AguaClara Wastewater
Granular Sequencing Batch Reactor (GSBR)

Fall 2015 Research Report

Amiel Middelmann, Nisarg Gohil, and Andrea Whalen

12/11/2015

Abstract

The AguaClara Wastewater team returned in Fall 2015 with two teams investigating both anaerobic and aerobic wastewater treatment reactors. The GSBR (Granular Sequencing Batch Reactor) team worked to expand knowledge about this potentially innovative and sustainable wastewater treatment technology. The goal of the Fall 2015 semester was to investigate potential improvements to reactor operation that would improve nitrogen removal. Furthermore, the team was interested in the stability of granular sludge under lower aeration requirements. One continuously operating reactor was inherited by the GSBR team at the start of the semester, which had been inoculated during the summer 2015 by visiting student researchers from Brazil. Lastly, the team considered the feasibility of this technology in implementation.

Results from monitoring ammonium and nitrate concentrations through several cycles of operation indicated that improvements to nitrogen removal did not result from operational changes that were installed. Conclusions from the semester included a decreased nitrification efficiency under lower aeration supply. However, granule stability and chemical oxygen demand (COD) removal maintained under lower airflow conditions.

Table of Contents

Task List

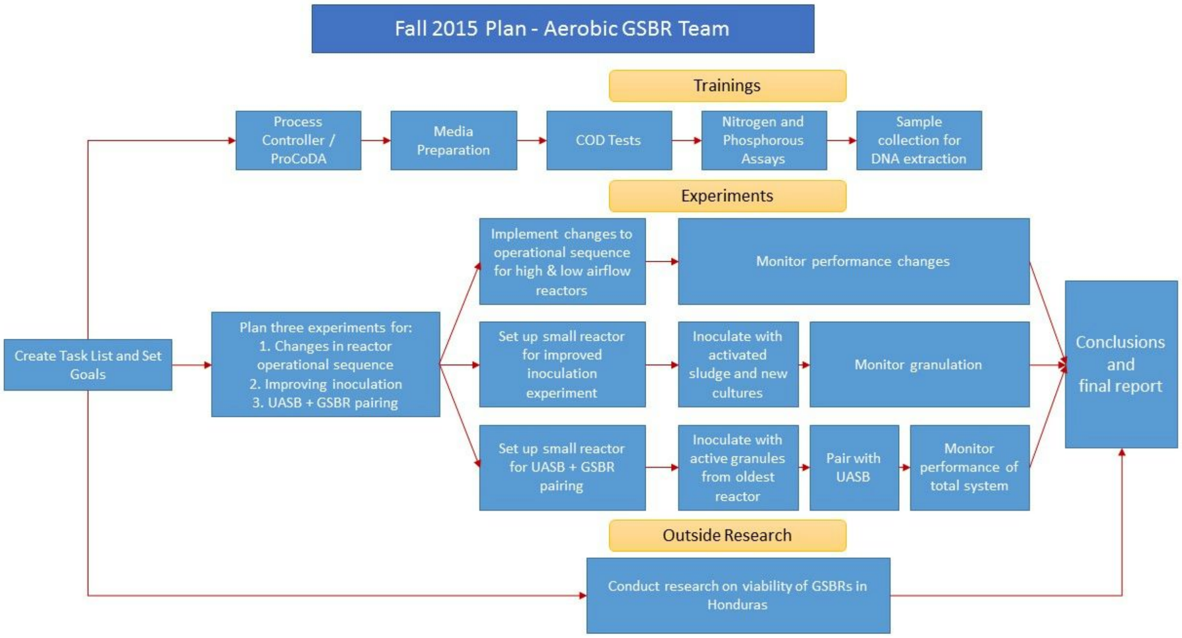


Figure 1: Task Map for Fall 2015

Note: **Week 1** is week of 9/7/15

Task Details

Trainings

1. Process controller / ProCoDA (9/14/15) - **Week 2**	**Complete**
2. Media preparation (9/16/15) - **Week 2**	**Complete**
3. GSBR operation and aeration setup (9/23/15) - **Week 3**	**Complete**
4. COD analysis (9/21/15) - **Week 3**	**Complete**
5. Dissolved Oxygen Measurement (9/30/15) - **Week 4**	**Complete**
6. Ammonium and Nitrate/Nitrite Assays	**Complete**
7. Collecting samples for DNA extraction (TBD later in semester)	**Incomplete**

Operational Optimization of GSBR - Andrea

The goal is to investigate what operational changes can be made to the GSBRs that will improve performance or reduce theoretical costs. Our aim will be to re-evaluate the operational sequence used for the reactors which we have held constant for nearly each granulation experiment.

1. Overview of reactor operation and maintenance (9/16/15) - **Week 2**

    a. This includes reviewing process control methods, media preparation, sampling, and cleaning the reactors. The focus will be on the two reactors operating with high and low air flow rates.
2. Present experiment goals and procedure (10/8/15) - **Week 4**
    a. Here we will propose a detailed list of objectives along with the experimental procedure. Preliminary plan is to implement a shortened aeration time and post-aeration anaerobic time to the sequence for both high and air flow reactors. Experimental procedure will outline methods of sampling and frequency of samples taken.
3. Implement changes to reactor sequence and begin sampling (10/5/15) - **Week 5**
    a. Sampling will be ongoing for at least 4-6 weeks before changes may be made to the experimental setup.
    b. Data will be analyzed to measure success of denitrification throughout experiment.
4. Present next experiment goals and procedure (11/5/15) - **Week 9**
    a. Preliminary plan is to implement either a higher strength influent mix (to mimic blackwater characteristics) or introduce temporal spikes in influent concentration and monitor performance effects. This experiment could be introduced to the reactors while they are still being tested for operational sequence changes.
5. Begin final data analysis for conclusions (11/23/15) - **Week 12**
    a. Give our team enough time at the end of the semester to begin data analysis and conclusions for final report.

UASB + GSBR Testing - Amiel

The goal is to monitor performance of a paired UASB / GSBR reactor system to see if the reactors could successfully operate in sequence. Furthermore, the next objective is to investigate whether the paired system could be net energy neutral or even positive.

Update 11/20/15: This experiment may be tested over the last week of classes if there is still interest and the UASBs are operating.

1. Coordinate with UASB team to discuss reactor setup (9/16/15) - **Week 2**
    a. This task includes deciding what size of reactor will be used for both the UASB and the GSBR as well as calculating the flow rate that will be used through both processes. Furthermore, a decision will need to be made for where this experiment will take place.
2. Present experiment goals and procedure (10/8/15) -**Week 5**
    a. Propose a full experimental procedure and objectives that outline what will be measured and how. This includes a full explanation of the setup for the paired reactor system.
3. Set up small airlift reactor to later be paired with UASB (10/19/15) - **Week 7**
    a. Before pairing the GSBR with the UASB, set up in the lab the GSBR on its own in order to operate for at least 1-2 weeks with synthetic media.

4. Inoculate small reactor with active granules from oldest reactor (10/26/15) - **Week 8**
    a. Perform inoculation and startup with active granules. Operate this reactor and monitor performance for 1-2 weeks before pairing with UASB. One idea would also be to reduce the strength of synthetic influent mix to match the COD levels of previous UASB effluent data.
5. Pair GSBR with UASB and measure performance through entire process (TBD when UASBs are operating) - **Approx Week 9**
    a. This task is dependent on the timing for UASB operation, which should operate for some time separate from the GSBR before pairing as well.
6. Perform energy balance between potential energy from methane and required energy for operation and aeration (11/23/15) - **Week 12**
    a. Data to be conducted throughout the semester with final calculations completed near end.
    b. Analyze methane generation data and aeration requirements in order to perform and energy balance on the system. Also make preliminary estimates for operation costs of other processes including pumping and controls

<u>Improving Inoculation - *Now future plans*</u>

The goal is to experiment with methods to reduce the startup time for granulation by imposing operational changes to the reactors as well as inoculating with new specific cultures of microorganisms other than activated sludge.

1. Present experiment goals and procedure
    a. The goal will be to inoculate most likely a small reactor for solely testing the granulation phase. This also includes deciding what added cultures we will be utilizing and what changes to the influent mix and operational parameters we might consider.
2. Set up small reactor and conduct “dry-run” without biomass
    a. This includes setting up a fridge for stock, pumping, and tubing, as well as process controller.
3. Explore new parameters for measuring granulation
    a. This includes finding new methods for measurement of granulation success beyond images of biomass and treatment performance.
4. Inoculate small reactor and begin observing granulation phase
    a. This task will include travel to the Ithaca Area WWTP and retrieval of activated sludge.
    b. Ongoing observation and sampling of granulation phase with performance (COD removal at the least) and granule imaging. Possibly will include new methods for granule assessment.
5. Time permitting, operational changes to the reactor may be made based on findings from other experiments

Viability of GSBRs and other Wastewater Treatment Systems in Honduras - Amiel

The goal is to research how viable GSBRs are in a resource limited setting like Honduras compared to other wastewater treatment methods. Efforts will include investigating low cost ways to operate and maintain a GSBR system in Honduras including low cost aeration methods. Furthermore, research should be conducted into other potential wastewater treatment methods for AguaClara to explore. Lastly, by the end of the semester a plan should be made for Wastewater Team members traveling to Honduras in January including what type of research they could conduct while in country.

Introduction

Conventional aerobic wastewater treatment methods are not perfectly suited for exportation to developing countries. The activated sludge process, a conventional form of secondary wastewater treatment, necessitates a large area for reactors and clarifiers and requires a high energy input for aeration. With the growing inclination towards nutrient mining and the lack of availability of space, there is a need for improved technology that addresses all the concerns associated with conventional secondary treatment processes. Furthermore, the need for sustainable and low cost wastewater treatment in the developing world calls for new technologies to be researched.

Recently, by integrating a more compact approach, aerobic granular sludge technology has emerged as a way to decrease the scale of the wastewater treatment process. This is done through the use of sequencing batch reactors, for simultaneous chemical oxygen demand (COD) and nutrient removal [1]. Granular sequencing batch reactors (GSBRs) cycle through five stages during operation: fill, anaerobic, aerobic, settle, and discharge. There is promise that GSBRs could be a suitable aerobic technology sustainable application. This semester, however, the objective is to look into alternative methods of operation, in order to achieve a reduction in the aeration requirements of the reactor and an upsurge in denitrification.

Nitrogen Removal:

The importance of nitrogen removal in wastewater treatment has grown significantly, as understanding of the effects on public health and the environment of various nitrogen compounds has broadened. Inorganic nitrogen can contribute to algae blooms; as these blooms recede, the decomposing material can bring about low dissolved oxygen concentrations, in a process known as eutrophication. Ammonia also poses a toxicity risk for many aquatic species [2]. Lastly, according to the World Health Organization, elevated levels of nitrate is the leading cause of methemoglobinemia, also known as “blue baby syndrome”, in infants that can ultimately lead to death if left untreated [3].

Wastewater treatment employs microorganisms to convert the various nitrogen species from one to another. Traditionally, the ammonia in the influent is changed to nitrate, and nitrate eventually to nitrogen gas, through the microbial processes of nitrification and subsequent denitrification, respectively. In nitrification, nitrifying bacteria convert ammonia, and nitrite to nitrate. Nitrification is an aerobic process, and requires oxygen to proceed. In denitrification, facultative bacteria convert the nitrate back into nitrite, and ultimately to nitrogen gas [4]. Denitrification is an anoxic or near anaerobic process, and will be limited if there is a high dissolved oxygen level in the water. Nitrogen removal can be achieved faster if partial nitrification (in which ammonia is oxidized to nitrite, only) is allowed to occur or anammox (ammonium oxidizes directly to nitrogen gas) is involved.

One of the advantages of the GSBR is the makeup of an aerobic granule. The granule structure has concentric layers (Figure 2) where different conditions exist, which promotes simultaneous nitrogen and COD removal [5]. Figure 3 is a more detailed look into the nitrogen cycle that includes the three processes discussed principally above.

