COMPARING THE RESPONSES OF NITRIFIERS TO DIFFERING AMMONIA LOADINGS

BY

BLAKE W. ANDERSON

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Engineering in Civil Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Adviser:
Professor Eberhard Morgenroth

Abstract

Return active sludge from the Danville Sanitary plant (Danville, IL) was fed 10 mg NH_3-N/L per cycle of separated swine manure to allow the biomass to adapt to a new influent in a sequencing batch reactor. The biomass was then separated into three reactors. A control reactor was exposed to 10 mg NH_3-N/L . A medium stress reactor was exposed to ammonia loading increasing from 10 mg NH_3-N/L to 120 mg NH_3-N/L. A final high stress reactor was exposed to the same increasing loading as the medium stress reactor with periodic shock loads. Biomass was regularly taken from each reactor and batch respirometer tests were performed with initial ammonia loading varying from 20 mg NH_3-N/L to 550 mg NH_3-N/L. Modifications to Activated Sludge Model 3 (ASM3) were used to attempt to model the OUR curves generated, but the curves could not be fitted. Despite the inability to model the OUR curves, the biomass from each reactor was found to have qualitatively different OUR curves from each other.

TABLE OF CONTENTS

Chapter 1: Introduction…… 1

Chapter 2: Materials and Methods……3

Chapter 3: Model…… ……..9

Chapter 4: Results and Discussion……13

Chapter 5: Conclusions……25

References……26

Appendix A Combined OUR Curves for Each Respirometer Batch Test……28

Appendix B Sample pH Curve……36

Appenidx C Generating Oxygen Uptake Rate Curves……37

Appendix D Summary of Attached Files……40

Chapter 1: Introduction

The primary purpose of wastewater water treatment plants is to prevent urban wastewater from degrading local water quality. In recent years, water quality and discharge standards for both nitrogen have become more stringent necessitating additional treatment at wastewater treatment plants in order to protect and improve water quality. In order to reduce nitrogen levels in water bodies, wastewater treatment plants oxidize ammonia using nitrifying bacteria to nitrite and then nitrate. Subsequently, the nitrate is used to oxidize biologically available organic carbon which reduces the nitrate to nitrogen gas. The nitrogen gas will then bubble off because the solution with be saturated with nitrogen gas thereby reducing nitrogen available for algae and bacterial growth in surface water. Therefore, eutrophication might be prevented, and water quality might not be degraded or could improve.

One difficulty with nitrification is that ammonia oxidizing bacteria can also be inhibited by high ammonia levels which can shut down nitrogen removal in treatment plants (Pambrun et al. 2006 and Anthonisen et al. 1976). Many waste streams from industrial sources and some process streams in municipal plants can have ammonia levels above 400 mg N/L which is more than order of magnitude above most public owned treated works (Weisman 1994; Gupta and Sharma 1996). In many cases, Lower nutrient effluent limits necessitate the nitrification of these high strength waste streams which might be difficult due to substrate inhibition of ammonia oxidizing bacteria (AOB). Startups in particular might be difficult because seed biomass might have trouble adapting to high ammonia levels, but most nitrifying populations should be able to adapt to higher ammonia levels (Buitron and Gonzalex 1996 and Antileo et al. 2002).

Nitrifying cultures from animal manure and marine sediments were found to be able to adapt to high ammonia loads (Antileo et al 2002). The progression of a nitrification culture's adaption from low to high ammonia loading was not studied in detailed. The kinetic parameters of the nitrifying cultures were determined before and after acclimation to high ammonia loading, but the kinetic parameters were not determined during the acclimation process (Antileo et al 2002). Performing batch tests on nitrifying cultures during acclimation

would give information about how nitrifying biomass adapts to high ammonia levels which can be of use in startups of treatment systems.

The objective of the current study was to determine how nitrifiers adapted to high ammonia levels. It was hypothesized that the substrate inhibition of ammonia oxidizing bacteria would become negligible and that the half saturation constant of AOBs for ammonia would be unchanged. In order to determine how biomass adapts to high ammonia levels, the same biomass originating from a municipal wastewater treatment plant was independently exposed to different levels of ammonia low level, high level, and high level with ammonia shocks. The OUR curves of these different nitrifying populations were examined qualitatively and quantitatively, in order to gain insight into how biomass containing nitrifying bacteria adapts to higher ammonia levels.

Chapter 2: Materials and Methods

2.1 Substrate and Seed Biomass

A complex feed of separated swine manure from the Illinois State Farm was used as the feed to all three reactors (Walker, 2003). Separated Swine manure is raw swine manure that has had at least 99% of its solids removed via a gravity belt thickener utilizing polyacrylamide as the polymer. The resulting solution contains ammonium between 250 and 1600 mg NH_4-N/L a. The COD of the separated manure varied between 2500 and 9000 mg COD/L. The high variability of the influent was due to the changing numbers of hogs at the ISU farm and weather which affects the hogs' water intake. From days 0 to 70 of the experiment, separated manure was directly feed. After day 70, the influent was diluted to 500 mg N/L as $NH_{3\ with}$ tap water in order to have stable influent ammonia levels. The seed material for this experiment was nitrifying activated sludge taken from the Danville wastewater treatment plant taken on April 1st 2008. The initial solids concentration was 1823 mg VSS/L for the activated sludge. The sludge was exposed to influent ammonia concentration of 20 mg N/L as NH_3 at the Danville Treatment Plant.

2.2 Reactor and Operations

During this experiment, three reactors were operated. Two of the SBRs were implemented using two identical glass reactors that have a water jacket to maintain a constant temperature of 25°C (Applikon). The working volume of each reactor was 5 L. Overhead mixing and 4 vertical baffles generated mixing and prevented vortexing inside the reactor. These two reactors will be referred to as medium stressed and high stressed. A third SBR was implemented using a plastic reactor with a water jacket to maintain a constant temperature of 25°C that was constructed at the University of Illinois. Aeration was provided using aquarium pumps that were automatically controlled. Dissolved oxygen concentrations were continuously measured (WTW Oxi 340, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), and dissolved oxygen concentrations were automatically controlled between 6 and 8 mg O_2/L by on/off aeration. The pH was measured via a pH probe (WTW SenTix 41-3, -Technische Werkstätten, Weilheim, Germany).The pH was automatically controlled by the addition of 2 M NaOH and 4 M HCl to maintain a pH between 7.2 and 7.8 The reactor setup is shown in figure 1 below. Influent, effluent, sludge withdrawal, base additions, and acid additions were fed to the reactor via peristaltic pumps (Masterflex, Cole-Parmer, Vernon Hills, IL, USA). The reactors were controlled using Labview (National Instruments, Austin, Texas).

Table 1 Comparison of the Reactors Used in This Experiment

	Control Reactor	High stressed Reactor	Medium stressed Reactor
Temperature	25°C	25°C	25°C
Working Volume	8.0 L	5.0 L	5.0 L
Water Jacket Material	Plastic	Glass	Glass
Mixing	Overhead	Overhead	Overhead
Baffles	4 built into the reactor wall	4 overhead baffles	4 overhead baffles

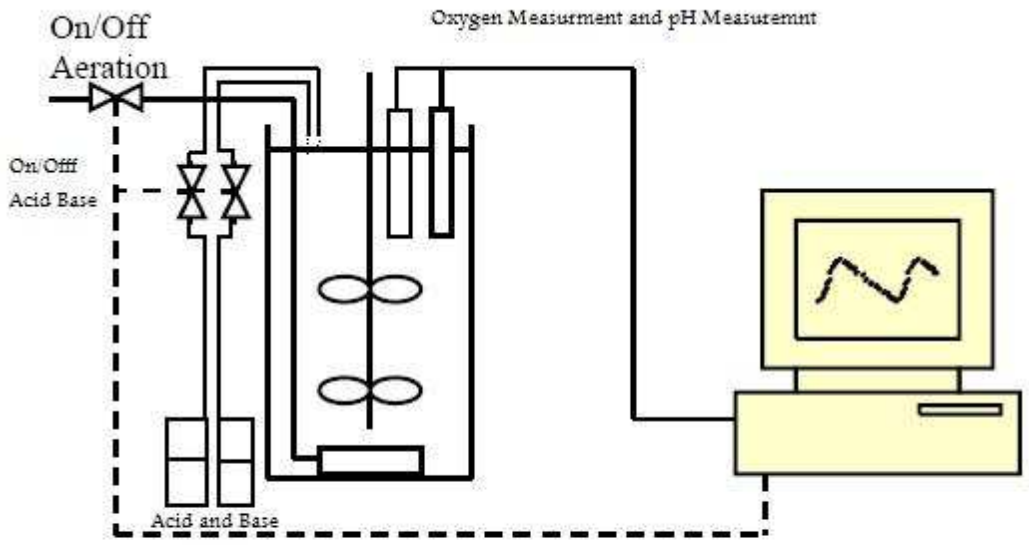

Figure 1 Reactor Diagram

The reactors were operated as sequencing batch reactors with a 6 hour total cycle time. The activated sludge SBR was operated as follows: The influent was added during the first 10 minutes of the cycle during which there was no mixing. The reactors were idle for the next ten minutes. Aeration, pH control, and mixing were on from 0.33 to 5.5 hours. Sludge was wasted between 5.25 and 5.50 hours. The reactor's biomass was allowed to settle from 5.5 to 5.8 hours. The effluent was withdrawn between 5.8 hours and 6.0 hours. The activated Sludge reactor was operated with an average solids retention time (SRT) of 12 days to retain the nitrifying bacteria. The biomass that was removed for batch respirometer tests was taken into account and at times batch wasted in order to maintain the 12 day SRT. Figure 2 contains the SRTs for each reactor on a daily running average.

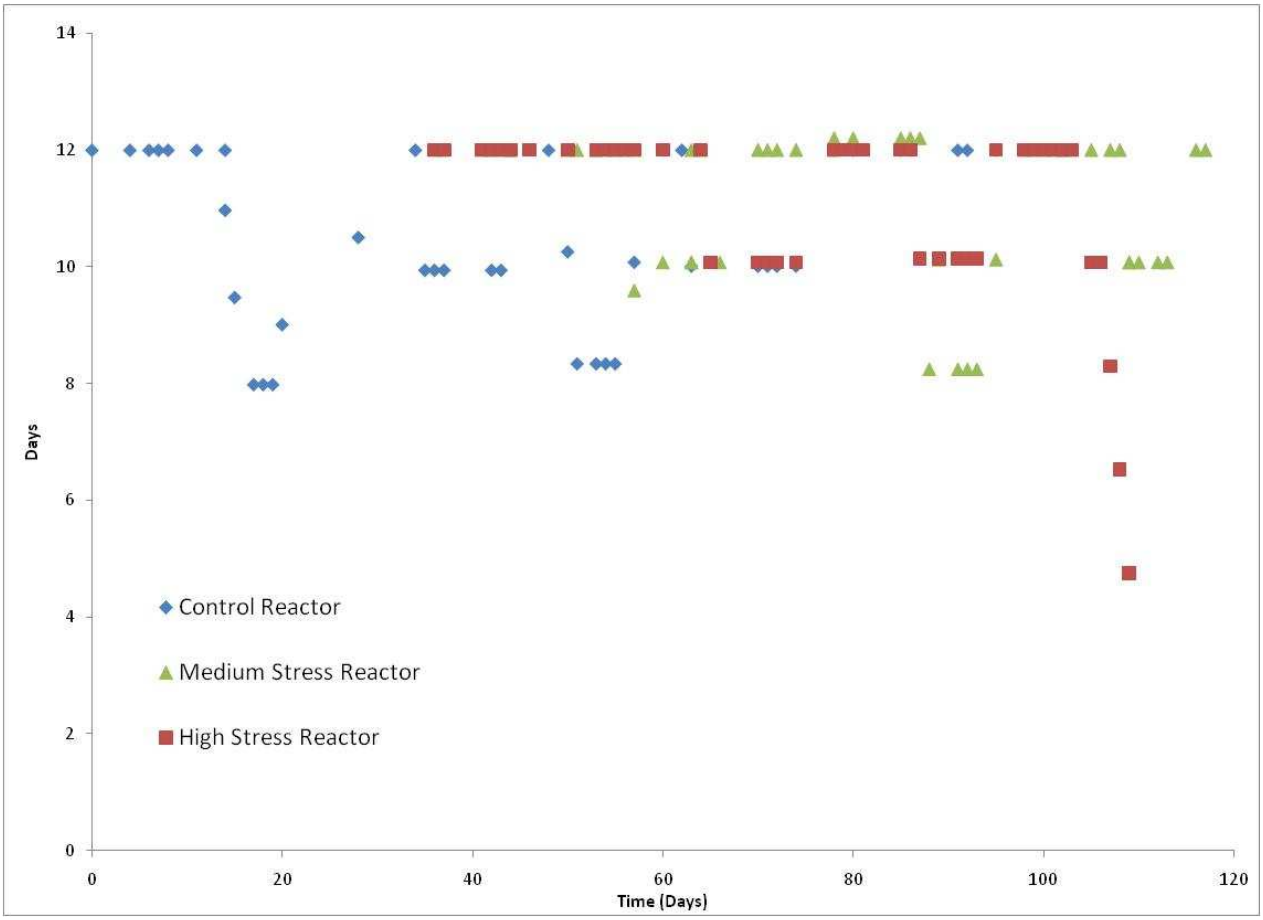

Figure 2 SRT in days versus Experiment Time for All Three Reactors; the SRT is a Daily Moving Average

The hydraulic retention time (HRT) varied according to the desired nitrogen loading and the ammonia concentration of the influent; figure 3 shows the HRT averaged ever day.

