotrophic bacteria are also capable of this process. Heterotrophic bacteria will readily use nitrate as the electron acceptor under anoxic/anaerobic conditions for their metabolism of biodegradable. However, they will use oxygen before nitrate for their metabolism, so for heterotrophs to be denitrifiers, the oxygen concentration in the bulk water must be minimized (Tchobanoglaus & et.al, 2013). Autotrophic bacteria will convert ammonium to nitrogen gas using nitrite as the electron acceptor under a process known as ANAMMOX (Zhou, Weili, & et.al, 2011). The ANAMMOX process is done by specific groups of bacteria and is still being researched.

Denitrification is impacted by availability of carbon, type of carbon source, and temperature. Denitrifiers require carbon sources to reduce nitrate into nitrogen gas. Without enough carbon in the system, the microbes will not grow sufficiently to denitrify the water. Additionally, denitrification has been proven to work best with methanol or acetic acid as the carbon source, although glucose has been shown to work as well. Denitrifying bacteria work best in temperatures between 5 and 30 °C, but nitrate removal rates increase with increasing temperature (Tchobanoglaus & et.al, 2013).
2.1.3 Assimilation

Nitrogen assimilation is a process in which TAN or nitrate is consumed by microbes during reproduction. This step in the nitrogen cycle can produce results that appear to be nitrification or denitrification due to a steep decline in TAN or nitrate concentrations. However, since microbial cells are approximately 12% nitrogen, they must consume nitrogen from the wastewater to reproduce. This loss in TAN or nitrate results in an increase of organic nitrogen or biomass (Tchobanoglaus & et.al, 2013).

2.2 Conventional Wastewater Treatment

Nitrogen removal pathways include chemical oxidation (breakpoint chlorination), suspended and attached biological systems, air stripping, and ion exchange. While all these mechanisms can remove nitrogen, most wastewater treatment plants utilize suspended growth biological systems, and some treatment plants work with fixed-film systems (Tchobanoglaus & et.al, 2013). While biological systems can be unpredictable, they recreate the nitrogen cycle more efficiently than many engineered systems. Whatever treatment system is used, it must be able to meet the EPA nutrient limits of 10 mg-N/L for total nitrogen, and 1.9 mg-N/L for TAN.

Conventional wastewater treatment facilities achieve nitrification and denitrification through a minimum of two tanks, one being anoxic and the other aerobic. Nitrification occurs in the aerobic tank where the activated sludge breaks down organic matter and converts TAN into nitrates. This aerobic zone has a high oxygen content with steady mixing provided by the aerators. The nitrate rich water then flows into an anoxic tank, where oxygen is not supplied, and bacteria reduce the nitrates into nitrogen gas, which is then off-gassed to the atmosphere (Tchobanoglaus & et.al, 2013).
Pre-anoxic activated sludge set-ups involve the anoxic tank coming before the aerobic, with the return activated sludge (RAS) being resupplied to the anoxic tank. Ludzack and Ettinger developed the first pre-anoxic system in 1962 (Figure 2.2). This relies on the RAS delivering nitrates formed in the aerobic zone back into the anoxic tank. This greatly limits the amount of denitrification occurring, as it is fully dependent on the nitrate concentration in the RAS cycle. Ludzack and Ettinger systems are typically run with high RAS recycle rates to prevent build-up of sludge in the secondary clarifiers (Tchobanoglaus & et.al, 2013).

![Figure 2-2: The process flow for a pre-anoxic Ludzack-Ettinger biological treatment system.](image)

The Modified Ludzack-Ettinger (MLE) process was developed by Barnard in 1973 as an improvement on the existing Ludzack-Ettinger model. This process (Figure 2-3) added an internal recycle from the aerobic zone to the anoxic zone to provide more nitrate to the anoxic zone. MLE is more adaptable for existing activated sludge facilities and the addition of an internal recycle allows the system to meet a typical effluent limit of 10 mg-N/L for total nitrogen (Tchobanoglaus & et.al, 2013).
The single sludge process is a post-anoxic nutrient removal system (Figure 2-4). This process, developed by Wuhrmann in 1964, involved the addition of a mixed anoxic tank after the conventional aerobic TAN removal unit. This system was common for retrofitting existing activated sludge treatment plants but requires a carbon source to be added to the anoxic tank for proper denitrification conditions. Additionally, TAN is produced during normal respiration in the anoxic tank, causing an increase in total nitrogen in the effluent (Tchobanoglaus & et.al, 2013).

Figure 2-3: The process flow for the modified Ludzak-Ettinger pre-anoxic process.

Figure 2-4: The process flow for a single sludge process, which is post-anoxic.
The Bardenpho process, developed in the 1970s, is a multi-stage post and pre-anoxic nitrogen removal system. The Bardenpho process (Figure 2-5) is traditionally four stages, with two anoxic tanks and two aerobic tanks, but can be adapted to have as many tanks as possible for the desired treatment. Along with nitrogen removal, this system has proven capable of removing phosphorus as well, leading certain wastewater treatment plants to adopt the Bardenpho process if both are constituents of concern for the plant. However, its large footprint, and need to incorporate additional carbon sources, makes it inefficient for conventional wastewater treatment (Tchobanoglaus & et.al, 2013).

![Figure 2-5: The process flow for the Bardenpho process.](image)

The four nitrogen removal processes described are just a few examples of the many ways wastewater treatment plants evolved to perform nitrification and denitrification. Some treatment mechanisms are more complicated than others, but ultimately, they all involve multiple large tanks with occasional chemical additions that can be burdensome to operators and municipalities. This is not always feasible for smaller systems or disadvantaged areas, requiring new ways to perform the nitrogen cycle in a smaller footprint.
2.3 Simultaneous Nitrification and Denitrification

Instead of removing nitrogen in a two-stage process, another removal pathway is simultaneous nitrification and denitrification (SNdN). SNdN has proven capable of nitrogen removal efficiencies as high as 98.3% and concentrations of total nitrogen as low as 3 mg-N/L in the wastewater effluent.

Both heterotrophic and autotrophic bacteria are capable of accomplishing SNdN (Tchobanoglaus & et.al, 2013). Certain heterotrophic bacteria are capable of denitrifying in aerobic conditions with limited dissolved oxygen (DO), which is beneficial for existing wastewater treatment plants that need to meet new nitrate limits. Paracoccus pantotropha is a heterotrophic bacterium capable of SNdN. This bacterium, and others like it, require high energy producing carbon sources, like methanol, to achieve SNdN (Uemoto & Saiki, 2000). Unfortunately, methanol and others like it are easily oxidized and, therefore, are not commonly found in aerobic systems (Novak, Goldsmith, Benoit, & O'Brien, 2019). This makes heterotrophic bacteria an unlikely source of SNdN in conventional wastewater treatment, although they may prove capable in other bioreactors.

ANAMMOX bacteria, from the family Planctomycetales, also perform SNdN. With temperatures above 20 °C, Planctomycetales will oxidize ammonia through the reduction of nitrite under anaerobic conditions. ANAMMOX has proven to perform SNdN six to ten times faster than other autotrophic bacteria such as Nitrosomonas europaea (Zhang & Narita, 2017). ANAMMOX is up-and-coming but can be costly and demanding for operators.

Previous studies show that autotrophic bacteria are the most capable at SNdN, but more reliable systems will involve a mixture of heterotrophic and autotrophic bacteria. This can
be difficult to accomplish in small footprints, due to the differing requirements of each bacteria.

2.3.1 Simultaneous Nitrification and Denitrification Mechanisms

SNdN is primarily achieved through two different mechanisms. The first is achieved within bacteria flocs inside low DO or minimized DO activated sludge settings (Figure 2-6). Traditionally for nitrification, air is diffused through the system to achieve DO concentrations around 4 mg/L (Tchobanoglaus & et.al, 2013). For systems achieving nitrification, it is possible to lower DO to approximately 1 mg/L to meet new denitrification requirements. The oxygen will diffuse through the bulk water and contact the sludge floc. At the surface, there will be enough oxygen to achieve nitrification. The ammonia oxidizing bacteria (AOBs) will consume this oxygen, leaving the middle of the sludge floc anoxic.

![Figure 2-6: Visualization of aerobic and anoxic zones within one sludge floc](image)

The other main SNdN mechanism is operational changes in mixing patterns, or mixing patterns purposefully designed, to provide aerobic and anoxic zones within one system. This is often accomplished with the aeration mechanism itself and causes a macro-SNdN environment (Daigger & Littleton). Systems like an oxidation ditch can accomplish
anoxic zones by lowering the power to the mixing system, causing the channel to be anoxic with the mixed area aerobic (Tchobanoglaus & et.al, 2013). The Orbal™ is another SNdN mechanism that involves three channels of varying DO levels (Figure 2-7). The first channel has zero to low DO (<0.3 mg/L), the second channel has 0.5 to 1.5 mg/L of DO, and the third channel has high DO (2 to 3 mg/L). The influent enters into the low DO system and loops through, with RAS and internal recycles providing the proper nitrate and carbon concentrations to the appropriate channels.

![Figure 2-7: Schematic of the Orbal™ system](image)

While these are the two primary pathways for SNdN, there are others being researched to promote the advancement of full nitrogen removal.
2.4 Membrane Aerated Bioreactor

The membrane aerated bioreactor (MABR) is a new biological system designed for SNdN. MABRs are traditionally comprised of a tank with hollow-fibered membranes along the entire length of the reactor (Figure 2-8). These membranes are often arranged in a dead-end configuration, forcing oxygen to diffuse through the membrane into the biofilm. The membranes serve a dual purpose, both as an oxygen distribution pathway, and as a surface for biofilm adhesion. Multiple materials have been tested and proposed as an ideal membrane, but silicon has proven the most capable. Nitrifiers have shown to easily adhere to the silicon’s surface without clogging the pores, allowing for oxygen to continuously diffuse through (Terada & et.al, 2003).

Figure 2-8: Schematic of a dead-end configuration MABR showing the ins and outs for both air and wastewater.
The mechanism occurring in the MABR is a macroscale version of floc SNdN (Figure 2-9). Air is pumped through the dead-end configured membranes, and oxygen diffuses through the pores into a biofilm. Initially, oxygen diffuses through the thin biofilm easily and makes its way into the bulk water. However, as the nitrifiers consume oxygen and oxidize ammonia, they multiply. As a result, the biofilm thickens, and oxygen is fully consumed within it. This allows for an anoxic zone in the bulk water that contacts the outer portion of the biofilm (Walter & Haase, 2005).

![Diagram of oxygen diffusion through the MABR membrane. Creation of aerobic and anoxic zones within the biofilm with the bulk wastewater providing substrate.](image)

*Figure 2-9: Oxygen diffusion through the MABR membrane. Creation of aerobic and anoxic zones within the biofilm with the bulk wastewater providing substrate.*

Studies on these systems have shown that DO concentrations at the membrane biofilm interface are approximately 8 mg/L, while the water biofilm interface sees concentrations around 0.2 mg/L. This results in the DO of the bulk water always being approximately 0 mg/L. This DO diffusion gradient causes SNdN to be achievable.
The biofilm in a MABR is more complex than typical attached growth seen in nitrification-only treatment units due to it being a multi-layered, multi-culture, and multi-reaction system. Ideal biofilm formation will have nitrifying bacteria in the aerobic zone with denitrifying heterotrophs in the anoxic zone. However, there are possibilities for the biofilm to improperly form due to the slow growth of nitrifiers versus heterotrophs (Terada & et.al, 2003). Often, nitrifiers are introduced to the system first and allowed time to grow before heterotrophic bacteria are introduced to the MABR. This prevents the fast-growing heterotrophic bacteria from out competing the nitrifiers. Systems that inoculate like this have proven successful in providing the nitrifiers the chance to take over the aerobic zone. However, this is not always possible for larger scale systems, so finding new ways to overcome this will be necessary.

MABRs have specific parameters that affect efficiency of the system. Like other biological systems, oxygen diffusion, carbon to nitrogen (C:N) ratios, pH, and temperature are vital to the functionality of the system. Oxygen diffusion is integral for the MABR due to the different zones of reactions. Oxygen needs to be around 3 to 4 mg/L at the surface of the membrane, but reach <0.2 mg/L by the bulk water. C:N ratios determine the ability of the heterotrophs to reduce nitrate, and if there is not enough nitrogen in the water, the overall yield will be minimal (Walter & Haase, 2005).

Nitrification and denitrification occur in set pH and temperature ranges, so monitoring and maintaining a pH of 6.5 to 8.0 and a temperature of around 17 to 20 °C is important.

Unique parameters to consider for the MABR are membrane material, intermembrane pressure, biofilm thickness, and liquid flow velocity (Terada & et.al, 2003). Membrane material affects biofilm adhesion and oxygen diffusion. Often, hollow fiber membranes made of silicon are used due to the balance of allowing adhesion, but not clogging the pores with microbes. Intermembrane pressure, the pressure the air is exerting on the
membranes, can affect the volume of oxygen diffused through the system. For MABRs, intermembrane pressure was determined to not have a large effect on the system’s performance. Biofilm thickness affects performance in multiple ways. The first being that if the biofilm is too thin, it will allow too much oxygen into the bulk water, reducing the size of the anoxic zone. The second is that if the biofilm is too thick, the biomass will slough off the membrane, and reduce the active biomass. Finally, liquid flow velocity is an important consideration to prevent disruption of the biofilm’s structure. If liquid flow velocity is too high, it can scour the biofilm. If it is too slow, the liquid can become stagnant, which also reduces performance.

MABRs have proven successful for carbon reduction, nitrification, simultaneous carbon and TAN removal, and the treatment of high-strength wastewater. To understand the full potential for SNdN or other nitrogen removal pathways in a MABR, further studies need to be conducted.