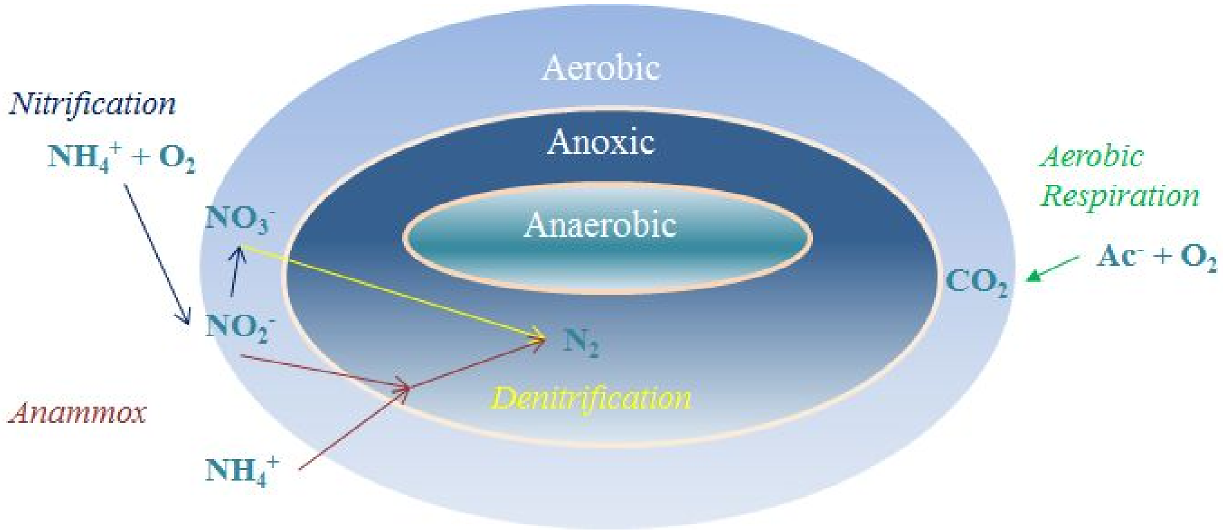


Figure 2: Granule makeup

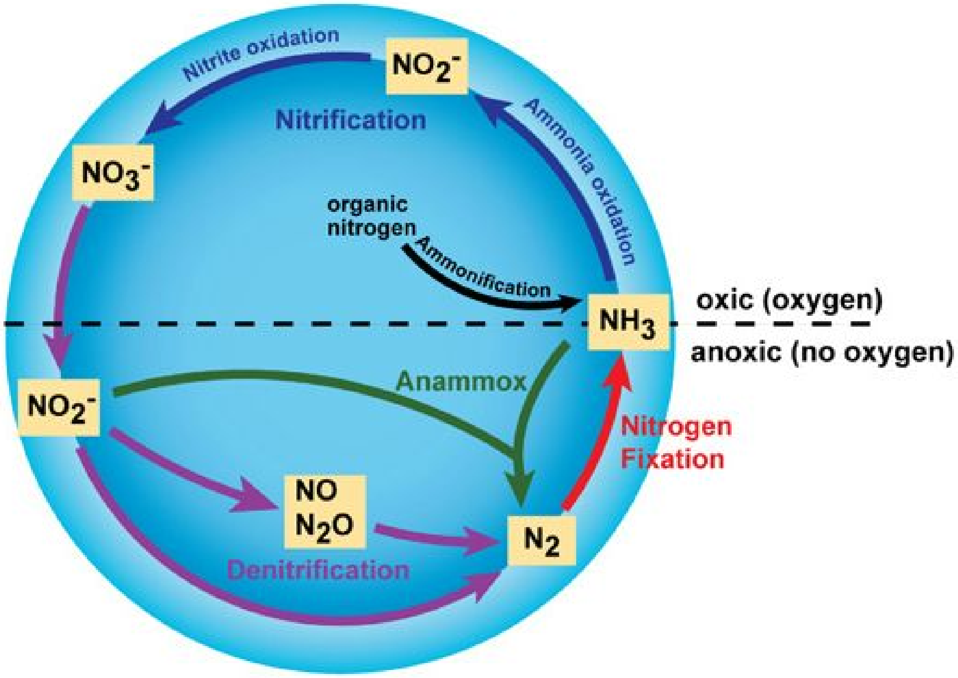


Figure 3: Nitrogen Cycle

Literature Review

Aerobic granules are formed by the interaction and aggregation of bacterial cells. Liu and Tay describe the aggregation process in four steps, summarized below [6].

1) Physical Movement (caused by external factors) : This step includes the movement of the bacterial cells which causes cell-cell contact. It can be achieved through application of a hydrodynamic shear, diffusion or simply by gravitational force

2) Initial Attractive Forces : In this stage, the contacted cells are kept in position due to some forces of attraction between them. Examples of such forces of attraction are van der Waals forces, and hydrophobicity.

3) Exo-polymer Formation : The third process is the formation of extracellular polymers, which are crucial in the maturation of the agglomerated cells. The exo-polymers provide stability to the amalgamated structures, which are the building blocks for granules.

4) Aggregate Shaping : The agglomerate shaping is the final process of the granule formation mechanism. This stage is primarily dependent on the hydrodynamic shear, as well as the substrate loading, type of species, and strength of interactions. The external shear causes the matured amalgamates to form a structured community.

Slow-growing organisms positively influence the stability of granules. These organisms are encouraged by the use of a feast-famine regime. The feast stage is the period in which external substrate is applied to the system; the famine stage is a period in which the slow-growing organisms consume their internally stored substrate [7].

Research by Moghaddam and Moghaddam examined the influence of a pre-anaerobic period before aeration on granulation on three reactors with pre-cultivated granules. The granules in the first, which operated under purely aerobic conditions, gradually disappeared, with filamentous bacteria beginning to dominate. However, the granules in their third reactor, with an extended pre-anaerobic time (175 minutes), also were negatively impacted; aerobic granules with a more fluffy, floc-like appearance were selectively formed, leading to poorer COD removal performance. The second reactor granules, subjected to a reduced pre-anaerobic time of 85 minutes, were the most stable and compact--illustrating the importance of balance in choosing the time of the famine stage in a GSBR [8].

Initial research into GSBRs had proposed the importance of high oxygen concentrations in growing stable granules [9]. However, maintaining high oxygen concentrations is costly. Moreover, if a goal is to observe both nitrification and denitrification (while maintaining high COD removal efficiency), these elevated concentrations will cause incomplete denitrification.

De Kreuk, Heijnen, and M. c. m. van Loosdrecht performed experiments geared toward observing the influence of oxygen saturation levels on nitrogen removal. Following a nearly two-month interval operating at a saturated oxygen concentration in order to mature the granules in their reactor, the concentration was lowered to 40% of the saturation level. The dissolved oxygen concentration in the reactor was controlled in the reactor by supplying Nitrogen gas along with air during aeration. This allowed for lower dissolved oxygen concentrations to be obtained while high shear levels were provided. Nitrate concentrations then slowly decreased, indicating a rise in denitrification efficiency, ultimately reaching a maximum nitrogen removal of more than 90%. However, after this initial favorable period, the granules’ shape transformed, and de Kreuk reported nitrogen removal efficiency dropping to around 60%. The next experiment the team performed was then at an oxygen concentration at 20% of the saturation level. After a period of almost 80 days of decreased nitrification efficiency, the reactor recovered and nitrogen removal was maintained at around 94% by the end of the experiment [10].

Subsequent experiments were performed by Lochmatter, Gonzalez-Gil, G, and Holliger centered on optimizing nitrogen removal in GSBRs. Various aeration strategies were examined, including (1) a constant DO concentration at around 25 - 40% of saturation, in order to allow the presence of ammonium, albeit in small concentrations, at the end of aeration, (2) alternating high and low DO concentrations, with the alternating high concentrations progressively decreasing over the course of the aeration cycle, and (3) intermittent aeration, which involved progressively shortened intervals of aeration at 60% of saturation, interrupted by non-aerated phases. The researchers observed the highest nitrogen removal for the intermittent aeration strategy; they concluded that this was due to this strategy allowing for both rapid and slow processes to proceed to a sufficient degree and the formation of a compact bed during the non-aerated phases to use excess COD for denitrification [11].

Previous Work

In Spring 2014, the AguaClara wastewater team began research on aerobic granular sequencing batch reactors. This was originally initiated by a realization of the need for additional polishing following anaerobic treatment (i.e. upflow anaerobic sludge blanket technology) to meet stringent effluent limits in the United States as set by the Clean Water Act through the National Pollutant Discharge Elimination System (NPDES) permit program, and the promise that GSBRs had shown in literature for nutrient removal capabilities. Unfortunately, due to difficulties in inoculating the GSBRs during AguaClara's first attempt, granular biomass did not survive following over a month of operation [12].

In Summer 2015, two Brazilian students, Maria Dias and Mirelly Manica, began an experiment to look into the the removal efficiencies GSBRs under both low and high airflow rates. The team successfully created granules, albeit small in diameter but in large concentrations. The team arrived at important conclusions regarding the performance of GSBRs. Although consistent in its high COD removal efficiency, at above 80% for all but one of the sample dates, the GSBR monitored over the summer showed limited phosphorus and nitrogen removal. The reactor achieved a minimum of 19% phosphorus removal efficiency, with a maximum below 60%. Additionally, the GSBR satisfactorily achieved nitrification, as the ammonium concentration decreased dramatically over the course of the cycle. However, denitrification was weak as nitrite/nitrate concentrations were elevated in the effluent and rarely showed any signs of removal [13]. It is an objective of this semester's project to observe adequate denitrification in the reactor; this will be done by imposing changes to the cycle schedule and airflow rates.

Methods

Lab Reactor Setup

This semester, the AguaClara GSBR team is operating the reactor inoculated during summer 2015 with low airflow rate by Mirelly Manica and Maria Dias. This reactor has kept the same experimental set up, using ProCoDA to control the GSBR operation and cycles. The same 4L bubble column type reactor is in operation. Furthermore, the aeration system is also similar using an accumulator, two solenoid valves, and a needle valve to supply a constant flow of air into the reactor. This set up is based off of the Oxygen Transfer laboratory experiment used in the CEE 4530 class. To give a constant flow of air to the reactor a solenoid valve connected to the bench air supply is controlled to open and close according to the needed air pressure in the accumulator. The accumulator air pressure remains relatively constant and is used to let a constant air flow rate into the reactor by the needle valve. A process schematic of the reactor and aeration system is shown in Figure 4.

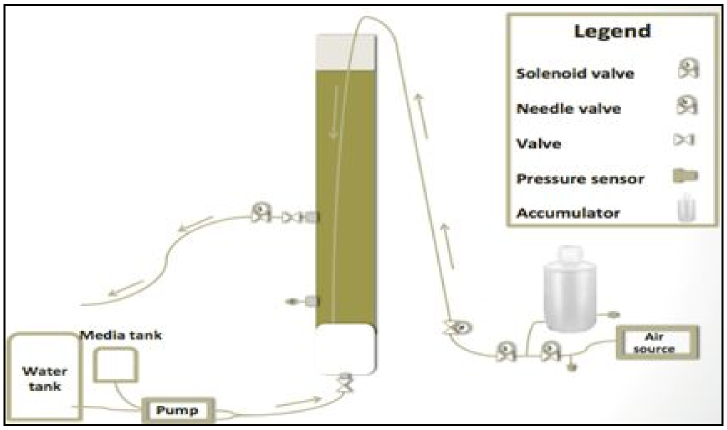


Figure 4: Reactor schematic

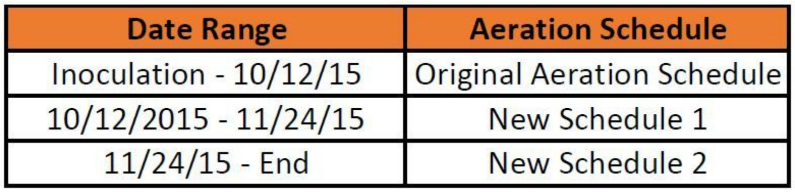
Denitrification Experiment and Aeration Schedules

The experiment to be completed is focused on improving nitrogen removal in the GSBR through operational changes. Nitrification has been successful in the reactor previously, but the nitrogen removal performance is lacking in denitrification. In order to encourage more denitrification, which is an anaerobic/anoxic process, the amount of oxygen supplied to the reactor was restricted in the latter part of the aeration process. Overall performance was monitored in order to show that the granule quality does not deteriorate and the COD removal is maintained at its current value even after making the above said changes. Samples were taken over an entire cycle and analyzed for COD, NH4, and nitrate in order to observe the effect on the amount of denitrification that occurs during the reactor cycle.