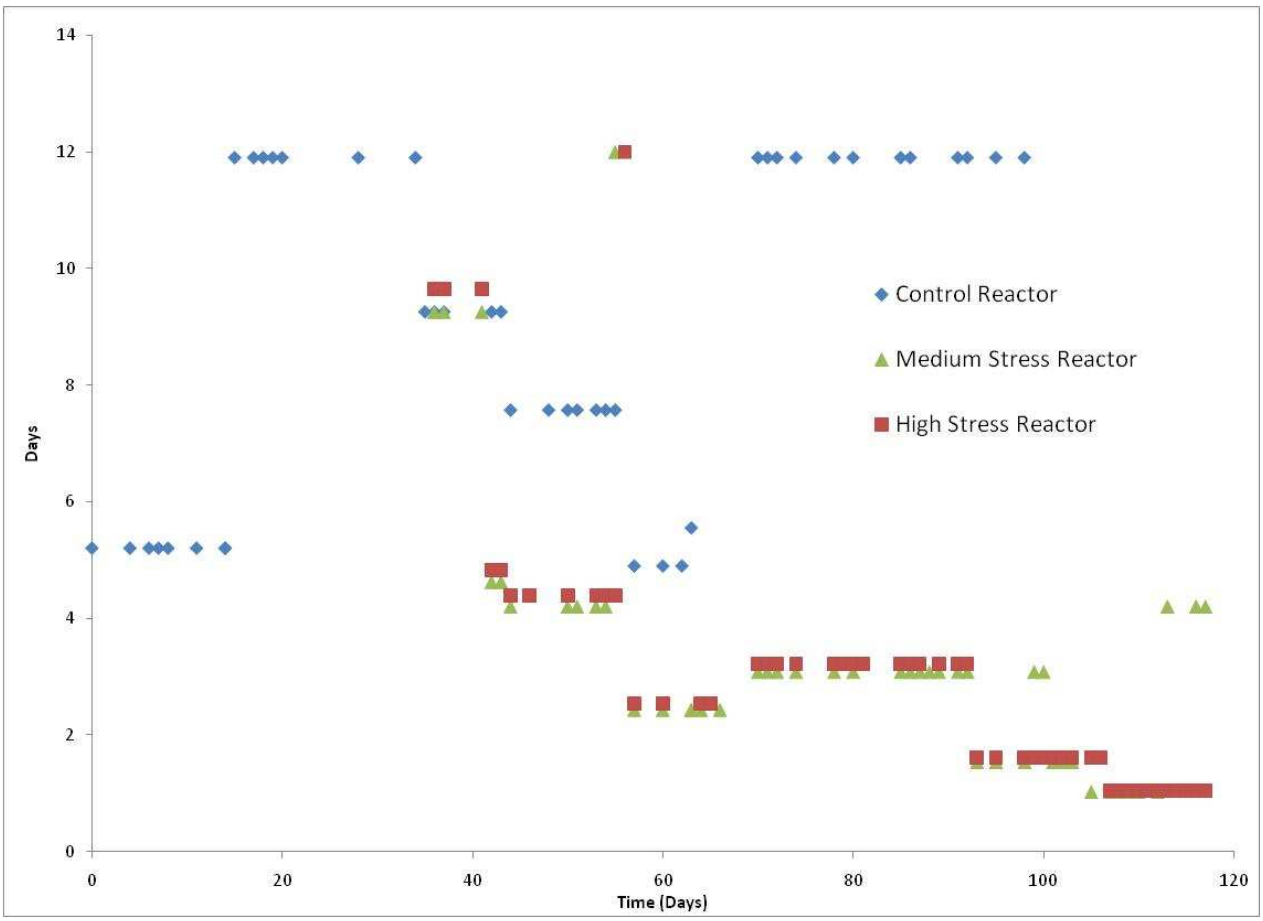

Figure 3 HRT in days versus Experiment Time for All Three Reactors; the HRT is a Daily Moving Average

The loading for each reactor was different for each reactor. The Control reactor was operated at a near constant loading of 10 mg N/L as NH_3. On day 36, 4 L of sludge was taken from the Control reactor. 2 L of sludge and 3 L of deionized water were used to start both the medium stressed and high stressed reactors. The loading for the medium stressed reactor was slowly increased stepwise, and the loading for the high stressed reactor was increased in the same manner as the medium stressed reactor but augmented with periodic high stress. Figure 4 gives the exact loading for each reactor.

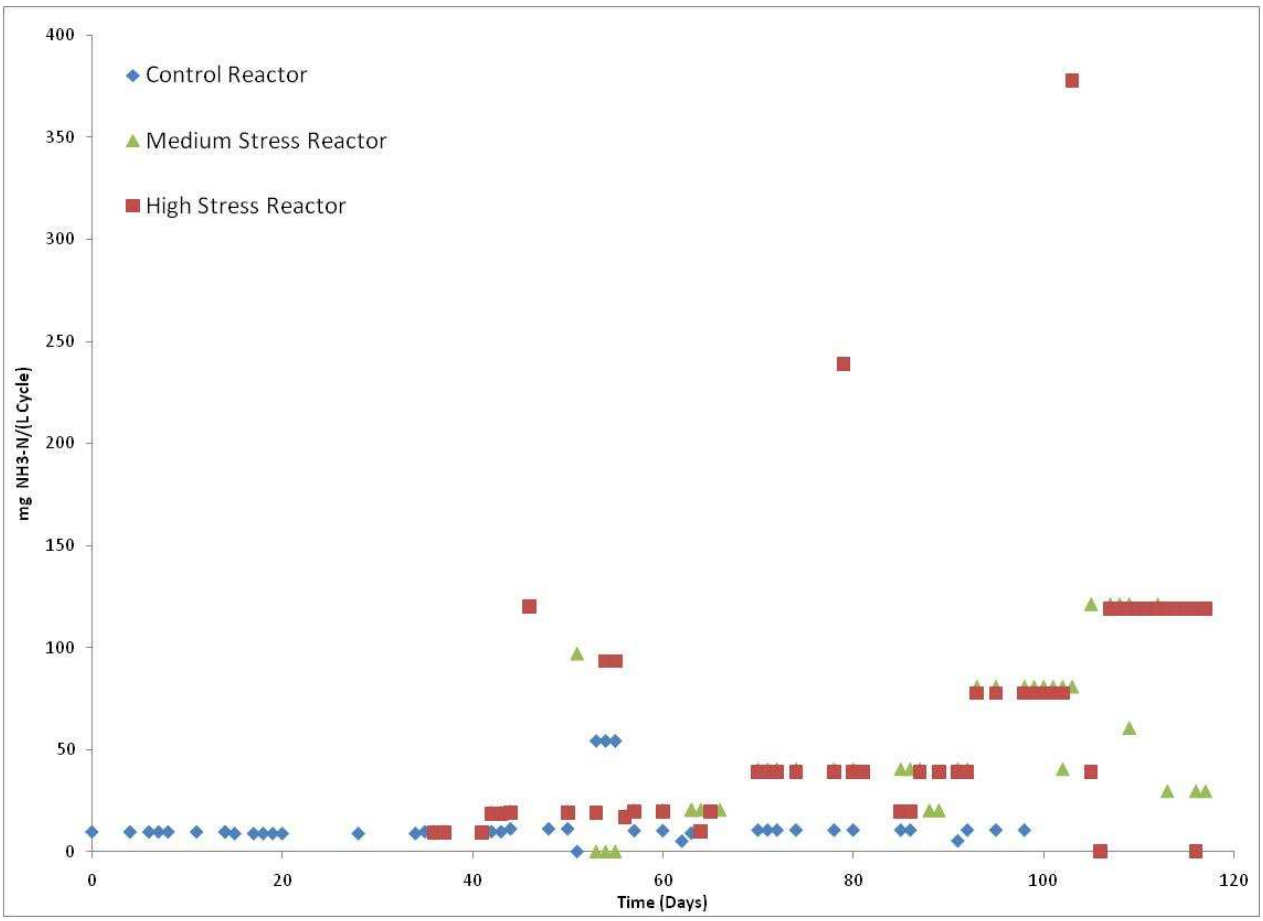

Figure 4 Reactor Ammonia Loading per Cycle During the Course of the Experiment

2.3 Respirometer Batch Tests

Respirometer batch tests were performed with a Challenge Environmental Systems, Model MS8-300 respirometer (Fayetteville, AR, USA) which contains 16 sealed bottles or cells with temperature controlled at 25°C. Biomass was added to each bottle after a starvation period between 8 hours and 2 days depending on the initial nitrite and ammonia concentration of the SBR supplying the biomass. Initially, no substrate was added to the bottles. After an endogenous period, nitrite and ammonia were added to the majority of the cells. Each level of nitrite or ammonia added had a replicate for each test. Buffer, acid, and base were added to obtain the desired starting pH which varied between batch tests from 7.75 and 8.05. Buffer was added to allow most of the ammonia to be oxidized while maintaining a pH above 6.5. Continuous SBR operations showed that nitrification become significantly inhibited around pH 6.5 and lower. The buffers used in these tests were tris(hydroxymethyl)aminomethane (Tris) which is a commonly used buffer for biochemistry experiments. The biomass eventually developed the

ability to metabolize Tris. The biomass did not develop the ability to metabolize 3-(N-morpholino)propanesulfonic acid **(**MOPS) which another common biochemistry buffer. Bicarbonate was also used as a buffer. Bicarbonate was added to prevent carbon dioxide limitation by the nitrifiers after tests on day 63 and 65. One issue is that CO_2 is removed from the sealed volume by dissolving into an alkaline solution within the respirometer bottles. CO_2 is scrubbed by the alkaline line from the bottles regardless of whether the CO_2 is respiration or equilibrium reactions. CO_2 is removed from the head space of the cell in order to allow a pressure drop from the metabolism of oxygen to draw in a precisely calibrated bubble of pure oxygen. The drawing of these calibrated bubbles allow for the OUR of each cell to be precisely calculated after the Loess method was used to smooth out the discrete addition of bubbles. Appendix C describes how the smoothed OURs were generated from the raw OUR data.

2.4 Chemical Tests and Other Methods

Ammonia levels were determined using Hach's Nessler method or a microplate ammonia method Rhine (1998). Nitrite and Nitrate levels were determined using a Dionex ICS-2000 chromatography system with an AS50 autosampler. The column was a Dionex's AS18 IonPac anion exchange column. For the SBRs oxygen uptake rates, OUR, were calculated, by determining the slope of the change in oxygen concentration, when the DO levels were decreasing. MLSS and MLVSS were calculated using standard methods (APHA et al., 1998).

Chapter 3: Model

3.1 Heterotrophic Model

Heterotrophs are bacteria that oxidize organic carbon to provide energy for cell growth. Oxygen is utilized in this process. Heterotrophs were modeled using Activated Sludge Model 3 (ASM3) (Henze et al. 1999) as shown in the equations below. ASM3 parameters were used except for K_{STO} and K_s. OUR higher than the estimated endogenous rates were assumed to be from stored COD being metabolized. Since the influent is a complex substrate, the K_s and K_{STO} are estimated from the OUR.

$$\frac{dX_{Het,growth}}{dt} = K_{STO} \cdot \frac{C_{O_2}}{K_{S,O_2,het} + C_{O_2}} \cdot \frac{C_S}{K_S + C_S} \cdot X_{Het}$$

3.2 Biomass Estimation

The active biomass concentrations of X_{Het}, X_{AOB}, and X_{NOB} were estimated by using the SBR's effluent and influent concentrations of NH_3, COD, NO_2, and NO_3, the yield coefficients for the heterotrophs, AOBs, and NOBs, and by assuming the reactor is at steady state (Rittman's Environmental Biotechnology).

3.3 Endogenous OUR

The decay constants of 0.15 d^{-1} for the nitrifiers (Rittman's Environmental Biotechnology) The resulting endogenous OUR is shown in equation below that uses activated sludge model 3's decay equations for biomass decay for Heterophes, ammonia oxidizers, and nitrite oxidizers.

$$OUR_{Endogenous} = K_{STO} \cdot \frac{C_{O_2}}{K_{O_2,Het} + C_{O_2}} \cdot \frac{S_S}{K_S + S_S} \cdot X_{Het} + b_{AOB} \cdot \frac{C_{O_2}}{K_{O_2,AOB} + C_{O_2}} \cdot X_{AOB} + b_{NOB} \cdot \frac{C_{O_2}}{K_{O_2,NOB} + C_{O_2}} \cdot X_{NOB} + b_{Het} \cdot \frac{C_{O_2}}{K_{O_2,Het} + C_{O_2}} \cdot X_{Het}$$

3.4 Nitrite Oxidizer

A modification of ASM3 where Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) were modeled separately was used. Nitrite Oxidizers are autotrophic bacteria that oxidize Nitrite to Nitrate see equation using oxygen as the electron acceptor that results in nitrate being formed.

Nitrite Oxidation was modeled using a Haldene-type equation (Anthonisen et al. 1976). The sludge in Jubany's work was shown to not be inhibited by nitrous acid (Jubany et al. 2005), so monod kinetics were used to describe nitrite oxidation see equation below.

$$\frac{dX_{NOB,Growth}}{dt} = \mu_{Max,NOB}\frac{C_{O_2}}{K_{O_2}+C_{O_2}}\frac{C_{NO_2}}{K_{S,NO_2,NOB}+C_{NO_2}}X_{NOB}$$

NOB's yield and NOB's $K_{O_2,NOB}$ were 0.083 mg VSS_a/mg N as NO_2 and 0.68 mg O_2/L, $\mu_{\max,NOB}$ =1.1/day (Rittman's Environmental Biotechnology.). $K_{S,NO_2H,NOB}$ was determined from batch experiments.

