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Partial nitrification and oxygen transfer analysis utilizing hollow fiber membrane aeration

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by

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ABSTRACT

Excess nutrients in lakes, rivers, and streams harm aquatic ecosystems. Advanced treatment at wastewater treatment plants can reduce this pollutant load. An innovative nutrient removal system, ANAMMOX, anaerobically converts ammonium and nitrite to N_2 gas. Because this system requires a 1:1.31 NH_4^+ : NO_2^- ratio, a partial nitrification system is coupled with the ANAMMOX system to provide the necessary substrates. A hollow fiber membrane reactor was employed to create this partial nitrification system. Gas mass transfer was first evaluated to determine the K_La oxygen transfer coefficient. Reactor parameters, such as mixing speed, membrane length, and gas pressure, were evaluated to determine how K_La was affected by these variables. This hollow fiber membrane was then used to control oxygen delivery to the partial nitrification system, producing an effluent with ammonium and nitrite. Controlling dissolved oxygen at low concentration selects for ammonium oxidizing bacteria, while nitrite oxidizing bacteria are suppressed. This system effectively treated influent ammonium concentrations ranging from 50 mg/L NH_4^+-N to 250 mg/L NH_4^+-N. Real-time polymerase chain reaction (qPCR) was employed to verify the system preference for ammonium oxidizing bacteria. Hollow fiber membrane aeration is effective for controlling oxygen transfer.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Nutrients play an important role in the earth's ecosystem. Specifically, nitrogen is necessary for plant growth; this creates a dependence on nutrients for all species in our ecosystems. However, like many natural systems, there exists a natural balance necessary for maintaining healthy conditions.

When discharged into waterways, nutrient pollution negatively impacts lakes, streams, rivers, and coastal areas, upsetting the nutrient balance. Nitrogen and phosphorus cause eutrophication. Eutrophication is the presence of excess nutrients in an ecosystem, which leads to algal blooms and hypoxia (low dissolved oxygen conditions). Hypoxia and algal blooms harm the aquatic ecosystem, killing fish, reducing water quality, and changing the ecosystem characteristics. The presence of nutrients is attributed to wastewater, agriculture, and the burning of fossil fuels.[1] In the United States, 78 percent of coastal areas have been identified as demonstrating signs of eutrophication.[2]

To mitigate environmental problems caused by nutrient discharge into waterways, regulators are looking to increase restrictions on point sources for nutrients. Aquatic nitrogen-based nutrient contribution from sewage is estimated to be 12% in the United States.[1] These point sources include wastewater treatment plants. More stringent nutrient limitations are likely, requiring advanced nutrient removal treatment systems.

Conventional nutrient removal systems employed in many wastewater treatment plants require an energy intensive aeration system to supply oxygen. A reduction of the oxygen requirement will result in operational cost savings.

Additionally, isolating specific microorganisms improve operational efficiency. A mixed-culture system requires excess aeration. Varying biomass yields prevent slower-growing bacteria from flourishing and performing efficiently. Developing an enriched culture of target organisms allows the system parameters to meet the direct needs of these organisms.

Oxygen transfer was studied on a silicone hollow-fiber membrane. Operation parameters were modified to determine the oxygen transfer coefficient for the membrane. This membrane is then used in a partial nitrification study to serve as an aeration source and a surface for attached bacterial growth.

Oxygen transfer study

Oxygen transfer coefficient (K_La, units d^{-1}) is a combination of "K_L," the liquid film transfer coefficient, and "a," the ratio of membrane surface area to water volume. This gas mass transfer coefficient allows for accurate integration of the membrane aerator into the system. The procedure will be performed at conditions representative of the partial nitrification study to calibrate the system as accurately as possible. The gas transfer can be affected by many variables, such as tank mixing, reactor size, wastewater characteristics, and membrane properties.

Oxygen transfer is essential to the partial nitrification process. The nitrification process is controlled by monitoring the dissolved oxygen in the reactor. The oxygen transfer coefficient is used to determine these process characteristics.

The clean water oxygen transfer coefficient is standardized. This clean water transfer is used with experimental results to develop the K_La. Variations in wastewater characteristics, tank size, and mixing can be accounted for through the use of correlation factors to compensate for field oxygen transfer rates.

Nitrification

Nitrification is the process by which ammonium (NH_4^+-N) is oxidized first to nitrite (NO_2^--N) by ammonium oxidizing bacteria (AOB), and then to nitrate (NO_3^--N) by nitrite oxidizing bacteria (NOB). Due to a lower biomass yield, it is difficult for nitrifying organisms to compete with heterotrophic organisms required for BOD removal.[3] For this reason, a reactor specifically designed for nitrification can be optimized for ammonium oxidation.

Thesis Organization

Two of the chapters of this thesis document consist of journal papers formatted for submission to specific scientific journals. Chapter 2 discusses the oxygen transfer study. My participation in chapter 2 included oversight and advising of data collection, assistance with analyzing the collected data to calculate K_La, and statistical analysis of the collected data and Box-Behnken model. Chapter 3 examines the application of hollow fiber membranes in a partial nitrification system. My participation in chapter 3 included treatment system construction, seeding and startup, operational monitoring, water quality testing, and data

collection. Chapter 4 discusses additional data collected which was not included in the journal papers, as well as opportunities for future research. Chapter 2 and Chapter 3 are formatted for submission to specific journals.

Literature Review

Partial nitrification has been studied by other researchers in the past. The system is commonly coupled with the ANAMMOX process as a pre-treatment step providing ammonium and nitrite substrates. Many systems utilize high temperature or limited sparging aeration to select for ammonium oxidizing bacteria and suppress nitrite oxidizing bacteria. Few studies have involved the use of a membrane system for oxygen delivery. A literature review of some of these previous studies is covered in the appropriate sections of Chapter 2 and Chapter 3. Additional partial nitrification studies are summarized in this section.

The partial nitrification process requires the activity of ammonium oxidizing bacteria (AOB), while suppressing the activity of nitrite oxidizing bacteria (NOB). This can be accomplished by adding inhibitors for nitrite oxidizing bacteria, controlling dissolved oxygen and relying on oxygen affinity to suppress NOB, using pure cultures of AOB, controlling pH to inhibit NOB, or operating the system at high temperature to take advantage of differential kinetics.

The SHARON partial nitrification process utilizes high temperature to create a partial nitrification system. It has been studied by numerous researchers, and is the most extensively researched for coupling with the ANAMMOX system. The single reactor high activity ammonium removal over nitrite (SHARON) system was first studied by Hellinga et al., and operates with no solids retention. This creates a system in which SRT is equivalent to HRT.

HRT is selected to minimize growth of nitrite oxidizing bacteria. Cyclic aeration was employed to limit oxygen, using a two hour cycle of 80 minutes aerobic, and 40 minutes anoxic conditions. HRT for this system was 1.5 days, and the operational temperature is 30 to 40° C. The study reported that the SHARON system is most feasible for high ammonium concentrations, defined by the authors as "hundreds to thousands mg NH_4^+-N/L.[4]

The reactor conditions of the SHARON system are quite extreme compared to the aeration basin of an activated sludge system. If this system were employed in a large scale, maintaining a temperature of 35° C in colder climates may be an energy intensive endeavor. However, as noted by vanDongen et al., a possible application of the SHARON process is treating sludge digester effluent. Anaerobic sludge digesters typically operate in the temperature range of 30 to 40° C. However, if the SHARON process were to be used to treat domestic wastewater, the wastewater temperature would need to be raised. The SHARON process developed by van Dongen et al. converted 53% of the influent ammonium to nitrite, and when coupled with the ANAMMOX system, removed 80% of the total ammonium to dinitrogen (N_2) gas; the system operated at a pH of 6.7-6.8, treating sludge digester effluent with ammonium concentration of 1-1.5 g/L NH_4^+-N. van Dongen et al. note that the SHARON/ANAMMOX process minimizes the COD addition that is typically necessary in a nitrification/denitrification process treating high ammonium wastewaters because the nitrifiers and ANAMMOX organisms are autotrophic; however, the study claimed that the SHARON process is best designed for wastewaters containing greater than 500 mg/L NH_4^+-N.[5]

Khin et al. examined the optimal pH of the SHARON system, determining that the partial nitrification operated best at a pH between 7 and 8. A pH higher than 8 increases the free ammonium (NH3) concentration, which can have inhibitory effects on nitrifiers. The authors also compared the SHARON-ANAMMOX system to a conventional nitrification/denitrification system, and determined that "the combined system saves 50% on required oxygen and 100% on the external carbon source," making it 90% less expensive than conventional treatment.[6]

Mosquera-Corral et al. studied the inhibitory effects of salt on the nitrification process, suggesting the SHARON process for the treatment of fish canning industry wastewater from anaerobic digesters. The study determined that at carbon-nitrogen ratios greater than 0.3, ammonium oxidizing efficiency decreased significantly, and at total organic carbon concentrations higher than 0.3 g/L, heterotrophic organisms outcompeted autotrophs. The presence of salt (in the form of NaCl, KCl, or Na_2SO_4) also inhibited the nitrification process; 40% inhibition was observed at a 100 mM salt concentration. Inhibition was similar for the different salts. Optimal treatment in this system employed an HRT of 1.1 days, and a pH of 7.16, treating a wastewater with an influent ammonium concentration of 1000 mg/L NH_4^+-N.[7]

Guo et al. employed aeration control in an SBR system to create a partial nitrification process operating at lower temperature (12 to 25° C). Dissolved oxygen was controlled between 0 to 4 mg/L O_2, with an average value of 2.5 mg/L. During startup, nitrite was found in the effluent, but the main product was nitrate. After culture enrichment significantly more nitrite (23.4 mg/L NO_2^--N) was produced, and nitrate was a minor product of the reaction (3.67

mg/L NO_3^--N).[8] The study did not control pH, and found a pH gradient which decreased (as seen in Figure 1 below). The ammonium valley identified in Figure 1 was used as an operational control point to stop aeration. The authors suggest that aeration duration control can be used to specify the ammonium/nitrite ratio in the SBR effluent.

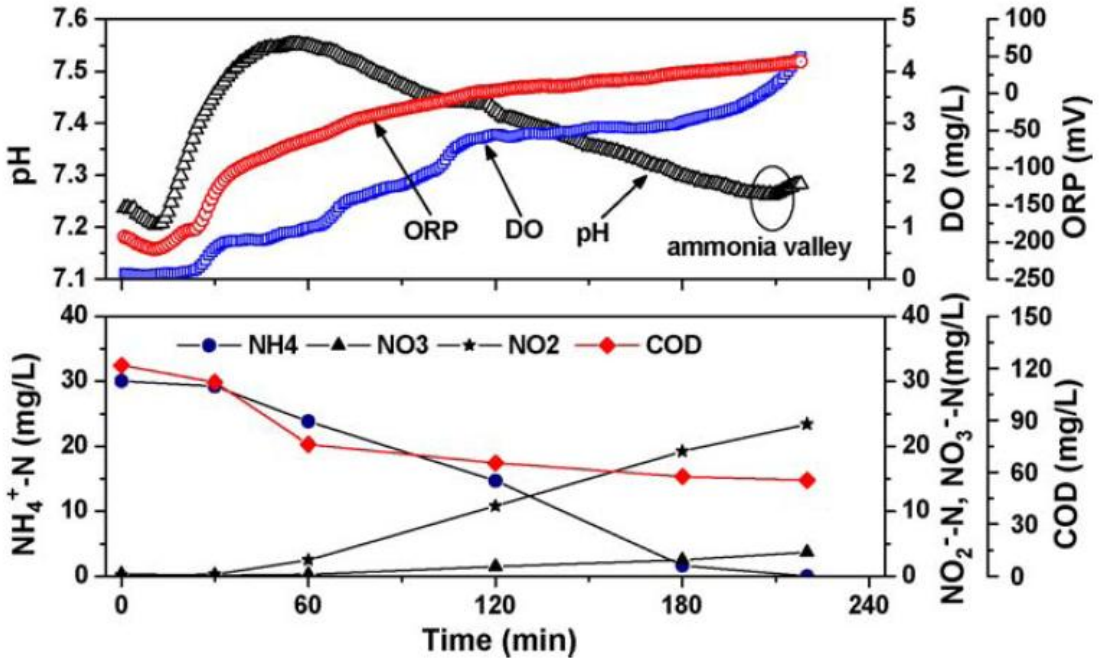

Figure 1. Air-sparged partial nitrification reactor after 26 days of culture enrichment, Guo et al. 2009

CHAPTER 2: OXYGEN TRANSFER STUDY USING A HOLLOW FIBER MEMBRANE FOR BUBBLELESS AIR TRANSFER BY BOX-BEHNKEN DESIGN: DEVELOPMENT OF AN INNOVATIVE PARTIAL NITRIFICATION PROCESS

Modified from a paper to be submitted to *The Journal of Membrane Science*

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Abstract

This research paper assesses oxygen transfer efficiency of a silicone hollow fiber membrane in an aqueous environment for wastewater treatment research. The K_La oxygen transfer coefficient was determined through lab testing. Box-Behnken statistical analysis was employed to assess the influence of three test variables: supply air pressure, mixing power, and membrane surface area. These variables were normalized for the reactor volume. Statistical analysis determined that membrane surface area and air supply pressure had a more significant effect on the K_La value than the mixing power variable. A mathematical model was developed to represent the oxygen transfer coefficient (K_La) as a function of the three test variables and their interactions.

Key words: Hollow fiber membrane; Oxygen transfer; Optimal experimental design; Box-Behnken design; Partial Nitrification

Introduction

As a result of human activity, particularly agriculture and sewage effluent, excess nitrogen (N) is accumulating in the environment.[1] Wastewater treatment plants release effluent into the environment without advanced treatment. Excess nitrogen in water bodies, can result in eutrophication. This causes a variety of problems such as the depletion of dissolved oxygen, toxicity toward the aquatic life, and a potential increase in public hygienic issues.[2, 3] The symptoms of eutrophication are observed in 78 percent of coastal area, 50 percent of lake area, and 60 percent of rivers in the United States.[4, 3] Consequently, more stringent nutrient limitations will be expected in the near future. In addition, water and wastewater facilities consume 3% of the electricity usage in the U.S., equivalent to approximately 56 billion kilowatt hours (http://www.epa.gov/waterinfrastructure/bettermanagement_energy.html). Installing biological nutrient removal to upgrade existing wastewater facilities to meet stringent nutrient limitations would require more than double the current electricity consumption.

ANAMMOX (anaerobic ammonium oxidization) is a newly discovered nitrogen removal process, in which ammonium is oxidized directly into nitrogen gas using nitrite as an electron acceptor.[5, 6] The stoichiometric ratio of the ANAMMOX reaction between reactants, ammonium and nitrite, and nitrate, is 1:1.31:0.22.[6] Most of the ammonium is converted into nitrogen gas, bypassing the formation of nitrate. Therefore, the process can provide a substantial reduction in energy use (saving up to 62.5% of air supply) and require no external carbon source. The slow growing characteristic of ANAMMOX bacteria adds another benefit in low sludge production, which reduces the sludge treatment and disposal need. The

ANAMMOX process provides a substantial reduction in energy use and requires no organic carbon source, because the bacteria responsible for the reaction are autotrophs. The ANAMMOX process requires the stoichiometric ratio of ammonium to nitrite approximately equal to 1. Therefore, a sustainable nitrite supply for the ANAMMOX application is a vital requirement. Many researchers have previously utilized pH and/or temperature as the control parameters to inhibit the nitrite oxidizers and to enrich the ammonium oxidizers for maintaining nitrite production, such as the SHARON process.[7, 8] However, the SHARON process requires influent waste with mesophilic temperatures or relatively low pH values. Consequently, the ANAMMOX application is limited to only treating reject waters with a high ammonium concentration such as industrial reject waters, anaerobic digester supernatant, or landfill leachate. Also, the SHARON process isn't designed to operate at a low dissolved oxygen (DO) condition for saving energy consumption from supplying oxygen. Thus, there is a need to develop a new energy sustainable, reliable, partial nitrification system for applications of a wider range of ammonium contain wastewaters.