Specifically, for the first fifteen minutes, the reactor is subjected to a high air flow rate of 3.3 L/min, in order to provide the shear necessary to ensure that we maintain granule shape. In the next hour, the air flow rate will be 0.5 L/min, due to the tendency for our reactors to reach close to saturation concentration as shown in our DO curves provided. Finally, in the last half hour of the aeration period, the air flow rate is reduced to 0.01 L/min, in order to keep the reactor somewhat mixed and prevent pure anaerobic conditions which would cause any PAOs to release phosphate just before discharge. Before settling, the air flow rate will be increased, in order to suspend the particles. Figure 5 below is a plot of the new aeration schedule in red, with the green horizontal line indicating the air flow rate initiated previously for the entire aeration phase of the cycle.

Later in the semester the aeration schedule was again modified, and named New Aeration Schedule #2, to increase the amount of time of normal aeration from 1 hour to 1.5 hours, while the anoxic period was reduced from 45 minutes to 15 minutes. Figure 6 shows this aeration schedule below. This decrease in the anoxic period in favor of normal aeration was an attempt to supply more air needed during nitrification in order to improve the ammonium removal. Data for the ammonium removal under the different aeration schedules is included in the analysis. Table 1 below shows the date ranges for implementing the three aeration schedules that were used throughout the semester.

Table 1: Date Ranges for Aeration Schedules



Date Range	Aeration Schedule
Inoculation - 10/12/15	Original Aeration Schedule
10/12/2015 - 11/24/15	New Schedule 1
11/24/15 - End	New Schedule 2

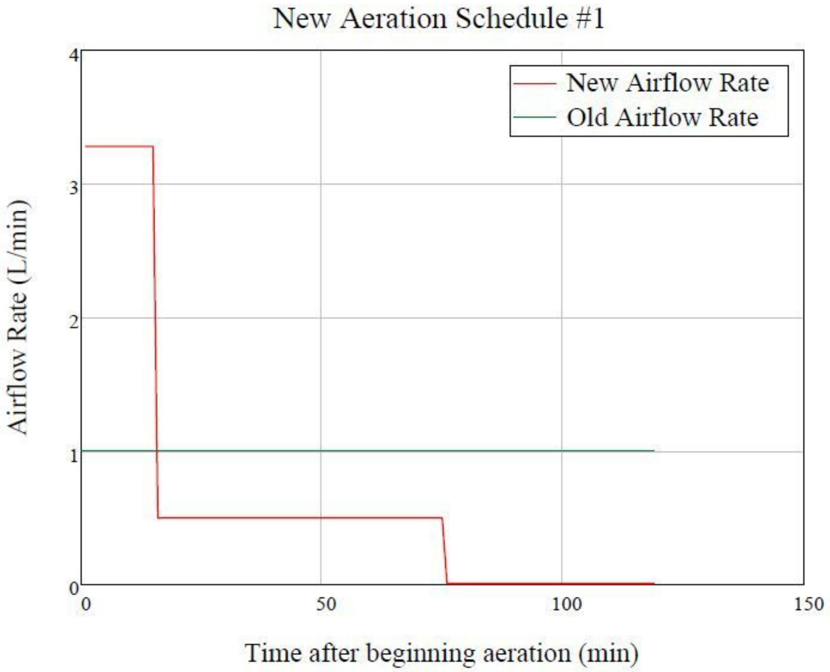


Figure 5: New Aeration Schedule 1

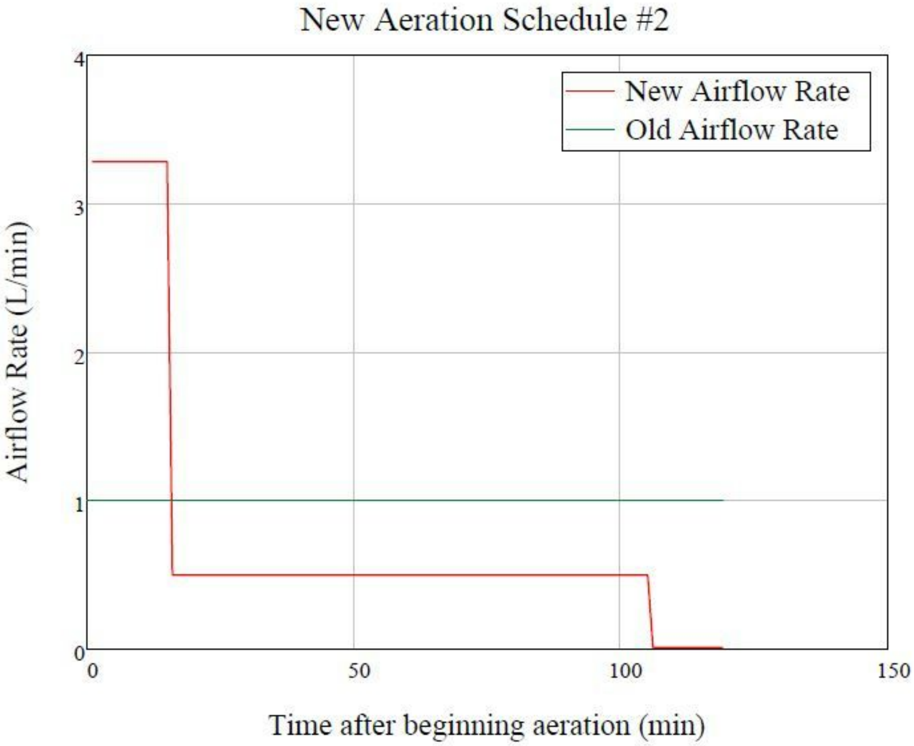
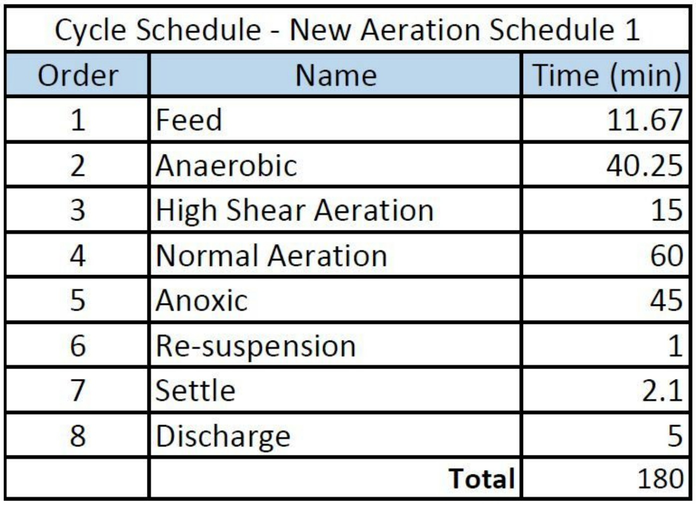


Figure 6: New Aeration Schedule 2

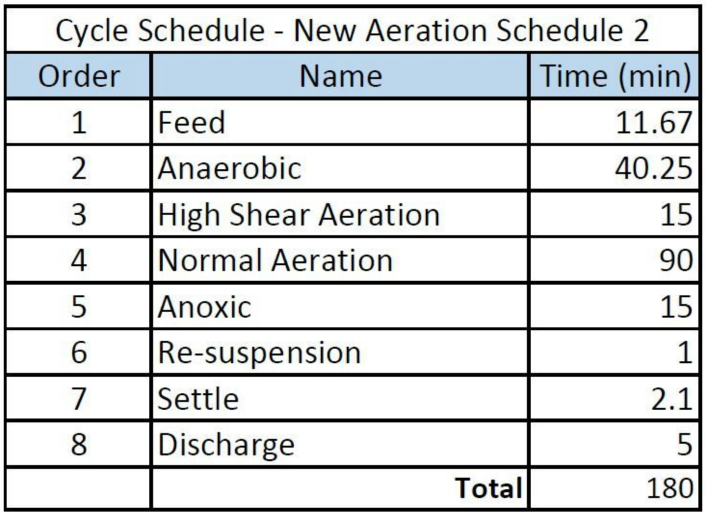
Tables 2 and 3 given below then show the full cycle schedules for both of the new aeration schedules. Similar values compared to the original schedule from the summer for the feed, anaerobic, settle, and discharge are used. One new addition is the Re-suspension state which is used to fully mix and stratify the biomass before settling and discharging.

Table 2: New Cycle Schedule 1



Cycle Schedule - New Aeration Schedule 1		
Order	Name	Time (min)
1	Feed	11.67
2	Anaerobic	40.25
3	High Shear Aeration	15
4	Normal Aeration	60
5	Anoxic	45
6	Re-suspension	1
7	Settle	2.1
8	Discharge	5
	Total	180

Table 3: New Cycle Schedule 2



Cycle Schedule - New Aeration Schedule 2		
Order	Name	Time (min)
1	Feed	11.67
2	Anaerobic	40.25
3	High Shear Aeration	15
4	Normal Aeration	90
5	Anoxic	15
6	Re-suspension	1
7	Settle	2.1
8	Discharge	5
	Total	180

Aeration Control

Using the needle valve to meter in a constant flow rate of air, we are also able to measure flow rate. Figure 7 displays the calibration curve for the flowmeter, in which the scale readings displayed for the silver, stainless steel and black glass balls give an indication of the flow rate of air into the reactor. Using this curve it is possible to confirm that the intended flow rate is correctly being supplied.

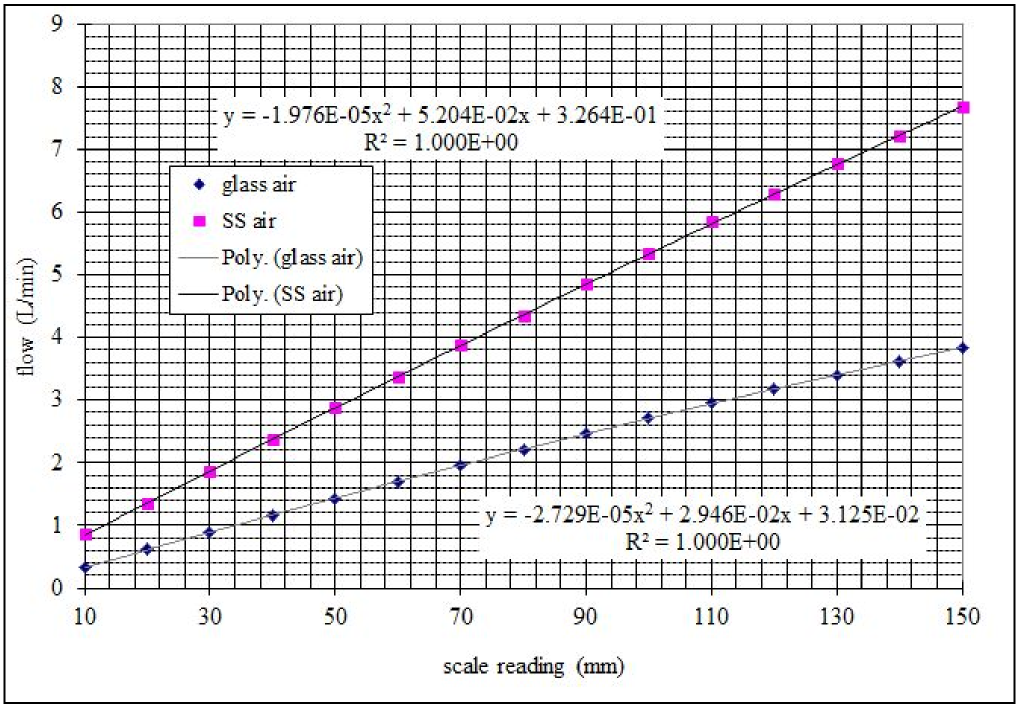
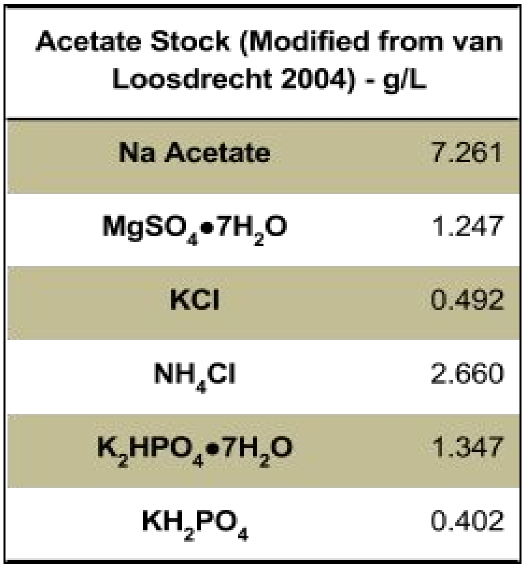