Nitrite Oxidization

$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^-$

3.5 Ammonia Oxidizers

Ammonia oxidizing bacteria (AOB) are bacteria that oxidize ammonia to nitrite consuming oxygen and generating hydrogen ions see equation below. In this model, free ammonia is considered to be the substrate for the ammonia monooxygenase enzyme in AOB (Susuki et al. 1974). Between pH 8.05 and 6.50 which was the pH window for the batch tests, ammonia is less than 10% of the total ammonia/ammonium present because the majority of the ammonia/ammonium is present as ammonia. A specific facet of ammonia oxidation is that AOBs can be substrate inhibited. The Haldane model gives the growth rate of AOB bacteria can be represented by equation below with a pH modification (Andrews 1968). The maximum growth rate of the AOB bacteria might be sensitive to the solution's pH (Dochain et al. 2001). During batch respirometer tests, the pH was found to change because the solution could not be buffered sufficiently without inhibiting bacteria to the point where oxygen was not taken up. AOB's yield and AOB's $K_{O_2,AOB}$ were 0.33 mg VSS_a/mg N as NH_3 and 0.50 mg O_2/L, $\mu_{max,AOB}$=1.02/day (Rittman's Environmental Biotechnology.). $K_{S,NH_3AOB}, pKa1, pKa2, and\ K_{i,NH_{3,AOB}}$ were determined from batch experiments

$NH_5^+ + 1.5\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$

$$\frac{dX_{AOB,growth}}{dt} = \frac{\mu_{\max,AOB}}{1+10^{pKa1-pH}+10^{pH-pKa2}} \cdot \frac{C_{O_2}}{K_{O_2,AOB}+C_{O_2}} \cdot X_{AOB} \cdot \frac{C_{NH_3}}{K_{S,NH_3,AOB}+C_{NH_3}+\frac{\left(C_{NH_3}\right)^2}{K_{i,NH_3,AOB}}}$$

3.6 pH Modeling

Since the AOB activity and the fraction of ammonia that is free ammonia are pH dependent, modeling pH change during batch tests is crucial. Many pH modeling methods attempt to take into account all species in a system, but the feed for this system was not buffered with phosphate or bicarbonate. The feed was buffered by the alkalinity present in separated swine manures. The exact concentration of buffering compounds in separated swine manure is unknown. In order to model pH, biomass was taken from the reactor before each batch test and mixed with the same concentration of buffer as was present in the batch respirometer test. The pH of the sample was raised to 8.50 and acid was added slowly while recording the pH change. A curve relating acid added versus pH was created with acid added used as a surrogate for ammonia nitrified. An algebraic expression was then fitted to the titration curve to generate a function where pH is equal to a function of acid produced or added. This method did not prove to effectively model pH changes in the respirometer bottles. The predicted pH was higher than the model pH particularly at higher ammonia levels. Since each test was replicated helping to eliminate abnormal tests, a function was generated from plotting the ending pH of the function versus ammonia consumed. These functions for each batch test were found to predict the ending pH of each cell to within 0.10 pH units see appendix B for an example.

3.7 Kinetic Parameter Determination

The parameters for endogenous respiration were determined from the two control bottles in each test. The $K_{S,NO2,NOB}$ was found from the bottles that were fed nitrite by fitting the OUR curves to the NOB model using aquasim. Determining ammonia oxidizer parameters was not as straight forward. The initial attempt to determine the AOB parameters was to find the parameters for each of the bottles independently in a single batch test. Next, the goal was to reconcile the parameters from each bottle of

a batch test to obtain one set of parameters which would fit all the initial concentrations or all of the bottles in a batch test. Reconciliation was performed by taking the parameters from batch tests with similar initial ammonia levels and attempting to find one set of parameters that fit similar ammonia level bottles. This process was then repeated until all the bottles in a batch tests were modeled using the same set of parameters. This final set of parameters would most likely represent the true parameters of the nitrifiers in the biomass sample because the set fits all of the OUR curves in a batch test.

Chapter 4: Results and Discussion

4.1 Sample Kinetic Parameter Determination

As an example of the parameter fitting process for a single bottle of a batch test, figure 5 shows the aquasim individual parameter fit for one of the bottles with an initial concentration of 40 mg/L ammonia-N. The parameters fitted were $K_{I,NH3,AOB}$, $K_{S,NH3,AOB}$, $K_{S,NO2,NOB}$, pKa1, pKa2, and $X_{HET,ini}$.

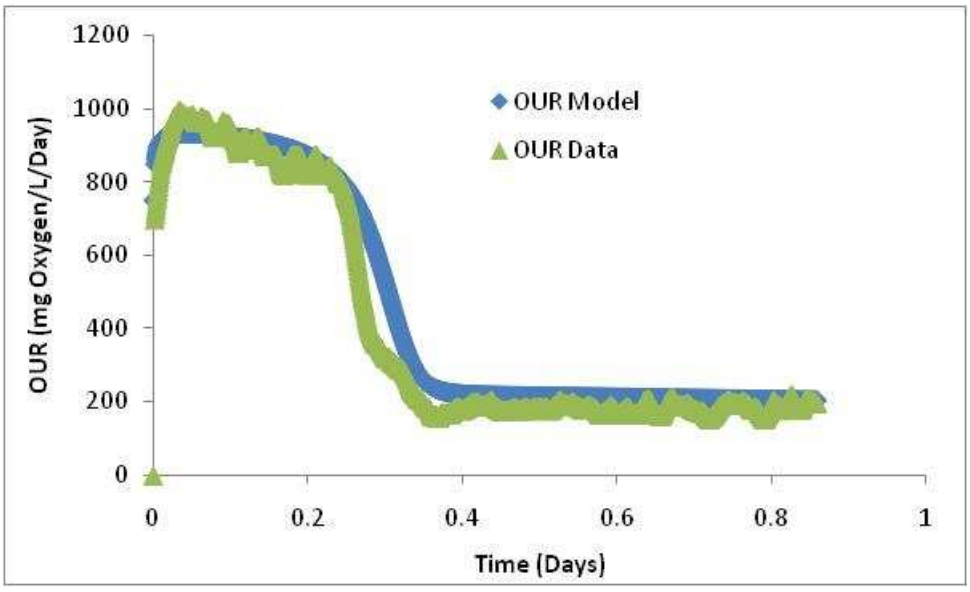

Figure 5 Oxygen Uptake Rate (OUR) Versus Time When the Initial Concentration is 40 mg NH_3-N/L For the Aquasim Model and the Smoothed Respirometer Data. Biomass was taken from the Control reactor on Day 111.

The figure 5 demonstrates that the model was able to fit an OUR curve that matched up with the smoothed experimental data indicating that the kinetic parameters generated represent the actual characteristic of the biomass being tested. The parameters were found to be: $X_{Het,ini}$=1404 mg/L, pKa2=10.3, pKa1=7.82, $K_{S,NO2,NOB}$=0.37 mg/L Nitrite-N, $K_{S,NH3,AOB}$=0.0556 Ammonia-N, and $K_{I,NH3,AOB}$=551.35. Next, the model was used to fit a bottle from the higher concentration. Figure 6 shows the best fitted aquasim curve and the smoothed experimental data together.

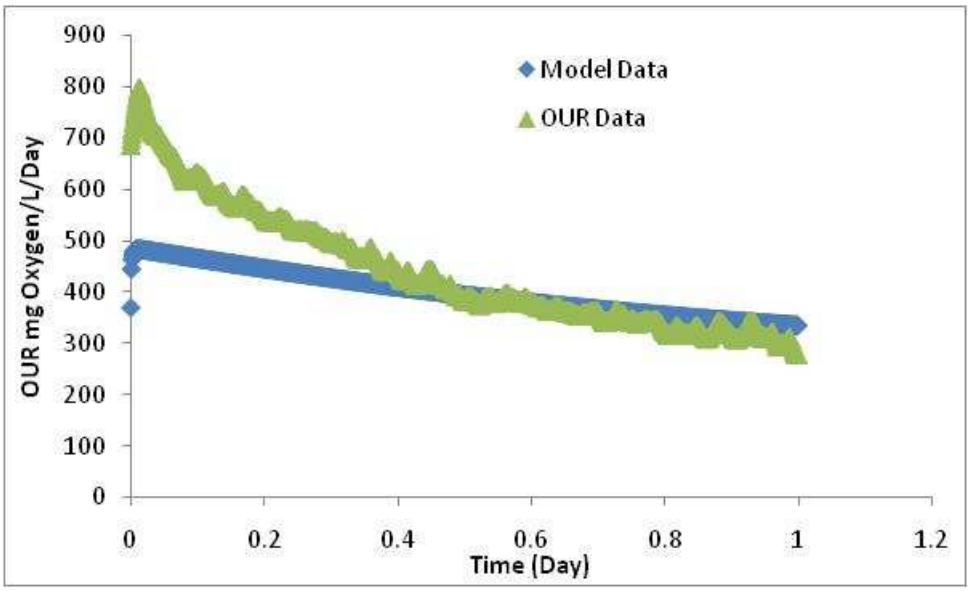

Figure 6 Oxygen Uptake Rate (OUR) Versus Time When the Initial Concentration is 515 mg NH_3-N/L For the Aquasim Model and the Smoothed Respirometer Data. Biomass was taken from the Control reactor on Day 111.

The parameters used to create this fit of the smoothed data were: $X_{Het,ini}$ =91.399 mg/L, pKa2=99.96, pKa1=8.05, $K_{S,NO2,NOB}$ =0.12 mg/L Nitrite-N, $K_{S,NH3,AOB}$ =4.97 Ammonia-N, and $K_{I,NH3,AOB}$=914.6. In addition, the above fit is not particularly good because there is systematic bias. The early parts of the test have the greater error than the later parts. Aquasim attempted to fit the higher level ammonia tests by decreasing the heterotrophic biomass levels to decrease the endogenous respiration and increased pKa1 in order to decrease the initial OUR relative to the low ammonia level bottle. A third bottle in the same test with V shaped data was modeled. Figure 7 shows the best fit and the raw data after 2000 iteration of aquasim.

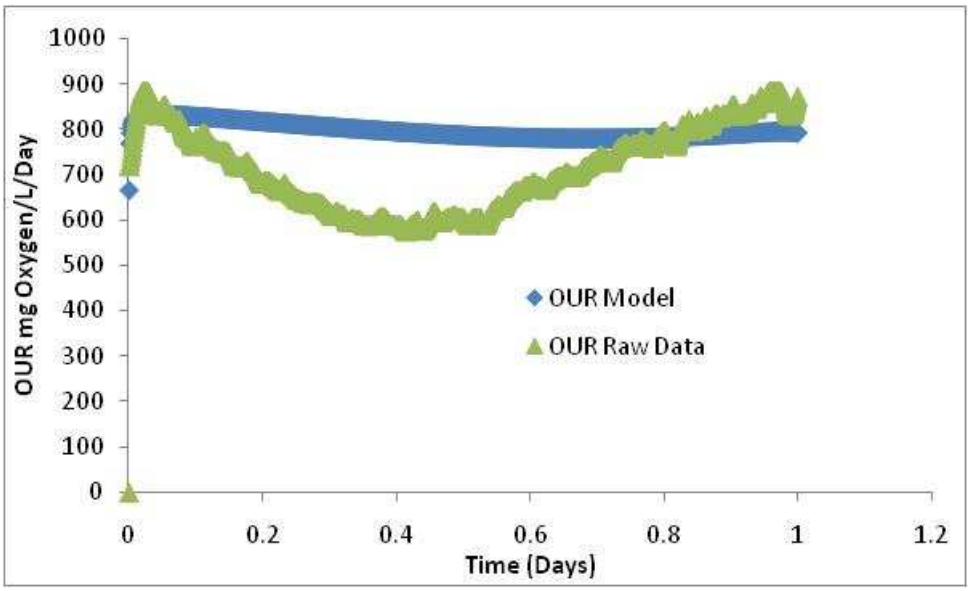

Figure 7 Oxygen Uptake Rate (OUR) Versus Time When the Initial Concentration is 150 mg NH_3-N/L For the Aquasim Model and the Smoothed Respirometer Data. Biomass was taken from the Control reactor on Day 111.

The model generated a straight line rather than replicating the V shape of the raw data in order get the best fit. This modeled data also has a systematic bias with higher differences between the model and smoothed data in the middle of a batch test than at the beginning or the end. The aquasim model was able to generate V-shaped data see figure 8. Despite the model being able to generate V-shaped data, aquasim found that the straight our curve was the optimal fit for the smoothed OUR that contained V-shaped data. Overall, the aquasim model used was able to model low ammonia levels accurately but not medium and high ammonia levels in this batch test and some but not all other batches tests.

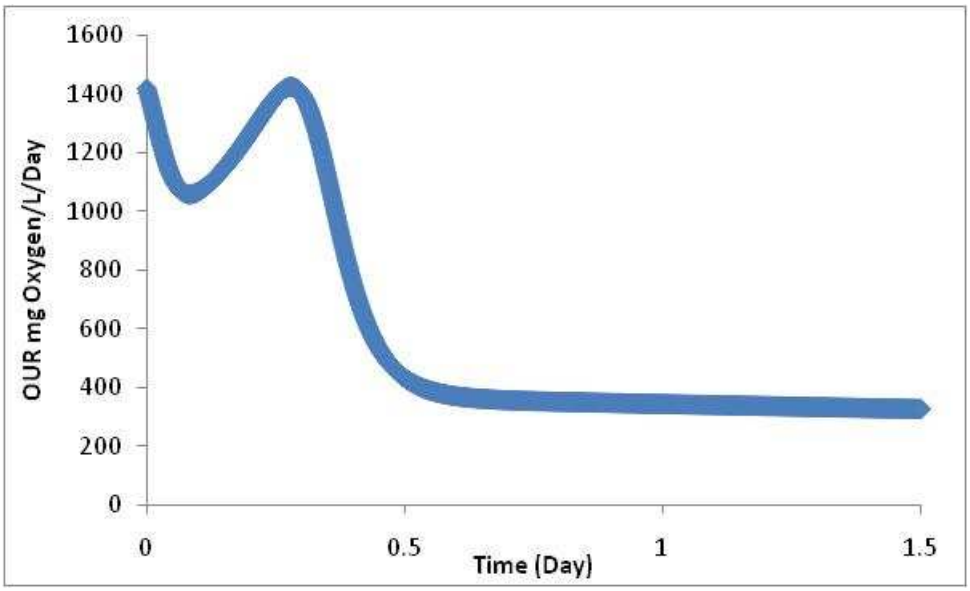

Figure 8 Model Generated V Shaped Data of OUR Versus Time

The step of reconciling the parameters in different bottles of the same batch test was attempted. But the highly variable parameters for bottles with different ammonia levels were not able to be reconciled completely for the above batch test. Similar attempts were made to find parameters for the remaining 10 batch tests. Many of the aquasim OUR curves fitted to one bottle in a batch tests showed systematic biases suggesting that the model was ill fitting. Using the aquasim model, no batch tests was found to have the same kinetic parameters for all the bottles that were the same biomass but exposed to different ammonia levels. Attempts were made to modify the model to make the AOB inhibition parameter pH dependent because one batch test was fitted using only a variable $K_{I,NH3,AOB}$. Additional attempts to generate a mathematical expression that could fit the data from an entire batch test without concern for biological mechanisms were also unsuccessfully attempted. Therefore, model parameters cannot be used to compare the biomass from different reactors and determine how the biomass adapted to different ammonia levels.