2.4.1 Biofilms

The biofilm formed on the membrane itself is essential to the treatment of wastewater. Microbes will behave differently in a biofilm formation than they do during suspended growth. This is due to the interconnectedness and reliance on other bacteria to support the overall process (Montana State, 2010). Biofilms are often stronger and less susceptible to shock loadings but can grow thick and slough off.

Nitrifiers tend to grow in thinner biofilm formations, which can increase the kinetics of TAN oxidation. AOBs and NOBs will form in layers as close to the oxygen source as possible. These thin layers (10 to 20 μm) are beneficial for a strong attachment to the membrane itself, and for increased contact with the TAN rich wastewater (Araki, Akiyoshi, Machdar, & Harada, 1999). Heterotrophic bacteria show similar tendencies but will form a thicker layer (20 to 30 μm) (Moreau, Liu, & Capdeville, 1994).
While the individual microbial groups perform this way in separate biofilms, this is not how they perform in the MABR. Thinner biofilms are better for more turbulent flows because it lowers the chance of scouring. However, MABRs have a less turbulent environment and combines nitrifiers and heterotrophs together into one biofilm. This causes the biofilm to be thicker, which makes it prone to sloughing off. The complexities of this multi-layered biofilm are not fully studied yet, but some progress is being made on the understanding of biofilm behavior (Moreau, Liu, & Capdeville, 1994).

2.4.2 Oxygen Diffusivity with a Silicone Membrane

Studied MABRs used pure oxygen with a dead-end configuration to ensure oxygen diffuses into the system. Dimethyl silicon membranes have been shown to have a 2:1 diffusivity ratio of oxygen to nitrogen, meaning that oxygen diffuses more readily than nitrogen (Haibing & Cloud, 2004). Diffusion rates of 6,600 cm²/s*cmHg have been observed for oxygen, with nitrogen diffusing at a rate of 2,800 cm²/s*cmHg. This high oxygen diffusivity makes silicon membranes ideal for the MABR’s application.

While MABRs typically use pure oxygen, therefore reducing the need to worry about diffusivity rates, the 2:1 ratio allows for the use of compressed air as the main oxygen source. The largest barrier to this is the dead-end configuration that was standard in previous models. In dead-end configurations, the added pressure gives nitrogen the chance to diffuse into the system which reduces the concentration of oxygen. Dead-end configurations also cause condensation to occur within the membrane itself which reduces the amount of transmissive area available (Haibing & Cloud, 2004). A study conducted in 2017 at the University of Notre Dame found that having an open-end configuration, with a steady leakage rate of air, allows oxygen to diffuse through the membrane, and nitrogen to vent off outside the system (Perez-Calleja, et al., 2016).
ability to use compressed air in an open-ended configuration, allows the system to be scalable at a lower cost for both technology and energy.

2.5 BiOWiSH

BiOWiSH® Aqua (Aqua), is a proprietary mixed microbial seed intended to enhance carbon/nitrogen degradation in surface water and wastewater treatment. The idea behind this product is to provide wastewater systems with a faster growing, sturdier, bacterial system consisting of heterotrophic bacteria from the *Bacillus*, *Pediococcus*, and *Lactobactus* genera. Alongside carbon removal, Aqua has been found to achieve nitrification and denitrification (Gorsuch, Roberts, & Showell, 2012). Aqua contains less than 1% of active ingredients, with the bulk of the product being dextrose (BiOWiSH Technologies, 2016). This results in it being a safe way to inoculate a new wastewater treatment system with the bacteria needed for carbon removal, nitrification, and denitrification.

Aqua relies on heterotrophic bacteria for nitrogen removal, which does require a carbon source for energy. Heterotrophic bacteria grow faster than typical nitrifiers, which can be beneficial for wastewater systems as it allows for lower solids retention times. Some heterotrophic strains of *Bacillus* (particularly the phylum Firmicutes) were observed to perform aerobic nitrification and denitrification at a higher rate than normal denitrifying heterotrophs (Gorsuch, Roberts, & Showell, 2012).

The bacterial strains in Aqua expected to perform nitrification and denitrification were isolated and tested in DI water reactors and wastewater reactors. The results from a BiOWiSH study in Table 2-1 and Table 2-2 show that at high DO concentrations (6 to 7 mg/L), nitrification and denitrification was performed by multiple isolate strains (BiOWiSH Technologies, 2016).
Table 2-1: Ammonia oxidation rates for isolated Aqua strains.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>DI Water Reactor</th>
<th>Wastewater Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate (mM/hour)</td>
<td>ΔDO (ppm)</td>
</tr>
<tr>
<td>B. licheniformis (B5)</td>
<td>0.655</td>
<td>2.4</td>
</tr>
<tr>
<td>B. licheniformis (B11)</td>
<td>0.563</td>
<td>0.9</td>
</tr>
<tr>
<td>B. licheniformis (B13)</td>
<td>0.530</td>
<td>1.8</td>
</tr>
<tr>
<td>B. amyloliquefaciens (B6)</td>
<td>0.146</td>
<td>4</td>
</tr>
<tr>
<td>B. amyloliquefaciens (B10)</td>
<td>0.639</td>
<td>5.5</td>
</tr>
<tr>
<td>B. subtilis (B9)</td>
<td>0.507</td>
<td>0.4</td>
</tr>
<tr>
<td>B. subtilis (KLB)</td>
<td>0.141</td>
<td>2.3</td>
</tr>
<tr>
<td>B. mojavensis (B8)</td>
<td>0.737</td>
<td>0.7</td>
</tr>
<tr>
<td>B. pumilus (B12)</td>
<td>0.797</td>
<td>0.8</td>
</tr>
<tr>
<td>P. pentosaceous</td>
<td>0.133</td>
<td>2.8</td>
</tr>
<tr>
<td>P. acidilactici</td>
<td>0.639</td>
<td>1.1</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>0.001</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 2-2: Nitrate reduction rates for isolated Aqua strains

<table>
<thead>
<tr>
<th>Isolate</th>
<th>DI Water Reactor</th>
<th>Wastewater Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate (mM/hour)</td>
<td>ΔDO (ppm)</td>
</tr>
<tr>
<td>B. subtilis (KLB)</td>
<td>0.010</td>
<td>1.3</td>
</tr>
<tr>
<td>B. mojavensis (B8)</td>
<td>-0.010</td>
<td>1.7</td>
</tr>
<tr>
<td>B. pumilus (B12)</td>
<td>0.001</td>
<td>1.9</td>
</tr>
<tr>
<td>B. licheniformis (B13)</td>
<td>0.002</td>
<td>1.9</td>
</tr>
</tbody>
</table>

From these preliminary results, it appears that Bacillus strains are capable of both nitrification and denitrification. This study, conducted by BiOWiSH, was done once and not repeated, but these results are promising for the use of Aqua as a nitrogen removal
mechanism. Aqua has been used in a few case studies and is being researched in multiple projects, which is beginning to provide more data and results. However, these are currently in progress and the results are currently unknown.

2.5.1 BiOWiSH Case Studies

Two case studies of Aqua application include surface water clean-up in Tile, Turkey and a septic tank project in a Chesapeake Bay RV Park. Both involve augmenting existing biological systems with the bacterial strains found in Aqua.

In Tile, Turkey, the municipality wanted to build a recreation center near a lake. However, this lake was turbid and had problems with vegetative overgrowth and sludge accumulation from degrading plant matter. These issues produced odors and unwanted insects, causing complaints in the area. Aqua was directly applied to this lake by adding 10 kg to an 800 L tank that the lake water was pumped through. The Aqua digested the excess sludge on the bottom, reduced turbidity, and decreased odor issues. As a result, wildlife became more active in the area, and the lake was no longer an aesthetic issue. Aqua is still added every day at 5 kg per 800 L tank (BiOWiSH Technology, 2016).

An RV park in Chesapeake Bay, VA experienced capacity issues in their septic system and leachfield, along with odors. The objective of this study was to reduce BOD$_5$, total suspended solids (TSS), and nitrogen in the septic tank to reduce overall odors. Aqua was mixed with water outside of the system and incubated overnight. Three 18 L buckets with 100 g of Aqua were prepared. Two were poured directly into the septic tank, and one entered the system through the toilet. Within 34 days, the Aqua augmented systems had reduced sludge volume, and reduced odor. BOD$_5$, TSS, and total nitrogen concentrations were reduced by half. Long-term implication of this will be larger system capacity, lower odors, and longer system lifetime. BiOWiSH
recommended the addition of Aqua monthly to the system to maintain this level of treatment (BiOWiSH Technologies, 2016).

Both case studies show that Aqua has the capabilities of enhancing overall treatment capacity in biological systems. These are narrow case-studies, but research on Aqua’s enhancing capabilities in wastewater treatment units is being conducted.

2.6 Previous Research

Previous research on Aqua and it’s benefits to a MABR system was conducted by Kirk Waltz and Joelle Arakaki at California Polytechnic State University – San Luis Obispo.

Kirk Waltz studied a dead-end airline configuration with compressed air that proved to be inconclusive. The dead-end configuration showed decreases in ammonia that did not result in the production of nitrite or nitrate. This indicated issues with oxygen diffusion inhibiting the system (Waltz, 2009). This spurred Joelle Arakaki to produce a MABR system with an open-end configuration that allowed compressed air to vent off. TAN removal was seen in this system as well, again without the production of nitrite or nitrate. However, due to upscaling the system too quickly, contamination became an issue and the results from this research were similarly inconclusive (Arakaki, 2018).

Due to the issues found by the previous researchers, the MABR design for this research is an open-end configuration that was scaled back down to a 1 L bioreactor system to gain further understanding and control on system nuances.
Chapter 3 - System Design

A small-scale bioreactor of 1 L was set-up for testing to observe operational effects on the MABR’s functionality and to observe Aqua’s contribution to the system. Silicon tubing connected to a compressed air line was distributed throughout the system and used as the oxygen source. Length of tubing, air pressure, control systems, and growth media for the Aqua were designed based on previous research. Carbon sources and C:N ratios were altered and analyzed for differing results.

3.1 Membrane Aerated Bioreactor Design

The MABR design and the controls used changed three times. Initially, the MABR (MABR A) was set-up with 4 feet of thick-walled silicon tubing. MABR A was dismantled during a lab inspection and reset-up with 7 feet of thinner-walled silicon tubing (MABR B). The third set-up included another MABR (MABR C), in parallel with MABR B, with 7 feet of the same tubing. The length of tubing was determined by the amount that could fit inside the bioreactor itself and was measured after removal.

All three set-ups were capped with aluminum foil to prevent oxygen from the atmosphere from dissolving into the bulk water. The aluminum foil was nonreactive and maintained a low DO in the system. Figure 3-1 depicts which MABR was used for each experiment.

Figure 3-1: Schematic showing MABR to experiment connection
3.1.1 MABR A

Experiments 1 through 6 were done with a Kontes Cytolift bioreactor (catalog number 880600). This is a 1 L glass bioreactor. Growth media was placed in the system with the airline tubing and ran like a batch reactor (Figure 3-2).

![Figure 3-2: Image of the MABR A set-up with thick-walled silicon tubing.](image)

A thick-walled silicon tubing of 4 feet was placed randomly throughout the system to provide a larger surface area for oxygen diffusion. The airline was connected to a wall air compressor, with a needle valve to control pressure. For the first 6 experiments, a pressure of 15 psi was maintained. The end of the airline was clamped, but slightly open to slightly leak, preventing nitrogen gas buildup in the system.
Aqua was introduced to MABR A four days before Experiment 1 by inoculating 1 g of Aqua outside of the system with growth media. This growth media was then poured into the bioreactor and 500 mg of glucose was added to promote growth.

3.1.2 MABR B

Experiments 7 through 12 were conducted with the same Kontes Cytolift bioreactor, but new tubing and inoculate (Figure 3-3). The tubing used was a thin-walled silicon tubing, with 7 feet inside the bioreactor for oxygen diffusion. Air pressure was maintained at 15 psi.

Figure 3-3: MABR B set-up for Experiments 7 through 12.

Aqua was introduced to the system seven days prior to Experiment 7 by inoculating 1 g of Aqua outside the bioreactor with growth media. Growth media was poured into the system with added glucose for growth.
3.1.3 MABR C

Experiments 11 and 12 utilized the same bioreactor set-up as Experiments 7 through 12. MABR C was set-up alongside MABR B with the same tubing length and type. To connect both MABRs to the airline, a tee-splitter was added to the airline to promote equal distribution of air between the replicate systems. Pressure from the wall air compressor was increased to 30 psi prior to the tee-splitter to ensure each MABR’s membrane was operating at 15 psi.

3.2 Activated Sludge Bioreactor Design

For experiments 7 through 9, a bioreactor was set-up to model an activated sludge system. A Kontes Cytolift bioreactor was filled with 1 L of the same growth media, and a fish bubbler was used to diffuse oxygen into the system (Figure 3-4).

*Figure 3-4: Activated sludge bioreactor with suspended growth. This set-up was a control for the MABR.*
Aqua was inoculated into this system by mixing 1 g with the growth media outside the system and putting it into the bioreactor. The suspended growth grew for seven days before experimentation began.

3.3 Airline Configuration

The oxygen source for all experiments was the wall air compressor in Building 13 on the California Polytechnic State University – San Luis Obispo campus. The airline was controlled through a series of valves, rotameters, and pressure gauges (Figure 3-4). The air came from the wall, through the needle valve, rotameter, and pressure gauge. From there, it connected to the silicon tubing that was wound through the bioreactor and nitrogen gas vented off through the clamped end of the tubing outside of the MABR. When MABR C was set up in parallel with MABR B, the airline was split after the pressure gauge.