Figure 7: Flowmeter calibration curve

Influent Media

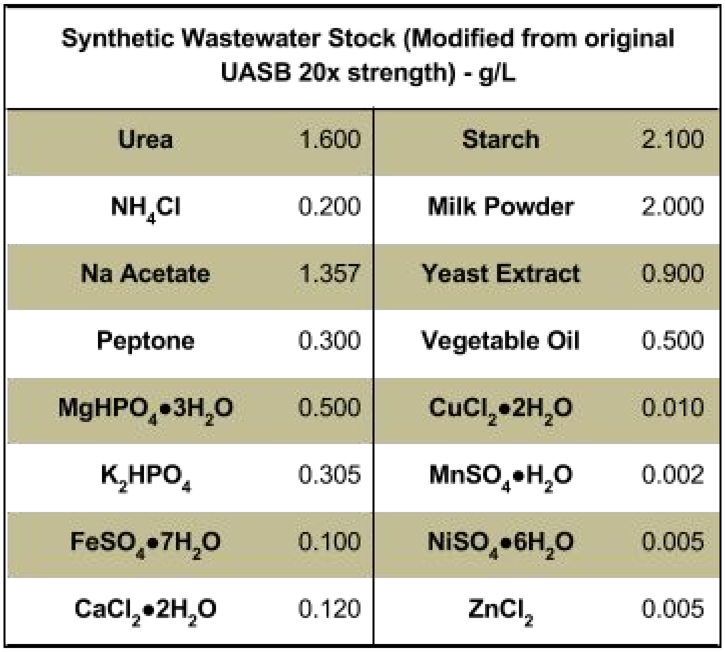
Two different medias (Acetate Stock and Synthetic Wastewater Stock) are used as a substrate for the reactor. These medias are then mixed with tap water with a dilution ratio of 14:1 (Tap water:Stock). The recipes for both media are shown below in Tables 4 and 5.

Table 4: Acetate Stock recipe



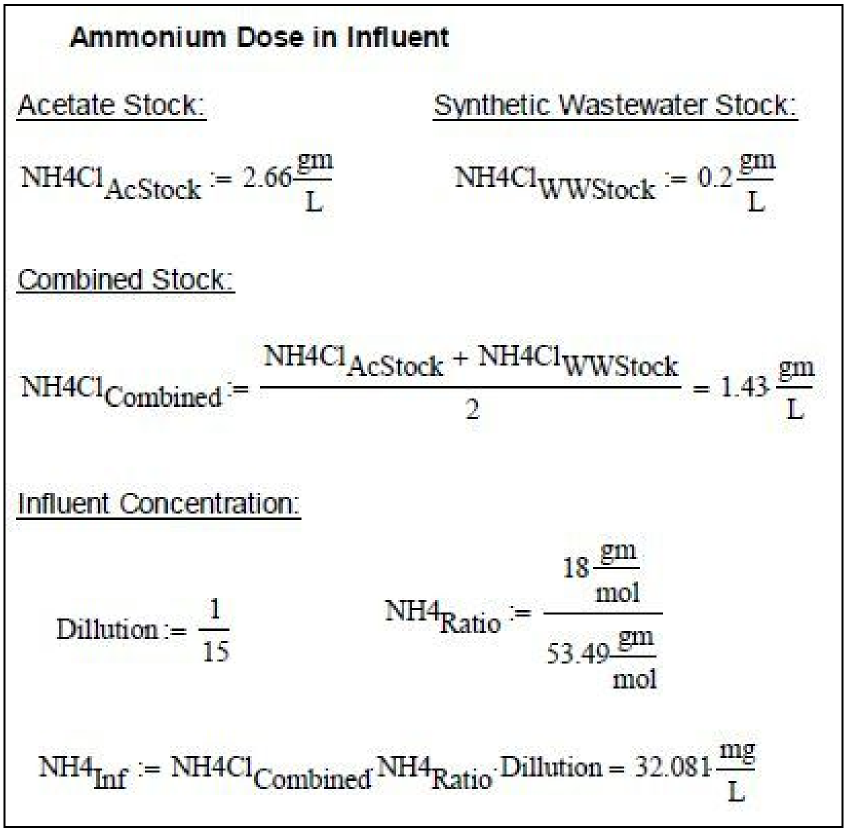
Acetate Stock (Modified from van Loosdrecht 2004) - g/L	
Na Acetate	7.261
$MgSO_4\bullet 7H_2O$	1.247
KCl	0.492
NH_4Cl	2.660
$K_2HPO_4\bullet 7H_2O$	1.347
KH_2PO_4	0.402

Table 5: Synthetic Wastewater Stock recipe



Synthetic Wastewater Stock (Modified from original UASB 20x strength) - g/L			
Urea	1.600	**Starch**	2.100
NH_4Cl	0.200	**Milk Powder**	2.000
Na Acetate	1.357	**Yeast Extract**	0.900
Peptone	0.300	**Vegetable Oil**	0.500
$MgHPO_4\bullet 3H_2O$	0.500	**$CuCl_2\bullet 2H_2O$**	0.010
K_2HPO_4	0.305	**$MnSO_4\bullet H_2O$**	0.002
$FeSO_4\bullet 7H_2O$	0.100	**$NiSO_4\bullet 6H_2O$**	0.005
$CaCl_2\bullet 2H_2O$	0.120	**$ZnCl_2$**	0.005

Since we are interested in Nitrogen removal we determined the ammonium dose in the influent. The concentration excluding the contribution from Urea was found to be 32.1 mg/L, shown in the calculation below.



Ammonium Dose in Influent

Acetate Stock:

$$\mathrm{NH4Cl}_{\mathrm{AcStock}} := 2.66 \cdot \frac{\mathrm{gm}}{\mathrm{L}}$$

Synthetic Wastewater Stock:

$$\mathrm{NH4Cl}_{\mathrm{WWStock}} := 0.2 \cdot \frac{\mathrm{gm}}{\mathrm{L}}$$

Combined Stock:

$$\mathrm{NH4Cl}_{\mathrm{Combined}} := \frac{\mathrm{NH4Cl}_{\mathrm{AcStock}} + \mathrm{NH4Cl}_{\mathrm{WWStock}}}{2} = 1.43 \cdot \frac{\mathrm{gm}}{\mathrm{L}}$$

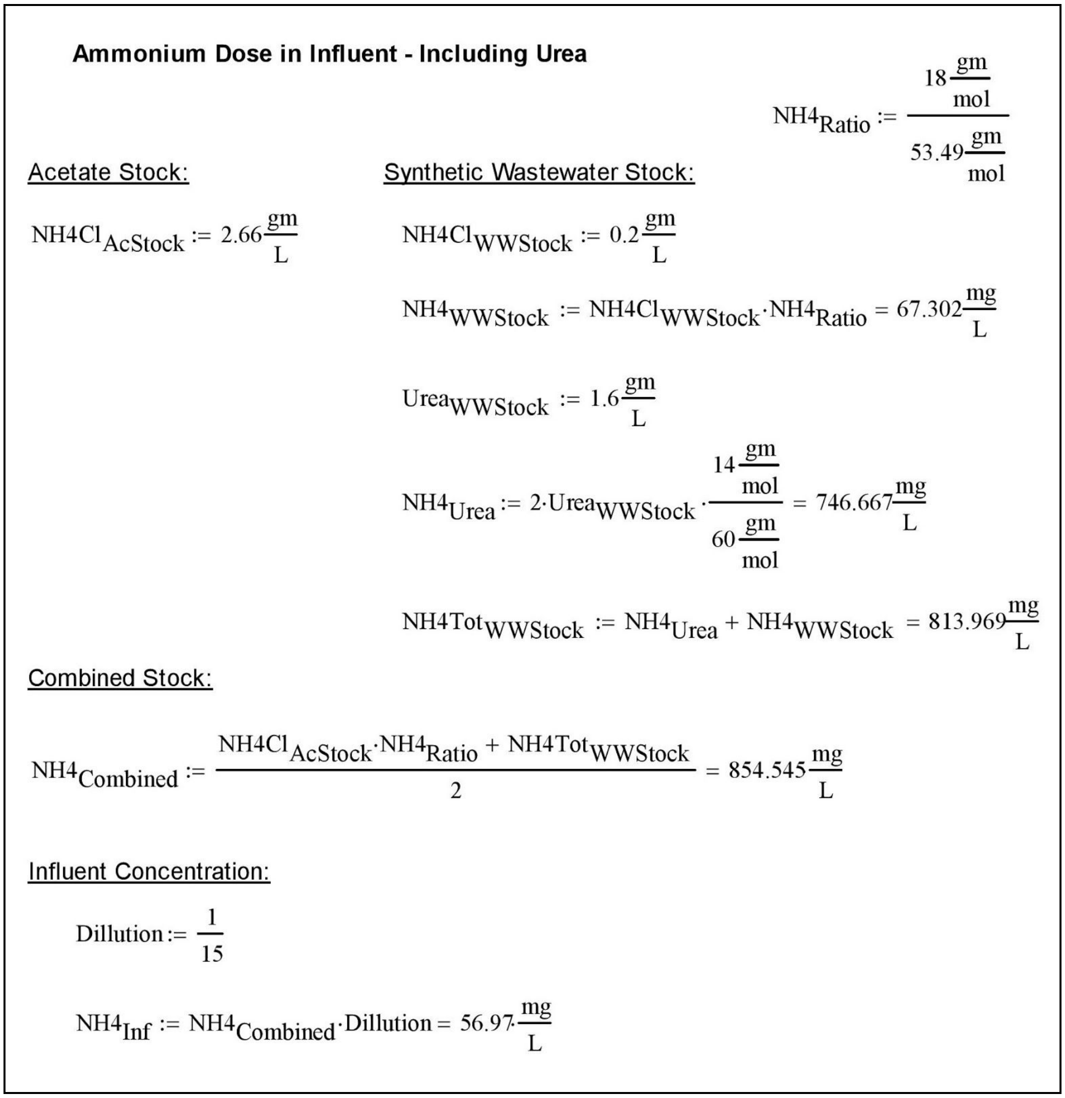
Influent Concentration:

$$\mathrm{Dillution} := \frac{1}{15} \qquad \mathrm{NH4}_{\mathrm{Ratio}} := \frac{18 \cdot \frac{\mathrm{gm}}{\mathrm{mol}}}{53.49 \cdot \frac{\mathrm{gm}}{\mathrm{mol}}}$$

$$\mathrm{NH4}_{\mathrm{Inf}} := \mathrm{NH4Cl}_{\mathrm{Combined}} \cdot \mathrm{NH4}_{\mathrm{Ratio}} \cdot \mathrm{Dillution} = 32.081 \cdot \frac{\mathrm{mg}}{\mathrm{L}}$$

Figure 8: Calculation of Ammonia in Influent

Including the contribution to ammonium from Urea, which is in the synthetic wastewater stock, the effective ammonium concentration dosed into the reactor is again calculated and shown below. This effective concentration was found to be 57 mg/L. This effective concentration will explain the increase in ammonium found during the beginning of a cycle as urea is converted into ammonium.



Ammonium Dose in Influent - Including Urea

$$NH4_{Ratio} := \frac{18\frac{gm}{mol}}{53.49\frac{gm}{mol}}$$

Acetate Stock:

$$NH4Cl_{AcStock} := 2.66\frac{gm}{L}$$

Synthetic Wastewater Stock:

$$NH4Cl_{WWStock} := 0.2\frac{gm}{L}$$

$$NH4_{WWStock} := NH4Cl_{WWStock} \cdot NH4_{Ratio} = 67.302\frac{mg}{L}$$

$$Urea_{WWStock} := 1.6\frac{gm}{L}$$

$$NH4_{Urea} := 2 \cdot Urea_{WWStock} \cdot \frac{14\frac{gm}{mol}}{60\frac{gm}{mol}} = 746.667\frac{mg}{L}$$

$$NH4Tot_{WWStock} := NH4_{Urea} + NH4_{WWStock} = 813.969\frac{mg}{L}$$

Combined Stock:

$$NH4_{Combined} := \frac{NH4Cl_{AcStock} \cdot NH4_{Ratio} + NH4Tot_{WWStock}}{2} = 854.545\frac{mg}{L}$$

Influent Concentration:

$$Dillution := \frac{1}{15}$$

$$NH4_{Inf} := NH4_{Combined} \cdot Dillution = 56.97 \cdot \frac{mg}{L}$$

Figure 9: Influent Ammonia Calculation Factoring In Initial Urea

Analysis

Dissolved Oxygen

After calibration of the dissolved oxygen (DO) probe, results for a typical cycle before implementing the new aeration schedule were analyzed and plotted below in Figure 10. According to the plot, the dissolved oxygen stabilizes after aeration is started until the microorganisms likely have finished consuming the COD around 60 minutes into the aeration cycle. The dissolved oxygen concentration is maintained at around 5.8 mg/L, and then sharply increases after about 60 minutes toward the saturation concentration, before finally dropping off during settling.