4.2 Qualitative Analysis

After the limitations of the current aquasim model were discovered in the sample kinetic parameter determination, qualitative analysis was decided upon to compare the affects of differing ammonia stresses on the same seed biomass. Since nitrifiers have been found in others studies to be pH

dependent, substrate inhibited, and substrate limited, kinetics parameters will still be used loosely when qualitatively describing the differences between OUR curves (Hulle 2007).

With respect to the control reactor, the relative shapes of the control OUR curves from batch tests for the control reactors did not significantly change despite one spike in loading see figure 4. This trend suggests that the differences between the batch tests from each reactor are likely to be the result of the differing loading. Figure 9 is a comparison of the endogenous respiration of the three biomasses. The control and high stressed biomass shows a linear decrease while the medium stressed biomass's OUR decreases in an exponential manner for a time and then decreases in a linear manner. The most likely cause of the initial exponential decrease by the medium stressed biomass is a small amount of residual slowly biodegradable COD being consumed. The medium stressed biomass has a much higher OUR than the high stressed and control biomasses due to higher levels of biomass present.

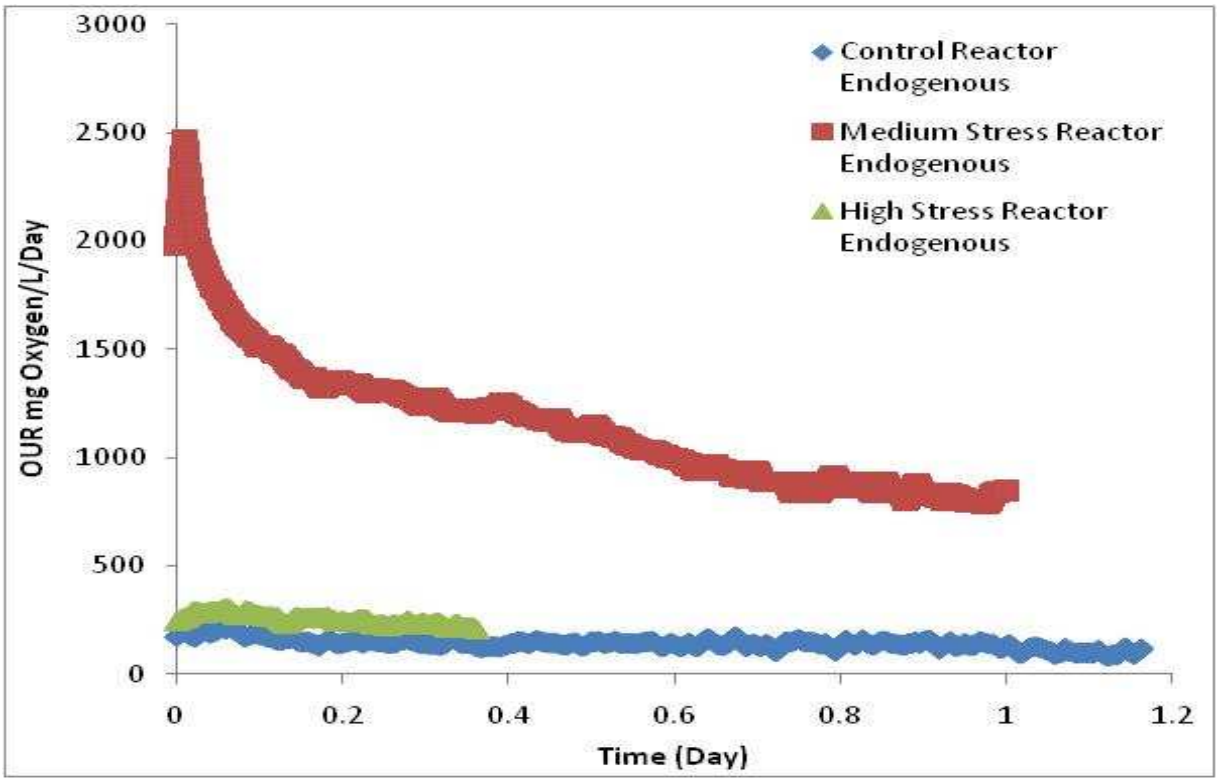

Figure 9 OUR Versus Time for Each Biomass with No Substrate Added. Control Biomass Was Taken on Day 111. Medium Stress Biomass Was Taken on Day 109. High Stress Biomass Was Taken on Day 116.

Figure 10 contains the OURs of the biomasses when nitrite was added. The high stressed reactor contained almost 60 mg/L Nitrite-N due a partial washout of NOBs due to sludge wastage from

repeated batch testing. The control biomass show a flat nitrite oxidation OUR followed by a rapid drop in the OUR to the endogenous OUR level. The medium stressed biomass shows a linearly decreasing OUR from the nitrite oxidation. The difference in the shapes of the OURs is most likely the result of the medium stressed reactor being exposed to higher nitrite levels due the buildup of nitrite as an intermediate during the oxidation of higher levels of ammonia. The high stressed OUR is flat due to the presence of more nitrite. Nitrite oxidation can be modeled using the aquasim model with the $K_{S,,NO2,NOB}S$ for nitrite being higher for the medium stressed biomass than the control biomass. This difference suggests that the medium stressed biomass was exposed to higher levels of nitrite due to the higher ammonia loading resulting in a higher $K_{S,NO2,NOB}$ for nitrite.

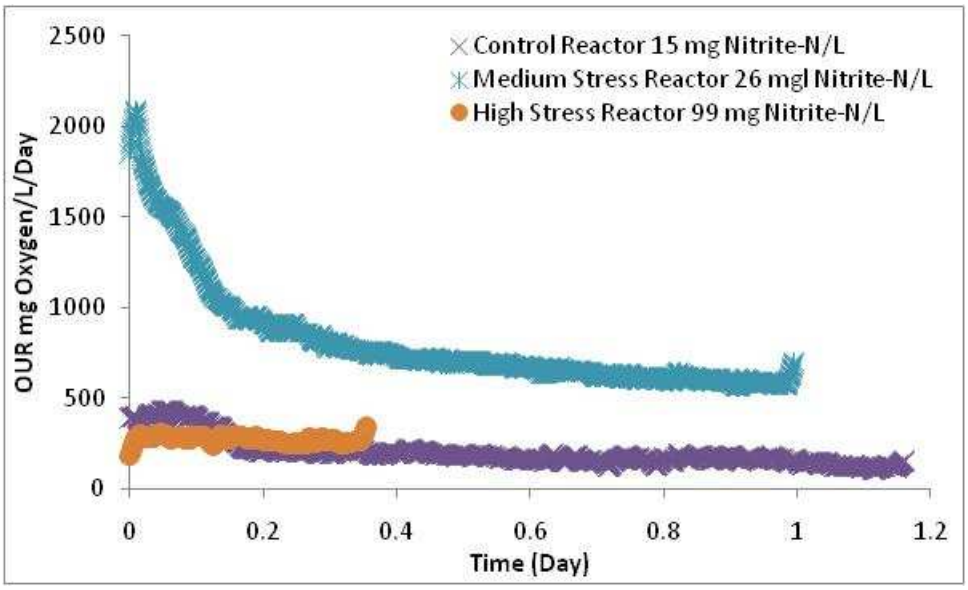

Figure 10 OUR Versus Time for Each Biomass with Nitrite Added. Control Biomass Was Taken on Day 111. Medium Stress Biomass Was Taken on Day 109. High Stress Biomass Was Taken on Day 116. Substrate Was Added at Time Equals Zero.

Figure 11 compares the OURs for each biomass when exposed to low levels of ammonia 35-50 mg NH3-N/L. The last batch test for each reactor was used for qualitative comparison. Qualitatively, there are strong differences between each biomass's OUR curves despite a similar initial ammonia levels. The control biomass showed a rapid increase that is a relic of smoothing, a flat OUR due to ammonia oxidation, and a rapid OUR decline when the ammonia nearly consumed, and then a low

endogenous level OUR after all the ammonia is consumed. The medium stressed OUR curve starts out at a high OUR that decrease rapidly until It reaches the endogenous OUR. The OUR curve that was generated from the batch test of biomass from the high stressed reactor, is entirely different because it shows a linear increase in OUR to a maximum OUR that then decreases linearly to endogenous levels. The control's OUR is most likely the result of a low $K_{S,NH3,AOB}$ for Ammonia causing the nearly flat maximum OUR with a rapid decline after the ammonia is oxidized. The medium stressed OUR curve is a linearly declining OUR curve most likely due to a higher $K_{S,NH3,AOB}$ for ammonia. Since the medium stressed biomass was exposed to higher ammonia levels in the reactor than the control biomass, the medium stressed biomass has less of a competitive advantage than the control biomass if the biomass has a low $K_{S,NH3,AOB}$ because more of the ammonia consumed is consumed when the background ammonia levels are higher. The highly qualitatively different $K_{S,NH3}$'s in the medium stressed and control mass is an interesting response to higher ammonia loading because the differing responses of the medium stressed biomass and the control biomass suggest that the ability of AOBs to absorb low ammonia levels also decrease the rate at which AOB can grow or metabolize ammonia otherwise the medium stressed data would still have the flat OUR curve due to a lower $K_{S,NH3,AOB}$.

The high stressed low level OUR curve increases as the test progresses which suggest something is slowing down the AOB ability to metabolize ammonia during the initial portions of the batches tests and not the later portions of the batch tests. One possibility is that the high stressed data which is exposed to more ammonia is more easily inhibited by ammonia. When ammonia is consumed, it might have less of an inhibitor effect. If the increasing OUR is due to ammonia inhibition, the higher level ammonia OUR curves will start at lower OURs. Another possible cause for the increasing OUR is due to the pH sensitivity of ammonia oxidation. But the OUR for the control test decreases slightly during probable ammonia oxidation. This suggests that the pH effects on ammonia oxidation cause a decreasing OUR, before the biomass is exposed to higher ammonia levels. So shock loads could cause the pH sensitivity of ammonia oxidation to reverse. The last option is that differing OUR curves of the control and high stressed biomass are the result of characteristics of the biomass that are not accounted for in the model. Helping to confirm the above analysis, the initial higher OUR is from ammonia and nitrite oxidation because oxygen consumption above the endogenous levels in that section match the amount of oxygen needed to oxidize nitrite and ammonia for each of the low level ammonia OUR curves for the control, high stressed, and medium stressed reactors.

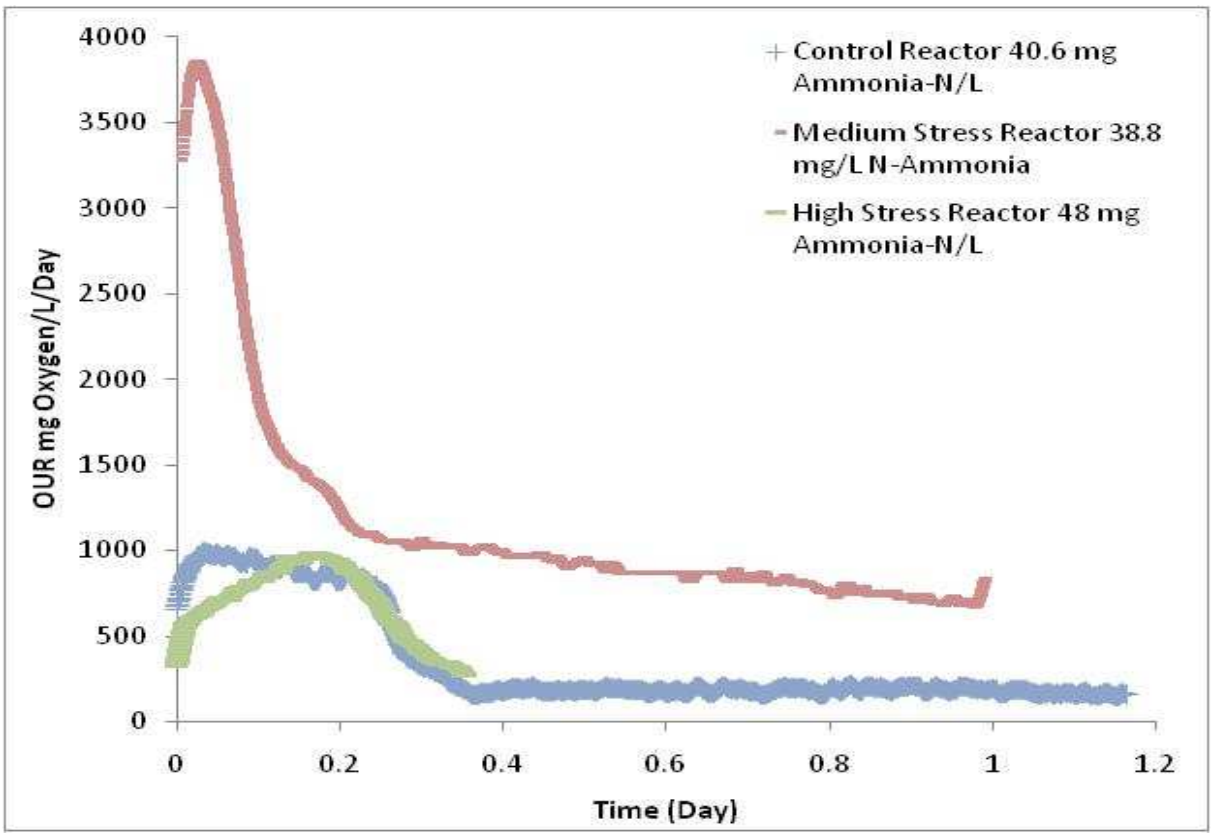

Figure 11 OUR Versus Time for Each Biomass with Ammonia Added. Control Biomass Was Taken on Day 111. Medium Stress Biomass Was Taken on Day 109. High Stress Biomass Was Taken on Day 116. Substrate Was Added at Time Equals Zero.