To transfer oxygen efficiently in the bulk solutions, using a gas permeable hollow fiber membrane (HFM) as an oxygen delivery diffuser has been evaluated in water and wastewater treatment processes.[9, 10, 11] However, it has not yet been applied in the partial nitrification process. Therefore, this study develops an innovative HFM partial nitrification biofilm process: employing a hollow fiber bubbleless membrane aeration reactor to limit dissolved oxygen and culture specific bacteria. Figure 2 shows the cross-section profile of the HFM partial nitrification system.[12] Oxygen is diffused from the inside of the HFM into the bulk solution, through a nitrifying biofilm. This biofilm consumes much of the oxygen before it

reaches the bulk solution. The process is implemented with a low DO operating strategy as a selection pressure to enrich ammonium oxidizing bacteria (AOB) on the membrane surface and to eliminate nitrite oxidizing bacteria (NOB), because the affinity values are within the range of 1-15 and 22-166 μM O_2 for AOB and NOB, respectively.[13] The objective of this study was to examine oxygen mass transfer from a silicone hollow fiber membrane for partial nitrification. The Box-Behnken method, a statistical experimental design method, was used to investigate the parameters of supplied air pressure (*P/V*), membrane surface area per volume (A_M/V) and power input per working volume for mixing (ω/V).

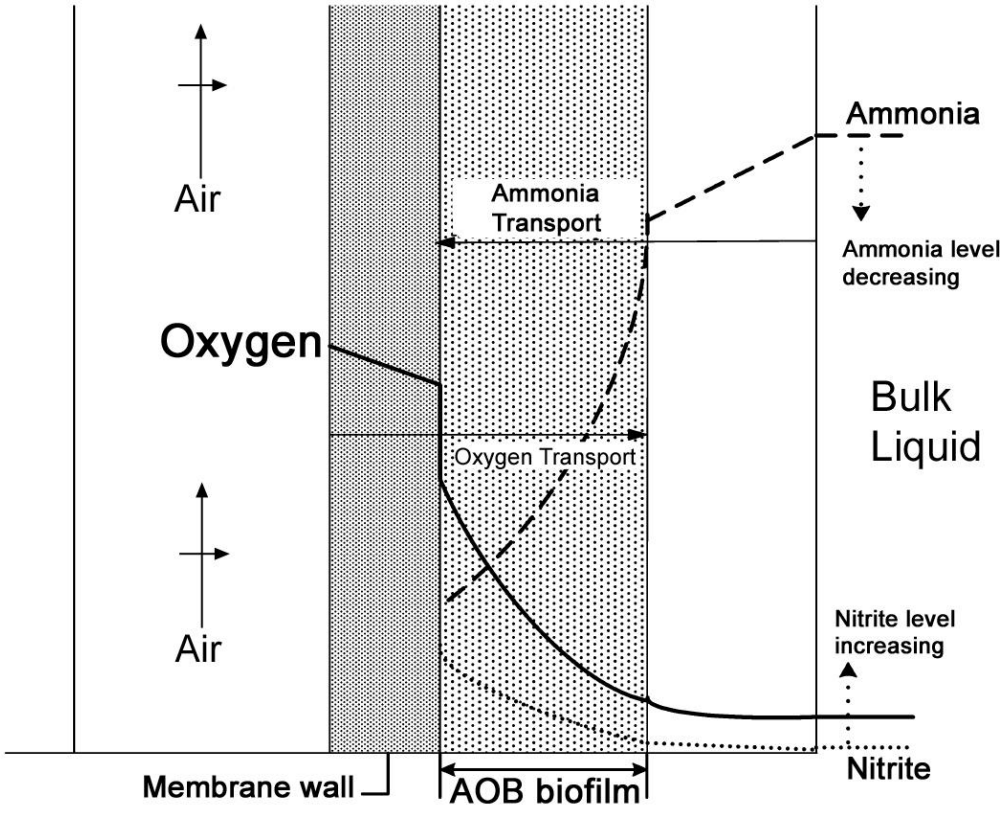

Figure 2. Schematic of silicone membrane oxygen transfer theory, adopted from Feng et al. 2007 [12]

Materials and methods

Membrane module

The membrane used for oxygen mass transfer in this study was the Gilbrane silicone hollow fiber membrane (silicone-rubber type) by Fuji Systems Corporation (Tokyo, Japan). The outer diameter and wall thickness of the nonporous silicone membrane was 2.0 mm and 250 μm, respectively. The length of the tested membrane was varied from 6 meters to 18 meters. The membrane was coiled around a supporting rack, and submerged in a 3-Liter acrylic plastic reactor. A BioFlo 2000 Fermentor was used as a variable-speed mixer. Nitrogen gas was used to purge oxygen from the reactor. Dissolved oxygen was measured using the Mettler Toledo O2 4100e oxygen probe. A schematic of oxygen transfer experimental setup is shown in Figure 3.

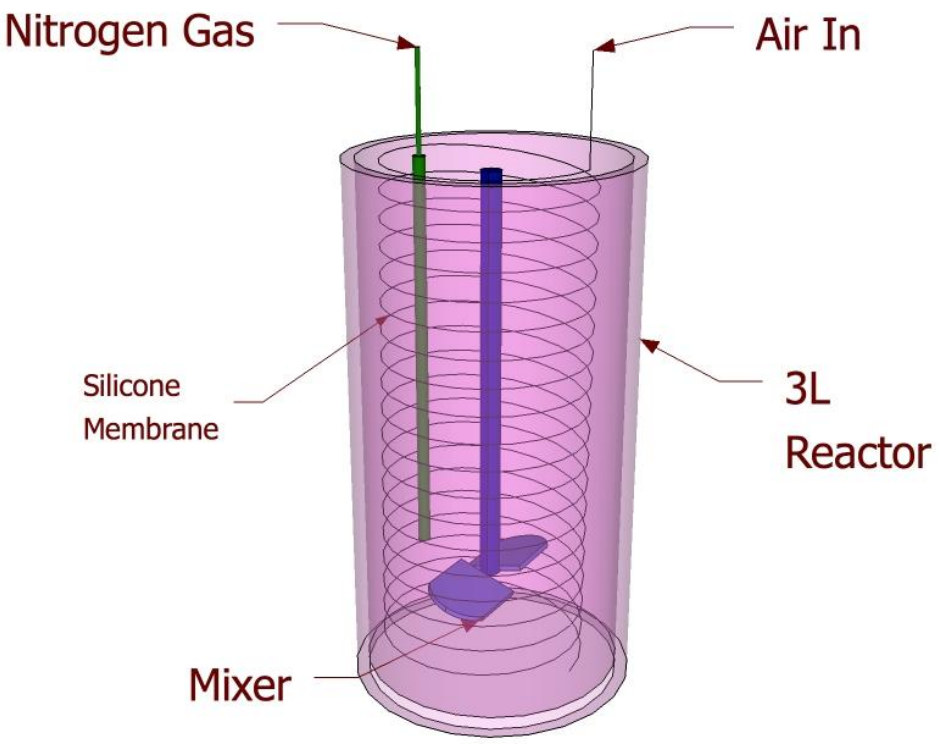

Figure 3. Schematic diagram of experimental setup

The reactor diameter was 12.70 cm, and the impeller diameter was 7.62 cm. The water height in the reactor was 25.00 cm. The reactor construction was acrylic clear plastic, and the volume of the reactor was 3 liters. This height to depth ratio matches an axial-flow 2-blade 45° impeller was chosen for this study because axial flow impellers have lower shear rates on cells than Rushton impellers.[14] This characteristic is necessary for supporting a viable biomass.

In the experiment, the membrane reactor was filled with distilled water at 20° C. The reactor temperature was consistently 18° - 22°C. The pH value of the water was constant throughout the experiment. The ungassed power input (ω) was not directly measured. Instead, the

reactor was mixed between 300 rpm to 500 rpm, and the ungassed power input per unit volume (ω/V) was calculated, resulting in values ranging from 189 to 874 W/m^3. It can be calculated by the following relationship:[15]

– ———— **(1)**

Where P_o is the power number for an axial-flow 2-blade 45° impeller (determined to be P_o= 1.8 in this study)[16], ρ is the water density (kg/ m^3), D is the impeller diameter (m), T is the reactor diameter (m), n is the mixing speed (rps), and H is the height of the water in the reactor (m).

Membrane surface area was another variable tested. Silicone membrane lengths of 6, 12, and 18 meters were installed in the reactor. The surface area of the membranes was normalized based on reactor volume, producing the variable of membrane area per unit volume (A_M/V), with units of m^2/m^3, or 1/m. Values for A_M/V ranged from 12.6 m^2/m^3 for the 6-meter long membrane, to 37.7 m^2/m^3 for the 18-meter long membrane.

Compressed air was transferred across the membrane into the bulk solution/water in the membrane reactor at a supply pressure ranging from 137.9 to 206.8 kPa (20 to 30 psi). This parameter is normalized for the reactor volume, resulting in a pressure per volume variable (P/V) ranging from 45965 to 68947 kPa/ m^3. The exhaust is vented from the top of the membrane reactor. The DO gradient was monitored with time by the DO probe connected to a computer for on-line recording. The experiment was operated at 22^o C. The oxygen transfer coefficient (K_La) was related to dissolved oxygen concentration using the following relationship:

(2)

where C^* = aqueous phase DO concentration in equilibrium with the gas phase (mg/L), 8.55 mg/L in this study, C = measured dissolved DO concentration (mg/L), k_La = oxygen mass transfer coefficient (1/hr) in the hollow fiber membrane reactor, and t = time (hour).

The manufacturer of the hollow fiber membrane (Fuji Systems Corporation, Tokyo Japan) provided the following equation to determine flow rate across the silicone membrane:

(3)

The solution is provided in mL gas per second. K is a constant dependent on the gas supplied (K=50 for O_2 gas, K=25 for N_2 gas, from manufacturer), L is the thickness of the silicone membrane, A is the available membrane area (cm^2), and (P-P') is the difference in partial pressure (cmHg). This equation was not used for the statistical model because the gas flowrate model cannot separate membrane area and gas pressure independently for analysis.

Experimental design

Box-Behnken design employs a response surface design will be implemented to systematically survey three variables (air pressure per volume, membrane surface area per volume, and mixing power input per volume) at three levels. The variable levels have been chosen under the conditions of the practical ranges. Table 1 shows the variables and their respective levels for this oxygen transfer study. The list of the experiments based on Table 1 is shown in Table 2. The experimental combination of variables was selected at random by the JMP statistical software. Experimental trials 2, 3, and 12 (Table 2) are the central

variable points; these trials have been repeated three times for calculating errors (mean and standard deviation) based on the Box-Behnken design.

Table 1. Variables and their respective levels for this membrane study

Variables	Levels		
	-1	0	+1
Supplied air pressure (P/V, kPa/m^3)	45965	57456	68947
Membrane surface area per volume (A_M/V, m^2/m^3)	12.6	25.2	37.7
Power input per volume for mixing (ω/V, W/m^3)	189	532	874

Table 2. List of experiments and response oxygen mass transfer determination

System Run	Pattern	Parameters			Response
		A_M/V (m^2/m^3)	(ω/V) (W/s)	P/V (kPa/m^3)	K_La (1/hour)
1	+ − 0	37.7	189	57456	2.0420
2	0 − +	25.15	189	68947	1.0845
3	− − 0	12.6	189	57456	0.8946
4	− 0 +	12.6	532	68947	1.1898
5	− + 0	12.6	874	57456	1.2482
6	0 0 0*	25.15	532	57456	1.1852
7	0 + +	25.15	874	68947	1.7436
8	0 + −	25.15	874	45965	0.9372
9	− 0 −	12.6	532	45965	0.7088
10	0 0 0*	25.15	532	57456	0.9015
11	+ + 0	37.7	874	57456	2.3230
12	0 − −	25.15	189	45965	0.7056
13	+ 0 −	37.7	532	45965	1.6257
14	0 0 0*	25.15	532	57456	1.4467
15	+ 0 +	37.7	532	68947	2.5859
		Statistical Errors		Mean*	1.1778
				STD*	0.2727

*Mean and Standard Deviation calculated for data points 6, 10, and 14.
A_M/V: Area of membrane per Volume (m^2 / m^3)
(ω/V): Power input per unit volume (W / m^3)
P/V: Air pressure per Volume (kPa / m^3)
K_La: Oxygen transfer coefficient (1/hr)

The Box-Behnken design among the response surface designs allows the estimation of an empirical second-order model shown below:

(4)

Where x_i are the variables, y is the response, b_0 is the constant term (intercept parameter), b_1, b_2, and b_3 are single factor effects, b_{11}, b_{22}, and b_{33} are quadratic terms, and b_{12}, b_{13}, and b_{23} are interaction effect terms.

The Box-Behnken design experimentations, statistical data analysis, and graphics in this study were conducted using the statistical software package JMP™ version 8.0.1 (©SAS Institute Inc., Cary NC).

Results and Discussions

Using the statistical model and experimental design listed above, the following full prediction model for K_La was determined by the equation (1), in the form of equation (4) above:

$$K_La = 1.1778 + .5669 \cdot A_M/V + .1906625 \cdot (\omega/V) + 0.3283125 \cdot P/V - .01815 \cdot (A_M/V) \cdot (\omega/V) + 0.1198 \cdot (A_M/V) \cdot (P/V) + .106875 \cdot (\omega/V) \cdot (P/V) + .4294875 (A_M/V)^2 + .0196625 \cdot (\omega/V)^2 - 0.08125 \cdot (P/V)^2 \quad \textbf{(5)}$$

Where

A_M/V: Area of membrane per Volume (m^2 / m^3)

(ω/V): Power input per unit volume (W / m^3)

P/V: Air pressure per Volume (kPa / m^3)

K_La: Oxygen transfer coefficient (1/hr)

The model in equation (5) includes all nine possible variable combinations in a 2nd degree prediction model. The fit of this model to the experimental data can be estimated using the R square value. The model listed in equation (5) has an R square value of 0.9648, indicating high statistical accuracy (see Table 3).

Table 3. Full model summary of fit

RSquare	0.964829
RSquare Adj	0.90152
Root Mean Square Error	0.182274
Mean of Response	1.37482
Observations (or Sum Wgts)	15

The model can be verified statistically through analysis of variance. By accounting for the total degrees of freedom of the model (7) and the degrees of freedom of the experimental design (14), a minimum F-ratio of 6.58 is established for 99.9% confidence. The calculated F-ratio of the model is 15.2401, greater than the minimum of 6.58; this indicates that the statistical significance of the model exceeds 99.9% confidence (see Table 4).

Table 4. Full model analysis of variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	4.5569760	0.506331	15.2401
Error	5	0.1661182	0.033224	**Prob > F**
C. Total	14	4.7230943		0.0040*

Variables (A_M/V), $(A_M/V)^2$ and (P/V) were most significant, with statistical probabilities less than 0.01. Statistical significance is identified as lower than 0.05 (95% confidence).

To increase the accuracy of the prediction model, low-significance variables can be removed to simplify the equation (see Table 5).