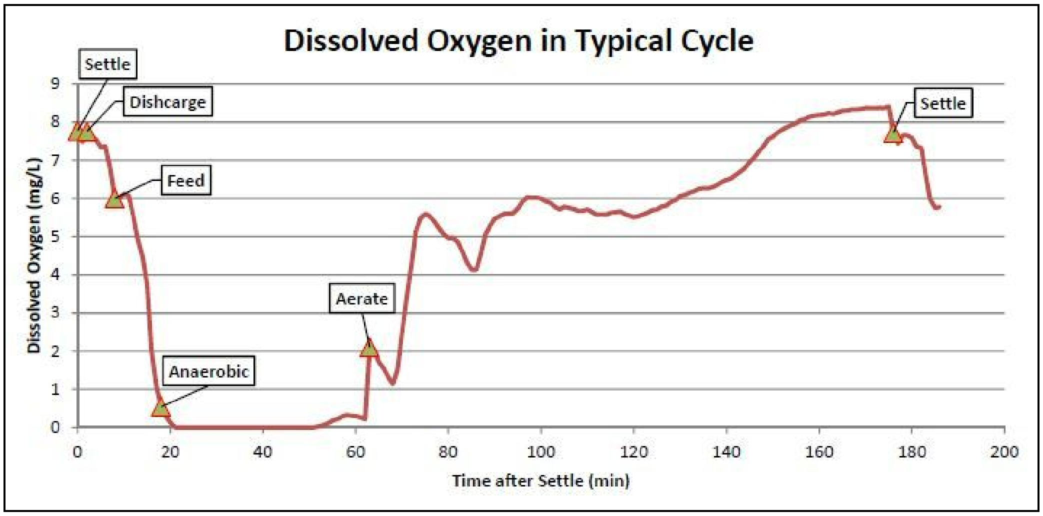


Figure 10: DO probe results following calibration

Following the first change in the aeration schedule to incorporate high shear and anoxic aeration states, the DO through a cycle was measured again. The results for a typical cycle following the changes are shown in Figure 11 below. Here, a quick increase in the DO concentration up to the range of 4 - 5 mg/L takes place immediately after high shear aeration begins. Then, during normal aeration the DO lowers to fluctuate between 1 - 2 mg/L which is in the typical range of operation for conventional activated sludge WWTPs. Finally our results confirmed that during the anoxic state the DO lowered to near zero levels through the remainder of the cycle.

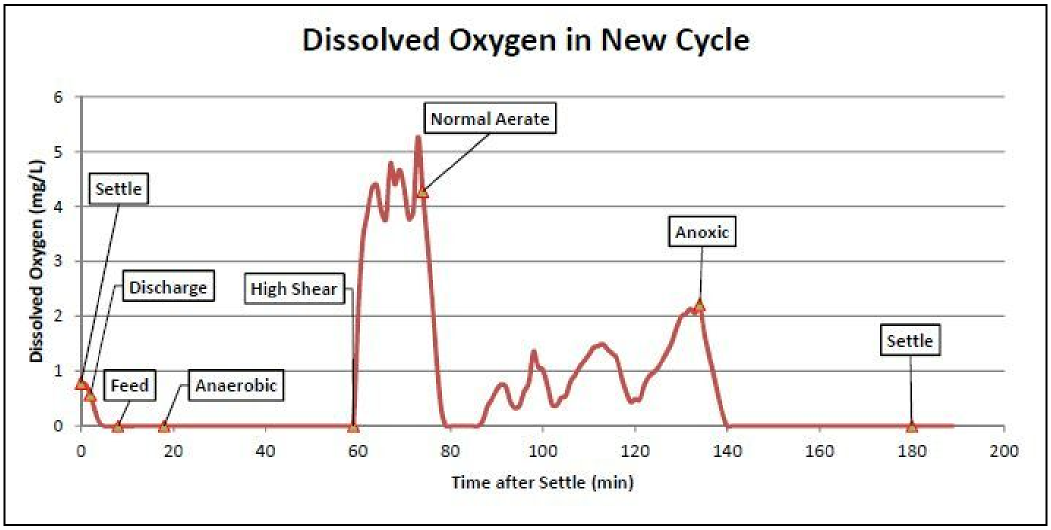
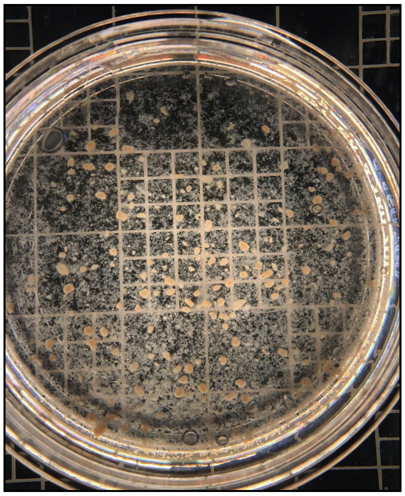


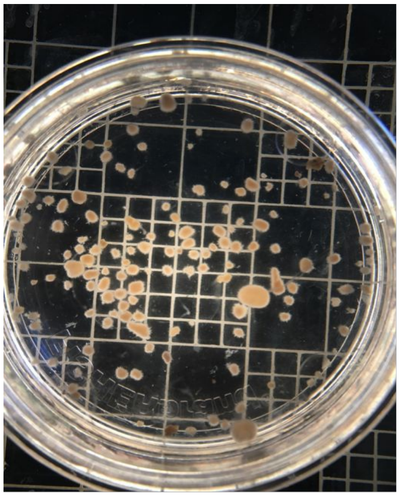
Figure 11: DO probe results for new cycle

Granule Growth

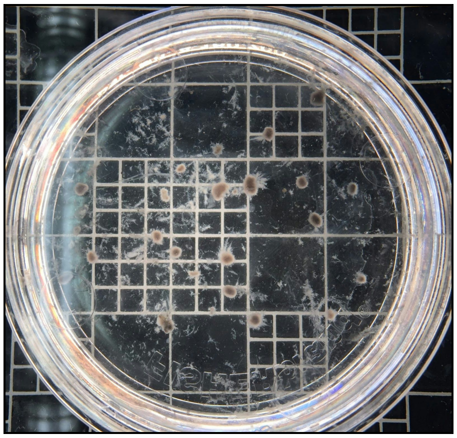
The granule size and quality was constantly monitored, before and after the change in the aeration regime. It was found that there was a significant difference in the granule size after modifications were made in the aeration cycle.(10/27/15) The granules grew in size and became clearer as compared to being small and hazy previously. This reduction in smaller particles was also aided by the reduction in the settling time from 200 seconds down to 150 seconds from the beginning of the semester.



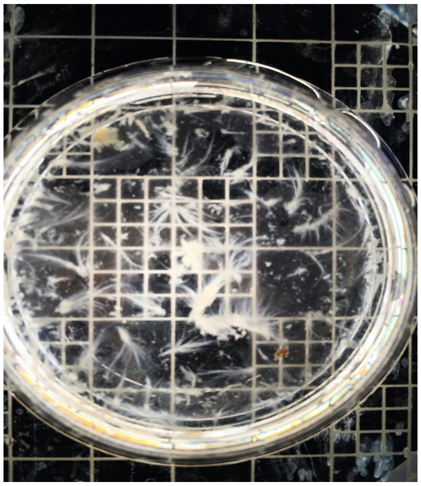
Granules - 10/16/15



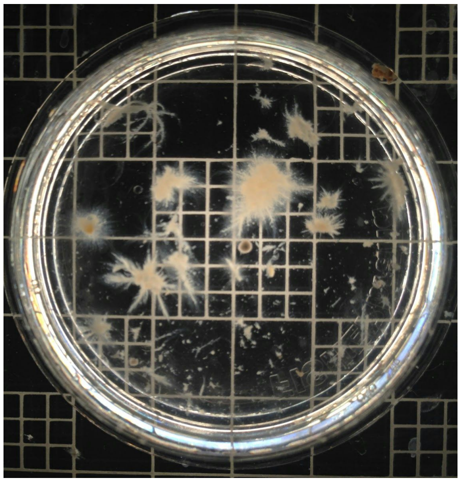
Granules- 10/27/15



Granules - 11/16/15



Granules - 11/30/15



Granules 12/4/15

From granule photographs on 11/16/15 it can be concluded that for reasons unknown, the quality of granules decreased after 10/27/15. They were both smaller in size and the outer ends were more filamentous compared to previous samples. Another new observation is that the granules became darker in color, which may be a result of decreased oxygen supplied to the reactor. Photographs taken on 11/30/15 and 12/4/15 showed that the filamentous ends of the granules had grown and the granules themselves were fewer in number.

Chemical Oxygen Demand (COD)

The samples taken during the reactor cycle were centrifuged for 10 minutes and the supernatant was used for analysis.

COD for New Aeration Schedule #1:

COD results for three sampling days under the New Aeration Schedule #1 are shown below. The overall removal efficiencies were 82%, 97%, and 24% for the three samples from the New Aeration Schedule #1, dating, 10/16, 10/28 and 11/11 respectively. The low COD removal on 11/11/15 of 24% is very strange, largely because the COD increased at the end of the cycle increased from near 10 mg/L up to 80 mg/L. This along with a surprising low value of the influent COD at 106 mg/L caused the removal efficiency to be quite low. Also for all three graphs, there seems to be a great deal of fluctuation throughout the cycle with increases in COD taking place at different times in all three sample cycles.