Figure 12 compares the OURs for each biomass when exposed to medium levels of ammonia. The high stressed OUR curve shows an initial OUR as high as the initial OUR of the low level tests despite have 3 times as much initial ammonia. If the high stressed curve's increasing OUR during ammonia consumption was the result of inhibition, the initial OUR for the medium ammonia level test could be up to 9 times higher. Since the initial OUR for the medium and low ammonia high stressed bottles, the increasing OUR cannot be due to less ammonia inhibition due to ammonia oxidation leaving pH as a factor or unaccounted for characteristics.

The medium stressed data shows a bit of a flat OUR initially after a rapid increase in medium stressed OUR which is likely the result of smoothing. The small flat region could be the result of the ammonia saturating the $K_{S,NH3,AOB}$ of the medium stressed biomass. The slowly decreasing medium stressed OUR is likely the result of the $K_{S,NH3,AOB}$ from the AOB. Speculatively, the small hump at about

0.4 days is produced from nitrite accumulation because the hump occurs where nitrite generally accumulates in a test. The control OUR curve is V shaped. The initial decrease in OUR, for the control biomass, is likely due to pH decreases that decrease the maximum rate at which ammonia can be oxidized. In the middle of the ammonia oxidation, the effects of decreased pH that decreases OUR and ammonia inhibition that increase OURs are balanced which is why the OUR has a relative minimum and then starts to increase due to lessening inhibition. The last portion of the control OUR curve is a rapid decrease to endogenous respiration due to the small $K_{S,NH3,AOB}$ of the control Biomass.

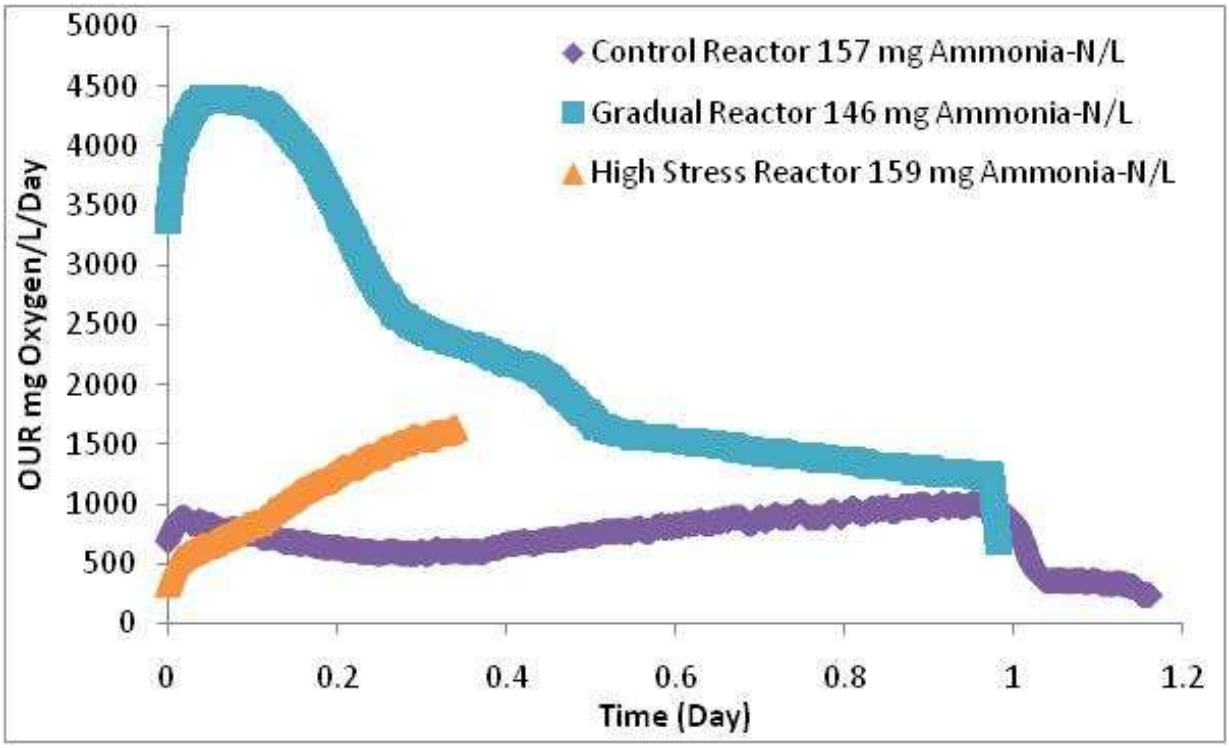

Figure 12 OUR Versus Time for Each Biomass with Ammonia Added. Control Biomass Was Taken on Day 111. Medium Stress Biomass Was Taken on Day 109. High Stress Biomass Was Taken on Day 116. Substrate Was Added at Time Equals Zero.

Lastly, figure 13 compares the OURs for each biomass when exposed to high levels of ammonia. The high stressed OUR curve for high ammonia lines up with the previous ones which suggests that the increasing OUR is not due to ammonia inhibition. The linear increase could be due to pH drops that might increase the ammonia oxidation rate or a property of the biomass not accounted for in the model. Other high stressed tests contained this trend so the trend was not a result of the nitrite initially present in the high stressed reactor. The increasing OUR is most likely due to unaccounted for biomass

properties because ammonia oxidation is generally slowed by lower pH and not increased (Hulle 2007). Finally, the aquasim model was unable to fit the OUR data suggesting that the biomass has characteristics that are not prevent in the aquasim model.

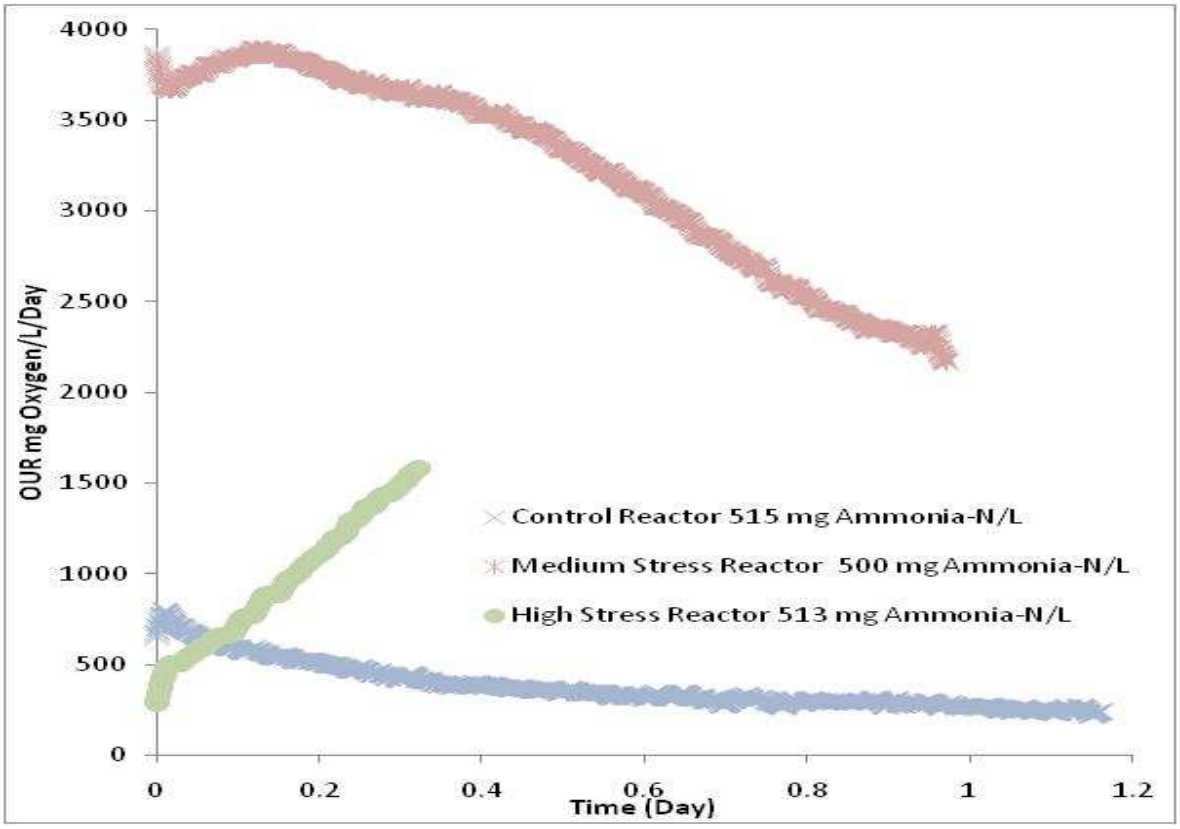

Figure 13 OUR Versus Time for Each Biomass with Ammonia Added. Control Biomass Was Taken on Day 111. Medium Stress Biomass Was Taken on Day 109. High Stress Biomass Was Taken on Day 116. Substrate Was Added at Time Equals Zero.

The medium stressed data shows a slowly decreasing OUR curve most likely to a decreased lower pH decreasing the rate at which ammonia can be oxidized. In addition, the medium stressed OURs are shown to drop as the initial ammonia in the batch test increases suggesting that there is a small inhibition effect present in the medium stressed biomass see figure A-3. The control OUR curve starts out at a high levels and decreases to an asymptote which is above the endogenous respiration OUR see figure A-2. The control biomass is most likely sensitive to pH drops, so the acidification of the bottles due to ammonia oxidation is dropping the ammonia oxidation rate. The control OUR decreases

and then the OUR levels off. The control OUR's leveling out is likely the result of less ammonia being present, so substrate inhibition is reduced. To confirm that some ammonia inhibition occurs, figure A-2 shows the lowest initial control OUR when the initial ammonia levels are highest.

Appendix C contains the complete OUR curves for each batch test performed on the high stressed OUR curves. Comparing the high stressed versus medium stressed OUR curves; the medium stressed biomass has a different response to ammonia qualitatively than the high stressed biomass. The difference between the OUR curves begins at the first batch tests after a shock has been applied to the high stressed reactor suggesting that the difference between the two nitrifier populations is exclusively the rest of the ammonia shocks because the reactors had the same ammonia loading. The changes in the shape of the high stressed OURs from the control's OUR curves became apparent in the 2^{nd} batch test suggesting that early shocks might be more influential than later shocks.

Overall, each biomass had different OUR curves at each of the different initial concentrations. The control OUR curves at the beginning and the end of the loading test are for the most part the same indicating that the differences between the different biomass OUR curves are the result of the different ammonia loadings because the control was constant. The OUR data indicates that the $K_{S,NH3,AOB}$ for AOB increases when the seed biomass is exposed to the medium stressed reactor's ammonia loading. The $K_{I,NH3,AOB}$ appears to be smaller (has a larger inhibitor effect) for the control biomass than the medium stressed biomass because the difference between the initial OURs that have differing ammonia levels for the control reactor are greater than the difference between the initial OURs for the medium stressed reactor see appendix C. The changes in the medium stressed biomass's AOB to pH sensitivities could not be deduced qualitatively from the OUR curves. Qualitatively, the additional ammonia stress caused the biomass in the medium stressed reactor to adapt and have different OURs most likely caused by differing biological parameters.

The high stressed biomass also appears to have a qualitatively different response to ammonia than the control or medium stressed biomass. The high stressed $K_{I,NH3,AOB}$ appears to be very high due because the high, medium, and low ammonia OURs line up together which indicates that no significant substrate inhibition occurs. Other High stressed tests demonstrate an OUR curve having a maximum OUR with approximately 70 mg/L ammonia-N still present suggesting that the AOB's half saturating constant is decreasing the OUR see appendix C. But the high stressed curves for most batch tests show an increasing OUR initially that cannot be modeled, and that OUR trend is not present in the other biomass's OUR curves. Therefore, the high stressed biomass shows qualitative differences from both

the medium stressed and the control biomass. Most likely the ammonia loading shocks caused the biomass in the high stressed reactor to have different biomass properties that caused the biomass to behave differently to ammonia addition than the other reactors. The differing loading and shocks produced three sets of biomasses with different OUR curves during a batch test indicating that a community of nitrifiers from a common source biomass can change their response to ammonia and their kinetic parameters in response to different ammonia stresses.

In addition, the method of batching testing in a respirometer revealed that models such as haldene model with pH sensitivity are not developed enough to model all of the trends in an OUR curve for a biomass source simultaneously exposed to different ammonia levels. In Antileo et al.'s and V Pambrum et al. work, they were performing their batch tests in the reactor containing all of the reactor's biomass with each addition of ammonia done in a sequence (Antileo et al. and Pambrum et al.). Since biomass adapts and changes depending on the concentrations that the biomass is exposed to, the biomass used in sequential testing has been subjected to extra stresses that are purely the result of the parameter tests, and the use of the whole reactor's biomass results in fewer ammonia concentrations tested. A respirometer batch tests allows each bottle's biomass to be disposed of after testing preventing the exposure of each reactor's biomass to extra stresses simply due to the need to perform batch tests. The respirometer also allows for more ammonia concentrations to be tested simultaneously allowing a more accurate OUR curves to be generated due to the ability to perform replicates. This additional data did generate complications because every OUR curve should be fitted by the model with the same kinetic parameters for each batch test which was not the case for these experiments. It appears that by testing additional ammonia levels across a broad range of ammonia concentrations simultaneously that additional biomass properties were revealed that are not easily observed in sequential testing and that could not be modeled using standard models. Additional testing and study could help to identify and model these additional nitrifier properties and confirm that they do exist possibly increasing the understanding of nitrifiers and communities of nitrifiers.