Table 5. Full model parameter estimates

Term	**t Ratio**	**Prob>|t|**
Intercept	11.19	<.0001*
AreaPerVolume	8.80	0.0003*
PowerPerVolume	2.96	0.0316*
PressurePerVolume	5.09	0.0038*
AreaPerVolume*PowerPerVolume	-0.20	0.8500
AreaPerVolume*PressurePerVolume	1.31	0.2457
PowerPerVolume*PressurePerVolume	1.17	0.2937
AreaPerVolume*AreaPerVolume	4.53	0.0062*
PowerPerVolume*PowerPerVolume	0.21	0.8440
PressurePerVolume*PressurePerVolume	-0.84	0.4389

Variables $(A_M/V)\cdot(\omega/V)$ and $(\omega/V)^2$ had probability ratios of 0.84 and 0.844, respectively. These probability values are significantly more than the 0.05 limit for a confident model. These variables were removed to create a simplified model, shown in equation (6) below.

$$K_La = 1.1899 + .5669\cdot A_M/V + .1906625\cdot(\omega/V) + 0.3283125\cdot P/V + 0.1198\cdot(A_M/V)\cdot(P/V) + .106875\cdot(\omega/V)\cdot(P/V) + .4294875(A_M/V)^2 - 0.08125\cdot(P/V)^2 \quad \textbf{(6)}$$

The model listed in equation (6) has an R square value of 0.9642, indicating high statistical accuracy. Utilizing analysis of variance, the model is analyzed by accounting for the total degrees of freedom of the model (7) and the degrees of freedom of the experimental design (14), a minimum F-ratio of 7.08 is established for 99.9% confidence. The calculated F-ratio of the model is 26.9699, greater than the minimum of 7.08; this indicates that the statistical significance of the model exceeds 99.9% confidence. The calculated F-ratio of the simplified model of equation (6) is higher than the full model shown in equation 4, indicating improved statistical accuracy of the model fit. Variables (A_M/V), $(A_M/V)^2$ and (P/V) continued to be most significant in the model, with probability values of 0.0001, 0.0011, and 0.0006, respectively.

The statistical accuracy of the model can be analyzed by evaluating the actual vs. predicted plots for the full and simplified models. Figure 4 shows the actual vs. predicted plot for the full model. The collected data points are plotted against the line drawn by the mathematical model. It shows that many of the collected data points are very close to the mathematical model.

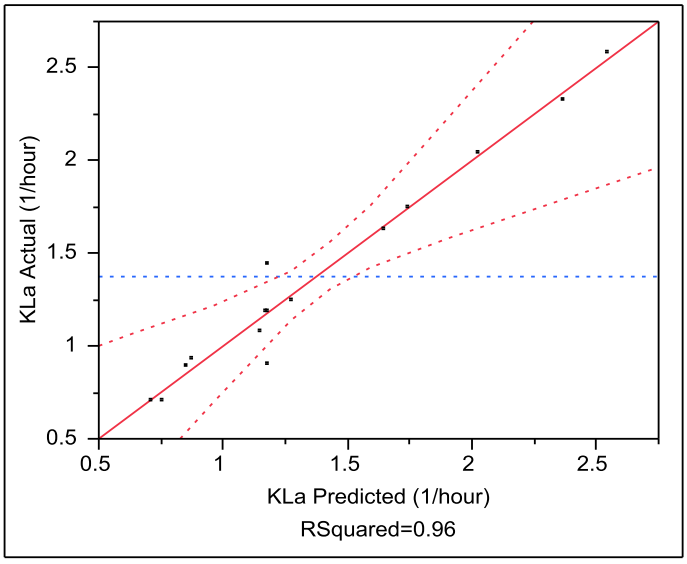

Figure 4. Full model actual by predicted plot

Likewise, the simplified model can be evaluated for fit by examining the actual by predicted plot (Figure 5). The experimental data are plotted against the model, and fit the mathematical model more accurately than the full model, indicating that removing variables improved model accuracy.

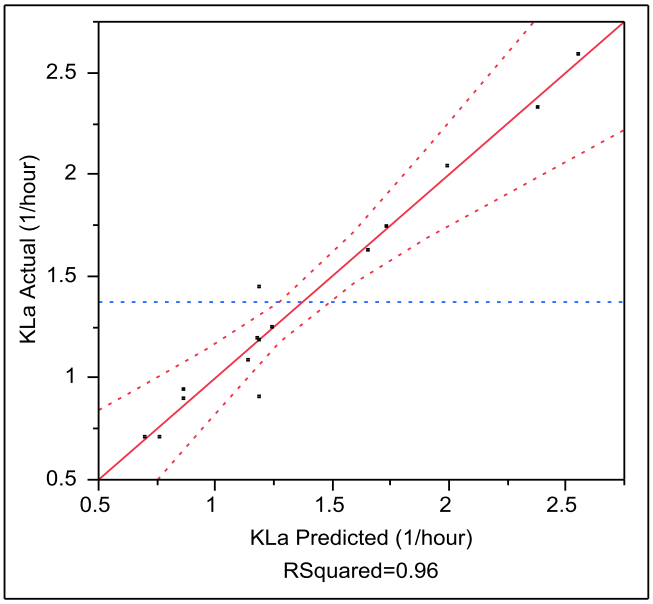

Figure 5. Simplified model actual by predicted plot

The significance of each variable can be evaluated through the use of a contour plot. The two-dimensional contour plot compares two variables and displays how the response variable (K_La) changes as the variables are modified. PressurePerVolume is compared to AreaPerVolume in Figures Figure 6, Figure 7, and Figure 8. These contour plots indicate that AreaPerVolume has a more significant impact on K_La value; for a given PressurePerVolume, the K_La changes more significantly with a change in AreaPerVolume.

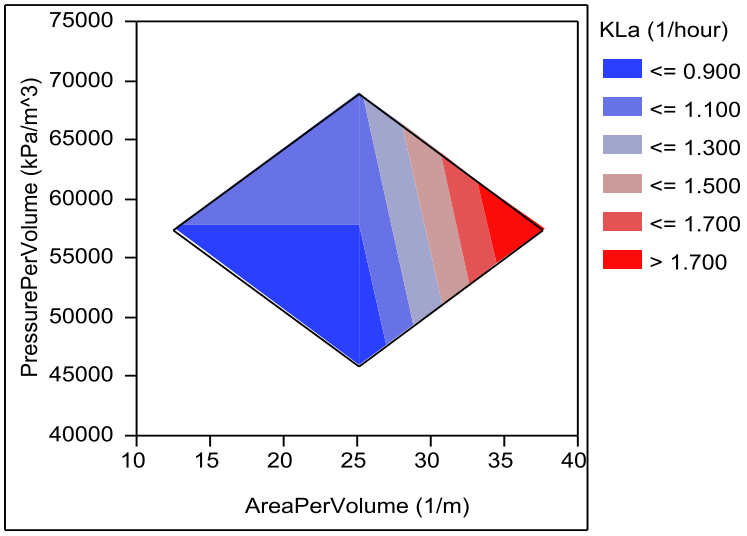

Figure 6. Contour plot for K_La for interaction of PressurePerVolume and AreaPerVolume with PowerPerVolume=189 W/m^3

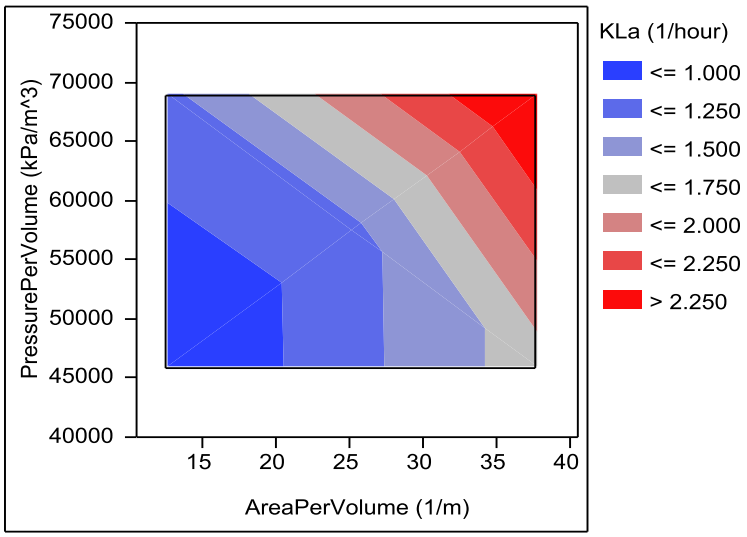

Figure 7. Contour plot for K_La for interaction of PressurePerVolume and AreaPerVolume with PowerPerVolume=531.5 W/m^3

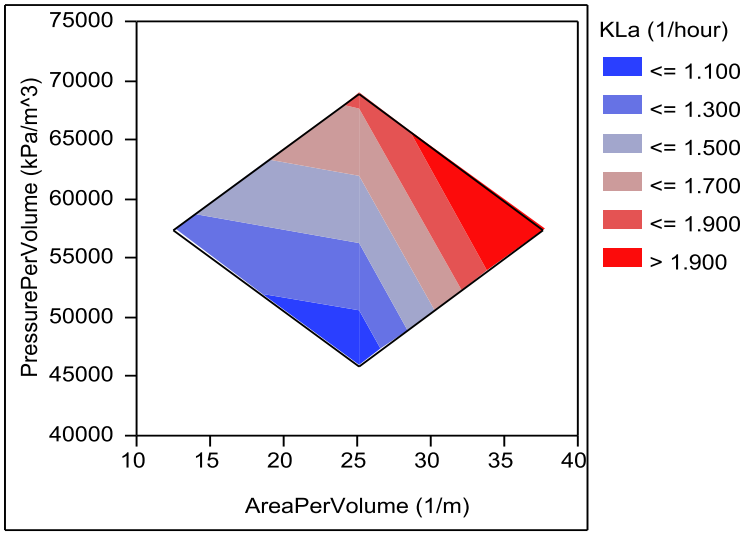

Figure 8. Contour plot for K_La for interaction of PressurePerVolume and AreaPerVolume with PowerPerVolume=874 W/m^3

PowerPerVolume and AreaPerVolume are compared in Figures Figure 9, Figure 10, and Figure 11. These contour plots also display the significance of AreaPerVolume, which is more significant than PowerPerVolume. At a given power value, the K_La changes significantly as AreaPerVolume increases.

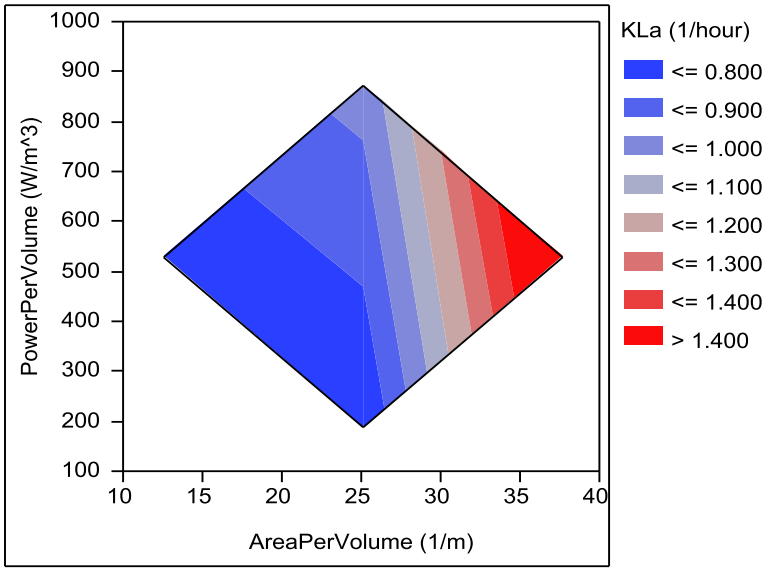

Figure 9. Contour plot for K_La for interaction of PowerPerVolume and AreaPerVolume with PressurePerVolume=45965 kPa/ m^3

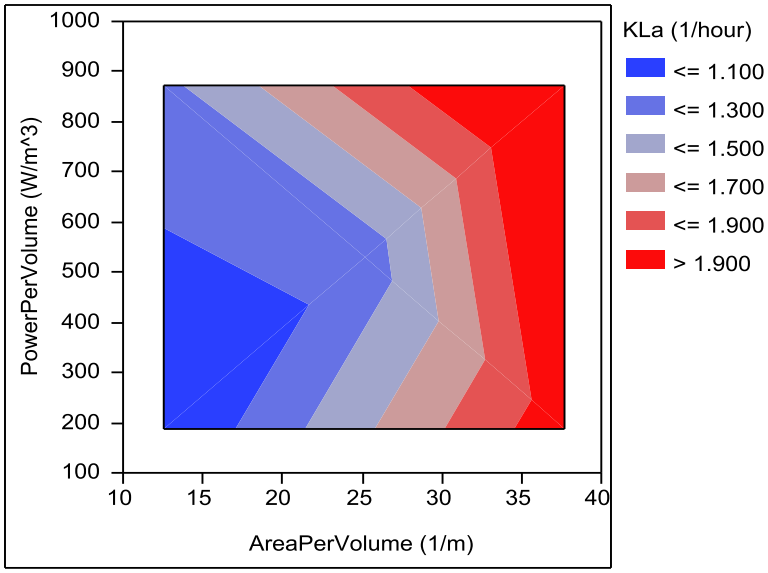

Figure 10. Contour plot for K_La for interaction of PowerPerVolume and AreaPerVolume with PressurePerVolume=57456 kPa/ m^3

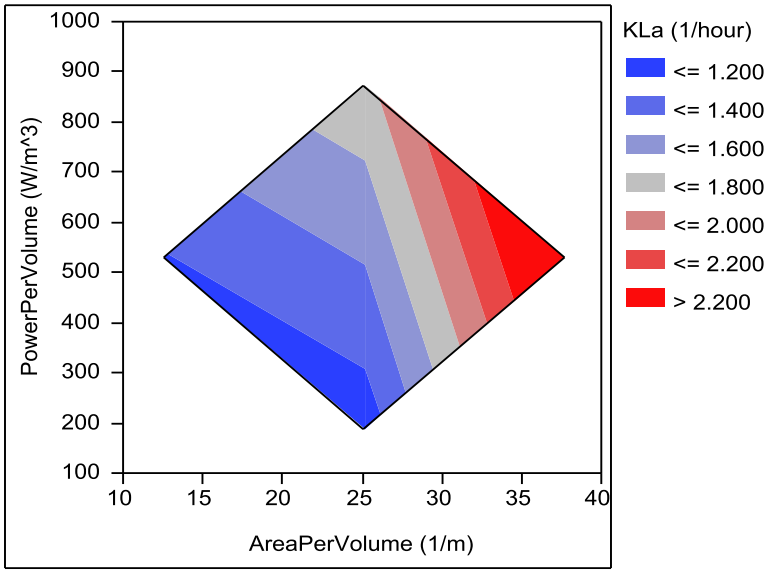

Figure 11. Contour plot for K_La for interaction of PowerPerVolume and AreaPerVolume with PressurePerVolume=68947 kPa/ m^3

In Figures Figure 12, Figure 13, and Figure 14, PressurePerVolume is compared to PowerPerVolume, and indicates that PressurePerVolume is more significant than the PowerPerVolume variable. At a given power value, the K_La changes dramatically as PressurePerVolume is increased. From these contour plots, it is observed that PowerPerVolume is less significant than the variables of PressurePerVolume and AreaPerVolume. This conclusion is also supported by the mathematical model: the coefficients for AreaPerVolume and PressurePerVolume are greater than the coefficient for PowerPerVolume.