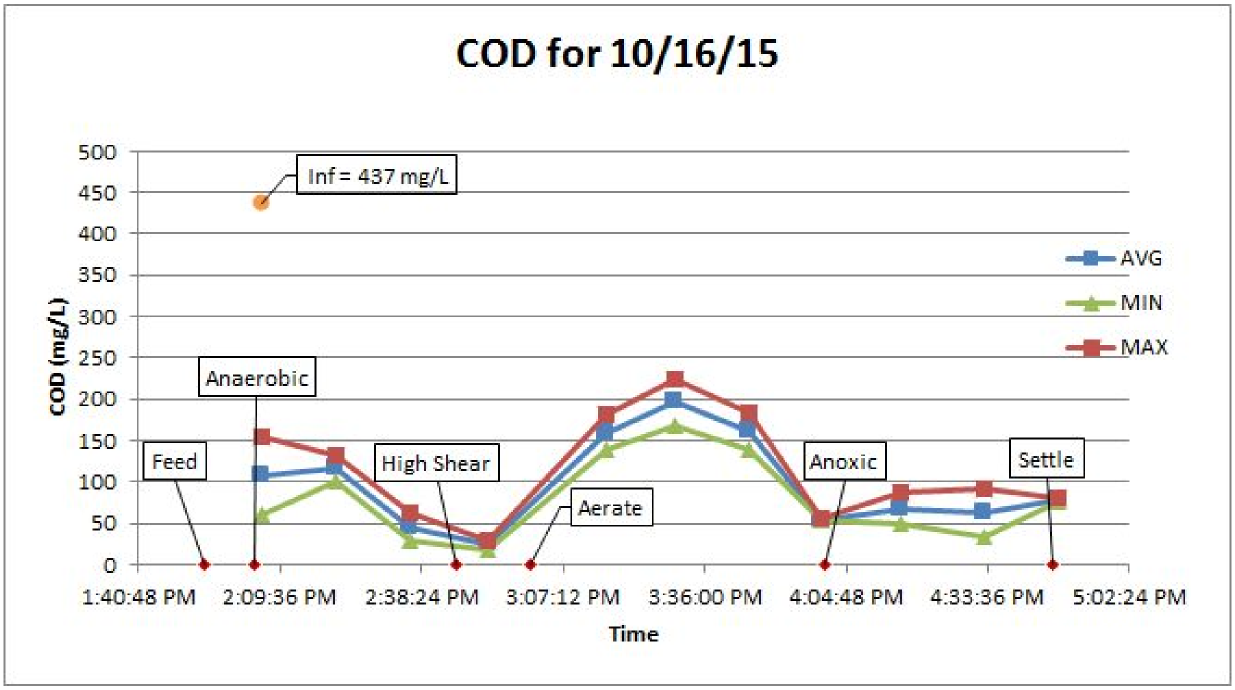


Figure 12: COD results from 10/16/15

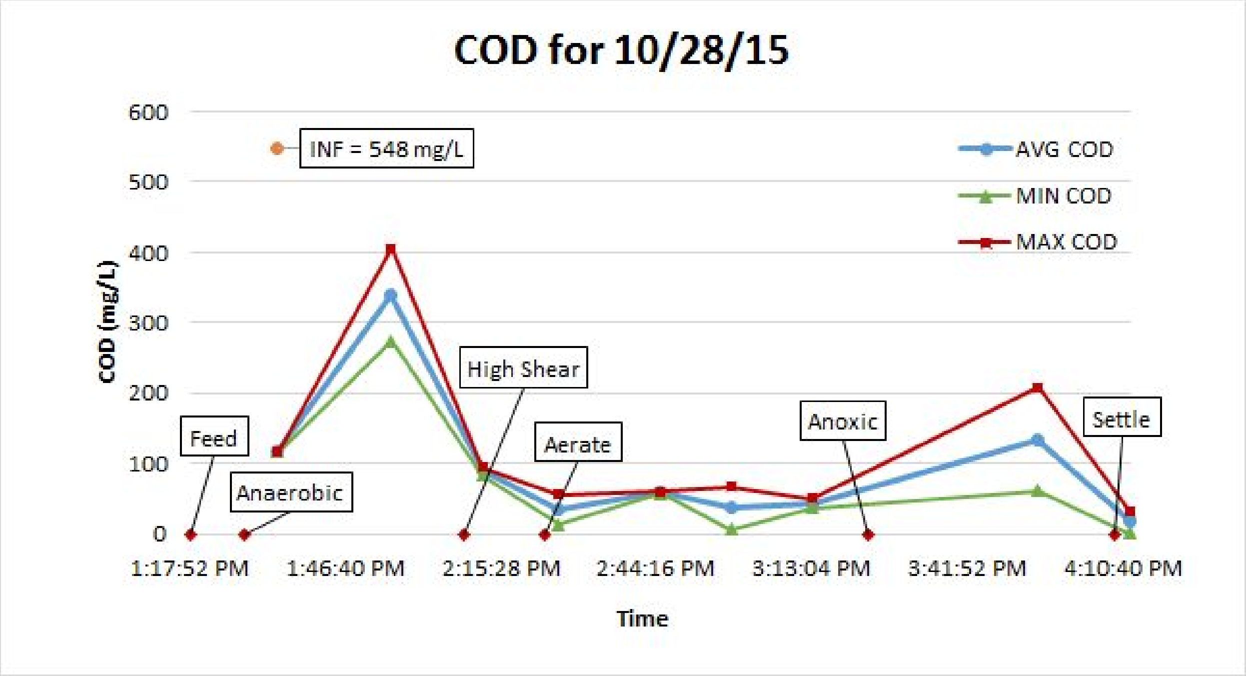


Figure 13: COD results for 10/28/15

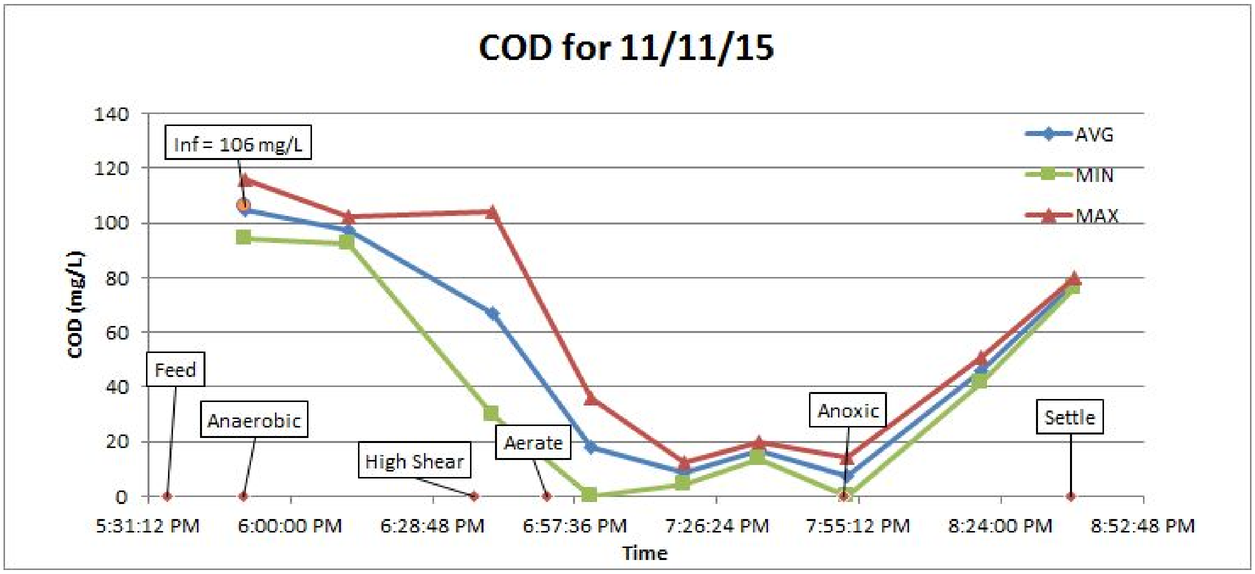


Figure 14: COD results for 11/11/15

COD for New Aeration Schedule #2:

The COD concentrations were measured during the reactor cycle after the New Aeration Schedule #2 was implemented. The COD for the cycle sample from 12/2/15 is shown below. The removal efficiency was 97% with COD rapidly decreasing and remaining near zero mg/L through the entire aeration period. These results help to show that the reactor was still digesting COD at a very high rate near the end of the semester.

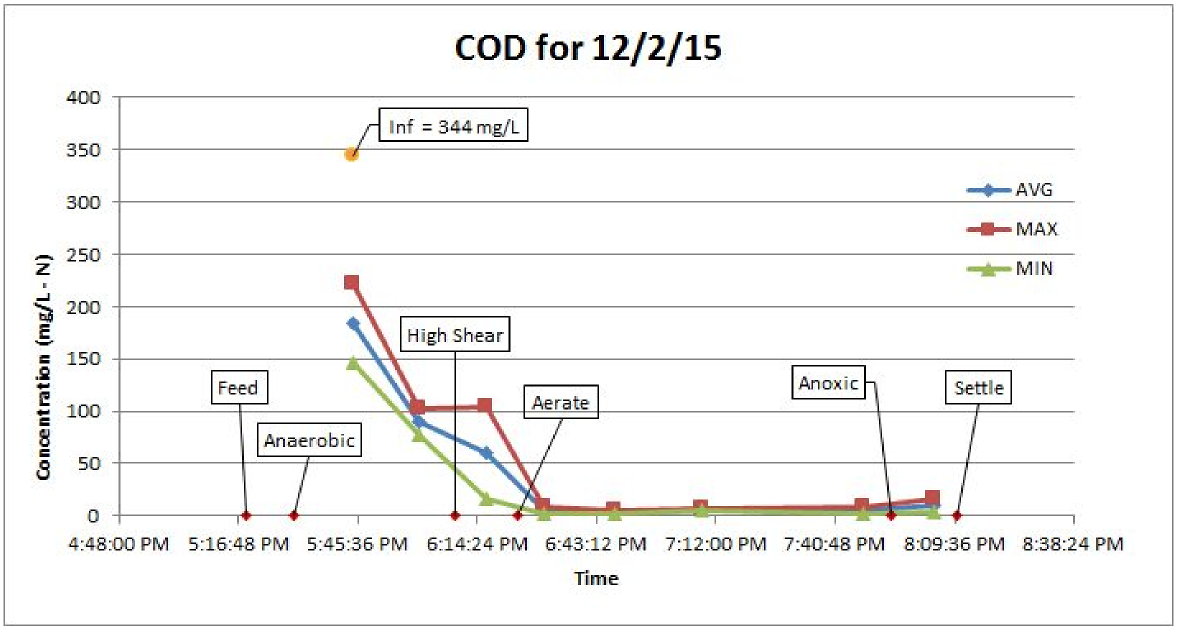


Figure 15: COD results for 12/2/15

Ammonium and Nitrate/Nitrite Assays

Below is the most recent result of the ammonium (NH4) standard curve. Results from the assay on 12/2/15 were obtained with an R-squared value of 0.96. However, the results at low concentrations of ammonium show some variability, including an increase in absorbance from the lowest standard (0.06 mg/L) to the blank standard (zero concentration).

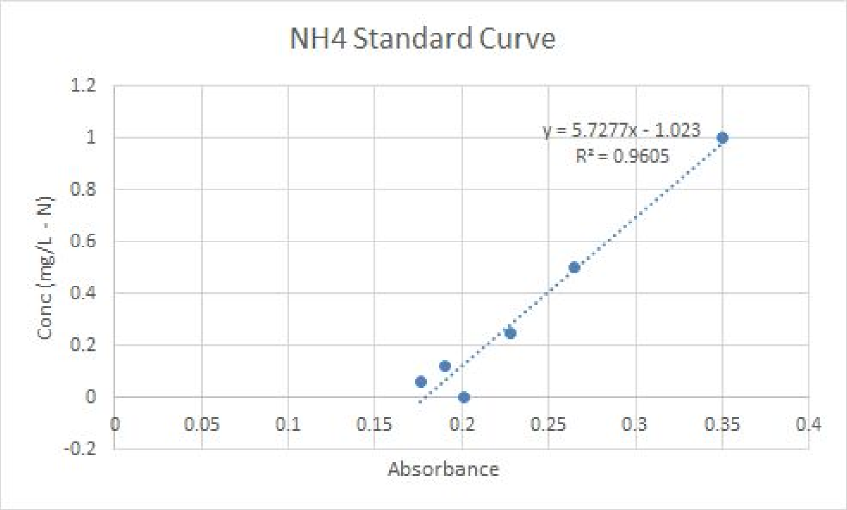


Figure 16: NH4 Assay Standard Curve

The standard curve for the nitrate/nitrite assay is also shown below from 10/28/15. The standard curve was completed with an R-squared value of 0.97.

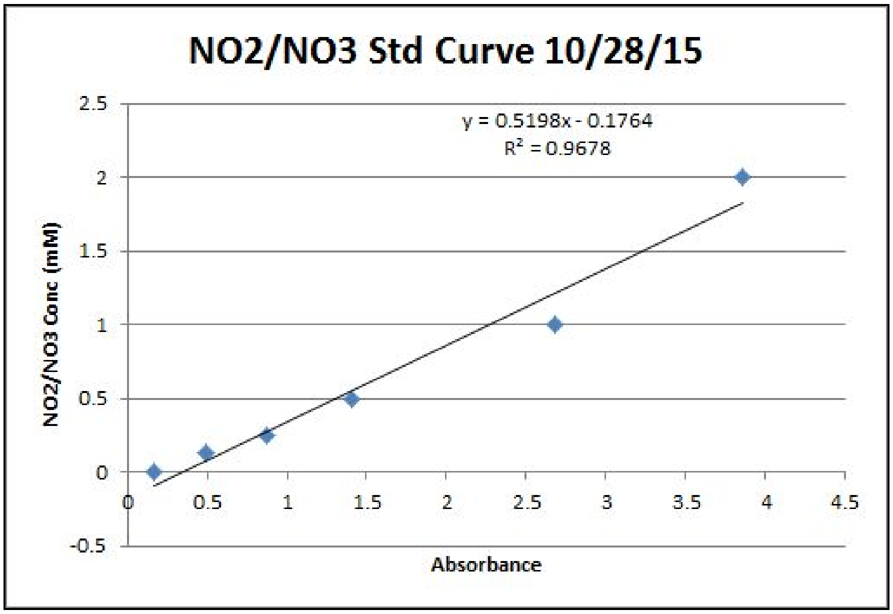


Figure 17: NO2/NO3 Assay Standard Curve

The results for ammonium through the cycle on 10/3/15 are also shown below. This cycle was the earliest cycle sample that was collected, which was before changes in the aeration schedule were made. The data show an increase in ammonium during the anaerobic period which could be related to conversion of urea into ammonium. Following the anaerobic period, the decrease in ammonium indicates that nitrification was taking place throughout the aerobic period, with an overall decrease in ammonium by 30 mg/L as N during that time.