Chapter 5: Conclusions

Differing ammonia loading and ammonia shocks were found to create different biomass properties from the same initial biomass in an SBR. Biomass exposed to ammonia shocks and not exposed to ammonia shocks despite both reactors being exposed to increasing ammonia loading was found to have different properties after the high stress biomass was exposed to the first ammonia shock until the end of the test. The two reactors exposed to higher ammonia loading had different properties than the control reactor exposed to low ammonia loading. These tests also confirm that nitrifiers can adapt to very high ammonia levels if the biomass is not washed out.

A pH modification and a haldene inhibition kinetic modification to activated sludge model three were found to be unable to model the respirometer generated OURs of the biomass from all three reactors. Additional testing could be useful in determining if a more complex and detailed model would be able to model the OURs generated by nitrifiers in a respirometer tests where the biomass is simultaneously exposed to several ammonia concentrations. A more complex model fitting the generated OURs might give additional insight into nitrifiers.

References

American Public Health Association Inc. (APHA), *Standard Methods for the Examination of Water and Wastewater.* **20** Washington DC. (1998).

Andrews JF. "A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates." *Biotechnology Bioengineering.* 707-723 (1968).

Anthonisen A, Loher R, Prakasam T, and Srinath E. "Inhibition of Nitrification by ammonia and nitrous acid". *Journal of Water Pollution Control Federation*. **48**:835-825 (1976).

Antileo C, Aspé E, Urrutia H, Zaror C, and Roeckel M. "Nitrifying Biomass Acclimation to High Ammonia Concentrations". *Journal of Environmental Engineering*. **128:** 367-375 (2002).

Buitrón G, and González A. "Characterization of the microorganisms from an acclimated sludge degrading phenolic compounds." *Water Science and Technology*. **34**:289-294 (1996).

Dochain D, and Vanrolleghem PA. "Dynamic Modeling and Estimation in Wastewater Treatment Process." *IWA Publishing*, London (2001).

Gupta SK, and Sharma R. "Biological oxidation of high strength nitrogenous wastewater." *Water Research.* **30**:593-600 (1996).

Henze M, Gujer W, Mino T, van Loosdrecht M. "Activated Sludge Models, ASM1, ASM2, ASM2d, and ASM3." *IWA Publishing, London.* (200)

Hulle I, Volcke E, Teruel J, Donckels B, Loosdrecht M, and Vanrolleghem P. "Influence of temperature and pH on the kinetics of the Sharon Nitration process". *Journal of Chemical Technology and Biotechnology.* **82**:471-480 (2007).

Jubany I, Baeza A, Carrera J, and Lafuente J. "Respirometric calibration and validation of a biological nitrite oxidation model including biomass growth and substrate inhibition." *Water Research.* **39**: 4574-4587 (2005).

Pambrun V, Paul E, and Spérandio M. "Modeling the Partial Nitrification in Sequencing Batch Reactor for Biomass Adapted to High Ammonia Concentrations". *Biotechnology and Bioengineering*. **95** (2006).

Rhine E, Sims G, Mulvaney R, and Pratt E. “Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Science Society of America Journal.* **62**:473-480 (1998).

Rittmann B, and McCarty P. Environmental Biotechnology: Principles and Applications. McGraw Hill. (2001).

Susuki I, Dular U, and Kwok S. “Ammonia or ammonium ion as substrate for oxidation by *nitrosomonas europea* cells and extracts.” *Journal Bacteriology*. **120**: 556-558 (1974).

Walker P, and Kelley T. “Solids, organic loading and nutrient concentration reduction in swine waste slurry using polyacrylamide (PAM)-aided solids flocculation treatment. *Bioresource Technology.* **90**:151-158 (2003).

Wiesmann U. “Biological nitrogen removal from wastewater.” *Advanced Biochemical Engineering*. **51**: 113-154 (1994).

Appendix A Combined OUR Curves for Each Respirometer Batch Test

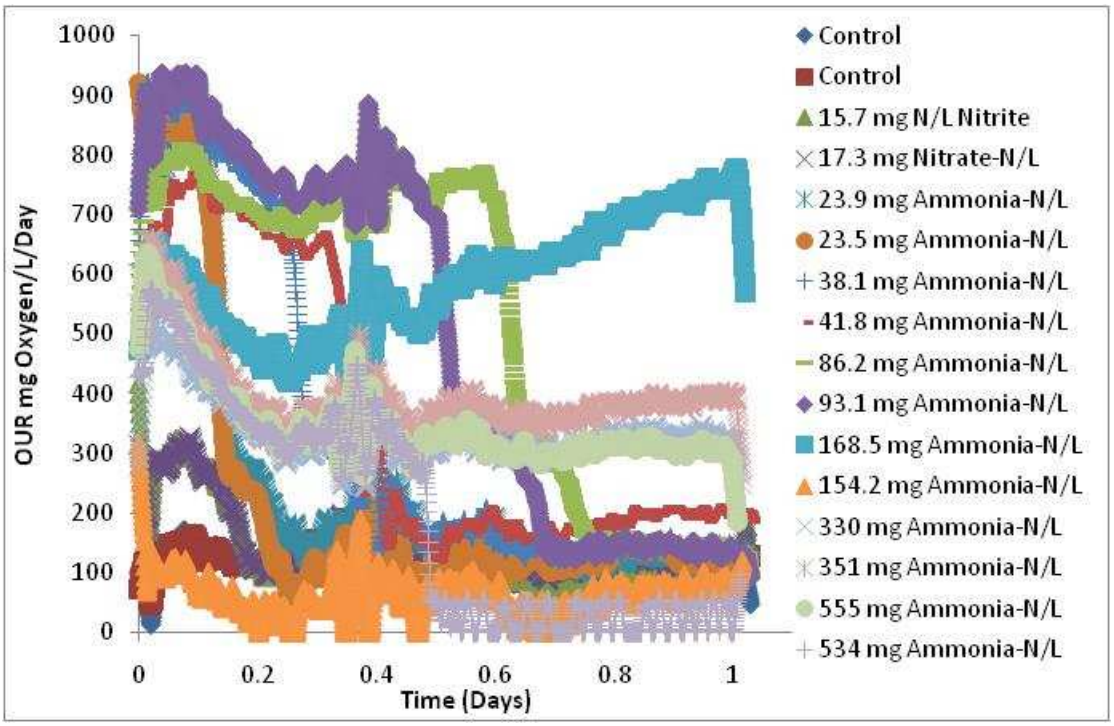

Figure A-1 OUR versus Time for a Respirometer Batch from the Control Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 91.

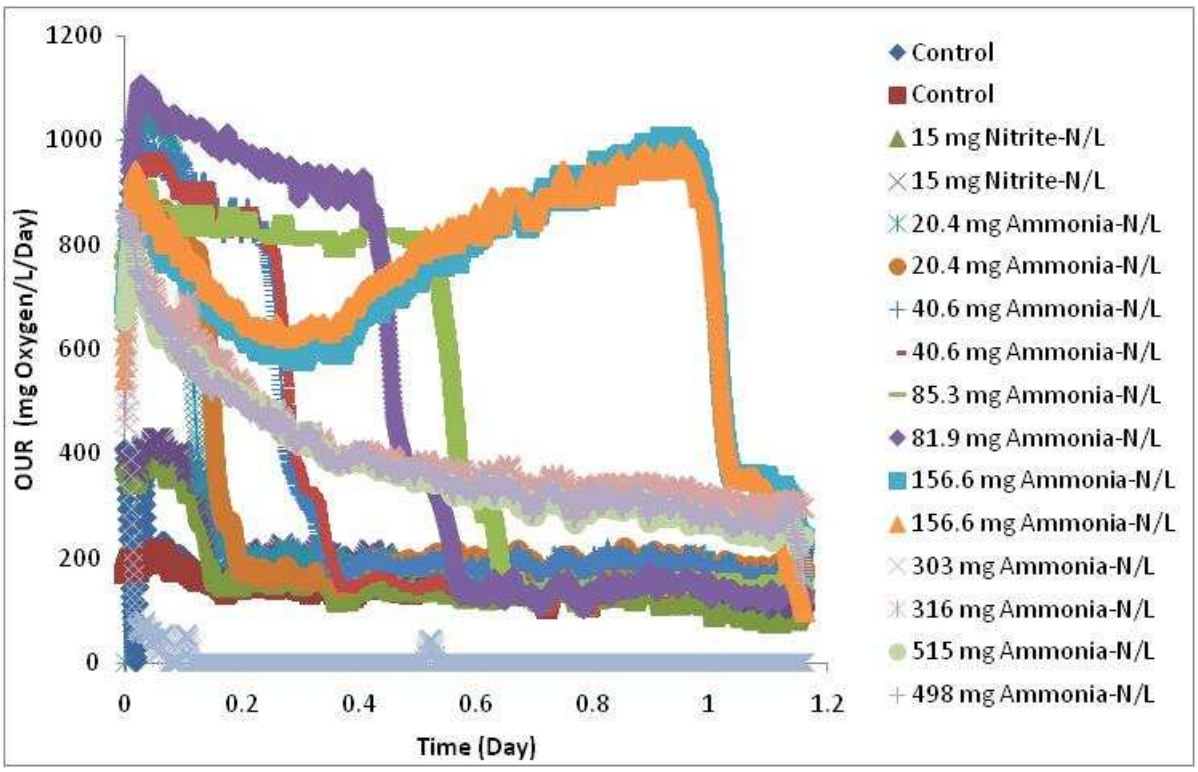

Figure A-2 OUR versus Time for a Respirometer Batch from the Control Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 111.

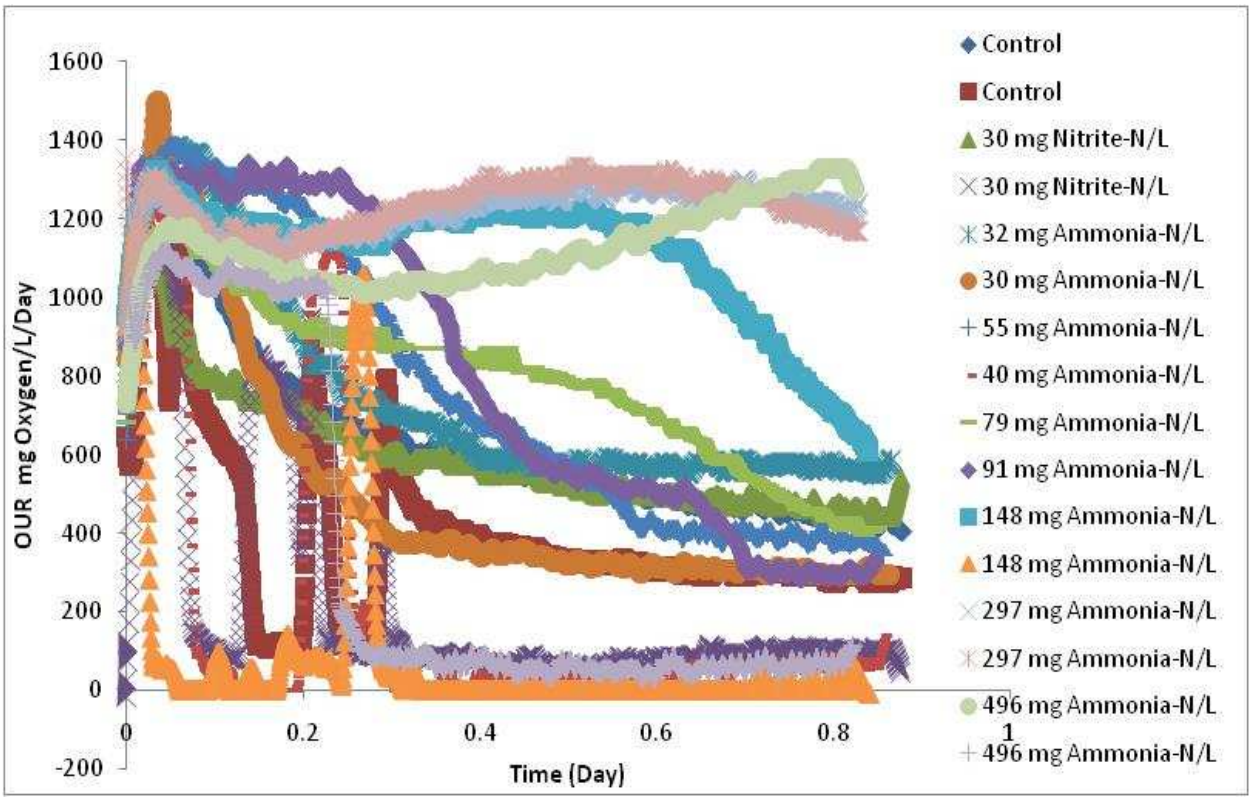

Figure A-3 OUR versus Time for a Respirometer Batch from the Medium Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 87.