![Figure 3-4: Schematic of the airline set-up.](image)

The needle valve was connected directly to the air outlet from the wall to appropriately control air pressure. This allowed for easier set-up as the wall valve can be fully open, and the needle valve lowers the pressure to the desired value. Air from the wall comes out at approximately 100 psi, which could damage the small-scale membranes. The rotameter in place was there to ensure a consistent flow of air was maintained throughout the experiment. A vacuum gauge was attached before the tubing entered the
system to monitor any buildup of gas within the membrane itself. This eased the process of determining the level of venting that needed to occur from the airline outlet.

The silicon tubing was randomly distributed throughout the system to achieve an overall length of 2.1 m (7 ft) for MABR B and C. At 0.32 cm diameter, this provided 0.021 m² of surface area for oxygen to permeate through. A peroxide cured tubing was used with a permeability for oxygen gas of $6.579 \times 10^{-11}$ cm²/s·cmHg and a nitrogen gas permeability of $2.763 \times 10^{-11}$ cm²/s·cmHg (Cole-Parmer, 2018). Nitrogen gas did not build up in the bulk water due to the twice as high permeability of oxygen alongside allowing the air to vent off.

3.4 Growth Media Constituents

The growth media used for all experiments was developed and tested in previous research conducted by Joelle Arakaki. It is a blend of phosphorus buffer and micronutrients to promote a stable pH and a healthy environment for microbial growth (Arakaki, 2018).

3.4.1 Buffer

The growth media had a phosphorus buffer to maintain a pH between 6.5 and 7.5. Previous research showed that it was difficult to maintain the pH range due to excess hydrogen ions being released during nitrification. This concern was resolved by increasing the concentration of buffer within the growth media. Table 3-1 details the concentration of each constituent in the buffer portion of the growth media.
Table 3-1: Phosphorus buffer constituents and concentrations

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Chemical Formula</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipotassium phosphate</td>
<td>K₂HPO₄</td>
<td>1,068</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>KH₂PO₄</td>
<td>526</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>NaHCO₃</td>
<td>500</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>Na₂HPO₄·7H₂O</td>
<td>33</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>CaCl₂·2H₂O</td>
<td>28</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>MgSO₄·7H₂O</td>
<td>23</td>
</tr>
<tr>
<td>Ferrous chloride</td>
<td>FeCl·6H₂O</td>
<td>0.25</td>
</tr>
</tbody>
</table>

This buffer solution maintains an initial pH of 7.0 to 7.1 and is resistant to large fluctuations in pH that can occur during nitrification. Concentrations for each chemical were determined using the Henderson Hasselbach Equation.

3.4.2 Micronutrients

Micronutrients were added to the growth media in accordance with recommendations from BiOWiSH® and common microbiology practice. Each mineral and micronutrient either provides vital ions for the bacteria’s growth or promotes the ability to perform nitrification and denitrification. Table 3-2 details the concentrations of each micronutrient added to the growth media.
### Table 3-2: Micronutrient concentrations in the growth media

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Chemical Formula</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulfate</td>
<td>MgSO(_4) \cdot 7\text{H}_2\text{O}</td>
<td>3,000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>1,000</td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>MnSO(_4) \cdot \text{H}_2\text{O}</td>
<td>500</td>
</tr>
<tr>
<td>EDTA</td>
<td>EDTA</td>
<td>500</td>
</tr>
<tr>
<td>Ferric sulfate</td>
<td>FeSO(_4) \cdot 7\text{H}_2\text{O}</td>
<td>100</td>
</tr>
<tr>
<td>Cobalt nitrate</td>
<td>Co(NO(_3))_2 \cdot 6\text{H}_2\text{O}</td>
<td>100</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>CaCl(_2)</td>
<td>100</td>
</tr>
<tr>
<td>Aluminum sulfate</td>
<td>Al(_2)(SO(_4))_2</td>
<td>100</td>
</tr>
<tr>
<td>Nickel chloride</td>
<td>NiCl(_2) \cdot 6\text{H}_2\text{O}</td>
<td>20</td>
</tr>
<tr>
<td>Boric acid</td>
<td>H(_3)BO(_3)</td>
<td>10</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>Na(_2)MoO(_4) \cdot 2\text{H}_2\text{O}</td>
<td>10</td>
</tr>
<tr>
<td>Sodium tungstate</td>
<td>Na(_2)WO(_4) \cdot 2\text{H}_2\text{O}</td>
<td>10</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>Na(_2)SeO(_3)</td>
<td>1</td>
</tr>
</tbody>
</table>

The micronutrients chosen are designed to promote the growth of heterotrophic bacteria alongside the nitrifiers (Sigma-Aldrich, 2019). Trace metals help with bacteria’s natural metabolism and growth rates. The other chemicals in Table 3-2 are added to model the environment the bacteria would grow in naturally to promote growth within this system.

#### 3.5 Aqua Dosing Methods

Aqua was inoculated by pulling 50 mL of the growth media and mixing 1 g of Aqua in. This was then reintroduced to the system at room temperature. This method was developed in previous research and maintained consistent throughout the experimentation phase.

#### 3.6 Carbon Sources and Dosing Techniques

Glucose and wastewater were used as the carbon source for these experiments. Since an objective of the project is impact of carbon dosing, this design aspect varied the most
from experiment to experiment. Table 3-3 outlines the carbon source, mass added, and C:N ratio for each experiment.

Table 3-3: Carbon source and C:N ratio for each experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Carbon Source</th>
<th>Amount added</th>
<th>C:N Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>250 mg</td>
<td>1.5:1</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>250 mg</td>
<td>1.5:1</td>
</tr>
<tr>
<td>3</td>
<td>Glucose</td>
<td>250 mg</td>
<td>1.5:1</td>
</tr>
<tr>
<td>4</td>
<td>Glucose</td>
<td>250 mg</td>
<td>1.5:1</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0 mg</td>
<td>0:1</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>0 mg</td>
<td>0:1</td>
</tr>
<tr>
<td>7</td>
<td>Glucose, Wastewater</td>
<td>500 mg, 10 mL</td>
<td>3:1</td>
</tr>
<tr>
<td>8</td>
<td>Glucose, Wastewater</td>
<td>5,000 mg, 10 mL</td>
<td>30:1</td>
</tr>
<tr>
<td>9</td>
<td>Glucose, Wastewater</td>
<td>500 mg, 10 mL</td>
<td>3:1</td>
</tr>
<tr>
<td>10</td>
<td>Glucose</td>
<td>500 mg</td>
<td>3:1</td>
</tr>
<tr>
<td>11</td>
<td>Wastewater</td>
<td>200 mL</td>
<td>1:1</td>
</tr>
<tr>
<td>12</td>
<td>Wastewater</td>
<td>400 mL</td>
<td>2:1</td>
</tr>
</tbody>
</table>

The ability for the MABR to process the available BOD$_5$ in the wastewater is important for real world applications of this system. Varying the amount of carbon added initially to the system allows for determination of the limits of the MABR to process carbon and nitrogen simultaneously. This impacts the eventual placement and application of this system within wastewater treatment plants.
Chapter 4 – System Experimentation and Implementation

Twelve experiments were conducted with the small-scale, 1 L systems described in Chapter 3. Additionally, one microbiology experiment and one screening experiment were done. This chapter will detail sample collection and nitrogen concentration testing methods. Along with methods and procedures, each of the experiments are described with their objectives.

4.1 Experimental Methods

The methods for the bioreactor set-up, sampling, sample preparation, and nitrogen concentration analysis were developed prior to the beginning of experimentation. Sampling procedures varied from the early experiments to the later experiments to gain a more representative sample.

4.1.1 Bioreactor Design

Despite having different lengths of silicon tubing, the set-up for MABR A, B, and C was identical. The MABR was set-up three different times, and one activated sludge control was set-up.

Prior to set-up, the glass bioreactor and silicon tubing were autoclaved to fully sanitize each system and ensure Aqua was the only contribution to the microbial biomass. Growth media was prepared in liter batches that were also autoclaved to prevent contamination of the media prior to use. After all materials cooled to room temperature, the tubing was attached to the airline and randomly distributed throughout the MABR until approximately 7 feet of tubing was inside. MABR A used a thicker-walled, 4 foot tubing. Experiment’s done within the same MABR maintained the same tubing and biomass throughout.
With the silicon tubing inside, the MABR bioreactor can hold approximately 675 mL of growth media. Initially, 625 mL was poured into the MABR and 50 mL was held back to inoculate the Aqua in. Once the 1 g of Aqua was inoculated, this was poured back into the system. MABR A was allowed four days of growth prior to experimentation beginning. MABR B and C were allowed seven days of growth prior to experimentation.

The activated sludge control did not have silicon tubing inside of it. Instead, after the bioreactor cooled to room temperature, the fish bubbler was attached to the bottom inlet of the system with PVC tubing and allowed to bubble air up through the system. While this system could hold more growth media, the same volume of 675 mL was used for consistency.

4.1.2 Sampling and Storage Methods

Two sampling methods were used during the research. Initially, samples were taken from the bottom of the bioreactor using a tubed opening. The first 2 mL were wasted at each sampling event to ensure the sample was representative of the current time point and not the previous one. Each sample was at least 15 mL, but if possible, 20 mL was taken to conduct all the analyses with proper quality control measures. This method proved to be unrepresentative of the first 30 hours of each experiment due to the slow mixing within the MABR.

After noticing the inconsistencies with the previous sampling method, samples were pulled from the top of the bioreactor, where the nitrogen was introduced to the system. This was done by removing the tinfoil lid and using a 10 mL micropipette to remove 20 mL of sample from the bioreactor. The bulk water was gently stirred prior to extracting the sample. The lid was never removed longer than it took to take the sample itself. The pipette was inserted a few inches into the water to get a representative sample of the bulk water's nitrogen concentrations.
Samples were collected in 50 mL falcon tubes and stored in the freezer. Previous research found that the addition of sulfuric acid before storage caused degradation of nitrogen compounds, skewing results. For this reason, samples were not acidified prior to storage. Samples were stored in the freezer at -4 °C to maintain a temperature low enough to stop microbial activity until the samples could be tested.

Every time a sampling event occurred pH was taken from the bulk water with an Oakton Acorn series pH 5 meter. If pH was below 6.5, sodium bicarbonate was added to the system to stabilize pH to the ideal range of nitrification and denitrification which is 6.5 to 8.5. This mainly occurred during the first 30 hours of experimentation, when most of the TAN was oxidized. The mass of sodium bicarbonate added to the system was determined to be insignificant and not cause dilution.

4.1.3 Sample Preparation

Prior to analyzing the samples, each sample was filtered through a glass filter set-up. At least 15 mL of each sample was filtered. For samples with 20 mL, 5 mL was left unfiltered for the total nitrogen analysis. Samples were initially filtered through a 1.2 micrometer (µm) glass fiber filter (Millapore Sigma Catalogue Number: RAWP02500) to prevent clogging the 0.22 µm nitrocellulose filter (Millapore Sigma Catalogue Number: GSWP04700). The filter set-up was rinsed 3 times between samples to prevent contamination. The apparatus was also dried between samples to prevent dilution.

4.1.4 Quality Assurance and Quality Control

Quality assurance and quality control measures were taken during each experiment to ensure all water quality tests reported accurate and precise results. The main quality assurance techniques came from careful recording of all steps and observations during research. Each experiment was laid out before beginning, and each analysis was
prepared according to standard operating procedures recommended by the manufacturer.

Quality control techniques included blanks, standards, splits, and spikes. Method blanks were used to determine any losses or gains from the preparation of the samples. Method blanks showed little interference from the method preparation except for the Hach total nitrogen test which showed approximately 16% interference. Standards of ammonium, nitrite, and nitrate were prepped in bulk and serially diluted from the initial solution. The bulk solution was prepared to have a concentration of 1,000 mg-N/L. Standards were used to prove the accuracy of the analyses and to create calibration curves. Calibration curves ranged from 10 mg-N/L to 100 mg-N/L and needed to have an $R^2$ greater than 99.9% to be considered accurate. Standards were determined to pass if their percent error was less than 10%.

Splits were performed on random samples for each analysis. The sample was shaken and then split evenly between two test tubes. A split was considered passing if their relative percent difference was less than 10%. Matrix spikes were sporadically used during experimentation. This was done by adding a known volume of a known concentration of nitrogen to an unknown sample. Matrix spikes were typically performed on early samples or end of experiment samples for more accurate assumptions on the sample’s concentration. A matrix spike was considered passing if the percent recovery was between 85% and 110%. Due to the variable nature of this research, matrix spikes did not pass consistently.

Additional quality control measures involved control systems and replicate systems. An activated sludge model was set-up to provide results that could be modeled with existing design equations. Later experiments had two MABR set-ups running in parallel to see if results were consistent and repeatable between MABRs.
4.1.5 Ammonia Analysis

Samples were analyzed in a TL-2800 Auto Ammonia Sampler from Timberline Instruments to determine TAN concentrations (Timberline Instruments, 2017). Each sample was filtered, and 6.5 to 7 mL was put in a 15 mL falcon tube. Sulfuric acid was added dropwise until the pH of the sample was below 2. Decreasing the pH will cause all ammonia in the system to reduce into ammonium.

The TL-2800 operates under principles of gas diffusion. The sample is combined with a caustic solution until the pH is raised between 11 and 13. This converts the ammonium into ammonia gas. The sample with dissolved ammonia gas is sent through a membrane that allows the gas to permeate and rejects the liquid. That ammonia gas is absorbed into a boric acid solution with a pH of 6.5 and passed through a conductivity meter. The meter reads the change in conductivity which is proportional to the concentration of TAN (Timberline Instruments, 2017).