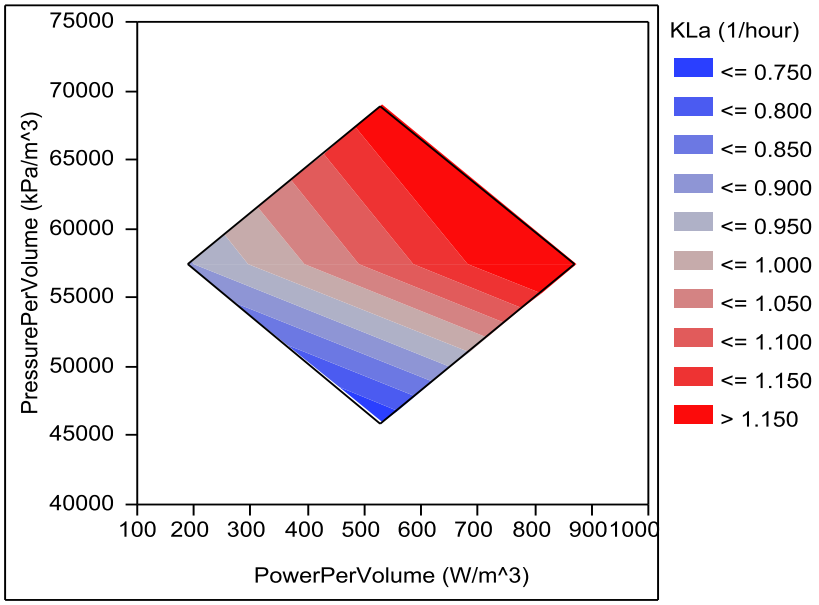

Figure 12. Contour plot for K_La for interaction of PressurePerVolume and PowerPerVolume with AreaPerVolume=12.6 m^2/m^3

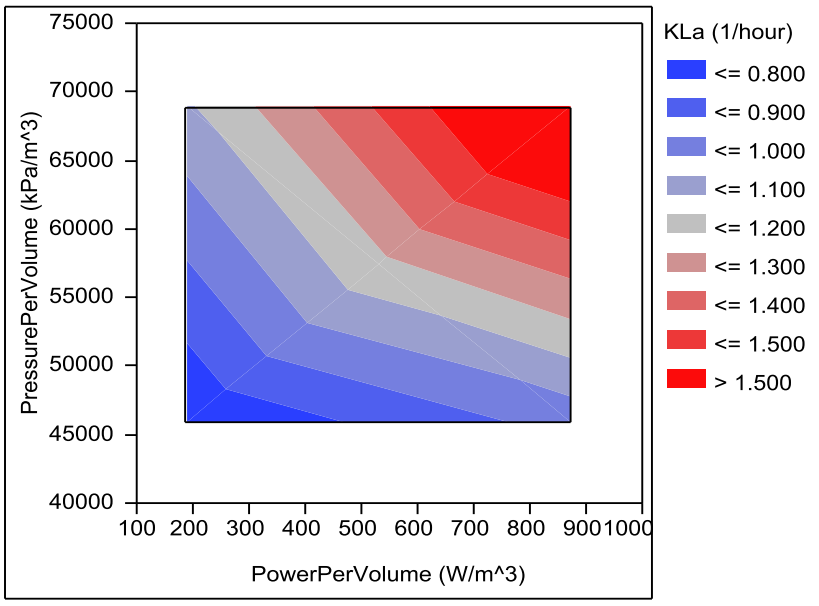

Figure 13. Contour plot for K_La for interaction of PressurePerVolume and PowerPerVolume with AreaPerVolume=25.15 m^2/m^3

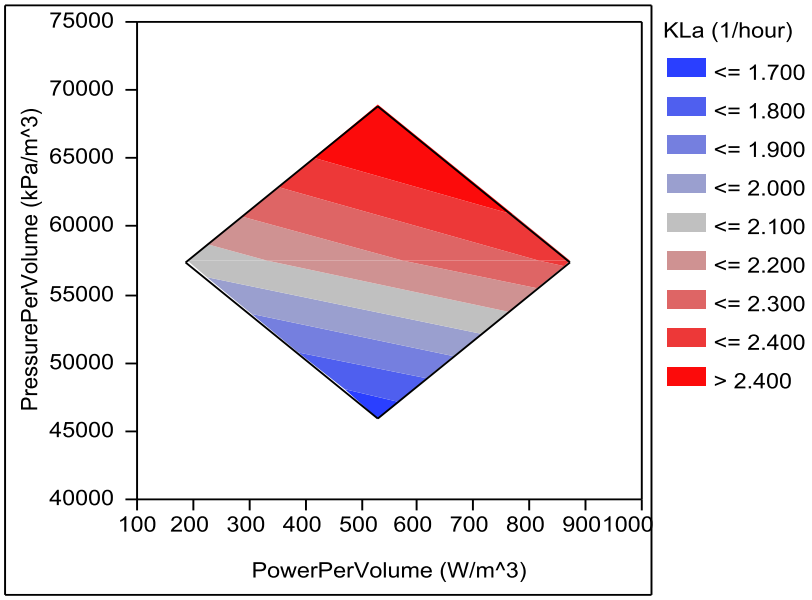

Figure 14. Contour plot for K_La for interaction of PressurePerVolume and PowerPerVolume with AreaPerVolume=37.7 m^2/m^3

These three variables and their interactions are also displayed graphically in Figure 15, the interaction profile plot for the simplified model. This plot shows how the K_La value is affected by each variable. The AreaPerVolume and PressurePerVolume plots display a second-degree trend, while the PowerPerVolume plots are more linear. This is reflected in the mathematical model (equation 6), as both variables $(A_M/V)^2$ and $(P/V)^2$ are included in the model. The lower statistical significance of the PowerPerVolume variable is also shown in the AreaPerVolume and PressurePerVolume plots which display the trends at the extremes of the PowerPerVolume variable (189 to 874). The increase in PowerPerVolume between these two curves does not have a significant effect on the K_La value determined from the plot.

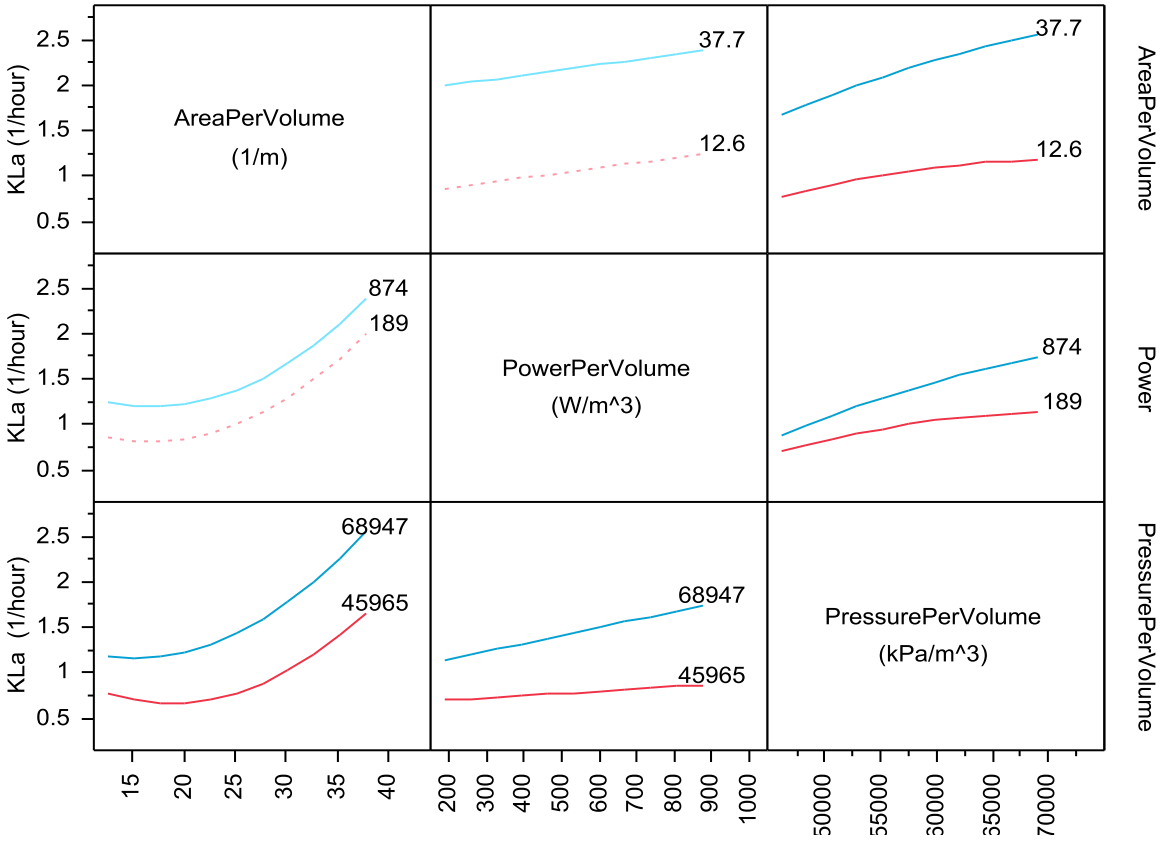

Figure 15. Simplified model variable interaction profile

Finally, the K_La data can be summarized by surface contour plots. These plots compare two variables and the resulting K_La value in a three-dimensional surface. Therefore, the full range of the two compared variables can be displayed on a single plot. PressurePerVolume is compared to AreaPerVolume in Figure 16, PowerPerVolume is compared to AreaPerVolume in Figure 17, and PressurePerVolume is compared to PowerPerVolume in Figure 18. The most notable surface plot among these three figures is Figure 16, which again indicates the significance AreaPerVolume has, and the relative insignificance of the PowerPerVolume variable; at a given PowerPerVolume value, AreaPerVolume changes much more drastically than PowerPerVolume changes at a given AreaPerVolume value.

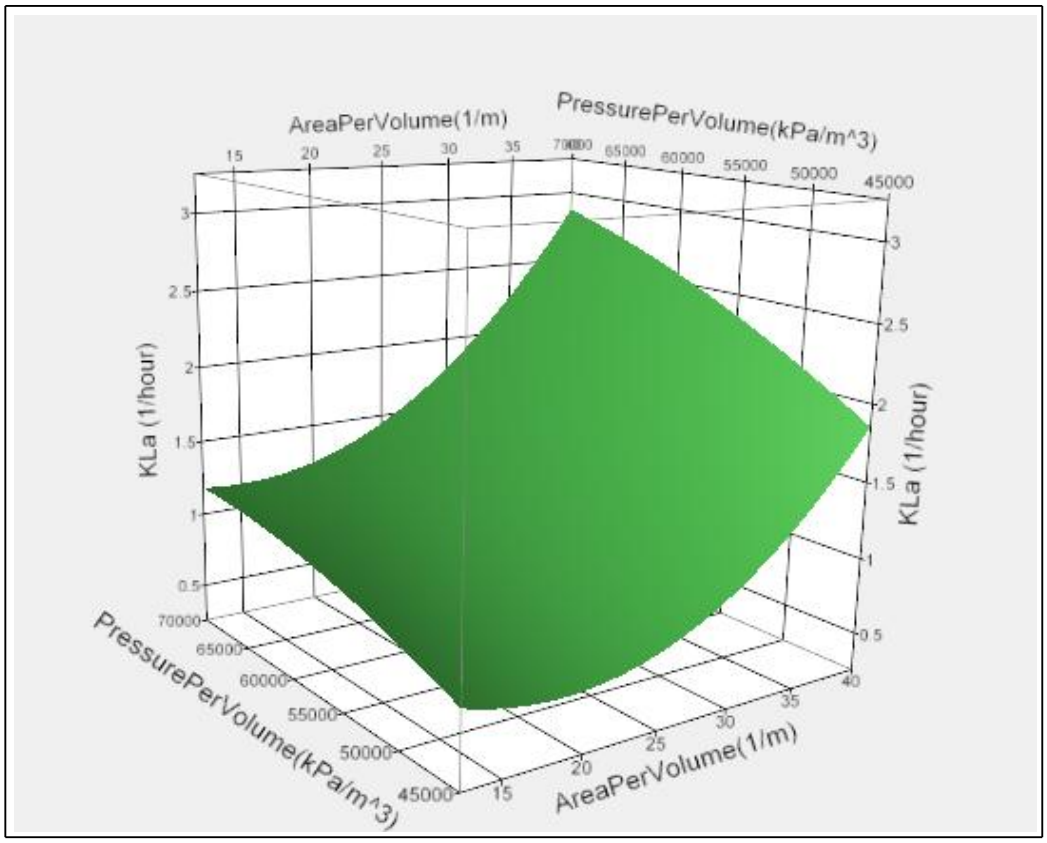

Figure 16. Response surface simplified model PowerPerVolume: 531.5 W/m^3, AreaPerVolume: 25.15 m^2/m^3, PressurePerVolume: 57456 kPa/ m^3

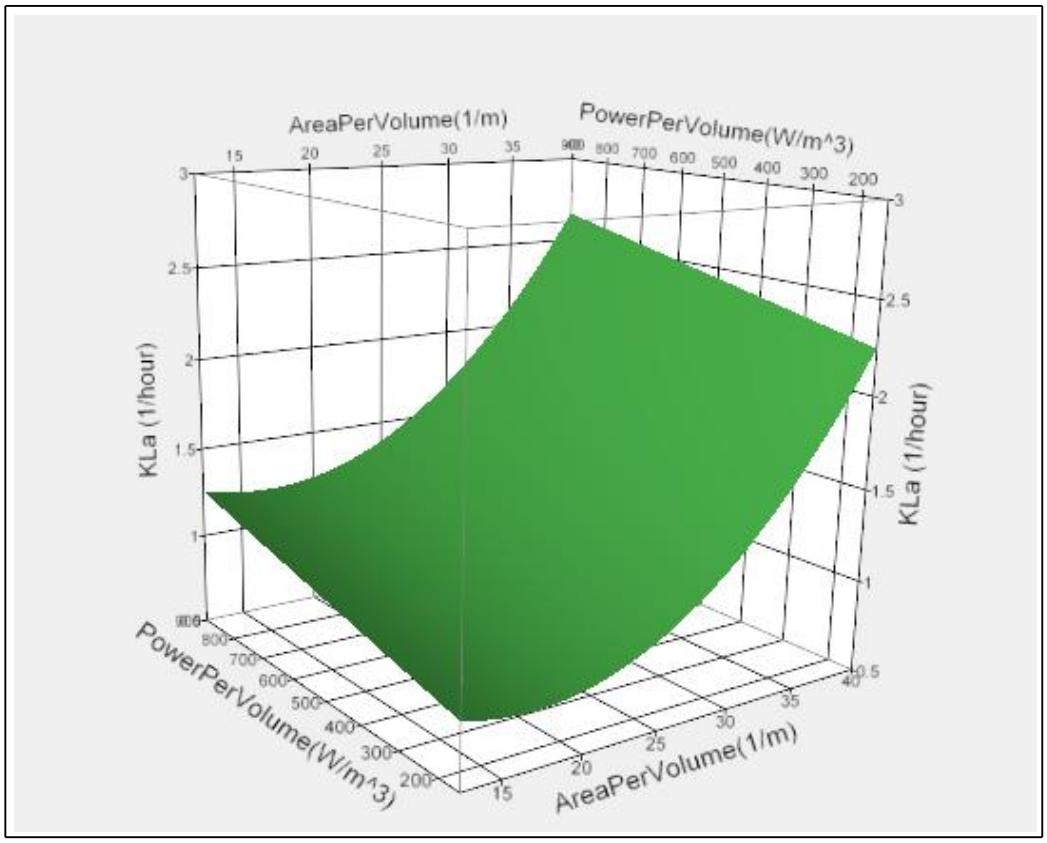

Figure 17. Response surface simplified model PowerPerVolume: 531.5 W/m^3, AreaPerVolume: 25.15 m^2/m^3, PressurePerVolume: 57456 kPa/ m^3