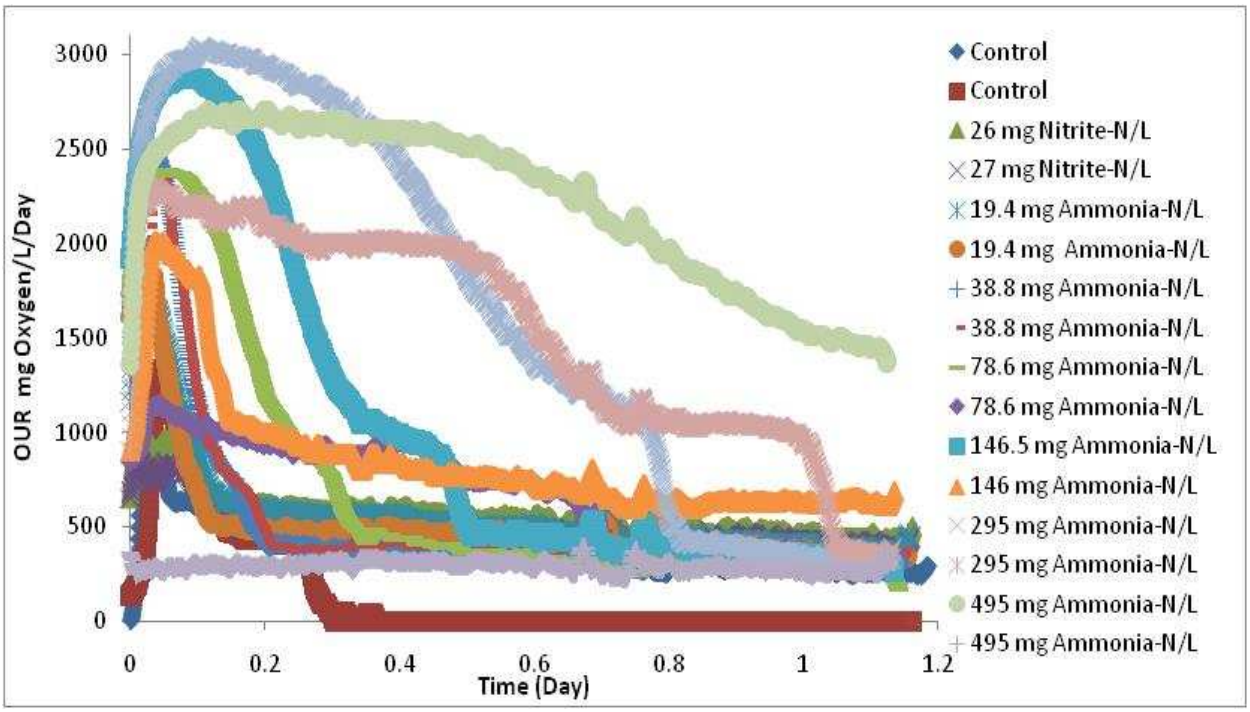

Figure A-4 OUR versus Time for a Respirometer Batch from the Medium Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 103.

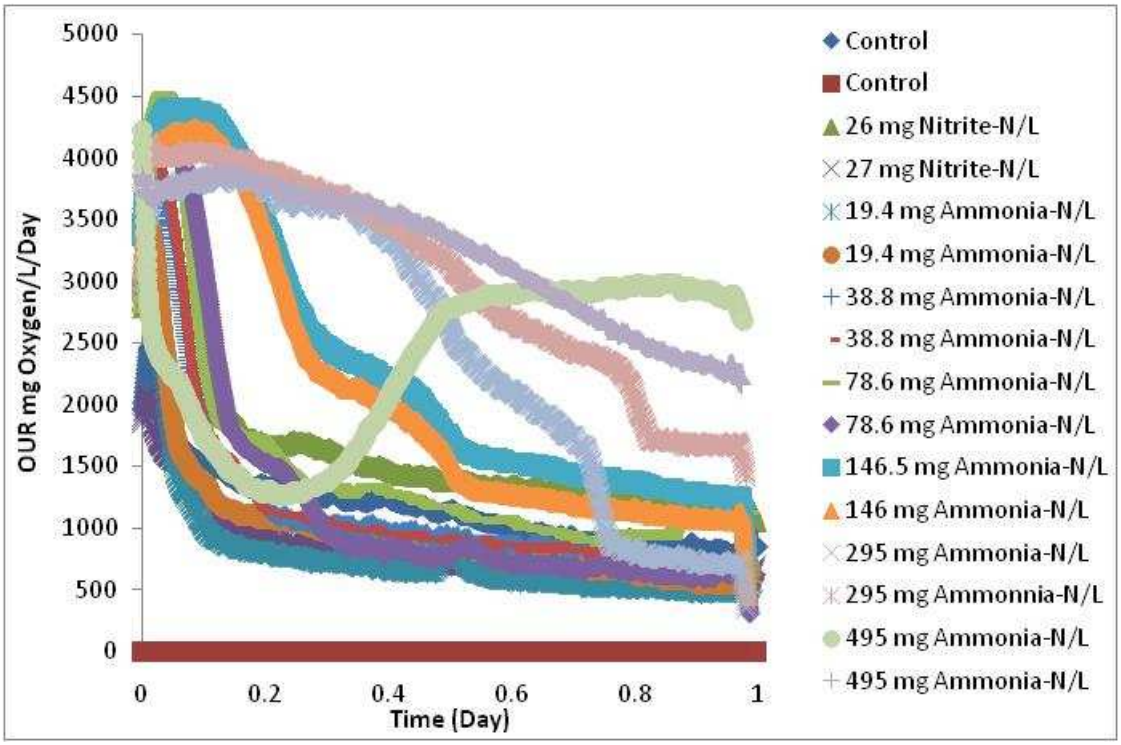

Figure A-5 OUR versus Time for a Respirometer Batch from the Medium Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 109.

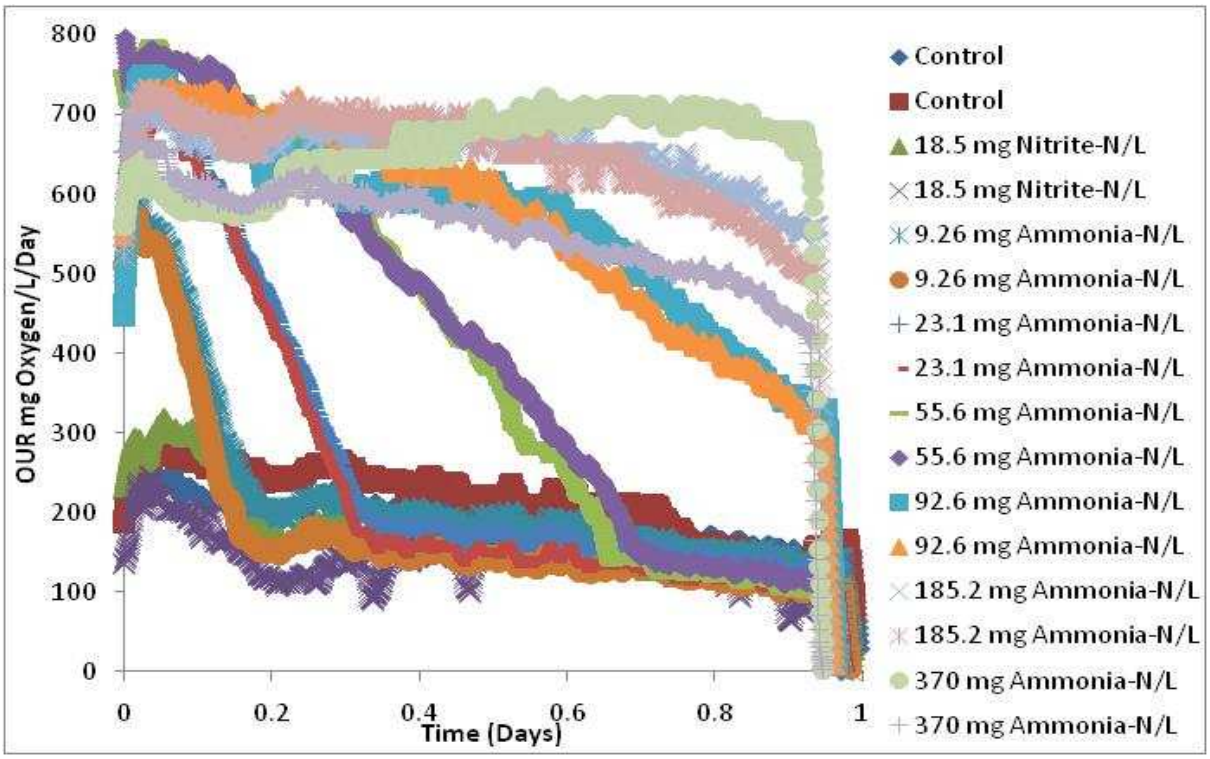

Figure A-6 OUR versus Time for a Respirometer Batch from the High Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 65.

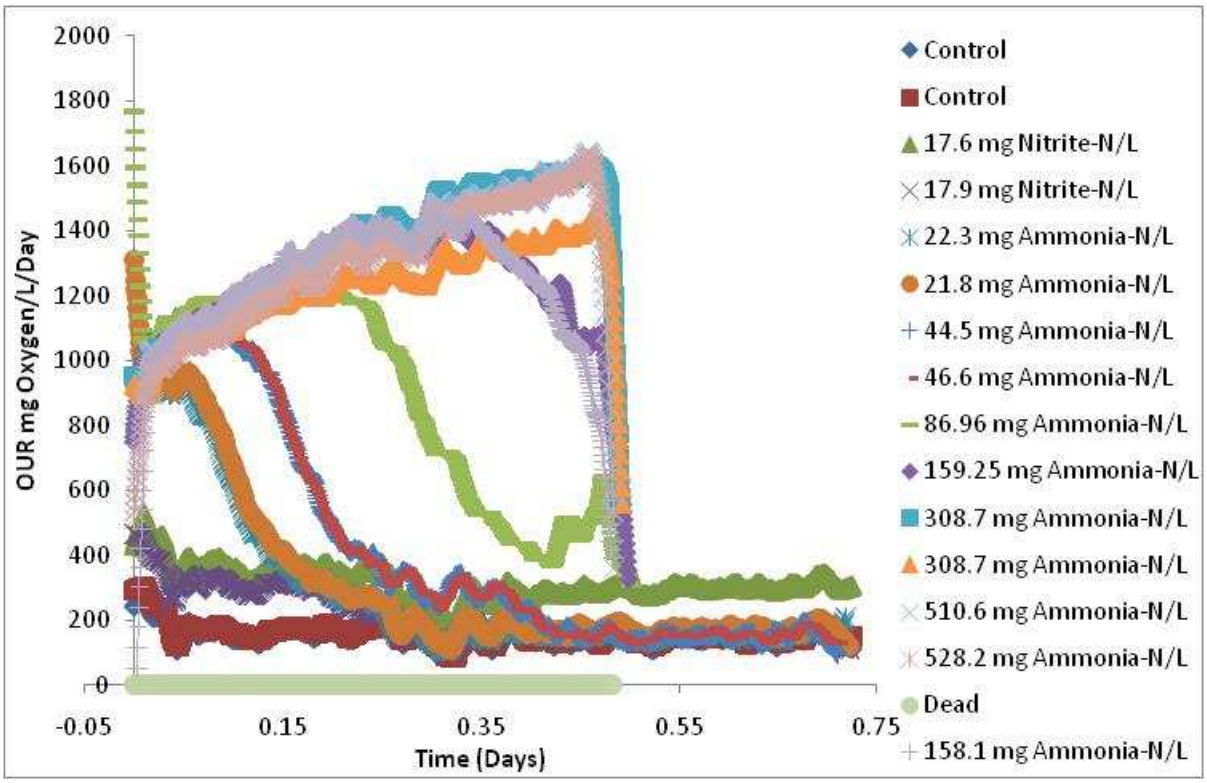

Figure A-7 OUR versus Time for a Respirometer Batch from the High Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 86.

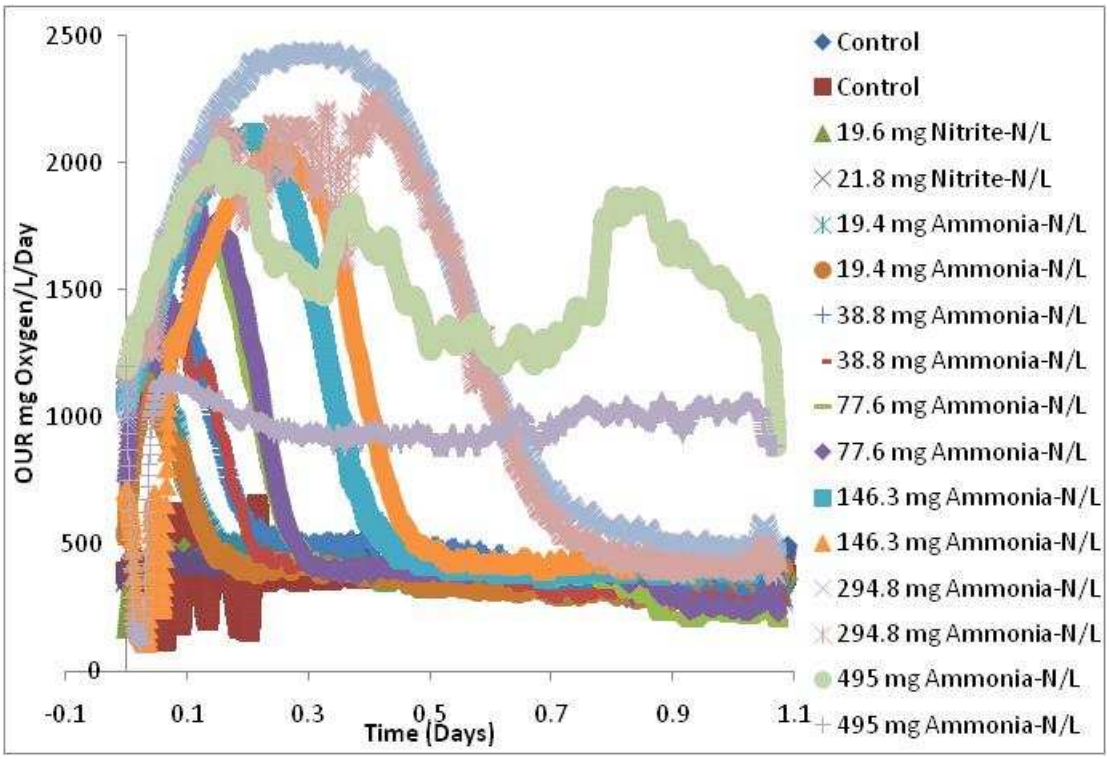

Figure A-8 OUR versus Time for a Respirometer Batch from the High Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 106.