Before running the TL-2800, new DI water was provided for the autosampler’s cleaning cycle to prevent contamination from previous runs. Gain and post-attenuation factors were set to 10, and each sample was run for 2.5 minutes. A calibration curve was established in the TL-2800 Data Acquisition software by preparing and testing known concentrations of TAN. Typical ranges for the calibration curve were 0 mg-N/L to 100 mg-N/L, as those were the boundary conditions for most experiments. The TL-2800 Data Acquisition software would then relate the area of the unknown sample’s curve to the calibration curve prepared.

4.1.6 Nitrate and Nitrite Analysis

Nitrate and nitrite were analyzed using a Dionex ICS-1600 ion chromatograph (IC) from Thermo Scientific. The IC performs this analysis in 6 steps shown visually in Figure 4-1.
For a nitrate and nitrite analysis, the eluent was prepared using 956.5 mg of NaCO$_3$ in 1 L of DI water, or a 955 millimolar solution.

*Figure 4-1: Steps involved in the IC’s ion analysis (ThermoScientific, 2012)*

First the eluent carries the sample through the series. The sample is injected into the eluent stream and is pushed through the guard column. The guard column removes any contaminants that could potentially damage the separator column. The main principle within the separator column is ion exchange, where different ions will move and exit the column at different rates. The suppressor enhances the ion’s response allowing for detection from the conductivity cell. The Chromeleon Chromatography Management System identifies the ion based on retention time and quantifies the peak area (ThermoScientific, 2012).

Analysis for nitrate and nitrite can be done with the same 955 mmol eluent solution. Each sample takes 20 minutes to go through the six-step process. Samples were not
acidified prior to IC injection to prevent contamination from the strong hydrogen and sulfate ions. The results from the IC had 2 peaks, the first being nitrite, the second being nitrate. Calibration standards were entered into the Chromleon Chromatography Management System ranging from 10 mg-N/L to 100 mg-N/L to convert the peak areas into concentrations. Analysis of method blanks showed that the IC had a detection limit of 0.25 mg-N/L for nitrite and nitrate.

4.1.7 Total Nitrogen Analysis
A Hach total nitrogen kit was used to determine total nitrogen concentrations in multiple experiments. Ideally, these concentrations would be used to find organic nitrogen in the system by subtracting TAN, nitrite, and nitrate concentrations from the total nitrogen concentration. The Hach kit uses 0.5 mL of an unfiltered sample and multiple reagent packets to volatilize all nitrogen compounds into a homogenous mixture. Each Hach total nitrogen tube was then read in a DR-3800 Hach Spectrophotometer at a wavelength of 410 nanometers (nm). The Hach total nitrogen test has a 20% systemic error based on each step of the process and the accuracy of the DR-3800. Due to the large percent error in this analysis, concentrations from this test were used for a general visualization more so than concrete numbers.

4.1.8 Microbiology methods
When MABR C was set-up, a series of plates were run on the system to see if there were any significant changes in bacterial colonies. The plates were prepared by a biology undergraduate. Three plates received 100 microliters of the bulk water and were plated using the bead plating method where glass beads are used to create an even spread of bacteria throughout the agar plate. Three plates were diluted by a factor of ten to gain visibility and were plated using the same method.
4.2 Experiments

Twelve experiments were performed to determine proper C:N ratios, carbon sourcing, understand biofilm growth, and confirm Aqua’s ability to augment a biological treatment system. Table 4-1 gives a broad overview of each experiment, and further details are provided in the appropriate sections.

Table 4-1: Overview of key experiment parameters

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Carbon Source</th>
<th>C:N Ratio</th>
<th>Initial $\text{NH}_4^+\text{ Concentration (mg-N/L)}$</th>
<th>Initial $\text{NO}_3^-\text{ Concentration (mg-N/L)}$</th>
<th>Time Run (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>1.5:1</td>
<td>50</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>1.5:1</td>
<td>100</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>Glucose</td>
<td>1.5:1</td>
<td>50</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>Glucose</td>
<td>1.5:1</td>
<td>100</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0:1</td>
<td>100</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>0:1</td>
<td>100</td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>7</td>
<td>Glucose</td>
<td>3:1</td>
<td>100</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>8</td>
<td>Glucose</td>
<td>30:1</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Glucose</td>
<td>3:1</td>
<td>100</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>Glucose</td>
<td>3:1</td>
<td>100</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>11</td>
<td>Wastewater</td>
<td>1:1</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>Wastewater</td>
<td>2:1</td>
<td>100</td>
<td>50</td>
<td>75</td>
</tr>
</tbody>
</table>

Additional experiments included a one-hour screening experiment to estimate TAN assimilation and the agar plates made during Experiment 11.
4.2.1 Experiment 1 – Functionality

The purpose of Experiment 1 was to make sure the system was functioning properly. In particular, the airline and pressure levels. Experiment 1 was done in MABR A. This had 4 feet of thick-walled silicon membrane, operating at 15 psi.

Ammonium Chloride (NH₄Cl) was used as the TAN source. To get 50 mg-N/L of nitrogen in the system, 191 mg of NH₄Cl was mixed into 50 mL of growth media and introduced to the MABR’s bulk water along with 250 mg of glucose. The MABR was run like a batch reactor without a water flow in and out of the system.

Samples were taken twice a day for 48 hours. While pH was measured at every sampling event, DO was not measured as literature review proved that oxygen should be depleted in the bulk water. Samples were analyzed for TAN, nitrite, and nitrate.

4.2.2 Experiment 2 – Stabilization

After proving the system was functioning, it was important to promote bacterial growth that could perform nitrification and denitrification. This was done with a series of 24-hour experiments with a consistent C:N ratio. This should promote specific bacteria capable of performing the nitrogen cycle.

Experiment 2 was performed in MABR A. Before beginning, the system was drained and filled with a new batch of 650 mL of growth media. For a concentration of 100 mg-N/L, 254 mg of NH₄Cl was mixed into 50 mL of growth media and introduced to the MABR’s bulk water along with 250 mg of glucose. The MABR was set-up to run as a batch reactor, without water inflow or outflow. Over the 24 hours, five samples were taken, and pH was monitored. Pictures were taken at the beginning and end to visually observe biofilm growth. Samples were analyzed for TAN, nitrite, and nitrate after the experiment ended.
4.2.3 Experiment 3 – Stabilization Continued

Continuing the preliminary stabilization of the system, Experiment 3 was another 24-hour experiment. The system was drained and refilled with 650 mL of new growth media. This time, only 191 mg of NH₄Cl was added to the MABR due to residual TAN in the system from the previous experiment. Procedurally, this experiment was identical to Experiment 2.

4.2.4 Experiment 4 – Stabilization Continued

Experiment 4 was the final stage of the stabilization phase of the research. The system was drained, and 650 mL of new growth media was added. It lasted for 24 hours, with five samples taken throughout. Ammonia was depleted from the system prior to this experiment, so 254 mg of NH₄Cl was added with the growth media along with 250 mg of glucose. Procedurally, this experiment was identical to Experiment 2.

4.2.5 Experiment 5 – No Carbon Source

After using a 1.5:1 C:N ratio for the first four experiments, Experiment 5 observed the system’s behavior without an external carbon source. Without a carbon source, autotrophic bacteria would dominate the system and microbial death should be observable. The MABR was completely drained and 650 mL of new growth media was added. Ammonia was added to the system by mixing 254 mg of NH₄Cl with the growth media. No glucose was added. The experiment ran for 24 hours, with five samples taken throughout. Samples were tested for TAN, nitrite, and nitrate at the end of the experiment.

4.2.6 Experiment 6 – No Carbon Source

After observing the autotrophic bacteria’s activity in Experiment 5, Experiment 6 was performed with a 0:1 C:N ratio to further understand the nitrification process within the MABR. Both ammonia and nitrate were added to the system at 100 mg-N/L and 50 mg-
N/L respectively. To achieve these concentrations, 254 mg of NH₄Cl and 190 mg of Sodium Nitrate (NaNO₃) were mixed into the new 650 mL of growth media and added to the MABR. The experiment ran for 120 hours, with two to three samples taken daily in the morning and evening for representative samples.

Samples were tested for TAN after the experiment was completed. The suppressor on the IC broke during this experiment, so samples were not tested for nitrite and nitrate until 1.5 months after experimentation. Samples were filtered and frozen before the IC became non-functional, so results were considered representative of the experiment.

4.2.7 Experiment 7 – Low Glucose Concentration

Before Experiment 7 began, MABR B had to be set-up along with an activated sludge control system. MABR A was dismantled during an annual lab cleaning and the tubing used was displaced. The activated sludge control was used to see if Aqua was capable of nitrification and denitrification by providing modellable results.

Experiment 7 used a C:N ratio of 3:1 by adding 500 mg of glucose and 254 mg of NH₄Cl. The experiment ran for 120 hours with samples taken twice a day from each bioreactor. Wastewater was micro dosed (10 mL) at hour 55 to see if the system could adapt to the wastewater as a new carbon source. Samples were analyzed for TAN, nitrite, nitrate, and total nitrogen after the experiment was finished.

4.2.8 Experiment 8 – Large Glucose Concentration

Experiment 8 was performed in MABR B. This time, a 30:1 C:N ratio was done by adding 5,000 mg of glucose to the growth media. This was done to determine if the carbon dosing was the main limiting factor in the SNdN process. If the C:N ratio was too low at 3:1, then by using a 30:1, there should be vastly different results in removal rates and concentrations. This experiment used 100 mg-N/L as a starting concentration by
adding 254 mg NH₄Cl to the new 650 mL of growth media. The experiment ran for 100 hours, with a 10 mL dose of wastewater at hour 55 for consistency with previous experiments. Samples were taken twice a day for the duration of the experiment and analyzed for TAN, nitrite, and nitrate after the experiment ended.

4.2.9 Experiment 9 – Low Glucose Concentration Replicate

Experiment 9 was identical to Experiment 7, although it was run for 200 hours instead of 120 hours. MABR B was drained and refilled with 650 mL of growth media. The starting concentration of 100 mg-N/L was achieved by adding 254 mg of NH₄Cl to the system. Wastewater was added at hour 55 for consistency with previous experiments. While the MABR typically operates with aerobic and anoxic zones, an anaerobic zone formed during this experiment and produced sulfur byproducts. At hour 55, the bulk water became black and DO measurements showed 0 mg/L of DO. pH measurements showed elevated pH levels from 7.5 to 8.5. Samples were still taken twice a day despite this change, and were analyzed for TAN, nitrite, and nitrate at the end of the experiment.

4.2.10 Experiment 10 – Further Study on MABR B

After observing the results from MABR B in Experiment 9, Experiment 10 was run with similar parameters. A 3:1 C:N ratio was achieved by adding 500 mg of glucose to the new 650 mL growth media. For a concentration of 100 mg-N/L, 254 mg of NH₄Cl was mixed into the growth media. The experiment ran for 128 hours. The main difference between Experiments 9 and 10 is that no wastewater was added to MABR B during Experiment 10. In part, this was to see how the system would react under the new conditions, with just the Aqua’s bacterial cultures and glucose influencing nitrogen removal. Samples were taken twice a day and analyzed for TAN, nitrite, and nitrate.
4.2.11 Experiment 11 – Wastewater as the Carbon Source

After understanding how the MABR functions with glucose and micro doses of wastewater, the next experiments observed if Aqua could use the BOD$_5$ in wastewater as the sole carbon source. The wastewater available for this research came from the SLO WRRF’s primary clarifier. This primary effluent has a BOD$_5$ concentration of 370 mg/L and TAN concentration of 15 mg/L. Experiment 11 filled 1/3 of MABR B with wastewater and the other 2/3 with new growth media which is equivalent to 200 mL of wastewater and 400 mL of the new growth media. This achieved a 1:1 C:N ratio.

Before beginning Experiment 11, MABR C was set-up to run in parallel with the existing system. MABR C had minimal DO in the bulk water and had not produced black sulfates like MABR B. This was primarily to observe differences in nitrogen removal between the two systems operating with different conditions in the bulk water. Like MABR B, MABR C was set-up with 1/3 wastewater and 2/3 new growth media. Since MABR B was already observed with TAN removals, 50 mg-N/L was introduced as 191 mg of NaNO$_3$. MABR C had an additional 100 mg-N/L added through 254 mg of NH$_4$Cl. Samples were taken from each bioreactor twice a day for 100 hours and analyzed for TAN, nitrite, nitrate, and total nitrogen.

4.2.12 Agar Plate Series

After MABR C was set-up and allowed to grow for 7 days, 3 samples were taken and plated for observation of colony growth patterns. Samples were taken at time 0, 24, and 48 hours of Experiment 11. Each sample was plated at full strength and at a 10:1 dilution using a bead plating technique.

4.2.13 Experiment 12 – Increased Wastewater Ratio

Experiment 12 increased the amount of wastewater in the system to 2/3, which resulted in a 2:1 C:N ratio in both MABRs. MABR B was still producing sulfur byproducts and
MABR C was still turbid, but not black in color. The purpose of this experiment was to observe the effects of the increased wastewater, but also to see interactions with a mix of ammonia and nitrate influent. Each bioreactor was filled with 400 mL of wastewater, 250 mL of new growth media, 254 mg of NH₄Cl and 191 mg of NaNO₃. This experiment ran for 75 hours, with samples taken twice a day. Samples were analyzed for TAN, nitrite, nitrate, and total nitrogen.