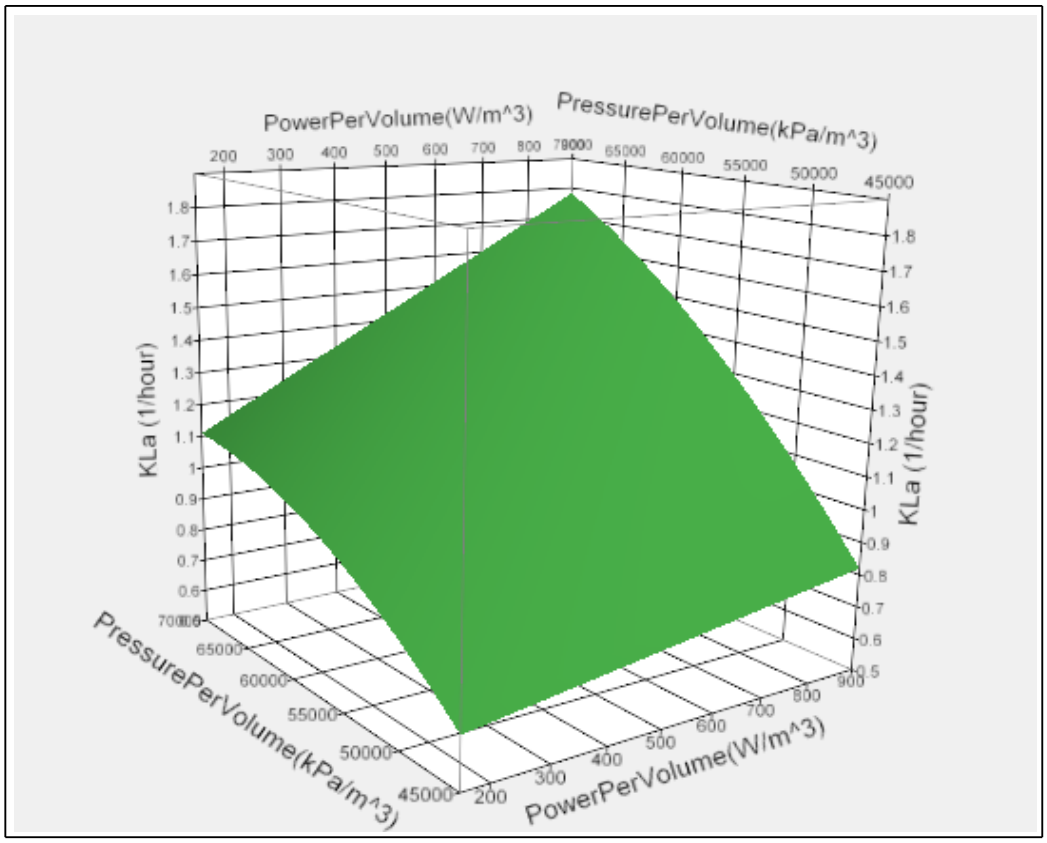

Figure 18. Response surface simplified model PowerPerVolume: 531.5 W/m^3, AreaPerVolume: 25.15 m^2/m^3, PressurePerVolume: 57456 kPa/ m^3

Conclusions

Oxygen transfer was quantified by determining the K_La value at varying reactor conditions. The gas mass transfer study successfully compared the influence of three variables (AreaPerVolume, PowerPerVolume, and PressurePerVolume) on the gas mass transfer response (K_La); a mathematical model representing these relationships represents their interaction. It was found that the variables AreaPerVolume and PressurePerVolume have a more significant effect on K_La determination than the mixing PowerPerVolume variable.

For partial nitrification, the oxygen transfer must be limited. The oxygen transfer results from this study indicate a K_La value ranging 0.71 to 2.59 h^{-1}, depending on reactor conditions. For fine bubble diffusers used in activated sludge treatment, K_La is reported to be 16.2 h^{-1}.[17] This lower oxygen transfer coefficient will assist in the oxygen limiting selection pressure to enrich ammonium oxidizing bacteria.

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CHAPTER 3: PARTIAL NITRIFICATION COMMUNITY SELECTION UTILIZING HOLLOW FIBER MEMBRANE AERATION

Modified from a paper to be submitted to *Environmental Science & Technology*

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Abstract

A hollow fiber membrane reactor was employed to create a partial nitrification reactor as a pre-treatment system for ANAMMOX nutrient removal. Using a silicone membrane to limit oxygen transfer, a biofilm treatment system was created, with biomass attaching on the membrane surface. The system operated at room temperature with a very low dissolved oxygen concentration (< 0.1 mg/L). Nitrite production was evident, with little nitrate produced in the system. The system treated high ammonium concentration (250 mg/L NH_4^+-N) and low ammonium concentration (50 mg/L NH_4^+-N). Ammonium oxidizing bacteria dominated the microbial community, while nitrite oxidizing bacteria were suppressed and growth was limited. Real-time polymerase chain reaction (qPCR) was employed to verify the dominance of AOB in the system. This verified that a low dissolved-oxygen condition selects for AOB, and the silicone membrane is an effective method of controlling oxygen transfer.

Keywords: Partial nitrification; Hollow fiber membrane; ANAMMOX; Selection pressure; Silicone membrane; Real-time PCR; *Nitrosomonas*

1. Introduction

Excess nutrients, particularly nitrogen, are discharged from wastewater streams directly into receiving water bodies without advanced treatment. These nutrients are causing eutrophication downstream, resulting in algal blooms and hypoxic conditions. This pollution changes aquatic communities and harms the natural ecosystem.[1] The symptoms of eutrophication are observed in 78 percent of coastal area, 50 percent of lakes, and 60 percent of rivers in the United States.[2,3] Consequently, more stringent regulation requiring greater nutrient removal is likely in the near future.

To mitigate environmental problems caused by nutrient discharge into waterways, regulators are looking to increase restrictions on point sources for nutrients. The USEPA is urging nationwide adoption of new numeric nitrogen and phosphorus discharge criteria. Upgrading existing facilities into biological nutrient removal system consisting of, at a minimum, anaerobic, anoxic, and aerobic tanks would increase treatment plant energy demands. Three percent of electricity consumption in the U.S. is from water and wastewater faculties (approximately 56 billion kWh).[4] Aeration of activated sludge secondary treatment typically accounts for 30 to 60 percent of total plant energy consumption. There is a need for developing a new sustainable, highly efficient treatment system.

Coupling a partial nitrification system with the Anaerobic Ammonium Oxidation (ANAMMOX) process would decrease energy consumption related to oxygen supply. By controlling oxygen delivery to the aerobic chamber and eliminating nitrite oxidizing bacteria,

the oxygen demands of a partial nitrification system are lower than an activated sludge system for nitrogen removal. This environmentally sustainable and cost effective system will be the most competitive solution to meet new nutrient discharge standards.

The anaerobic ammonium oxidation (ANAMMOX) process is an innovative process that oxidizes ammonium and nitrite directly to N_2 gas. The partial nitrification step can be combined with the ANAMMOX process to provide the necessary nitrite substrate. Together, ANAMMOX and partial nitrification are combined to create a more efficient nutrient removal system.

The stoichiometric ratio of the ANAMMOX reaction between ammonium, nitrite, and nitrate, is 1:1.31:0.22.[5] Most of the ammonium is converted into nitrogen gas, bypassing the formation of nitrate.

Partial nitrification has previously been achieved using a silicone membrane. Feng et al. addressed optimal alkalinity in a partial nitrification reactor. However, oxygen transfer and species microbiology were not thoroughly addressed in the study by Feng et al. The study addressed the alkalinity requirements of nitrifying organisms. The optimal alkalinity was determined to be 1500 mg/L $CaCO_3$ for 50% partial nitrification.[6] Additionally, previous studies have limited dissolved oxygen concentration to 0.5 mg/L.[6] Research on the process described in this article achieved consistent dissolved oxygen of 0.05 mg/L or less in the bulk solution, indicating lower maintained dissolved oxygen than described in the Feng et al. study.

2. System Operation

2.1 Stoichiometry

Nitrification is the process by which ammonium (NH_4^+-N) is oxidized first to nitrite (NO_2^--N) by ammonium oxidizing bacteria (AOB), and then to nitrate (NO_3^--N) by nitrite oxidizing bacteria (NOB). The conventional oxidation process is completed by the following stoichiometric reactions:

Ammonium Oxidation: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$

Nitrite Oxidation: $2NO_2^- + O_2 \rightarrow 2NO_3^-$

Complete Ammonium Oxidation to Nitrate: $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$

However, the pairing of the partial nitrification system and the ANAMMOX system yields a process which ultimately produces nitrogen gas and water, as described by the following reaction:

Anaerobic Ammonium Oxidation to Nitrogen gas:

$NH_4^+ + 1.31NO_2^- + 0.0425CO \rightarrow 1.045N_2 + 0.22NO_3^- + 1.87H_2O + .09OH^- + .0425CH_2O$

2.2 Selection Pressure

Isolating specific microorganisms improves operational efficiency. Unlike a mixed-culture system which requires excess aeration and retention time, an organism-specific system can be optimized to meet the needs of the target organism. The reactor operation was controlled in a manner that encouraged the dominance of ammonium oxidizing bacteria (AOB); nitrite oxidizing bacteria (NOB) were suppressed. This allowed for the accumulation of nitrite (NO_2^-) in the reactor because nitrite was not being oxidized by NOB to nitrate.

Previously, the SHARON process has been effectively employed to partially nitrify ammonium to nitrite, as a pre-treatment for the ANAMMOX process. This process operates

above 25°C, often operating between 30-40°C. SHARON treats wastewaters with high ammonium concentrations (above 500 g NH_4^+-N / L).[7]

The selection pressure for AOB was the low dissolved oxygen condition. The partial nitrification system was operated at anoxic conditions, with a dissolved oxygen concentration below 2.0 mg/L. This low DO condition encourages AOB growth because AOB have a higher affinity for oxygen.[8] The oxygen saturation coefficient values are within the range of 1-15 μM O_2 for AOB and and 22-166 μM O_2 for NOB.[9] The values for oxygen saturation coefficient in research performed by Ciudad et al. differs slightly: 30.94 μM O_2 for AOB and 43.75 μM O_2 for NOB (0.99 and 1.4 g O2/m3, respectively, as reported by the authors).[10]

Denitrification, the conversion of NO_3^- to nitrogen gas, operates best at low dissolved oxygen concentrations. Heterotrophic denitrification requires organic material to serve as an electron donor.[8] To discourage the growth heterotrophic organisms, the synthetic wastewater used in this study included very little organic carbon (see

Table **6**). Ammonium oxidizing bacteria are autotrophic organisms (genus *Nitrosomonas*), and therefore utilize an inorganic carbon source.

2.3 Hollow fiber membrane selection

A silicone membrane was selected for oxygen delivery based on its physical characteristics. Air diffuses through the silicone membrane and forms small bubbles on the silicone surface. The silicone membrane provides an aeration surface for oxygen transfer, without bubble release from the membrane, unlike a typical air-sparging aerator. A bubbleless reactor is thus created, and biomass is not blown away from the membrane by the air bubbles. This mechanism allows biomass to attach to the silicone membrane and consume the available oxygen before the oxygen reaches the bulk solution. Therefore, bulk

dissolved oxygen remains low (less than 0.1 mg/L), while oxygen-consuming organisms grow on the silicone membrane.

Additionally, attached growth is ideal for nitrifying autotrophs. Membrane aeration systems provide available surface area for biofilm attachment; a biofilm system takes advantage of long sludge age to aid in culturing nitrifying organisms.[11] Due to the slow-growing nature of nitrifying organisms, a biofilm or granule structure allows for an increase in biomass concentration and solids retention time (SRT).[8,12] Nitrifier biofilms also attach more securely than heterotrophic biomass.[11]

Experimentally, the biomass yield of fixed-film nitrification processes ranges from 0.044 to 0.097 g SS/ g NH_4^+-N.[11] Heterotrophic organisms for decomposition of organic compounds have a yield of 0.40 mg VSS/ mg OD.[13] The lower yield of nitrifier organisms indicates that heterotrophs must be eliminated for autotrophic nitrifiers to dominate and perform partial nitrification.

2.4 Microbial community analysis

Based on the observed results from experimental tests, the presence of high nitrite concentrations indicates the dominance of ammonium oxidizing bacteria. It is also assumed that little nitrite oxidation occurs. The experimental data support the assumption of an effective selection pressure favoring ammonium oxidizing bacteria. The theoretical selection pressure of oxygen affinity also supports the claim of ammonium oxidizing bacteria dominance, as explained earlier.

To verify these assumptions, a microbiological assessment, real-time PCR (polymerase chain reaction), was conducted on biomass samples collected from the partial nitrification reactor. Analysis has also been performed in previous research using standard PCR,

fluorescence in-situ hybridization (FISH) analysis, and terminal restriction fragment length polymorphism (T-RFLP).[14] Real-time PCR was selected based on accuracy of the test and efficiency of the lab procedures.

Previous studies have used PCR to identify ammonium oxidizing organisms. There are two approaches outlined in previous studies. One approach is to identify DNA sequences in the 16S rRNA which are specific for nitrifier genera; the other approach is to identify genetic material representing the genes that oxidize ammonium or nitrite. Both DNA target approaches were used in this research. The gene representing ammonium oxidation is the ammonium monooxogenase gene (*amoA)*, which is found in both bacteria and archaea. The gene representing nitrite oxidation is the nitrite oxioreductase gene (*nxrA)*. In the natural environment, AOB only account for 0.1% of the total bacterial community. Additionally, non-extremophilic archaea have been detected.[15] AmoA is the enzyme responsible for catalyzing the rate-limiting step in bacterial ammonium oxidation. It is therefore a reliable molecular marker for studying the AOB community.

The DNA amplicon size amplified by PCR in the 16S rRNA studies for *Nitrosomonas europea* and *Nitrobacter* genus was too long to use in the real-time qPCR analysis performed in this research. Therefore, the nitrifier-specific gene selection method was chosen for community analysis. The nxrA gene has not been sequenced in the *Nitrospira* genus of NOB; therefore, the 16S rRNA sequence was used to identify this organism.

Mintie et al. (2003) performed a study employing PCR and T-RFLP analysis to examine ammonium oxidizing bacteria in soil. The PCR thermal cycling program utilized was adopted from this Mintie et al. study.

3. Materials and Methods

3.1 Partial nitrification reactor configuration

The reactor configuration for the partial nitrification study employed a long glass tube reactor with a recirculation reservoir for sampling and monitoring pH and dissolved oxygen. The reactor is 122 cm long, 2.5 cm outer diameter, and 2.29 cm inner diameter, for a reactor volume of 500.4 mL. The reservoir provides 716 mL of additional volume. The reactor incorporates a single silicone membrane fiber. The silicone membrane is the length of the reactor (122 cm), and has an outer diameter of 2.0 mm, and a wall thickness of 0.25 mm. A recirculation pump cycled the wastewater to be treated through the membrane reactor and into the reservoir. This reactor configuration is seen in Figure 19 below.

Compressed air was applied to the silicone membrane at 137.9 kPa (20 psi). The manufacturer of the silicone membrane (Fuji Systems Corporation, Tokyo Japan) provided the following equation to determine flow rate across the silicone membrane:

$$\frac{K \cdot A \cdot (P - P')}{L}$$

The gas flowrate is calculated in mL gas per second. K is a unitless constant dependent on the gas supplied (K=50 for O_2 gas, K=25 for N_2 gas), L is the thickness of the silicone membrane (cm), A is the available membrane area (cm^2), and (P-P') is the difference in partial pressure (cmHg). Based on the supply pressure of 137.9 kPa (20 psi) and a membrane length of 122 cm, the oxygen flow rate was determined to be 9.458×10^{-14} mL/sec, or 5.675×10^{-12} ml/min.

The calculated flowrate is quite low. The low flowrate of the gas permeation through the silicone membrane creates an environment which is ideal for maintaining a low dissolved oxygen in the bulk solution. As gas is diffused, small bubbles form on the surface of the

membrane. This creates a system which can be classified as "bubbleless." Few previous partial nitrification studies have been performed using a bubbleless system. Previously, researchers have controlled the gas flowrate. In this research, the system was operated at a constant pressure, allowing for consistent operation of the system, and eliminating the need for aeration control.