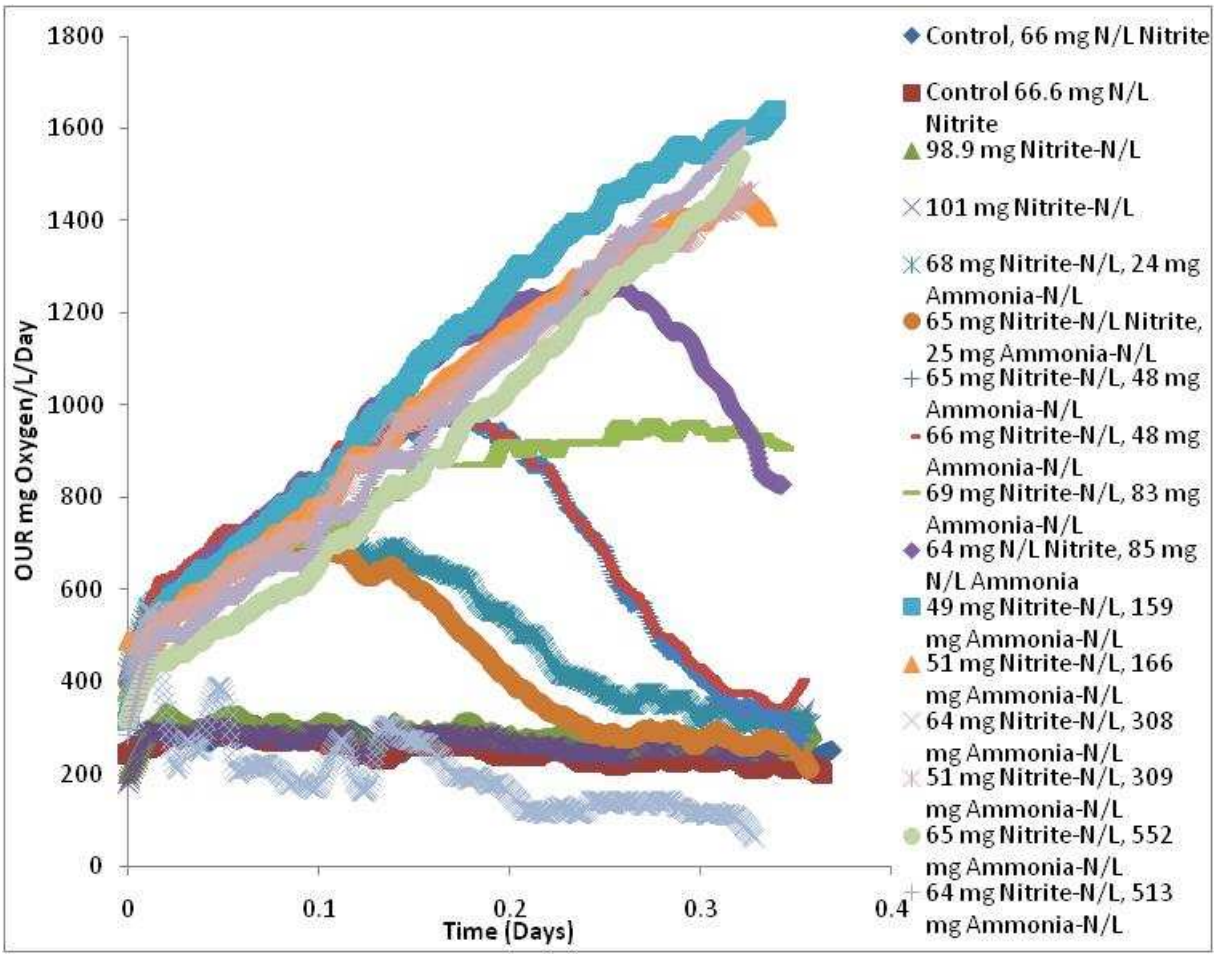

Figure A-9 OUR versus Time for a Respirometer Batch from the High Stress Reactor. Substrate was added at Time equal zero. Biomass was taken from the Control Reactor on Day 116.

Appendix B Sample pH Curve

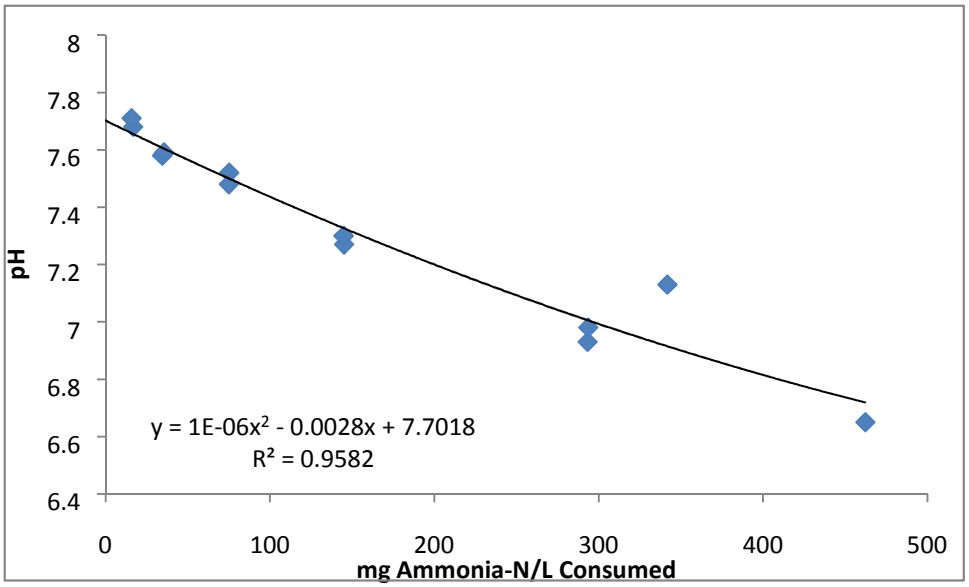

Figure B-1 pH versus mg Ammonia-N/L consumed in the Respirometer Batch Tests

Appenidx C Generating Oxygen Uptake Rate Curves

Oxygen consumption was measured by the total number of calibrated bubbles drawn into the bottle at every minute of the test. Figure C-1 gives an example of the raw respirometer data. One test will be used as example of the work performed on the data for each batch test.

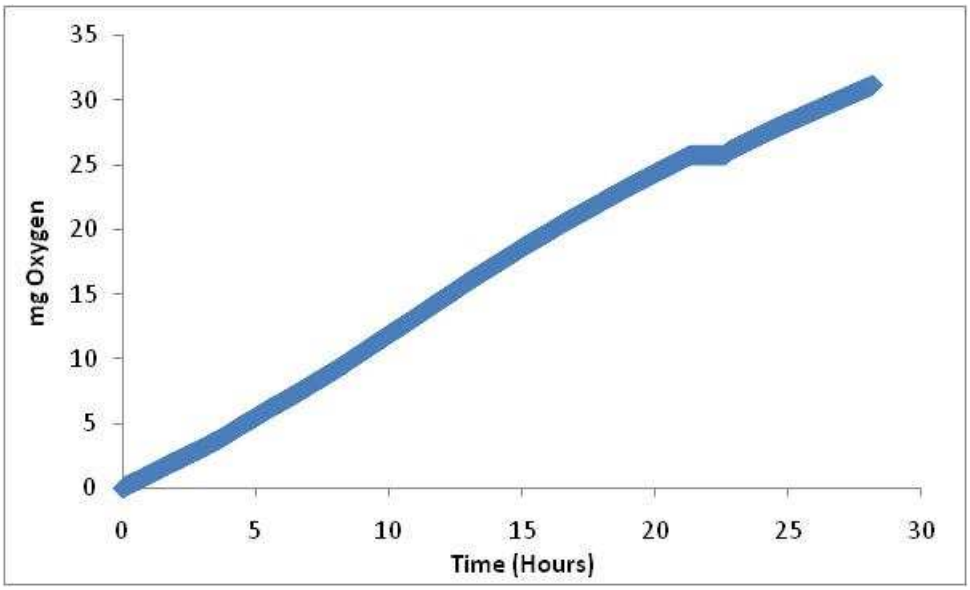

Figure C-1 mg of Oxygen Drawn versus Time

The total oxygen consumed is used to determine the rate of oxygen uptake. First, the oxygen consumed each minute is determined by finding the mg of oxygen taken up by the biomass each minute of a test. Figure C-2 shows oxygen taken up each minute of the experiment. The data contains only four discrete values with most of the data points being zero. The discrete nature of the data is a relic of the oxygen being added one bubble at a time to the bottles, and the number of bubbles added being measured every minute not instantaneously.

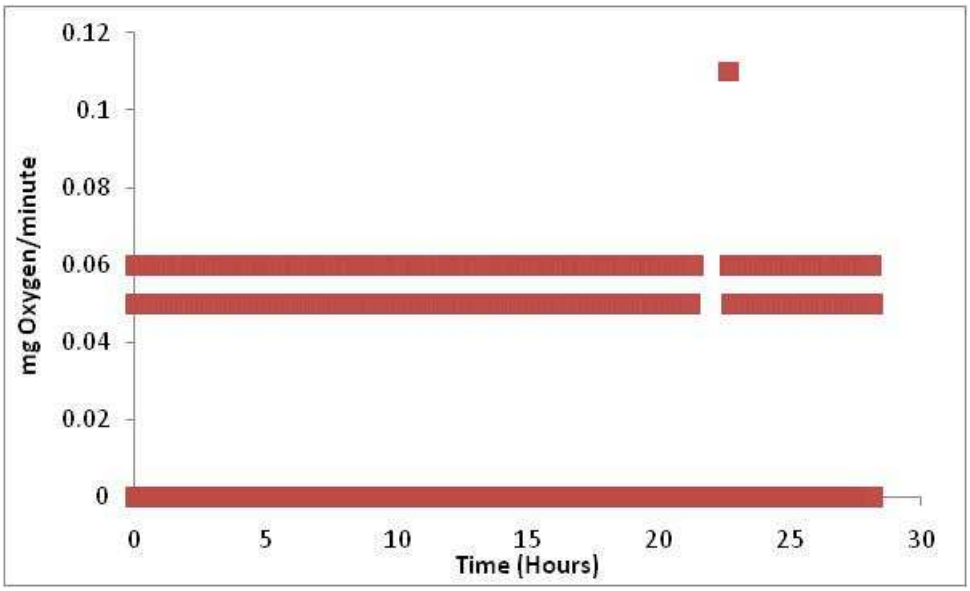

Figure C-2 mg oxygen consumed each minute versus time

The figure C-2 cannot be used to be directly used to determine the kinetic parameters of the OUR curve. In order to obtain an oxygen uptake rate that can be used for modeling, each bottle of each test's discrete data was smoothed. The data was smoothed using a form of local regression. Oxygen rates near a specific time are average using the points within 15 minutes of the specific time. Each point is not equally averaged as is the case with a moving average. Points farther from the specific time are weighed less and points closer are weighed more in the averaging. A 2nd degree polynomial model was used in the weighing in order to allow a larger range to be used in the averaged for data sets that had lower nitrifier concentration with lower oxygen draws. The same smoothing range was used in each test in order allow comparisons between tests to be more straightforward. The remaining OUR graphs are smoothed in this manner. Figure C-3 is an example of smoothed data that contains all of the OUR data for one batch test, and the amount and type of substrate added to each bottle at the beginning of the batch testing with the data of testing. The title indicates the date and source reactor for each batch test.

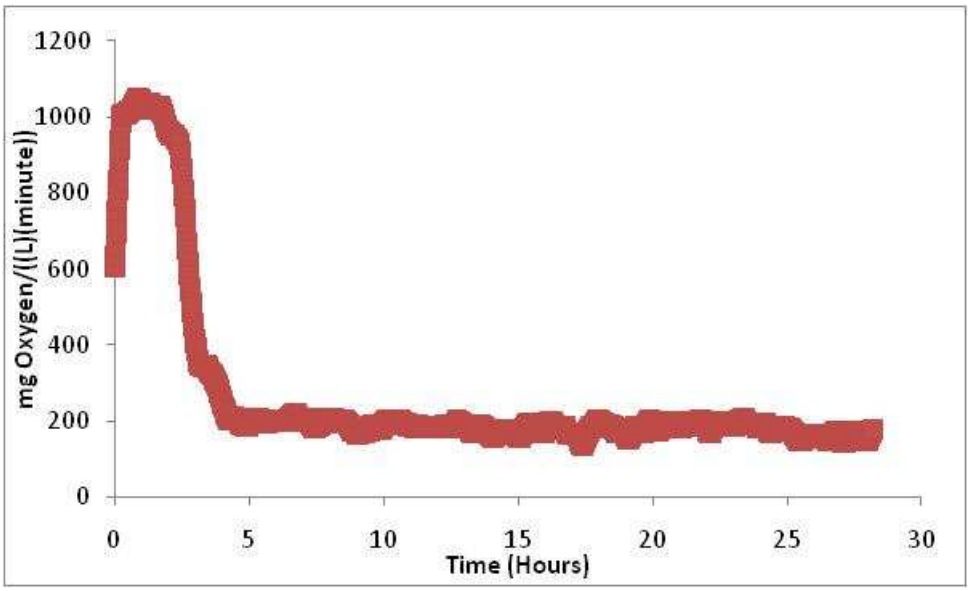

Figure C-3 mg Oxygen per L Oer Day Versus Time for a Batch Experiment (Smoothed Data)with 20 mg NH_3-N /L added a time equals zero.

Appendix D Summary of Attached Files

Appendix Graph 6_11s react cell All.xlxs

Appendix Graph 7_2s react cell all.xlxs

Appendix Graph 7_3g react cell all.xlxs

Appendix Graph 7_7c react cell all.xlxs

Appendix Graph 7_19g react cell all.xlxs

Appendix Graph 7_22s react cell all.xlxs

Appendix Graph 7_25g react cell all.xlxs

Appendix Graph 7_27c react cell all.xlxs

Appendix Graph 8_1s react cell all.xlxs

Charts in Thesis-Parameter fitting 1.xlxs

Charts in Thesis-Parameter fitting 2.xlxs

Graphes for Qualitative Analysis.xlxs

Kinetic Parameters Each Cell individually fit.xlxs

Kinetic Parameters Trying to each test to have the same parameters.xlxs

Model Data Used For V Shaped Graph.xlsx

Parameter Fitting Forced for every Respirometer Cell.zip

Raw Respirometer Data.zip

SBR Operating Data.xlsx

Smooth Data and Aquasim fit files for every Respirometer Cell.zip

Smoothed Data in Text Files.zip