4.2.14 Ammonia Assimilation Screening Experiment
A one-hour screening experiment was performed to understand the rates of ammonia assimilation in the biofilm formed by the Aqua. Literature review showed that Aqua is primarily comprised of Bacillus strain bacteria. The main removal pathway for Bacillus bacteria is assimilation. Two beakers were set-up for this experiment. One beaker received 1 mL of MABR B’s bulk water, the other received 10 mL. DI water was then added at 49 mL and 40 mL respectively. This solution was thoroughly mixed and 20 mg of NH₄Cl was added to each of them to achieve a concentration of 100 mg-N/L. Samples were taken every 30 minutes for 1 hour and tested for TAN.
Chapter 5 - Evaluation and Discussion

Each experiment in the batch MABR reactors provided insight into the conditions under which SNdN is possible, and if Aqua can enhance removal rates. These experiments helped narrow down the operational conditions of the system and provided clarification for future implementation. Figure 5-1(a) and 5-1(b) are provided as experiment schematics to provide context for each experiment’s results.

Figure 5-1(a): Schematic showing which MABR was used during each experiment
Figure 5-1(b): Experiment Schematic
5.1 Ammonia Assimilation Screening Experiment

During the twelve experiments, a discrepancy was observed between the concentration of nitrogen added to the system, and the sample concentration at hour 0, particularly for TAN. Often, the intended concentration for TAN would be 100 mg-N/L, but the hour 0 sample would show a concentration in the range from 20 mg-N/L to 50 mg-N/L.

The hour 0 sample was taken 5 minutes after introducing nitrogen to the MABR to allow some time for the added nitrogen to mix into the bulk water. To understand the discrepancy, a small screening experiment was performed. Two beakers were set-up with 50 mL each. One beaker contained 2% microbes from MABR B while the other beaker contained 20% microbes. TAN was introduced at 100 mg-N/L, and 3 samples were taken at 5 minutes, 30 minutes, and 60 minutes.

From this screening experiment, with TAN fully mixed throughout, the same discrepancy occurred, although it took the 20% mixture 30 minutes to reach the same concentrations as the 2% mixture (Figure 5-2). Both started at lower concentrations than intended, and plateaued at 14 mg-N/L. This indicated that the bacteria within the MABRs (primarily from Aqua on the biofilm and wastewater in the bulk water) were likely the cause of the lowered TAN concentrations.
Aqua is primarily comprised of heterotrophic *Bacillus* strain bacteria. Research on heterotrophic bacteria, particularly those in the *Bacillus* family was done to determine if Aqua was the cause of the missing nitrogen. From this, it was found that heterotrophic bacteria assimilate 70% of nitrogen compounds within the first 5 minutes of exposure (Meers & Pedersen, 1972). This indicated that if 100 mg-N/L of TAN was introduced to the system and only 25 mg-N/L shows up in the first sample, this is due to the rapid assimilation of TAN through heterotrophic respiration.

Conclusions from this finding indicate that Aqua’s main nitrogen removal pathway is assimilation. While an effective part of the nitrogen cycle, this is not typically the mechanism desired in wastewater treatment as it leads to a larger production of sludge that can cause operational issues later.
While this experiment showed a likely cause for the discrepancies in TAN concentrations, it does not explain the occasional discrepancies in initial nitrate concentrations. This may be due to adsorption capabilities of the microbes in the system. Bacillus strain bacteria and other microbes have a negatively charged surface and often behave like a negative particle. This negative surface area may be attracting the TAN particles (positively charged) leading to increased adsorption rates along with the potential assimilation. This could explain why nitrate does not seem to assimilate with the TAN, as it should per the nitrogen cycle.

Each of the twelve experiments show discrepancies in the initial concentrations despite passing QAQC measures, likely due to one or both of the aforementioned removal pathways. Further research needs to be done on assimilation as a removal mechanism before this discrepancy can be fully explained.

5.2 Experiment 1 – Functionality Results

The results of Experiment 1 indicated that the airline was diffusing oxygen through the system. TAN decreased within the first 15 hours (Figure 5-3). Despite the rapid decrease in TAN, little movement was seen in nitrite or nitrate indicating that nitrification was not occurring, but rather assimilation.
Despite not adding any nitrate to the system initially, the concentration of nitrogen from nitrate at hour 0 was approximately 98 mg-N/L. This discrepancy is attributed to the presence of nitrate in the growth media itself. The growth media contains approximately 63.7 mg-N/L from nitrate. However, this leaves 34.3 mg-N/L unaccounted for. The cause of this is unknown, but may be from within the Aqua itself, or from immediate nitrification.

5.3 Experiment 2 – Stabilization Results

The goal of Experiments 2 through 4 was to stabilize the biofilm and promote growth of nitrifying and denitrifying bacteria. Results from this initial phase showed that TAN declined rapidly in the first 5 hours before plateauing at 51 mg-N/L (Figure 5-4). The plateau in concentration is likely due to running out of carbon.
The decrease in TAN corresponded with the doubling in nitrate concentrations. Nitrification likely occurred causing this correlation. Nitrite remained steady the entire experiment. Nitrite conversion to nitrate is the fastest step in nitrification, so this was expected. Nitrate concentrations also plateaued 3.5 hours into the experiment. These plateaus likely indicate that the bacteria either ran out of carbon or oxygen or that the conditions within the system were not ideal for nitrification. Experiment 2 was conducted with a 1.5:1 C:N ratio, much lower than traditional systems, so the bacteria likely did not have enough carbon to continue the nitrification process.
During this experiment, MABR A grew more turbid (Figure 5-5), meaning that suspended growth was occurring alongside the attached growth. With the low C:N ratio, the biofilm was not growing consistently, diffusing more oxygen into the bulk water. This might be another cause of the plateaued nitrate concentrations as too much dissolved oxygen can limit denitrification.

![Figure 5-5: MABR A, showing turbidity and open spaces on the silicon membrane](image)

The open spaces on the membrane where biofilm did not grow allowed for bacteria to grow in the bulk water. This corresponded with a decreased pH from 7.1 to 4.6. This decline in pH and the movement of nitrogen from TAN to nitrate indicates that nitrification was occurring, but that denitrification was not achieved. At low pH, bacteria cannot complete the entire respiration cycle necessary for nitrification and other natural cycles and become slower moving. As such, the low pH is likely the cause for decreased removal rates.
5.4 Experiment 3 – Stabilization Continued Results

Experiment 3’s results (Figure 5-6) are relatively consistent with Experiment 2’s indicating that the MABR can produce replicable results. This experiment produced a more gradual removal in ammonia after the initial sharp decline. The nitrate concentrations initially increased, but then decreased very minimally over the course of 24 hours.

![Graph](image)

*Figure 5-6: Experiment 3’s results. 2\(^{nd}\) stage in stabilizing MABR A*

There was, again, minimal biofilm growth in MABR A and significant growth in the bulk water (Figure 5-7). The MABR became frothy in the top layer due to some gas bubbling out of the system. Since nitrate did not decline in a significant way, the bubbles are likely indicative of excess oxygen bubbling out of the water. Due to the thin biofilm formation during Experiments 2 and 3, oxygen was able to diffuse through the bulk water in excess, preventing proper conditions for denitrification.
Figure 5-7: Bubbles in MABR A during Experiment 3 indicating increased diffusion of oxygen because nitrate was not removed from the system.

Similar drops in pH were observed. Overgrowth of heterotrophic bacteria is likely the cause of the unstable pH. As heterotrophic bacteria grow, they can sour a system if they do not die quickly enough. This souring causes pH to fall and the acidic nature of the water will limit the natural cycles of other bacteria.

5.5 Experiment 4 – Stabilization Continued Results

The final step in the stabilization process showed similar values for TAN removal. The IC malfunctioned during this testing and was unable to read the samples for nitrite and nitrate.
The similarity in TAN removal to Experiment 2 and 3 implies that nitrate and nitrite are likely similar as well. Experiment 4 saw minimal growth of the biofilm but did have an increased turbidity.

5.6 Experiment 5 and 6 – No Carbon Source Results

Experiment 5 and 6 looked at how the MABR system behaved when there was no additional carbon added to the system. Results from these experiments were analyzed out of curiosity but did not add to the overall conclusions for the research. The results from Experiment 5 included rapidly declining TAN concentrations with nitrogen concentrations sharply increasing towards the end of the 24 hours. Experiment 6 saw no movement in either nitrate or nitrite but did have steadily increasing TAN concentrations.

Both experiments showed that there is a minimum carbon requirement for nitrification and bacteria growth. The bacteria in the MABR system need a minimum amount of carbon to support the natural reproduction cycle and without that, they instead begin to die off more rapidly.

MABR A did produce bubbles during Experiment 5 (Figure 5-8), but there is little indication that this was due to denitrification. Without a carbon source, the bacteria do not have the appropriate reduction/oxidation (redox) potential to perform nitrification or denitrification.
Additionally, without a carbon source, the biofilm from the previous experiments began to slough off. The bacteria in the attached growth began to die and without a proper carbon source to reproduce, the adhesion of the biofilm to the membrane began to fail. Overall, there was a net increase in nitrogen of 0.5 mg-N/L/hour during Experiment 6. However, little movement was seen in both nitrate and nitrite while TAN frequently increased and decreased. This indicates that without a carbon source, nitrification is likely not occurring, but instead nitrogen is converted between organic nitrogen and TAN to contribute to the bacteria’s natural reproduction cycle.
5.7 Experiment 7 – Low Glucose Concentration Results

Experiment 7 was run with both the activated sludge bioreactor and MABR B at a 3:1 C:N ratio. Despite using Aqua in both, different results were produced. At hour 55, 10 mL of wastewater was added to the system to promote increased removal of TAN.

In the activated sludge model, there was no movement in nitrite or nitrate (Figure 5-9). However, TAN decreased by 33 mg-N/L in the first 55 hours. The addition of wastewater promoted a rapid reduction in TAN, removing another 20 mg-N/L. After this initial reduction, TAN began to increase in the system and ended at hour 120 only 7 mg-N/L lower than the original concentrations. A potential source of this TAN could be decomposition of biomass. The extended aeration and low C:N ratio produced less biomass, but the total nitrogen results showed that there was an initial decrease in biomass concentrations that levels out. Since the total nitrogen results level out despite the increase in TAN, this may indicate decomposition of organic nitrogen. Since nitrogen did not leave the system, total nitrogen did not decrease.
In MABR B, there was a steady decrease in TAN from 6 hours to 120 hours (Figure 5-10). The initial increase in TAN is attributed to a concentration gradient between the bulk water and the biofilm membrane interface where residual TAN ions moved to the less concentrated bulk water. Despite the decrease in TAN from 117 mg-N/L to 41 mg-N/L, there was little movement in nitrite or nitrate showing that nitrification was likely still not occurring. The lack of movement in nitrite or nitrate corresponds to an increase in total nitrogen showing that TAN is likely being converted into biomass rather than being oxidized. In the last 20 hours of the experiment, nitrite and nitrate increased by approximately 10 mg-N/L. Since total nitrogen also began to decrease at this time, it likely indicates that nitrification was beginning to occur during these last hours.
Figure 5-10: MABR B results from Experiment 7

This experiment showed that a 3:1 C:N ratio is capable of providing the carbon necessary for TAN removal. This low carbon concentration means that the bacteria were operating in the stationary zone of growth much like a typical septic system which results in less biomass produced. Despite promoting a decreased biomass, TAN was still likely being assimilated rather than nitrified, but further studies would need to be conducted to confirm this.

Another potential explanation for the minimal movement in nitrate is that denitrification is occurring but cannot be visually captured. This may be because the growth media has an initial concentration of nitrate in it, so any additional nitrate production/removal is masked behind that baseline concentration. Future experimentation should remove that nitrate from the growth media to try and observe whether denitrification is occurring.
5.8 Experiment 8 – Large Glucose Concentration Results

After observing the MABR’s functionality under low carbon concentrations, a 30:1 C:N ratio was used to observe changes in nitrogen removal and biomass production. Wastewater was also added to this system at hour 55. TAN concentration decreased rapidly with 55 mg-N/L being removed in the first 55 hours at a steady rate (Figure 5-11). In previous experiments, TAN was removed similarly, but with intermittent plateaus and spikes in concentrations. While TAN was removed at a similar rate, the slope of removal decreased with the addition of wastewater. This is likely due to the BOD₅ in the wastewater having less redox potential than pure glucose.

![Figure 5-11: Experiment 8 results using a 30:1 C:N ratio](image)

Experiment 8 saw minimal movement in nitrite and nitrate concentrations, but a significant production of sludge at the bottom of MABR B. The high C:N ratio promoted rapid growth of bacteria as enough carbon was in the system to rapidly reproduce and replace any dead microbes. The increased sludge indicates heterotrophic bacteria
growth since they reproduce quickly and die quickly. If heterotrophic bacteria outcompeted other strains, it is possible that the bacteria capable of the fast removal rates seen in the first 55 hours died off, causing a slower removal in the later hours of this experiment.

There are a few potential explanations for the TAN removal and stable nitrate concentrations. The first is that TAN may be getting assimilated or adsorbed to the bacteria in the system. The second is that nitrification and denitrification may be occurring, but due to the baseline concentration of nitrate in the bulk water, it is not visibly captured. The third is that as pH drops due to nitrification, the system becomes stagnant in an acidic phase and cannot move forward with either nitrification or denitrification.

5.9 Experiment 9 – Low Glucose Concentration Replicate Results
Experiment 9 was a replicate of Experiment 7 to further study the movement in nitrite and nitrate that occurred in the last 20 hours. This time, a 3:1 C:N ratio was used, and the experiment ran for 191 hours. At hour 55, 10 mL of wastewater was added.