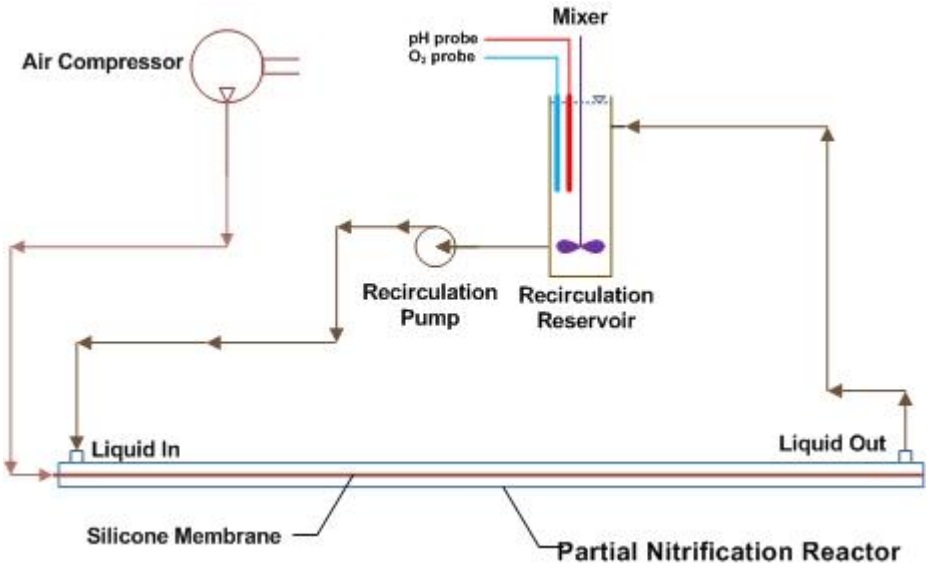

Figure 19. Partial nitrification reactor configuration

3.2 Water quality analysis and data collection

Dissolved oxygen was measured using the Mettler Toledo O2 4100e probe and the Mettler Toledo O2 4100 meter. The pH was measured using the Mettler Toledo 405-DPAS-8C-K8S/328 pH probe and the Mettler Toledo pH 2100 meter. pH and dissolved oxygen values were recorded on a twenty second interval using a National Instruments computer interface, and National Instruments LabView software. Ammonium data were collected using an Orion 95-12 ammonia selective probe according to procedure 4500 – NH_3

D. Ammonia-Selective Electrode Method of the Standard Methods for the Examination of Water and Wastewater.[16] Nitrite and nitrate were tested using a Hach DR3000 spectrophotometer using the NitriVer2 (ferrous sulfate method) and NitraVer5 (cadmium reduction method) reagents for 10 mL samples according to the DR/3000 testing procedures provided by the Hach Company.

3.3 Reactor startup and cycling

The reactor was inoculated with concentrated activated sludge from the Boone wastewater treatment plant in Boone, Iowa. The reactor was operated at room temperature, varying from 18 - 22° C.

High-strength synthetic wastewater was fed into the reactor to encourage nitrifier growth. Because the synthetic wastewater contained little organic carbon and high ammonium, the synthetic wastewater encouraged nitrifier growth, and discouraged denitrifier growth. The alkalinity in the synthetic wastewater was adjusted to account for alkalinity consumption by the ammonium oxidation process; AOB nitrifiers consume 7.41 g of alkalinity per gram of NH_4^+-N. Yeast extract was added as a micro-nutrients supplement and to determine if autotrophs could dominate the culture, despite a low level of COD available for heterotrophic growth. The diluent for the synthetic wastewater was tap water from the City of Ames, Iowa. The synthetic wastewater is summarized in

Table 6 below.

Table 6. Synthetic wastewater composition

NH_4HCO_3	0.249 - 1.410	g/L
$KHCO_3$	0.156 - 0.410	g/L

$NaHCO_3$	0.100 - 0.360	g/L
KH_2PO_4	0.050 - 0.150	g/L
Yeast Extract	0.015 - 0.040	g/L
Na_2HPO_4	0.030 - 0.090	g/L
$MgSO_4$	0.040 - 0.100	g/L

The reactor was operated as a batch process. The reactor liquid media was replaced by draining the recirculation reservoir and refilling with synthetic wastewater. Hydraulic retention time was varied based on experimental data collected for ammonium, nitrite, and nitrate. However, during the inoculation phase the HRT was approximately 40 hours. During low-ammonium testing, the HRT was decreased to 24 hours. Solids retention time (SRT) is approximated as infinite because sludge was not removed from the system, and the decant phase of reactor cycling was preceded by reservoir settling, minimizing solids loss during decant.

3.4 DNA extraction

DNA for qPCR analysis was extracted from biomass samples using a phenol/chloroform extraction procedure adopted from Cheng and Jiang (2006).[17] Biomass was suspended in 2mL of water and centrifuged at 8,000 *g* for 5 minutes. The biomass pellet was washed twice with 400 uL of STE buffer (100 mM NaCl, 10 mM Tris/HCl, and 1mM EDTA), centrifuged at 8,000 *g* for 2 minutes, and then resuspended in 200 uL of 1X SSC buffer. 100uL of Tris-saturated phenol was added, and the sample was mixed to lyse cells. The product was centrifuged at 13,000 *g* for 5 minutes, and 160 mL of the aqueous supernatant was transferred to a clean centrifuge tube. 40 uL of 1X SSC buffer and 100 uL of chloroform were added. This mixture was centrifuged at 13000 *g* for 5 minutes, and the aqueous supernatant was again transferred to a clean tube. 40 uL of 1X SSC buffer and 5 uL

of RNase were added, and the DNA was incubated at 37° C for 10 minutes. After incubation, 100 uL of chloroform was added, and samples were mixed and centrifuged at 13000 *g* for 5 minutes. 150 uL of the aqueous supernatant (containing the recovered DNA) was transferred to a new tube.[17] After DNA extraction, the DNA quantity in each sample was determined, and all samples were diluted to a standard concentration of 125 ng/µl. This concentration was used for the real-time PCR analysis.

3.5 Primer selection

The real-time PCR procedure requires specific DNA primers which isolate DNA sequences for amplification. Previous studies on nitrifier genes provided guidelines for primer selection. The bacterial *amoA* gene was isolated using the forward primer amoA-1F (5'-GGGGGTTTCTACTGGTGGT-3') and the reverse primer amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3').[14] The *nxrA* gene was isolated using the forward primer F1norA (5'-CAGACCGACGTGTGCGAAAG-3') and the reverse primer R1norA (5'-TCYACAAGGAACGGAAGGTC-3').[18] The *Nitrospira* genus was isolated using the forward primer EUB338f (5'-ACTCCTACGGGAGGCAGC-3') and the reverse primer Ntspa685r (5'-CGGGAATTCCGCGCTC-3').[19] The real-time PCR protocol employed a SYBR Green master mix for analysis. The thermal profile used consisted of: 10 min at 95° C; 30 cycles of 90 s at 95° C, 90 s at 55° C, and 90 s at 72° C; and a final elongation step of 15 min at 72° C.

4. Results and Discussion

The system effectively operated under low dissolved oxygen (DO) conditions. DO was not detected over 0.04 mg/L. This low dissolved oxygen concentration is essential if the

effluent of the partial nitrification is to be used as a substrate for the ANAMMOX process; the ANAMMOX process is inhibited at dissolved oxygen concentrations as low as 0.5% the air saturation concentration.[7]

At the beginning of the reactor operation, the system contained a mixed culture of heterotrophs and autotrophs. After 6 days of reactor operation, the controlled aeration did not provide sufficient oxygen to support nitrifier dominance (as evidenced by Figure 20 below). It is assumed that the mixed culture consisted mostly of heterotrophs in a primarily anoxic environment at this initial seeding stage. Dissolved oxygen was supplied locally at the membrane surface, but the bulk dissolved oxygen concentration was low.

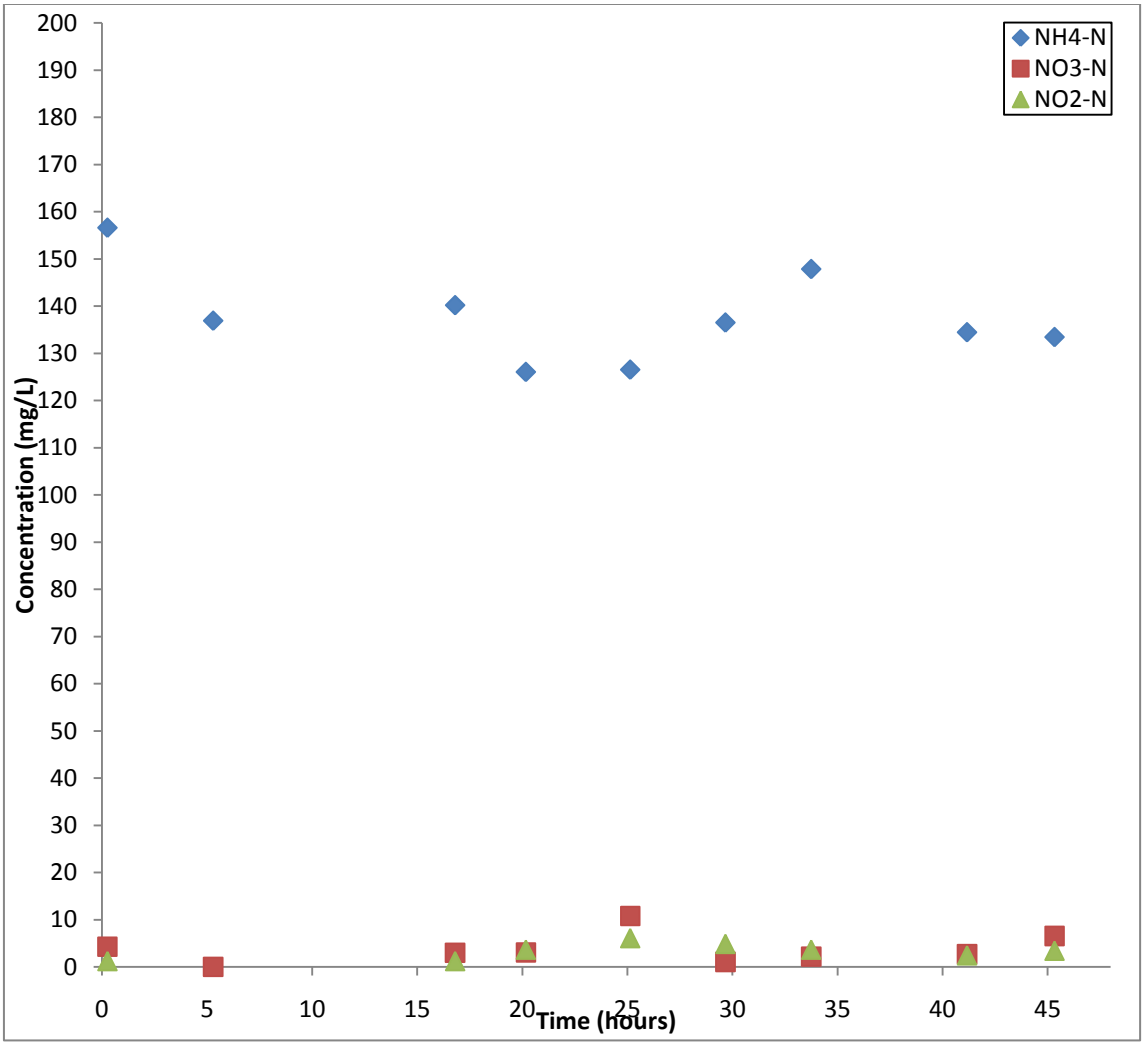

Figure 20. Nitrogen profile of partial nitrification reactor after 6 days of operation. ♦: NH_4^+-N; ■: NO_3^--N; ▲: NO_2^--N

After 14 days of reactor operation, ammonium was oxidized, but little nitrite and nitrate was detected in the reactor. Because bulk dissolved oxygen was low and endogenous decay provided organic carbon, it can be assumed that denitrification converted nitrate to N_2 gas, causing total nitrogen in the system to decrease as N_2 was released from the system. This decrease in ammonium can be seen in Figure 21 below.

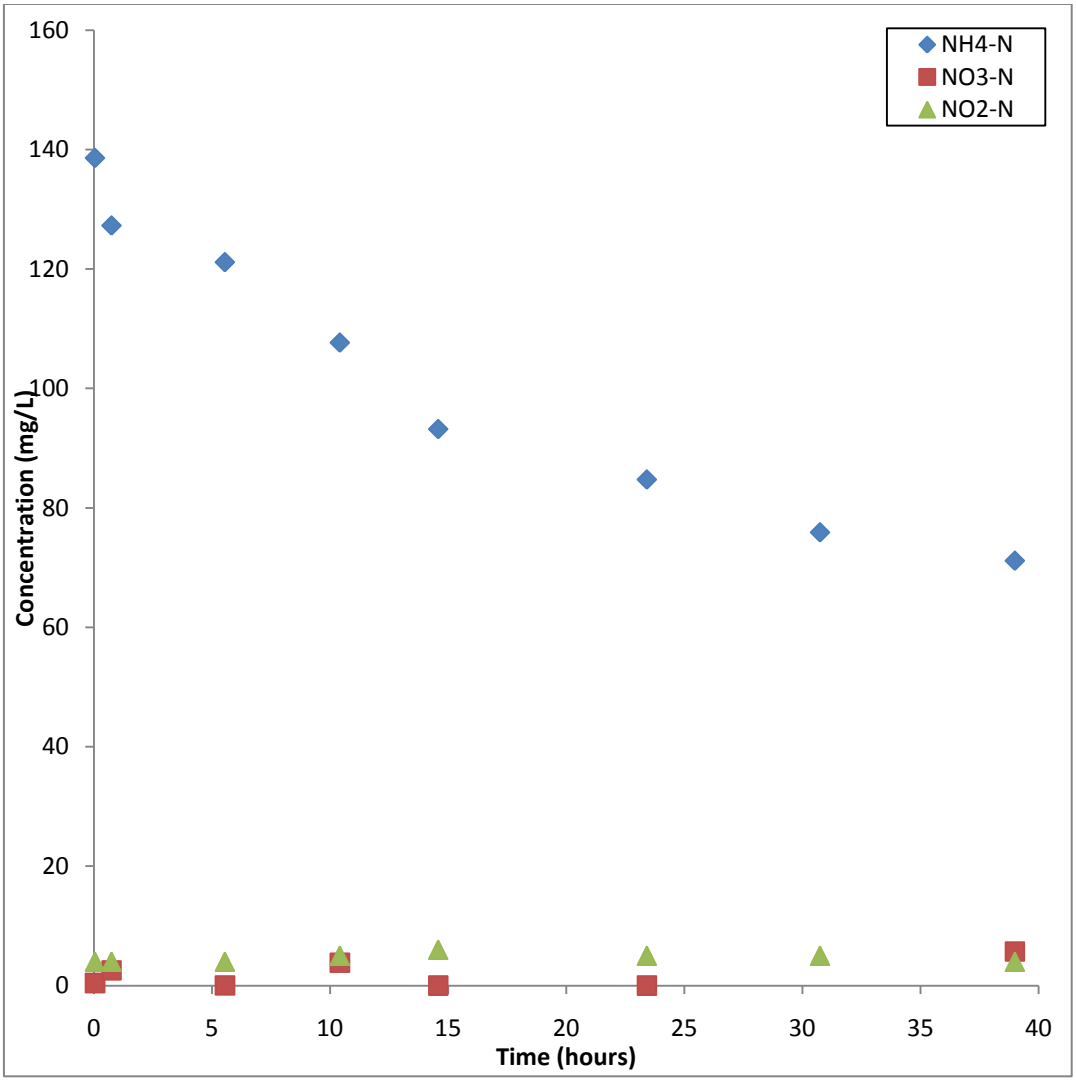

Figure 21. Nitrogen profile of partial nitrification reactor after 14 days of operation. ♦: NH_4^+-N; ■: NO_3^--N; ▲: NO_2^--N

After 26 days of operation, the reactor produced a significant amount of nitrite after 40 hours of operation. Increased influent ammonium concentration aided in the nitrifier selection process. Nitrite concentrations above 180 mg/L NO_2^--N were observed at the end of the reactor cycle. This trend is shown in Figure 22 below.