NH4 and NO3 for Original Aeration Schedule:

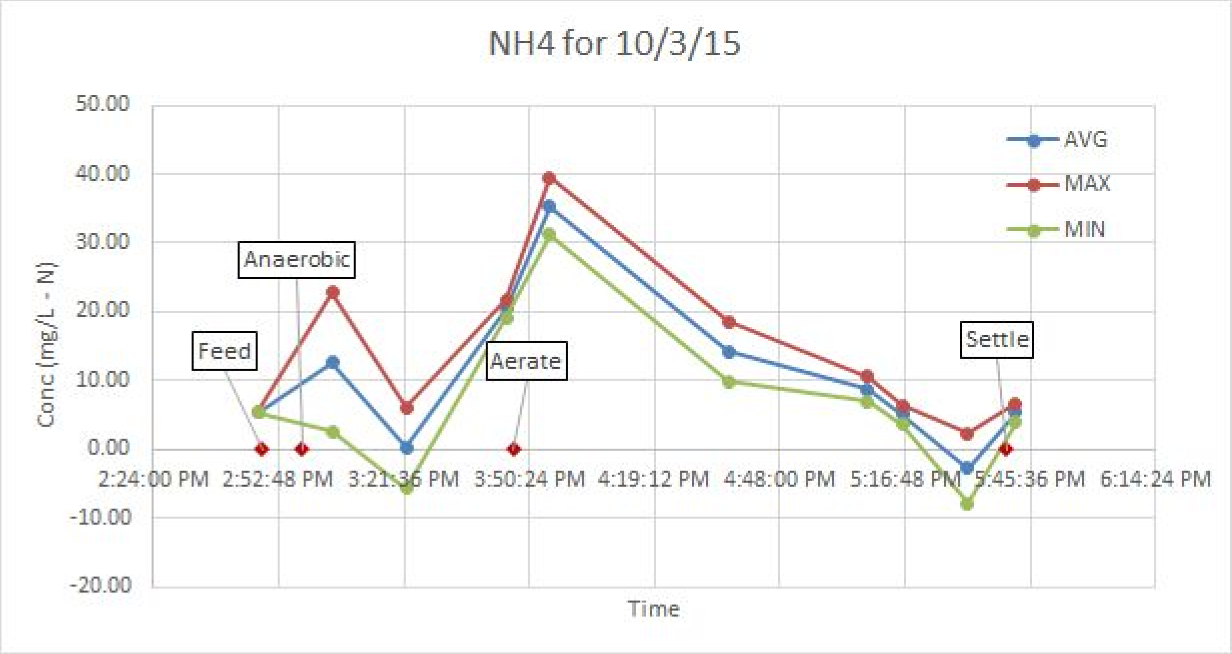


Figure 18: NH4 results for 10/3/15

The corresponding results for nitrate and nitrite in the same cycle do not show an equivalent amount of nitrate production. Looking just at the aeration period, there is only an increase in about 10 mg/L of nitrate and nitrite. This might be evidence of simultaneous nitrification and denitrification during the aerobic phase. One strange result, however, is the decrease in ammonium and increase in nitrate during the anaerobic period. It is unclear how nitrification would be taking place during that time under anaerobic conditions. A graph that compares both ammonium and nitrate is also shown below.

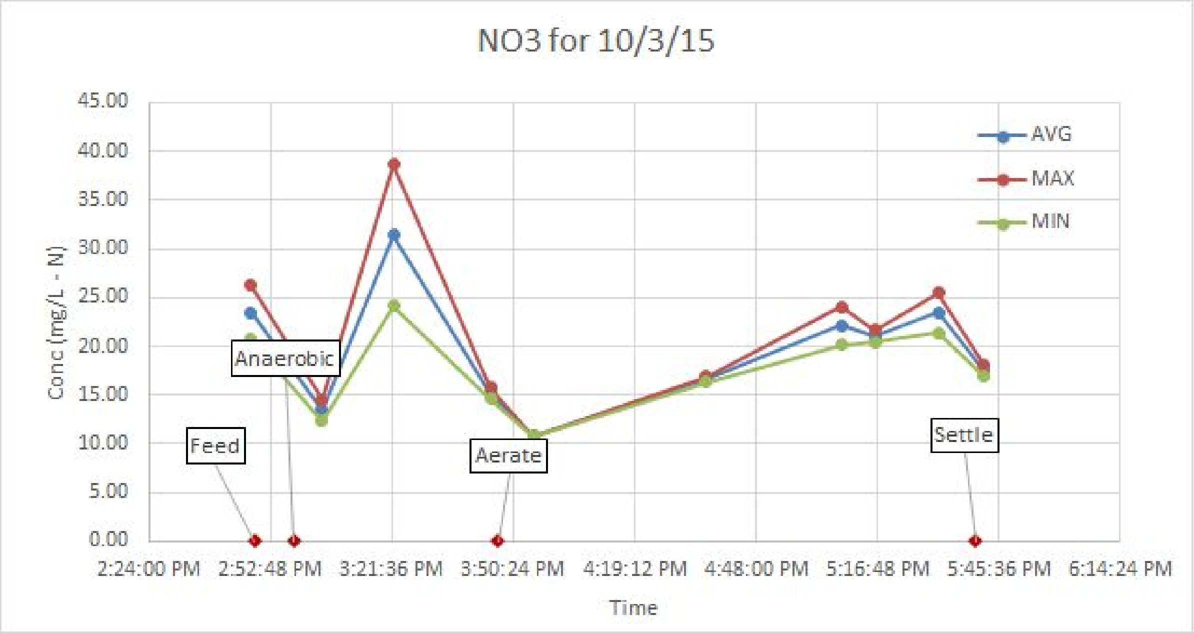


Figure 19: NO3 results for 10/3/15

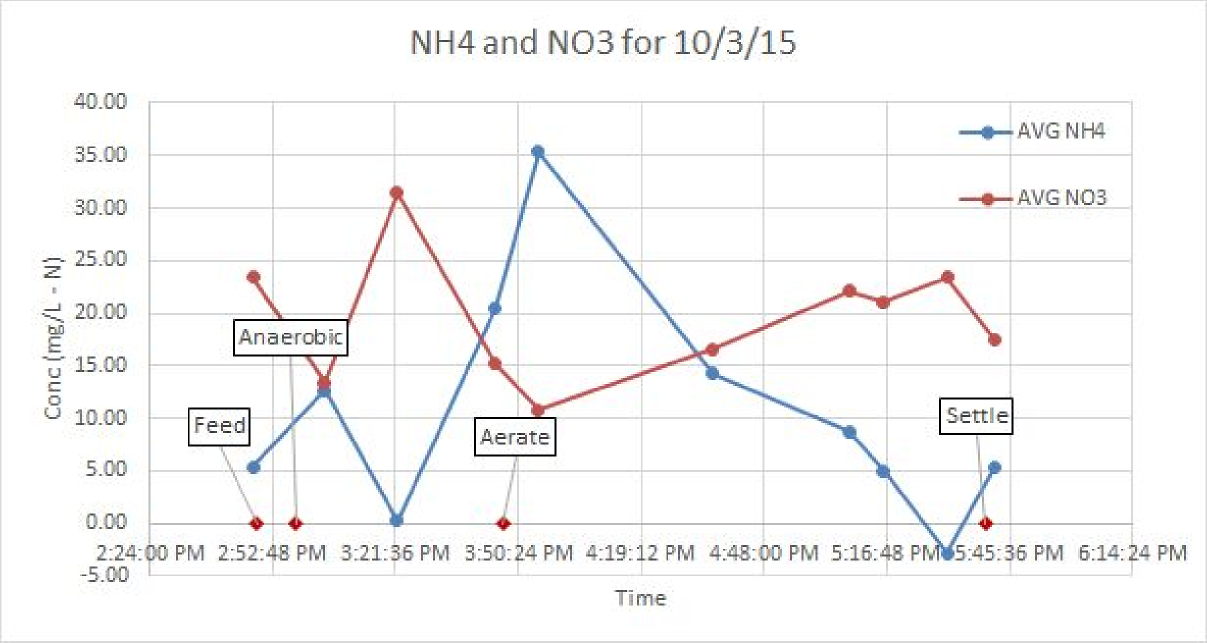


Figure 20: NH4 and NO3 comparison for 10/3/15

NH4 and NO3 for New Aeration Schedule #1:

Results for ammonium and nitrate for 11/11/15 are both shown below. This cycle was recorded almost a month after changes to the aeration schedule were made. The results for ammonium show a large increase to above 60 mg/L during the anaerobic period until aeration begins when ammonium concentration decreases moderately. Through the anoxic period, the decrease in ammonium is less as the concentration increases and subsequently decreases once again. Through the aeration period there is about 20 mg/L of ammonium degraded. Unlike in the cycle before the anoxic period was implemented, the nitrate and nitrite concentration recorded in the reactor is far lower. Fluctuating between 0.1 to 0.5 mg/L as N, the nitrate concentrations do not increase while ammonium is degraded. This is evidence of some simultaneous nitrification and denitrification taking place in the reactor.

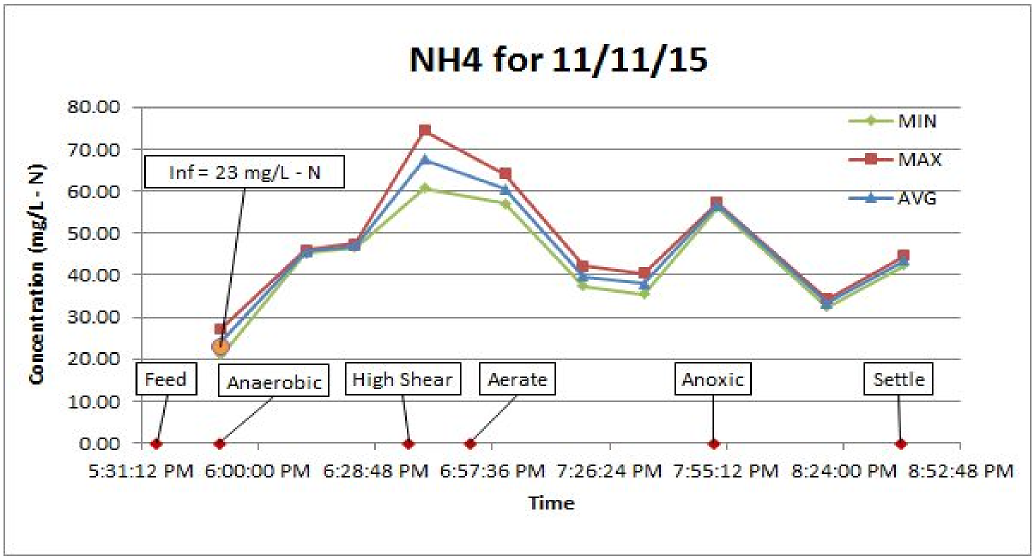


Figure 21: NH4 results for 11/11/15

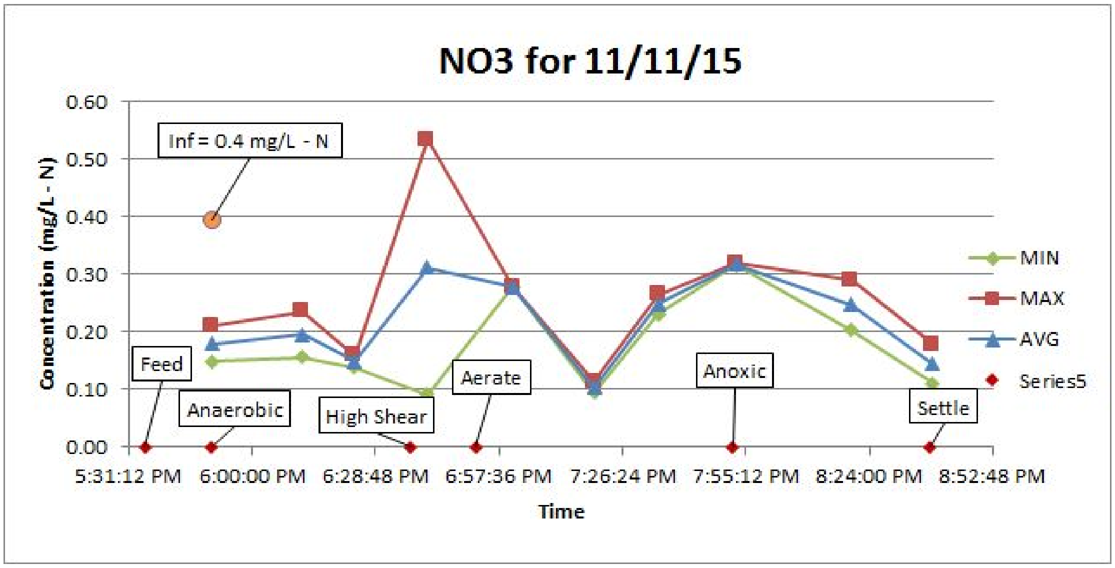


Figure 22: NO3 results for 11/11/15

Results for nitrate and nitrite during a cycle on 10/28/15 were also obtained, however at this time the ammonium results have not successfully been recorded. Nonetheless, there is similar evidence of low levels of nitrate and nitrite in the reactor at that time.