Experiment 9 produced results that had not been seen before (Figure 5-12). TAN concentrations jumped from 10 mg-N/L to 35 mg-N/L until plateauing at 10 mg-N/L from 55 hours to 191 hours. When TAN plateaued, nitrite grew exponentially from 0 mg-N/L to 83.6 mg-N/L indicating that TAN was being converted into nitrite. At hour 126, nitrite began to decline. Despite the movement in nitrite, nitrate stayed steady at 0 mg-N/L, meaning that the background nitrate concentration of approximately 63 mg-N/L was either denitrified or assimilated. From this data, one conclusion is that the bacteria in the system are capable of partial nitrification but cannot convert nitrite into nitrate.
Additionally, the TAN removal seen in the first 55 hours stopped from hour 55 to hour 191. This could indicate that the bacteria ran out of carbon and were unable to continue converting the remaining TAN to nitrite. Previous experiment showed that carbon in the system was consumed by hour 36, which could explain the decline in TAN concentrations around that time and the eventual plateau.

The main development of Experiment 9 is that at hour 55, MABR B began producing what were likely black sulfur byproducts and a DO reading showed the bulk water had 0 mg/L of dissolved oxygen (Figure 5-13). A slight odor was detected that aligned with the “rotten-egg” smell associated with sulfur. From this, it was determined that MABR B had likely developed an anaerobic zone alongside the aerobic biofilm membrane interface. Readings for nitrite and nitrate on the IC showed an ionic spike after the nitrate reading that had not been seen before. Literature on sulfur IC readings showed a similar
retention time to the ones seen in Experiment 9’s samples, indicating that there are likely sulfur compounds being produced in MABR B (Krol, 2000).

Alongside the sulfuric odors, MABR B began to release odors that smelled like acetone. This indicates that another mechanism may have been developing in MABR B. With the build-up of sludge at the bottom of the reactor, and the often low pH’s, MABR B may have begun operating as an aerobic digestor that was stalled in the acidogenesis phase. The acidogenesis phase produces acetates and volatile fatty acids that could give off the acetone odors (Tchobanoglaus & et.al, 2013). Both pathways could explain the new results seen in Experiment 9, but further investigation needs to be done before concluding the mechanism occurring within MABR B.
While it would be possible to set-up a new MABR to reset the system, this was the first time TAN was converted into nitrite successfully. These results were promising and MABR B continued to be used in the remaining experiments to further study the anaerobic zone’s nitrogen removal mechanics.

5.10 Experiment 10 – Further Study on MABR B Results

Experiment 10 was identical to Experiment 9 in that a 3:1 C:N ratio was used, and 100 mg-N/L as TAN was added initially. The main difference is that primary effluent wastewater was not added to MABR B. It is important to note that MABR B continued to produce “rotten-egg” and acetone odors during this experiment.

TAN steadily declined throughout the 128-hour experiment, removing a total of 12 mg-N/L (Figure 5-14). The minimal increases in TAN concentrations are likely attributed to the concentration gradient between the biofilm and the bulk water. Nitrite and nitrate concentrations were steady. Nitrite’s initial concentration was 26 mg-N/L, showing that the system continued removing nitrite after Experiment 9 ended with a nitrite concentration of 41 mg-N/L. Additionally, nitrate concentrations are 3 mg-N/L higher than the previous experiment, indicating that some nitrite was converted to nitrate during the transition process.
Since all parameters were kept identical between Experiments 9 and 10 except the addition of wastewater, the conclusion from Experiment 10 is that the primary effluent is adding bacteria capable of SNdN. In the transition between experiments, those bacteria were likely outcompeted by heterotrophic bacteria causing a stagnation in nitrite and nitrate concentrations, while TAN declined as expected. The bacteria capable of consuming TAN and nitrate have likely evolved in the wastewater. This conclusion indicates that while Aqua is capable of assimilating TAN, it may not be capable of performing complete nitrification and denitrification.

Figure 5-14: Experiment 10 results
During Experiment 10, MABR B became very black and light could no longer be seen through the bulk water (Figure 5-15). This resulted in an accumulation of black sludge at the bottom of the bioreactor set-up. When this sludge was removed, it was thick and shiny in nature indicating the precipitation of some compound. Further studies should be conducted on the sludge production of a MABR under anaerobic conditions to determine what this byproduct is.

Figure 5-15: Image of MABR B during Experiment 10

5.11 Experiment 11 – Wastewater as the Carbon Source Results

Experiment 11 was run with both MABR B and MABR C at a 1:1 C:N ratio with primary effluent wastewater. The primary effluent had an average BOD₅ concentration of 370 mg/L. MABR B received 50 mg-N/L of nitrate while MABR C received 100 mg-N/L of TAN. MABR B continued to produce sulfur byproducts and had a 0 mg/L DO
concentration. MABR C was newly set-up 7 days prior to experimentation and was not producing any sulfur byproducts.

MABR B produced results that indicated that nitrification occurred (Figure 5-16). Nitrite concentrations decreased by 119 mg-N/L corresponding with an 81 mg-N/L increase in nitrate concentrations. This shows that the primary removal pathway of nitrite within MABR B is the conversion to nitrate. This is confirmed by the low organic nitrogen concentrations that did not increase or decrease during this experiment. Additionally, TAN did not appear in the system which indicates the concentration gradient was minimized and little to no decomposition of organic biomass occurred.

![Figure 5-16](image)

*Figure 5-16: Results from MABR B during Experiment 11*

MABR C also indicated that nitrification was occurring (Figure 5-17), although it started with TAN as opposed to the nitrate in MABR B. TAN removal was complete with the initial concentration of 48 mg-N/L being reduced to below the detection limit. Additionally,
nitrite concentrations declined by 40 mg-N/L. Nitrate concentrations grew steadily, corresponding to the declining nitrite concentrations. In total, nitrate concentrations increased by 140 mg-N/L. This indicates that nitrification did occur in MABR C.

![Graph showing concentrations of different nitrogen compounds over time](image)

**Figure 5-17: MABR C results from Experiment 11**

To further understand the nuances between the long standing MABR B and the newer MABR C, each nitrogen compound was taken and compared side by side. TAN concentrations for MABR C show the gradual decline with a sharp decrease in the last 4 hours (Figure 5-18). MABR B did not have TAN introduced, and as such stayed relatively close to the detection limit for the entire experiment. This shows that TAN will not be produced in a system with low carbon concentrations that has adapted to the conditions of the wastewater being treated.
In terms of nitrite, the two MABRs performed similarly (Figure 5-19). In MABR B, nitrite was removed at a rate of 1.19 mg-N/L/hour while MABR C saw nitrite removal rates of 0.393 mg-N/L/hour. This indicates that the longer a MABR is operating, the faster nitrogen is removed from the system. This is a common phenomenon where the bacteria within a system gradually adapt to the conditions. This evolution allows the bacteria capable of converting nitrite to nitrate to grow more readily, resulting in more efficient treatment.
Both MABRs saw an increase in nitrate concentrations (Figure 5-20). MABR B had a more consistent, steady increase in nitrate concentrations of approximately 0.808 mg-N/L/hour. MABR C had a 1.39 mg-N/L/hour increase in nitrate. While MABR C had a much faster rate than MABR B, it was a less stable increase and indicates a stop-and-go transition of nitrite to nitrate.
Figure 5-20: Nitrate comparison between MABR B and MABR C

During Experiment 11, MABR B and MABR C were set up next to each other with a split airline (Figure 5-21). This visualized the difference between MABR B which continued to produce odors and black by-products and MABR C which is newer and had not yet undergone this change.
5.12 Plating Results

Results from the plating experiments were inconclusive. MABR C’s bulk water was extremely concentrated in microbes and even diluting the solutions by a factor of 100 did not produce significant results. From the plates, it was seen that a white colony is forming, but with the background interference, there is no way to conclude what microbes are forming in this colony.

5.13 Experiment 12 – Increased Wastewater Ratio Results

Experiment 12 added 100 mg-N/L as TAN and 50 mg-N/L as nitrate to both MABR B and MABR C with a 2:1 C:N ratio from the wastewater. The primary effluent had an average BOD$_5$ concentration of 370 mg/L. MABR B continued to produce black byproducts and odors while MABR C was still the typical brownish color seen in earlier experiments.
In MABR B, TAN concentration initially declined, but after a slight increase in the middle of the experiment, the final concentration of 22 mg-N/L was identical to the initial concentrations (Figure 5-22). This is likely the concentration gradient causing TAN to increase in the bulk water. Due to the MABR being operated as a batch reactor, the lack of mixing causes pockets of higher TAN concentrations, which can lead to concentration gradients forming. Nitrite increased by 38 mg-N/L likely from the initial TAN conversions. Additionally, nitrate was completely removed from the system in the first 6 hours, indicating that either denitrification or assimilation occurred. However, minimal sludge was produced in MABR B during Experiment 12 and small bubbles left the system indicating that denitrification was occurring with some residual nitrite produced.

![Figure 5-22: MABR B results from Experiment 12](image)

MABR C experienced the opposite results of MABR B. TAN gradually grew throughout the experiment, resulting in a net gain of 15 mg-N/L (Figure 5-23). This is likely attributed to TAN concentrations building up in the biofilm. MABR B experienced similar problems
when it was a newer reactor, indicating that MABR C is likely undergoing the same developments. Nitrite decreased consistently throughout Experiment 12 and reached the detection limit at hour 70. This correlated with an increase in nitrate. This indicated nitrite conversion into nitrate, meaning partial nitrification was occurring. Nitrite was lost at a rate of 0.494 mg-N/L/hour while nitrate increased at a rate of 0.266 mg-N/L/hour, showing some delay in the conversion process. Nitrate increased by 22 mg-N/L which does correlate with the decrease in nitrite, making this transition stoichiometrically sound.

![Figure 5-23: MABR C results from Experiment 12](image_url)

Both MABR B and MABR C experienced some growth in TAN during Experiment 12 (Figure 5-24). The concentration gradient found in both MABRs indicates that there is an uneven distribution of TAN in the influent wastewater causing there to be less concentrated zones. In the small, laboratory-scale bioreactor, this concentration gradient
results in diffusion that is noticeable and impactful. This incomplete mixing is inhibiting the reduction of TAN, which can cause issues for effluent total nitrogen concentrations.

![Comparison of TAN concentrations in MABR B and MABR C](image)

*Figure 5-24: Comparison of TAN concentrations in MABR B and MABR C*

Nitrite behaved differently in MABR B and MABR C (Figure 5-25). In MABR B, nitrite increased significantly throughout the 74 hours while MABR C saw complete removal over the same time period. Interestingly, MABR B gained nitrite at a rate of 0.511 mg-N/L/hour while MABR C lost nitrite at a rate of 0.494 mg-N/L/hour. This indicates that the nitrite is coming from nitrate in MABR B. Nitrite is converting into nitrate in MABR C. This shows that the conversion rate of nitrite to nitrate for the bacteria inside these MABR's is consistent at approximately 0.5 mg-N/L/hour.
Nitrate also behaved differently between MABR B and MABR C (Figure 5-26). MABR B removed all the nitrate within the first 6 hours and maintained a concentration below the detection limit (0.25 mg-N/L) for the rest of the experiment. MABR C experienced a 22 mg-N/L increase in nitrate. The only difference between the two MABRs is the black byproducts in MABR B indicating that the removal mechanisms are different between the two MABRs.
Multiple factors limit nitrogen removal in a MABR including pH and temperature, DO concentrations, C:N ratios, and general growth kinetics and behavior of the attached and suspended growth. Additional complications were experienced during this research with experimental methods such as the IC and the Hach total nitrogen kits.

5.14.1 pH and Temperature Impacts

Limiting factors such as pH and temperature primarily affect the behavior of bacteria in the system. In general, nitrification and denitrification can occur in a pH range from 6.5 to 8.0 and temperatures from 17 °C to 25 °C (Environmental Protection Agency, 2009). While this is a good guideline to follow, each biological system will form different colonies that behave ideally under certain pH and temperature ranges.
The MABR was set-up with the goal of maintaining a pH between 6.5 and 8.0 and each experiment had a different pH during it. When a new MABR was set-up, there were initial issues with pH dropping too rapidly (from 7.0 to 4.3) and that was alleviated by the addition of sodium bicarbonate to prevent souring the system. Through the twelve experiments and the total of 3 different MABRs, a correlation between nitrogen removal rates and pH levels was seen. In general, the MABRs were unable to effectively remove nitrogen (often gaining more nitrogen) at the lower and upper extremes of the typical pH ranges. Table 5-1 compares the average pH of each experiment to the nitrogen removal rates. Positive rates indicate nitrogen gained, while negative rates indicated nitrogen removed.
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<th>Nitrite Removal (mg-N/L/hr)</th>
<th>Nitrate Removal (mg-N/L/hr)</th>
<th>Net Nitrogen Removal (mg-N/L/hr)</th>
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<td>-1.62</td>
<td>-7.61</td>
<td>0.02</td>
</tr>
<tr>
<td>MABR A</td>
<td>6.51</td>
<td>-1.62</td>
<td>-7.61</td>
<td>0.02</td>
<td>-9.21</td>
</tr>
<tr>
<td>Experiment 6</td>
<td></td>
<td></td>
<td>0.0022</td>
<td>0.0022</td>
<td>-0.015</td>
</tr>
<tr>
<td>MABR A</td>
<td>7.1</td>
<td>0.0022</td>
<td>0.0022</td>
<td>-0.015</td>
<td>-0.011</td>
</tr>
<tr>
<td>Experiment 7</td>
<td></td>
<td></td>
<td>-0.17</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>MABR B</td>
<td>7.2</td>
<td>-0.17</td>
<td>0.07</td>
<td>0.04</td>
<td>-0.06</td>
</tr>
<tr>
<td>Experiment 8</td>
<td></td>
<td></td>
<td>-0.45</td>
<td>-0.0013</td>
<td>0</td>
</tr>
<tr>
<td>MABR B</td>
<td>7.3</td>
<td>-0.45</td>
<td>-0.0013</td>
<td>0</td>
<td>-0.45</td>
</tr>
<tr>
<td>Experiment 9</td>
<td></td>
<td></td>
<td>-0.081</td>
<td>0.14</td>
<td>0</td>
</tr>
<tr>
<td>MABR B</td>
<td>6.9</td>
<td>-0.081</td>
<td>0.14</td>
<td>0</td>
<td>0.059</td>
</tr>
<tr>
<td>Experiment 10</td>
<td></td>
<td></td>
<td>-0.061</td>
<td>0</td>
<td>-0.061</td>
</tr>
<tr>
<td>MABR B</td>
<td>7.3</td>
<td>-0.061</td>
<td>0</td>
<td>0</td>
<td>-0.061</td>
</tr>
<tr>
<td>Experiment 11</td>
<td></td>
<td></td>
<td>0</td>
<td>-0.766</td>
<td>0.53</td>
</tr>
<tr>
<td>MABR B</td>
<td>7.4</td>
<td>0</td>
<td>-0.766</td>
<td>0.53</td>
<td>-0.239</td>
</tr>
<tr>
<td>MABR C</td>
<td>6.7</td>
<td>-0.29</td>
<td>-0.26</td>
<td>0.9</td>
<td>0.35</td>
</tr>
<tr>
<td>Experiment 12</td>
<td></td>
<td></td>
<td>0.0055</td>
<td>0.33</td>
<td>-1.15</td>
</tr>
<tr>
<td>MABR B</td>
<td>7.2</td>
<td>0.0055</td>
<td>0.33</td>
<td>-1.15</td>
<td>-0.82</td>
</tr>
<tr>
<td>MABR C</td>
<td>7.55</td>
<td>0.14</td>
<td>-0.32</td>
<td>0.17</td>
<td>0</td>
</tr>
</tbody>
</table>