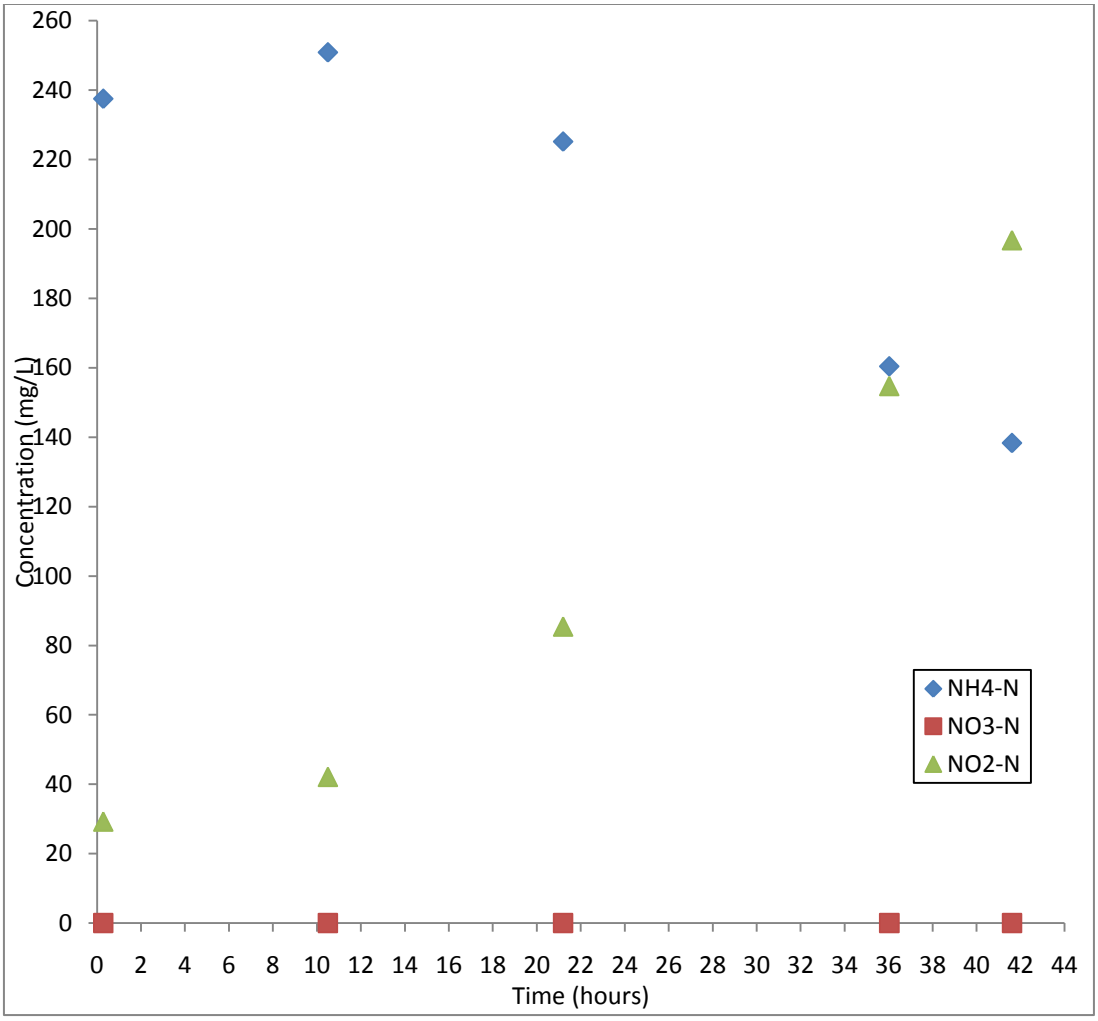

Figure 22. Nitrogen profile of partial nitrification reactor after 26 days of operation. ♦: NH_4^+-N; ■: NO_3^--N; ▲: NO_2^--N

High influent ammonium concentrations are found in industrial applications, landfill leachate, and anaerobic digester supernatant. However, domestic wastewater contains 50 mg/L NH_4^+-N or less. After successful selection of nitrifying organisms and effective production of nitrate at a high ammonium concentration, the system was tested at a low influent ammonium concentration. HRT was decreased to 24 hours, and effective

ammonium oxidation and nitrite production were possible with minimal production of nitrate (Figure 23 below).

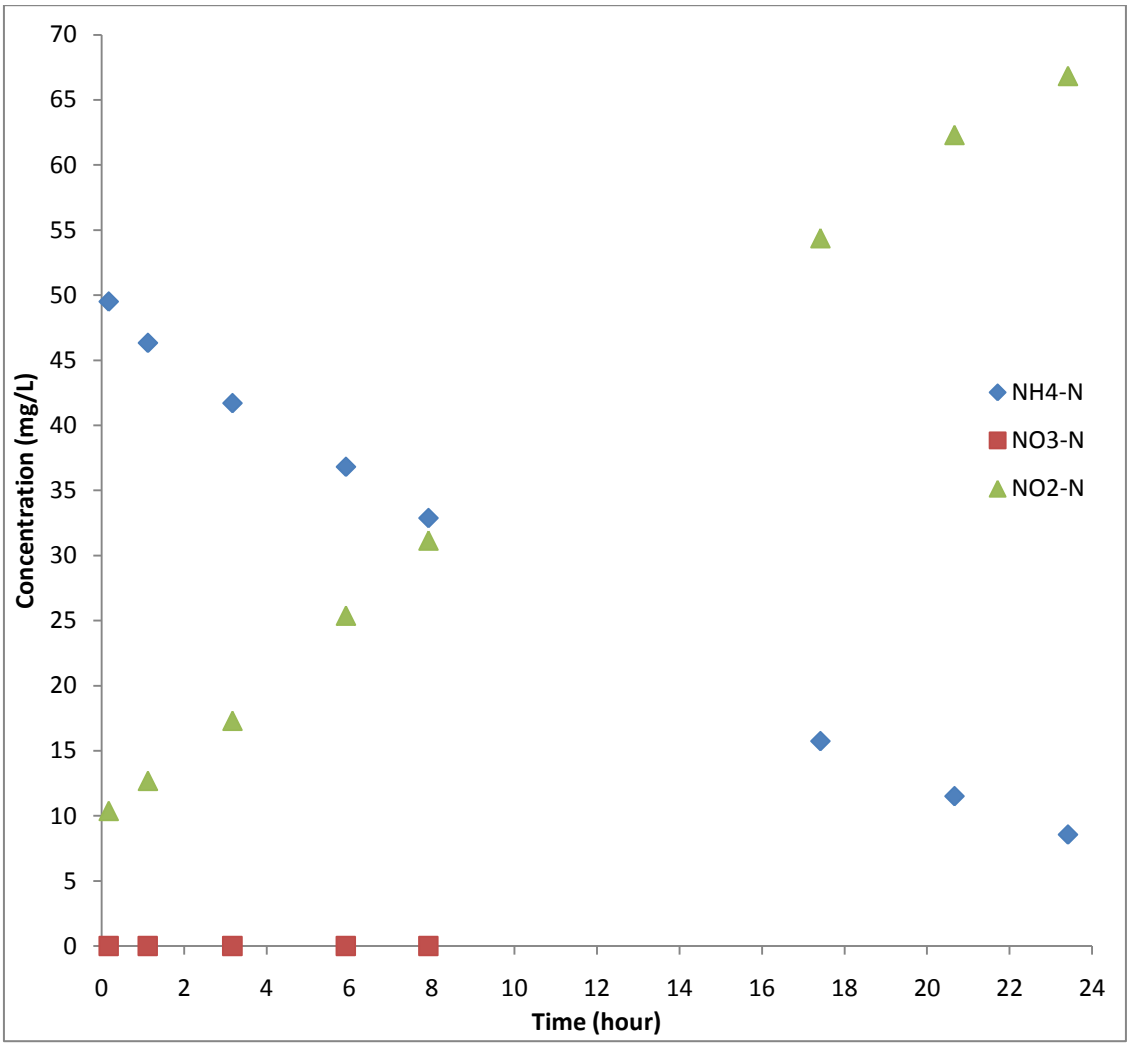

Figure 23. Partial nitrification at low influent ammonium concentration ♦: NH_4^+-N; ■: NO_3^--N; ▲: NO_2^--N

The attachment of the ammonium oxidizing bacteria on the silicone membrane surface was a topic of interest. To investigate this, sections of the silicone membrane with the developed attached biofilm were analyzed by the Microscopy and Nanoimaging Facility at Iowa State University. Scanning electron microscopy was employed to view microorganism attachment

on the silicone surface. Figure 24 below shows the biomass growth attached to the surface of the silicone membrane at 200x magnification. Individual nitrifying organisms cannot be seen at this resolution.

Figure 25 shows individual nitrifying organisms attached to the silicone membrane surface at 10,000x magnification. A closer look is found in Figure 26. Even at 20,000x magnification, pores could not be identified in the silicone membrane. Individual organisms are shown attached to the top of this smooth silicone surface, indicating that attachment is quite delicate.

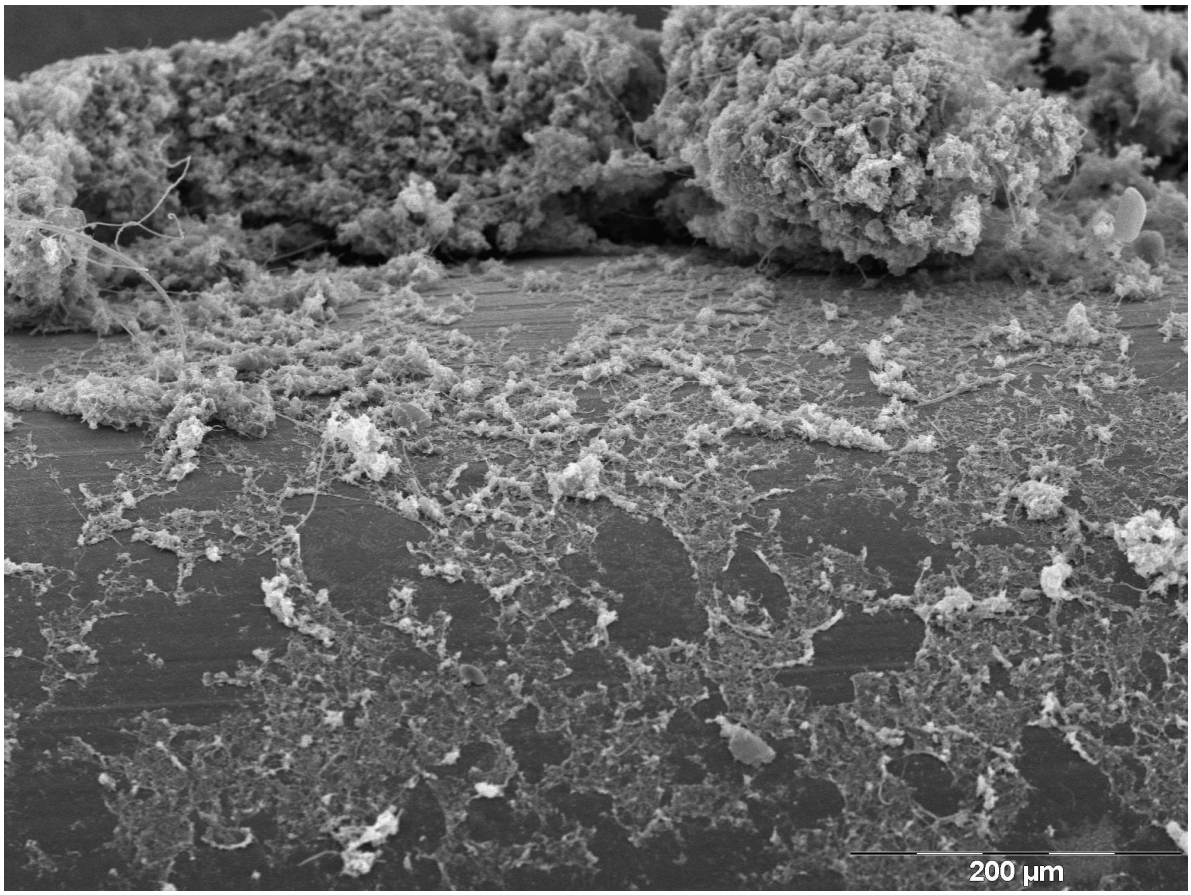

Figure 24. 200x scanning electron microscope magnification of biomass attached to silicone membrane surface.

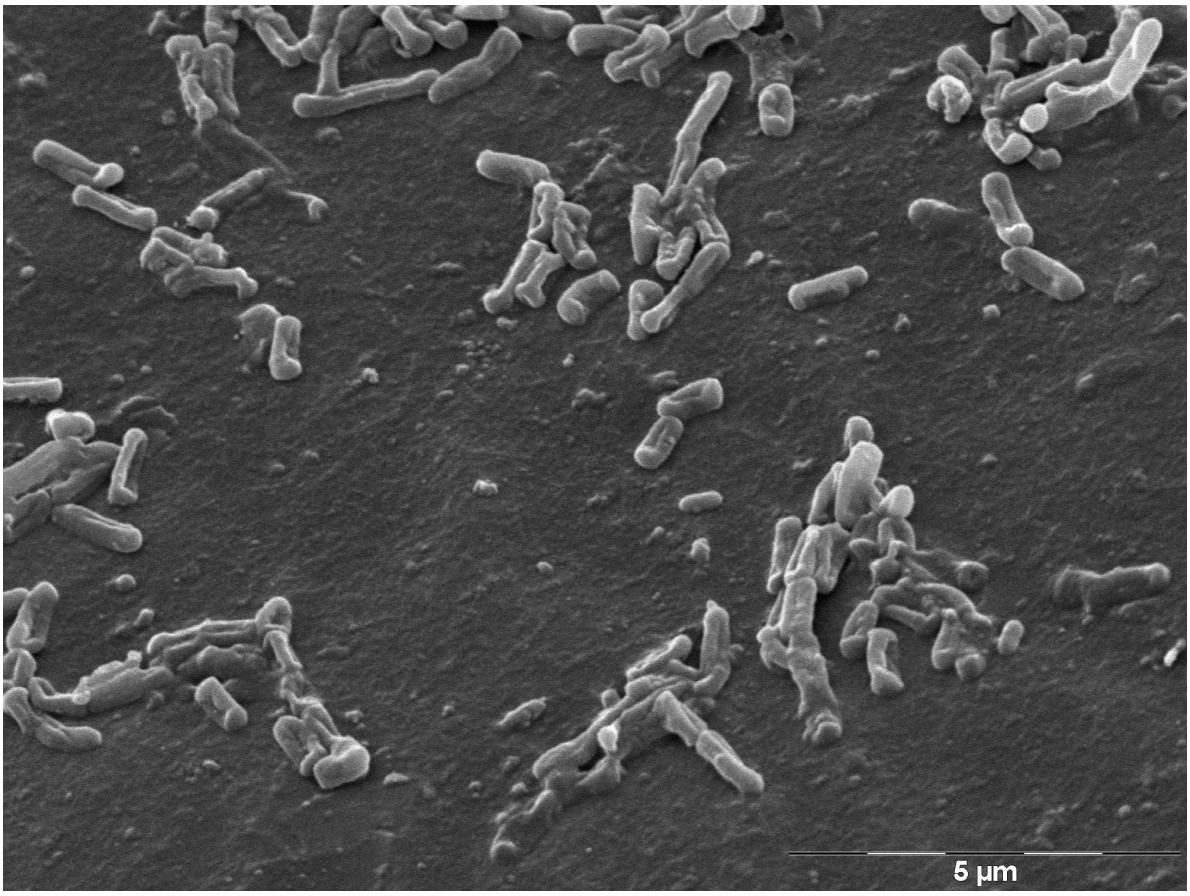

Figure 25. 10,000x scanning electron microscope magnification of individual organisms attached to silicone membrane surface.