In general, the MABR removed nitrogen the most consistently at a pH range of 7.0 to 7.4. While Experiment 3 and 5 showed the fastest removal rates, this is likely due to the
rapid growth of bacteria leading to increased assimilation rates. Additionally, the MABRs had unstable pH levels during initial startup, but once experimentation continued, all three MABRs evened out at a pH between 7.0 and 7.4 indicating this is the ideal operational range for a MABR system. This shows that future applications of this system will not require alkalinity additions after the initial startup phase.

In terms of temperature, the MABR seemed to operate within the typical range from 17 °C to 25 °C. Temperatures in the lab never rose above 20 °C, but there was one instance where temperature fell to 15 °C, which did result in microbial death. Future applications of this technology should require the MABR to be set in the ground to prevent freezing temperatures from interfering with nitrogen removal.

5.14.2 Dissolved Oxygen

Another concern with the MABR is whether oxygen will properly diffuse through the membrane. Nitrification requires oxygen to occur, and without enough DO, TAN oxidation will be inhibited. Additionally, to achieve a proper anoxic and or anaerobic zone in the bulk water, there cannot be excess dissolved oxygen. Ensuring the oxygen diffusing through the membrane is enough for nitrification, but not too much to inhibit denitrification is a careful balance.

To properly measure the DO inside the biofilm, a microDO probe is required. However, a microDO probe was not available during this research. Theoretical design calculations were done to understand the mass transfer of oxygen required for nitrification and the theoretical mass transfer of oxygen occurring from the membrane.

First, Equation 4 was used to determine the mass of oxygen diffusing through the membrane per unit of area (Tchobanoglaus & et.al, 2013). Permeability was assumed to
be $6,579 \times 10^{-11} \frac{\text{cm}^2}{\text{s cmHg}}$ from the literature review. Area of the silicone membrane was 210 cm$^2$ using MABR B as a reference point.

$$\text{Volume} = \frac{\text{Permeability} \times A \times t \times (P_2 - P_1)}{\text{thickness}} \quad \text{................................................................. (4)}$$

This showed that the membrane could produce a volume of 9,525 cm$^3$ over 5 days. Converting this to a mass using density showed that the membrane was capable of diffusing 1,429 grams of O$_2$ per m$^3$. Using Equation 5, the rate of diffusion was found (Tchobanoglaus & et.al, 2013).

$$\text{Rate of Diffusion} = \frac{\text{Mass of gas per area}}{\text{Time}} \quad \text{................................................................. (5)}$$

Equation 5 showed the silicone membrane can diffuse 152 grams of O$_2$ per day. To fully understand if this was enough for nitrification, theoretical design equations for oxygen requirements in a biological treatment system were used to determine the bacteria’s requirement for nitrification (Tchobanoglaus & et.al, 2013). Equation 6 and Equation 7 were used with Experiment 12’s data.

$$R_o = Q^* (S_o - S) - 1.42P_{xbio} + 4.57Q(NO_x) \quad \text{................................................................. (6)}$$

Where: $R_o =$ oxygen requirement for nitrification

$Q =$ flowrate

$S_o =$ initial carbon concentration

$S =$ final carbon concentration

$P_{xbio} =$ active biomass concentration

$NO_x =$ concentration of TAN converted into nitrate
\[
P_{X_{\text{bio}}}=\frac{QY(S_o-S)}{1+(k_d)SRT} + \frac{(f_d)(k_d)QY(S_o-S)SRT}{1+(k_d)SRT} + \frac{QY_n(NO_2)}{1+(k_{dn})SRT} \tag{7}
\]

Where: \( Y \) = yield of heterotrophic bacteria

- \( K_d \) = decay rate of heterotrophic bacteria
- \( SRT \) = solids retention time
- \( F_d \) = fraction of biomass that is non-volatile
- \( Y_n \) = yield of nitrifying bacteria
- \( K_{dn} \) = decay rate of nitrifying bacteria

Flow rate (or volume displaced from the batch reactor per day) was estimated as 325 L/day, initial carbon concentration was 300 mg/L, final substrate concentration was 0.7 mg/L, and concentration of TAN converted into nitrate was 34.4 mg/L after accounting for the portion of TAN assimilated into biomass. Using these values in Equations 6 and 7 showed that the biofilm requires 150 grams of \( O_2 \) per day.

Theoretically, the silicone membrane is diffusing just enough oxygen for the nitrification process to occur, with approximately 2 grams of \( O_2 \) in excess. This is supported by the consistent removal of TAN within the MABR systems. However, theoretical design equations for biological treatment are often imprecise due to the variable nature of bacteria and environmental factors. To completely confirm proper DO diffusion through the MABR, a microDO probe will need to be purchased and an oxygen profile will need to be created.

5.14.3 Carbon Source and Concentration

Another limiting factor for biological treatment is the C:N ratio and where the carbon is coming from. This is due to nitrification and denitrification being driven by redox
potentials. Using a purer form of carbon, such as glucose, has a higher redox potential than typical BOD$_5$ found in wastewater. Experiments 11 and 12 looked at using wastewater as the carbon source (BOD$_5$ concentration of approximately 370 mg/L) to determine if the bacteria in the MABRs could utilize BOD$_5$ as the main carbon source. Experiments 8 and 9 also used wastewater, although not as a carbon source, but as a way to introduce wastewater bacteria to the system. Table 5-2 shows the average nitrogen removal rates for each C:N ratio and carbon source.

*Table 5-2: Carbon to Nitrogen Ratio Impacts on Nitrogen Removal Rates*

<table>
<thead>
<tr>
<th>C:N Ratio</th>
<th>Carbon Source</th>
<th>TAN Removal (mg-N/L/hr)</th>
<th>Nitrite Removal (mg-N/L/hr)</th>
<th>Nitrate Removal (mg-N/L/hr)</th>
<th>Net Removal (mg-N/L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>Wastewater</td>
<td>-1.96</td>
<td>-0.790</td>
<td>1.10</td>
<td>0.114</td>
</tr>
<tr>
<td>1:1.5</td>
<td>Glucose</td>
<td>-9.96</td>
<td>1.04</td>
<td>39.1</td>
<td>30.2</td>
</tr>
<tr>
<td>2:1</td>
<td>Wastewater</td>
<td>0.109</td>
<td>0.008</td>
<td>-10.8</td>
<td>-10.7</td>
</tr>
<tr>
<td>3:1</td>
<td>Glucose</td>
<td>-0.400</td>
<td>0.056</td>
<td>0.037</td>
<td>-0.306</td>
</tr>
<tr>
<td>3:1</td>
<td>Wastewater</td>
<td>-0.329</td>
<td>1.052</td>
<td>0</td>
<td>0.724</td>
</tr>
<tr>
<td>30:1</td>
<td>Glucose</td>
<td>-1.02</td>
<td>0</td>
<td>0.002</td>
<td>-1.02</td>
</tr>
</tbody>
</table>

The rates shown in Table 5-2 indicate that the best removal of nitrogen occurred at a 30:1 C:N ratio with glucose and then at a 2:1 C:N ratio with wastewater. Overall, the wastewater removal rates are more promising because they consistently involve the removal of TAN with minimal increases in nitrite. The 3:1 C:N ratio with wastewater experienced an overall growth in nitrogen, but this was the first instance of TAN conversion to nitrite and is indicative of a healthy system, just one that is undergoing a change.
Ultimately, the MABR performed more ideally in experiments that had wastewater as either a micro dose or as the main source of carbon showing that the wastewater was introducing other constituents or bacteria that allowed nitrification and denitrification to occur more readily. Experiment 9 had the first instance where TAN readily transferred into nitrite. Experiment 11 and 12 both showed the fastest nitrogen removal rates for MABR B at a low C:N ratio. While MABR C performed differently under the same conditions, this is attributed to the newer bacteria in this system adjusting to the operational conditions.

The increased nitrogen removal with wastewater was likely due to the more diverse bacterial cultures formed. When a pure form of carbon like glucose is used, heterotrophic bacteria can overgrow and sour a system leading to nitrifying bacteria being outcompeted. This is due to heterotrophic bacteria being quick growing and utilizing the carbon to reproduce. When wastewater is used, with a lower redox potential, heterotrophic bacteria are less likely to overgrow due to the ability of other bacteria to process the BOD$_5$ in a more productive way. The wastewater promoted a more diverse biofilm culture and suspended growth, leading to increased nitrogen removal.

In conventional wastewater treatment, C:N ratios are typically between 10:1 to 20:1. This is typical in activated sludge basins where heterotrophic bacterial growth is promoted to increase BOD$_5$ oxidation. However, in the MABR, the closed environment makes it more difficult to remove sludge, and overgrowth of bacteria could damage the integrity of the biofilm, making a lower C:N ratio more desirable. Low C:N ratios would promote growth in the stationary phase where the growth rate of bacteria will equal the death rate. This creates a more stable biofilm.

Most of the experiments used a C:N ratio from 1:1 to 3:1, except for Experiment 8 that used a 30:1 ratio. While Experiment 8 did have the fastest removal rates, it primarily
removed TAN through assimilation which is not the ideal removal pathway. Additionally, significant sloughing of the biofilm was observed. When glucose was used as a carbon source, a 3:1 ratio was the most consistent in removing nitrogen without causing overgrowth of the biofilm. Minimum sludge production was seen and the MABR had a more stable pH. When wastewater was used as the carbon source at a 1:1 and 2:1 ratio, nitrogen removal occurred in MABR B. This indicates that when wastewater is the main carbon source, the C:N ratio can be low in well-established MABR systems. These experiments were conducted with high-strength wastewater (nitrogen concentrations of approximately 100 mg-N/L), but this will likely hold true for medium-strength wastewater as well.

5.14.4 Biofilm and Suspended Growth

The type of bacteria growing in the biofilm and bulk water is vital to the treatment process. While the microbiology experiments proved inconclusive, theoretical growth and removal equations were used to estimate heterotrophic and nitrifying bacterial growth in MABR B. Aqua is primarily composed of heterotrophic bacteria which is why this growth was also modeled.

Using Experiment 7’s nitrogen data, the time for carbon depletion and nitrogen removal was estimated using batch reactor design equations (Equation 8 and 9). Table 5-3 shows the conventional growth rate kinetics used for calculations.

\[ K_s \ln \left( \frac{S_0}{S} \right) + (S_0 - S_t) = X \left( \frac{\mu_m}{\mu} \right) t \]  

Where: \( K_s \) = half saturation constant

\[ X = \text{concentration of biomass} \]

\[ \mu_m = \text{maximum growth rate} \]
t = time of consumption

$$K_N \ln \left( \frac{N_0}{N_t} \right) + (N_0 - N_t) = X_N \left( \frac{\mu_{nm}}{Y_N} \right) t \quad .................................................. (9)$$

Where: $K_N =$ half saturation constant

$N_0 =$ initial nitrogen concentration

$N_t =$ nitrogen concentration at time $t$

$X_N =$ concentration of nitrifying biomass

$\mu_{nm} =$ maximum growth rate of nitrifiers

$Y_N =$ yield of nitrifiers

**Table 5-3: Growth Rate Kinetics Used in Calculations (Tchobanoglaus & et.al, 2013)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heterotrophic Bacteria</th>
<th>Units</th>
<th>Nitrifying Bacteria</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$</td>
<td>8</td>
<td>gbCOD/m³</td>
<td>0.5</td>
<td>gNH₄-N/m³</td>
</tr>
<tr>
<td>$\mu$</td>
<td>6</td>
<td>gVSS/gVSS*day</td>
<td>0.75</td>
<td>gVSS/gVSS*day</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.45</td>
<td>gVSS/gbCOD</td>
<td>0.15</td>
<td>gVSS/gNH₄-N</td>
</tr>
</tbody>
</table>

These calculations indicated that the carbon was fully removed from MABR B in Experiment 7 at hour 8.6, while the nitrogen removal seen should have occurred in 2.84 hours. In actuality, the nitrogen removed during Experiment 7 occurred over 120 hours. While theoretical equations have some inaccuracy within them, the actual removal rate should be closer to the calculated 2.84 hours. Since the discrepancy between the design equation and the actual removal rate is so large, this indicates the bacteria promoted in Aqua are not capable of nitrification.
Next, the biomass growth rate for different C:N ratios was found for MABR B to see whether operating at a low C:N ratio was inhibiting growth significantly or not. Equation 10 models heterotrophic growth and Equation 11 models nitrifier growth (Tchobanoglaus & et.al, 2013).

\[
\mu = e^{(T-15)}Y \left( \frac{S}{K_s+S} \right) - k_d \quad \text{.............................................. (10)}
\]

\[
\mu_N = e^{(T-15)}Y \left( \frac{N}{K_{sN}+N} \right) - k_{dN} \quad \text{.............................................. (11)}
\]

Experiment 8, 9, 11, and 12 were all modeled using Equations 10 and 11 to see growth rate kinetics at a 30:1, 3:1, 1:1, and 2:1 C:N ratio. Table 5-4 shows the results of these calculations.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>C:N Ratio</th>
<th>Growth Rate (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>30:1</td>
<td>1.89</td>
</tr>
<tr>
<td>9</td>
<td>3:1</td>
<td>1.8</td>
</tr>
<tr>
<td>11</td>
<td>1:1</td>
<td>1.75</td>
</tr>
<tr>
<td>12</td>
<td>2:1</td>
<td>1.82</td>
</tr>
</tbody>
</table>

These calculations show that a C:N ratio of 1:1 result in a much lower growth rate than other C:N ratios. However, a C:N ratio of 30:1 is not significantly higher than a C:N ratio of 2:1, indicating that MABRs and the biofilm’s growth are not inhibited at low C:N ratios. This means the MABR can treat high strength wastewater, or medium strength wastewater, both with relatively low sludge production.

However, growth rate kinetics of nitrifying bacteria were calculated to be 0.431 days⁻¹. This is less than half the growth rate of heterotrophic bacteria. While the MABRs were operated with a 7 to 10 day solids retention time, long enough for nitrifier growth, Aqua is
primarily heterotrophic bacteria. These heterotrophic bacteria were promoted within the system, allowing them to outcompete the nitrifiers. This explains the inconsistent results seen in the MABR in the first experiments after set-up. Likely, the newer the MABR was, the more unstable the biofilm was due to the fast-dying heterotrophic bacteria. After the first few experiments, the biofilm stabilized with the slower growing nitrifying bacteria, allowing for more consistent nitrogen removal.

5.14.5 Experimental Methods

During experimentation, the IC’s suppressor broke multiple times. The first instance occurred prior to testing Experiment 6’s data. Luckily, those samples were already filtered and frozen, allowing for minimum decay in nitrate and nitrite. The suppressor was replaced, and analysis continued like normal. However, after testing Experiment 7, the suppressor began to leak, indicating the membranes were broken and the eluent was flooding the suppressor. The suppressor was once again replaced, and the IC functioned ideally for the rest of the research. After each suppressor was replaced, a trial sample set was run that included blanks and standards to ensure the IC was reading concentrations properly and new calibration curves were created.

Additional issues include the systemic errors seen in the Hach total nitrogen test. The Hach test itself has an approximately 16% error due to the multiple reagent packets and inconsistent heating of samples. The spectrophotometer used to analyze the absorbency added an additional 4% error. This meant that the total nitrogen data often did not pass quality assurance and quality control procedures, including those for blanks and standards. Total nitrogen concentrations were included for visualization of data, but the actual total nitrogen concentration is unknown, and therefore no conclusions can be drawn about the mass of nitrogen lost to the atmosphere.
Chapter 6 – Conclusion

The objectives of this research included observing the effects of carbon source and concentration on nitrogen removal rates, understanding attached and suspended growth rates and behaviors, using theoretical equations to see if the MABR is behaving in an expected way, and confirming Aqua’s ability to augment a biological treatment system.

The research done answered some questions, created some questions, and left some questions unanswered. This chapter will detail the conclusions drawn, potential improvements to the design and experimental methods, and future work recommendations.

6.1 Final Conclusions

C:N ratio and carbon source were concluded to have large impacts on the operational capabilities of a MABR. The two carbon sources compared were glucose and wastewater with C:N ratios ranging from 0:1 to 30:1. From the data analysis, low C:N ratios of around 3:1 for glucose and 2:1 for wastewater resulted in consistent nitrogen removal of approximately 0.306 mg-N/L/hour and 10.5 mg-N/L/hour respectively. This indicates that the MABR removes nitrogen at a faster rate with wastewater than glucose. This aligns with theory because glucose has a higher redox potential than wastewater which would result in heterotrophic bacteria overgrowth. Heterotrophic bacteria can remove nitrogen, but at much slower rates than bacteria that are genetically designed to.

Additionally, while the 30:1 C:N ratio (using glucose as the carbon source) removed TAN at a faster rate than other experiments, it averaged at a 1.02 mg-N/L/hour removal rate. This is approximately ten times less than that of the 2:1 wastewater system. From this, it was concluded that the MABR can handle lower C:N ratios effectively. This means that MABRs are capable of treating high strength or low strength wastewater alongside
traditional medium strength, giving this system versatility not often seen in other biological treatment systems.

Another conclusion from the C:N ratio experiments was that the biofilm was much more stable at lower C:N ratios. Experiment 8 (30:1 C:N ratio) was the only time the biofilm sloughed off, and a large accumulation of sludge occurred at the bottom of the bioreactor. Since the MABR can remove nitrogen at low C:N ratios, it is operationally ideal to keep that ratio low in future operations. This prevents the build-up of sludge which can be complicated to remove in a membrane system like a MABR.

Traditionally, carbon concentration has a relatively significant impact on the growth rate of bacteria. However, theoretical calculations showed that there was not a significant difference in growth rate between a 3:1 and a 30:1 C:N ratio. This further promotes the conclusion that a MABR is better suited to lower C:N ratios as this will be easier to operate and opens the door to more applications than just traditional domestic wastewater treatment.

During initial experimentation with a new MABR, pH would rapidly decline without the production of nitrite/nitrate. This indicates that the pH drop was not due to nitrification, but rather overproduction of heterotrophic bacteria. After approximately 3 experiments (estimated 3 to 4 weeks), the MABR stabilized and began to perform nitrification and denitrification like intended. While pH was used as an indicator of performance, it was not concluded to have a significant impact on the MABR as long as it was monitored effectively. Similarly, this shows that the MABR requires a longer start-up phase than conventional activated sludge systems. The MABR operated best when allowed a week-long inoculation period and did not treat nitrogen efficiently until an average of 3.5 weeks of operation. The start-up period should be further assessed prior to implementation.
The last aspect of this research focuses in on the bacteria introduced to the system. Aqua is primarily formed of heterotrophic bacteria, while wastewater will promote a large, biologically diverse environment. Experiments 1 through 8 were done using pure Aqua cultures (no wastewater introduced to the system). These experiments experienced variable removal rates, a lack of nitrification and denitrification, and averaged a removal rate of 1.30 mg-N/L/hour.

Mixed cultures of Aqua and wastewater (Experiments 9 through 10 where only small volumes of wastewater were introduced) resulted in the production of sulfur, indicating that some facultative anaerobes were introduced to the system. While this was not seen in previous research endeavors, this resulted in the first ideal conversion of TAN into nitrite, and later into nitrate indicating that the new bacteria were able to process nitrogen in a way that Aqua could not. These experiments resulted in nitrogen removal rates averaging 10.5 mg-N/L/hour. Full wastewater systems (where the main source is wastewater, with contributions from Aqua in the biofilm) had the most success in converting nitrogen to different forms and ultimately removing nitrogen at the fastest rates. While Experiments 11 and 12 still averaged around 10.5 mg-N/L/hour removed, these experiments saw the fastest removal rate, at a low C:N ratio, of 21.3 mg-N/L/hour.

Additionally, using theoretical batch reactor equations and activated sludge equations, the nitrogen removal seen in various experiments was expected to occur within 3 to 10 hours. However, in systems where Aqua was the sole contributor to the biomass, the time of removal was 120 hours. This indicates that Aqua is not capable of removing nitrogen at the rate necessary for traditional wastewater treatment systems. However, when wastewater was introduced to the system, the nitrogen removal rates and the time to achieve low concentrations did align more closely with theoretical equations. From
this, it was concluded that Aqua does not increase nitrogen removal rates significantly past that of typical wastewater bacteria.

6.2 Proposed Improvements to Design

The main problems experienced with the small-scale MABR design included sludge removal, incomplete mixing, and airline control. Additionally, the background concentration of nitrate in the growth media caused confusing results and should be replaced with a salt-based micronutrient. In future iterations of the MABR’s design, it is recommended to design an effective method for sludge removal that will not disrupt the system and biofilm. Currently, to remove all the sludge produced, the bioreactor needs to be unscrewed from the base which can cause jostling of the membrane. This can result in biofilm losses that may put back the experimentation a week or so to stabilize. Alongside removal, leaving the sludge at the bottom during the entirety of the experiment resulted in anaerobic gas production that lead to odors.

This research was conducted using the bioreactor as a batch treatment unit. This made it difficult to clean out between experiments and there was approximately 10 to 20 mL left of the bulk water from the previous experiment when the next experiment began. This was due to the way the bioreactor was attached to the base. Excess water not sampled was drained from the bottom, as dumping it out of the top would result in biomass loss. The exit valve for the bioreactor does not reach the full bottom of the system, resulting in some leftover water in the bottom. This can lead to unexpected nitrogen concentrations that cause a discrepancy in the nitrogen balances. In future iterations, it is recommended to operate it at a low flow rate to promote constant movement of water. This should also prevent the formation of the concentration gradient seen during this research.
The airline should be reconfigured to have a pressure gauge on the exit of the airline to observe head losses through the silicone membrane. This can lead to further analysis on oxygen diffusion and oxygen flow rates that is more precise and less estimated based on pressure. Additionally, the wall air compressor is efficient, but is occasionally shut off for department maintenance. It is recommended to find a more reliable source of compressed air for future experiments.

Next, the MABR should be scaled up in a controlled way to observe macroscale reactions. Many issues experienced with pH and DO were detrimental to the biomass in the small-scale MABR due to the microscale setting. If a larger system was used, changes would be less impactful to the overall constitution of the biomass and real-world situations could be tested.

6.3 Proposed Improvements to Experimental Methods

Experimental methods throughout this research were relatively consistent. However, microbiology experiments were inconclusive and total nitrogen analyses were rife with errors. For future work, it is recommended to collaborate with a microbiology researcher to accomplish and create a profile for the bacteria creating the biofilm versus those in the bulk water. This will lead to a deeper understanding of the mechanics for nitrogen removal in the MABR.

The Hach total nitrogen analysis has a 20% systemic error that drastically affects the viability of the results. Future researchers should consider running total suspended solids, volatile suspended solids tests, and carbonaceous oxygen demand (COD) tests to track biomass production. If biomass production rates were known, the organic nitrogen loading could be estimated from there. Additionally, it may be possible to test for total kjeldahl nitrogen, which can provide organic nitrogen concentrations when
tested alongside TAN. This should be investigated as a new experimental method for future research to complete a nitrogen balance on the MABR.

6.4 Future Work Recommendations

For future researchers looking into a MABR system, there are a few facets this research was unable to get to: oxygen diffusion and DO concentrations, effects of temperature on the system, and sludge production. Future work should also include scaling-up the MABR and experimenting with flow rates, along with exploring the removal pathways of assimilation, and the causes for why MABR B became either anaerobic or began experiencing aerobic digestion.

Oxygen diffusion and DO concentrations through the biofilm and bulk water cannot be properly analyzed without a microDO probe. While theoretical equations showed that enough oxygen is diffusing for nitrification, this is just an assumption, and future work should include a DO profile from the membrane to the center of the bulk water to develop a diffusion curve.

Temperature was largely ignored as a factor during this research. However, temperature can affect DO concentrations, oxygen diffusivity rates, and biological activity. Future work should include analysis of the MABRs performance under cold and hot temperatures to understand the full limitations of the system. This may indicate that a MABR needs external heating and or cooling during the winter and summer seasons.

Sludge production in the MABR will vary from other systems due to the combined nature of attached and suspended growth. The volume of sludge, characterization of sludge, and the moisture content should be determined in future studies. This will allow for understanding of how to integrate a MABR into a centralized wastewater treatment system where the operators will need to handle the sludge produced. Additionally,
analysis of the sludge’s constituents may yield potential byproducts that can be recovered.

Future researchers should consider scaling up the MABR system to analyze nitrogen removal under different flowrates. Finding optimal flowrates through the MABR system will increase its ability to treat nitrogen and other constituents. Additionally, the right flowrate will minimize the number of dead zones where no mixing occurs and should result in the concentration gradient seen during this research disappearing. Scaling up the MABR will present unique challenges that operators will experience upon implementation of a MABR. These will likely include finding a way to seal the system to prevent passive air diffusion, while allowing nitrogen gas to vent off from the system, securing the membranes in a larger tank, and general maintenance requirements for optimal functionality. Research should include membrane spacing, sizing, and piping required to accomplish treating nitrogen with a MABR in a full-scale setting.

The main questions developed during this research was whether assimilation was occurring, and the cause of and implications of MABR B becoming either anaerobic with sulfur compounds or stalled in the acidogenesis phase of aerobic digestion. These are two missing pieces that could increase understanding of the MABR’s removal pathways if studied further.

The MABR is a viable way to perform SNdN. It can treat high strength wastewater at low C:N ratios. This makes it a promising option for implementation in all settings, particularly domestic and industrial wastewater treatment or agricultural runoff treatment. Future research can scale this design up to conclude whether a MABR will be able to offset wastewater treatment land requirements and costs for communities needing to treat nitrogen.
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