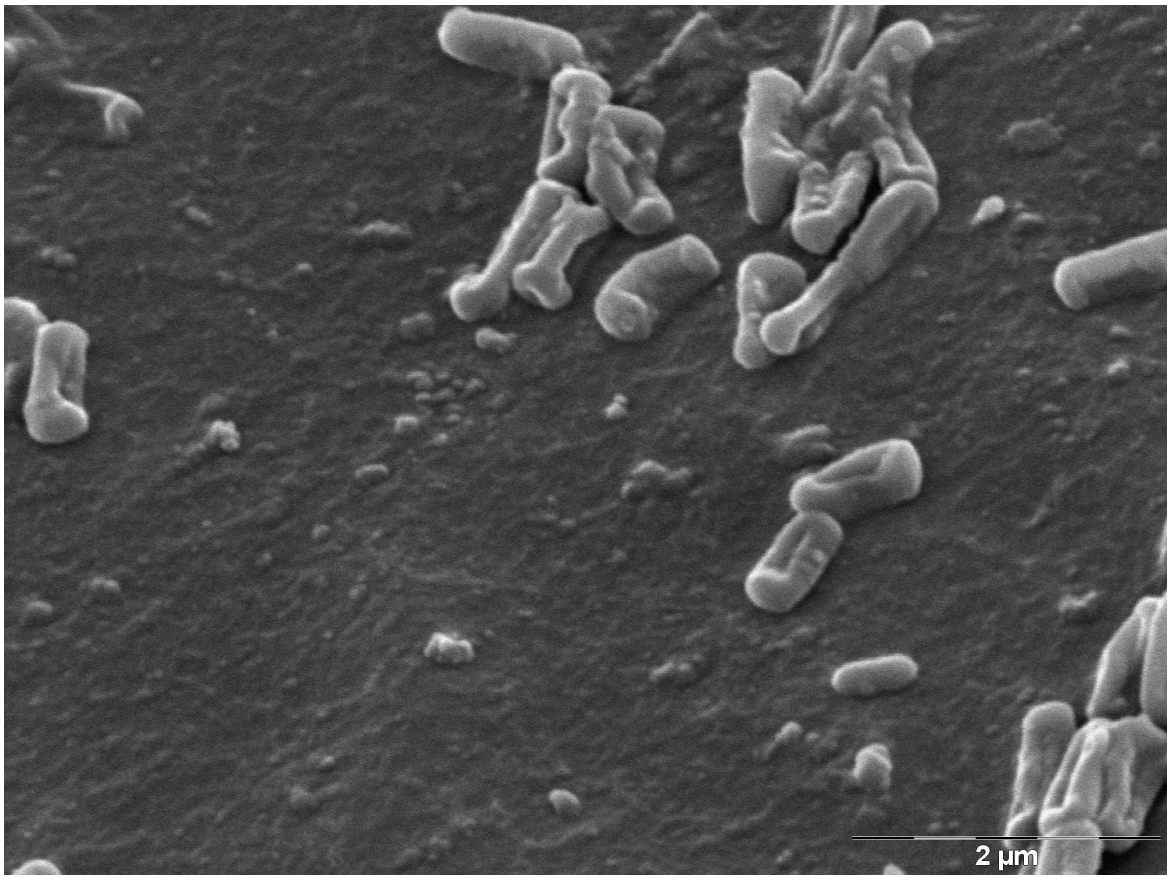

Figure 26. 20,000x scanning electron microscope magnification of individual organisms attached to silicone membrane surface.

The real-time PCR analysis concluded that ammonium oxidizing bacteria dominated the system, while nitrite oxidizing bacteria growth was suppressed. The SYBR green fluorescence detection system showed this culture enrichment by detecting fluorescence. Two samples from each day were tested using the DNA extraction and qPCR protocol outlined in the Materials and Methods. These results are observed in

Table 7 below.

Table 7. Ct values for real-time PCR analysis comparing ammonium oxidizing bacteria and nitrite oxidizing bacteria

	Nitrosomonas AOB (AmoA gene identifier)	*Nitrobacter* NOB (NorA gene identifier)	*Nitrospira* NOB (16S rRNA identifier)
Day 0 - Seed	29.96	30.50	24.29
Day 23	28.77	32.89	22.87
Day 73	27.36	33.27	22.14

This table displays the Ct values determined in real-time PCR assessment. The Ct value is the PCR cycle number at which a threshold level of fluorescence is detected. A lower Ct value indicates a higher concentration of the target organism in the culture. A higher Ct value indicates the target organism is being eliminated.

Table **7** shows that the Ct value for ammonium oxidizers is decreasing, indicating a significantly higher concentration of these organisms at day 73 than at day 0. Ct values for *Nitrobacter* nitrite oxidizing species increase, indicating these organisms are being eliminated. The increase observed in Ct value for *Nitrobacter* nitrite oxidizing organisms indicates an approximate tenfold decrease in population of these nitrite oxidizers. The Ct values for *Nitrospira* nitrite oxidizing bacteria is inconclusive; the qPCR identifier for this genus was different from the *Nitrosomonas* and *Nitrobacter* gene identifiers. Therefore, while the Ct value indicates an increase in *Nitrospira* population, it cannot be compared to the increase or decrease of *Nitrosomonas* and *Nitrobacter*.

5. Conclusions

The partial nitrification system developed in this research effectively oxidized ammonium to nitrite, without producing a significant amount of nitrate. The silicone membrane aeration system provided an attachment site for nitrifying organisms, and limited oxygen delivery to create a low dissolved-oxygen system which selected for ammonium oxidizing bacteria. Providing a long sludge age for these organisms allows them to flourish despite low biomass yield. Real-time qPCR analysis confirmed the dominance of ammonium oxidizers over nitrite oxidizers.

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CHAPTER 4: ADDITIONAL RESULTS AND CONCLUSIONS

Additional data collected

In addition to the silicone membrane system developed, another hollow fiber membrane system was implemented. This alternate system employed a polypropylene membrane bundle in a 0.7 liter reactor. A recirculation pump was installed to pump reactor solution from the bottom of the reactor to the top; a magnetic stir bar was used to mix the reactor.

This system was tested at various influent ammonium concentrations and hydraulic retention times to determine optimal conditions. Air flowrate was limited to 36.4 ml/min, and air pressure (4 psi) was lower than the silicone membrane supply pressure (20-30 psi). The system HRT was varied from 2 to 12 hours, depending on influent ammonium concentration and ammonium oxidation efficiency. The pH gradient was similar to the silicone membrane reactors, but also displayed the "ammonium valley" feature described in the Guo et al. (2009) study.[8] The system demonstrated partial nitrification capabilities, but effluent nitrates were higher than desirable. The effluent nitrate level reached 40 mg/L at the end of a 6-hour cycle, as shown in Figure 27 below. This excess effluent nitrate made this polypropylene membrane system undesirable for partial nitrification-ANAMMOX coupling application.

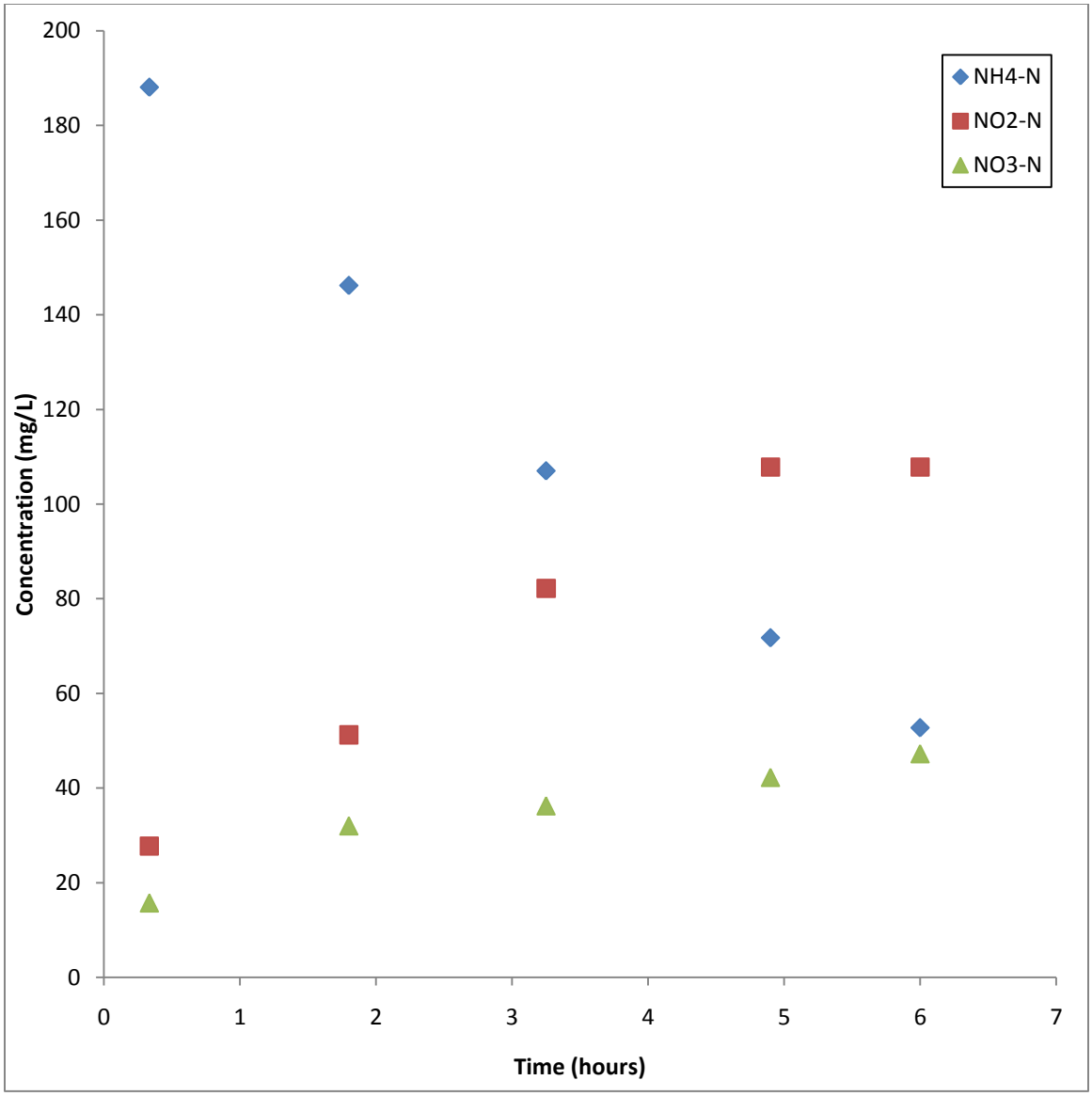

Figure 27. Partial nitrification utilizing a polypropylene membrane aeration system. ♦: NH_4^+-N; ■: NO_2^--N; ▲: NO_3^--N

The polypropylene aeration system does not limit oxygen to sufficiently suppress nitrite oxidizers. This is evidenced by higher operational dissolved oxygen in the bulk solution of this alternate reactor (0.1-0.4 mg/L O_2). However, this higher dissolved oxygen could be a

result of a broken polypropylene membrane, causing excess air to enter the aqueous solution. This polypropylene membrane reactor deserves further investigation.

Scanning electron microscopy was employed to examine the biofilm attachment to the polypropylene membrane surface. Figure 28 shows a detialed SEM photo of this biofilm attachment mechanism. The pores on the polypropylene are visible in the image, and the organisms appear to be attaching at the site of the pores, with some bacterial growth inserted into the pores.

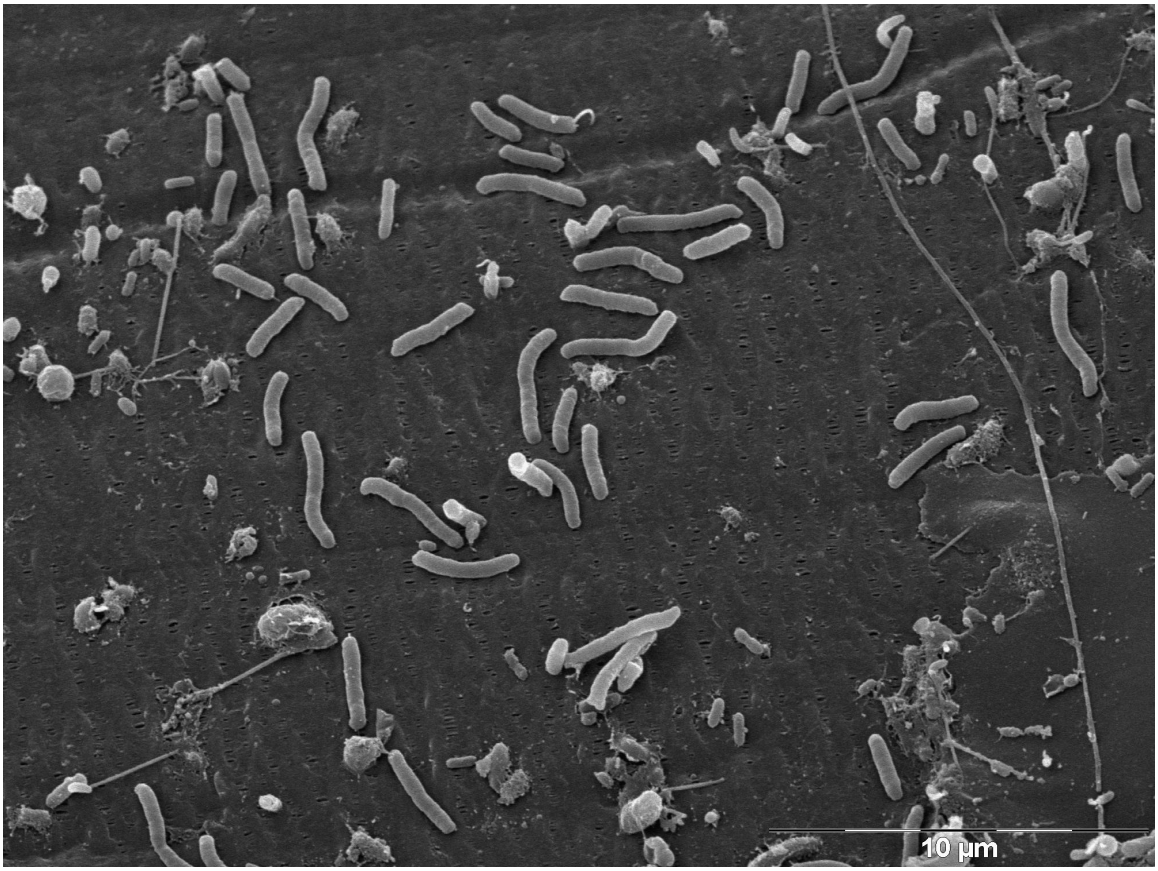

Figure 28. Scanning electron microscope image of biomass attachment on polypropylene membrane surface, 5000x magnification

Recommendations for Future Research

This research generates numerous questions which must be answered through further investigation. The kinetics of the partial nitrification study could be further investigated, especially with regard to temperature. If operated in a temperature-controlled environment, the ammonium removal rate could be correlated to operational temperature. However, if temperature is raised above 30° C, then the selection pressure changes from oxygen affinity to selection based on differential kinetics.

In order to reach the optimal ratio of 1:1.32 for coupling with the ANAMMOX system, an end-point of reaction must be determined to stop the system and cycle the SBR. Based on pH, aeration time, or alkalinity consumption, an end-point for optimal NH_4^+: NO_2^- ratio could be determined. Additionally, the effects of a fixed pH of the system could be evaluated. This may optimize the nitrification conditions, instead of allowing pH to decrease as ammonium is consumed. Alternatively, pH gradient could be utilized as an aeration end-point, or as a means of culture enrichment. For instance, the microbial community selected at various aeration cut-off points could be evaluated.

To improve removal efficiency, the density of the membranes in the reactor could be increased, thus maximizing the attached biomass in the system.

Continued investigation of hollow fiber membranes is recommended. Various materials and pore sizes could be investigated, and additional research with the polypropylene membranes is recommended.

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