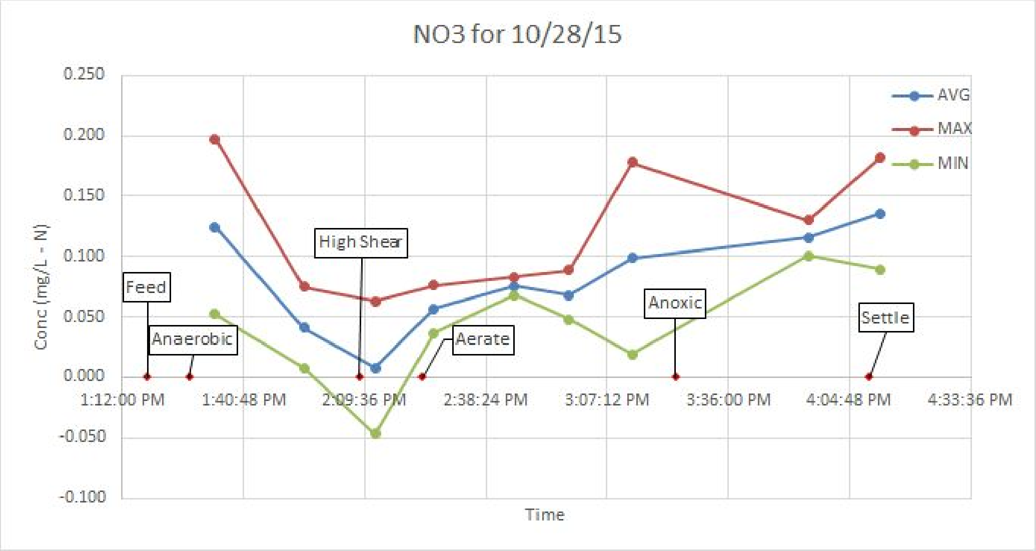


Figure 23: NO3 results for 10/28/15

After analyzing the results for ammonium and nitrate on 11/11/15, with evidence of simultaneous nitrification and denitrification taking place and yet poor overall removal of ammonium, changes to the aeration schedule were made as described previously. The results for the ammonium and nitrate under the New Aeration Schedule #2 from 12/2/15 are shown below. The results for ammonium removal indicate that increased aeration time did not lead to increased nitrification in the reactor. The ammonium concentrations through the cycle increased across the cycle and remained very high the entire time. The influent concentration measured was surprisingly high and remained high for this cycle, with influent concentration measured at 121 mg/L - N. The results for NO3 concentration again showed consistent levels under 0.5 mg/L - N through the cycle. Overall, the attempt made to increase nitrification by supplying air for a longer period did not work as planned.

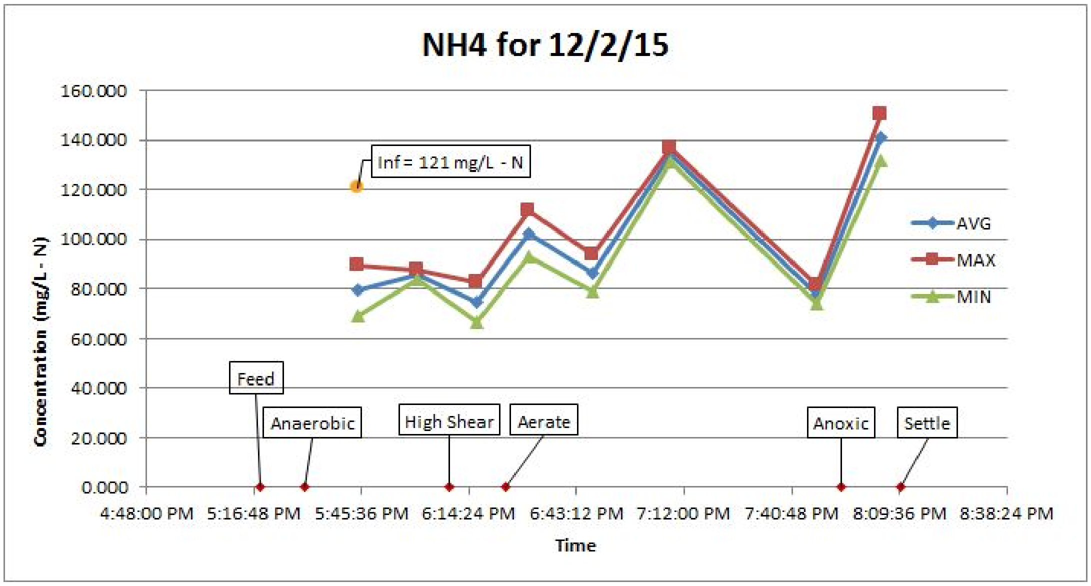


Figure 24: NH4 results for 12/2/15

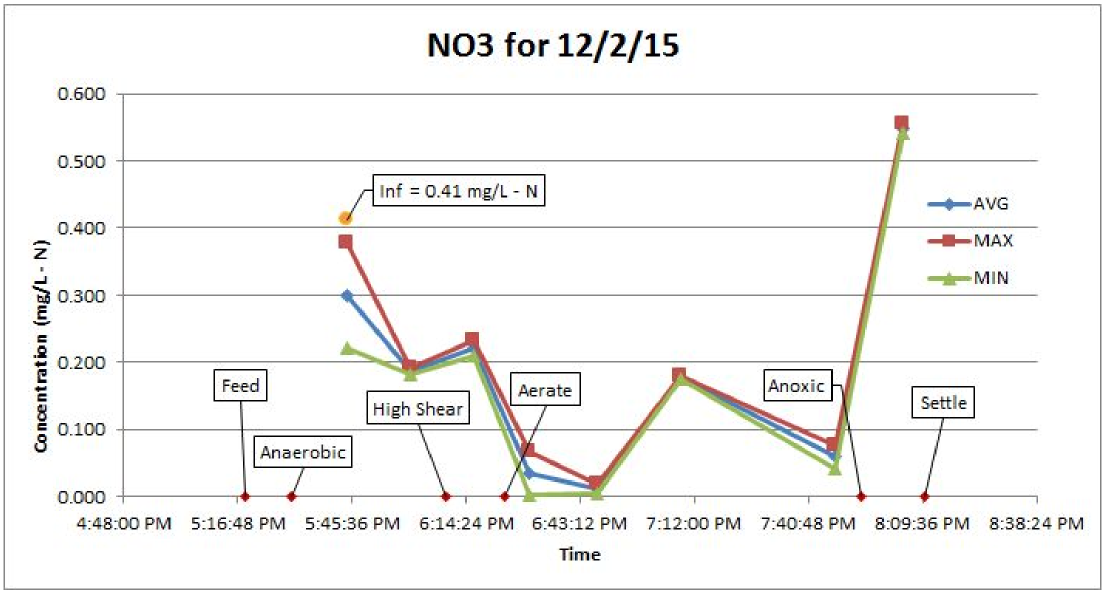


Figure 25: NO3 results for 12/2/15

Applicability of GSBRs in Honduras and India:

1. Regulations in Honduras and India:

In 2003, the Consejo Nacional de Agua Potable y Saneamiento [National Water and Sanitation Council] (CONASA) was created to formulate national strategies and policies regarding drinking water and sanitation. Under the direction of this agency, the Servicio Autónomo Nacional de Acueductos y Alcantarillados [Autonomous National Water and Sanitation Service] (SANAA) was given the responsibility to provide technical assistance for municipalities and local water boards [14]. High-quality effluent standards exist in the country, with maximum limits set on COD, TKN, and Ammonia as Nitrogen to be 200.0 mg/L, 30.0 mg/L, and 20 mg/L, respectively [15].

Unfortunately, even with the regulatory structure in place, these treatment goals are not reached. In 2004, it was reported that approximately 68% of Hondurans have access to some form of sanitation services. However, only 10% of the sewage thereafter collected is treated [16]. The level of treatment expected from regulation is just not financially possible for many areas in the country; consequently, there is little enforcement or monitoring of these standards.

In India, the Water Pollution (Prevention and Control) Act of 1974 and the Environment Protection Act of 1986 prohibit any individual or industry from directly disposing wastewater into a water body or on land.

According to guidelines provided by the Central Pollution Control Board in the Environmental Protection Act of 1986, the maximum permissible limits for COD, NH_4-N, and Phosphorus content in the treated effluent are as follows:

1. COD: 250 mg/L for Inland surface water and marine coastal areas
2. NH_4-N: 50 mg/L for inland surface water, sewers and marine coastal areas
3. Phosphorus: 5 mg/L for inland surface water
4. Total Kjeldahl Nitrogen (TKN): 100 mg/L for inland surface water and marine coastal areas.

As per a recent estimate from 6/24/15 out of 22,900 MLD (Million Liters per Day) of wastewater generated in the country, only about 5900 MLD (26%) is treated before discharge, the rest is disposed of untreated. Twenty seven cities have only primary treatment facilities and forty-nine have primary and secondary treatment facilities. The level of treatment available in cities with existing treatment plant varies from 2.5% to 89% of the sewage generated [17].

2. Feasibility:

Aerobic granulation is a novel and promising technology for sustainable wastewater treatment. To date, it still lacks in theoretical models to explain the mechanism of aerobic granulation although several hypotheses have been proposed. Furthermore, the majority of studies on aerobic granulation were carried out in laboratory-scale reactors or some were completed in pilot-scale reactors. Nonetheless, a small amount of full-scale plants have been built in the Netherlands, Portugal and South Africa with success [18].

Conclusions

The New Aeration Schedule #1 which utilized overall less cumulative airflow through each cycle than previous operation decreased the stability and structure of granules by the end of the semester. From the start of the experiment for over a month the structure of the biomass maintained granule like until the end of the semester when the granules became decidedly floc-like. This is result shows that low aeration rates may still inhibit the growth of dense granules.

Throughout the semester the reactor showed good COD removal except for one cycle that was lower than expected. For the cycles tested, there remained evidence of COD removal as high as 97%. This level of COD removal was expected in the reactor after high COD removal efficiencies have been recorded in GSBR experiments in the past. This result remains motivating for the continued use of complex synthetic wastewater media with GSBRs.

Overall, the experiment designed to improve denitrification through limiting airflow supplied to the reactor was inhibited by the reduced efficiency of nitrification following the change. The decreased nitrification in the reactor after the New Aeration Schedule #1 was implemented was most likely due to a lack of air supply to nitrifying bacteria and subsequent decline of the population. Nonetheless, some ammonium removal was recorded during the semester while almost no nitrate/nitrite had been produced, which shows that simultaneous nitrification and denitrification took place in the reactor, albeit less than hoped for. Lastly, the subsequent increase in aeration supply with the New Aeration Schedule #2 did not aid in increasing the nitrification. This showed that a short term change in operation like aeration time did not lead to an immediate increase in nitrifiers converting ammonium to nitrate.

Future Work

More work must be done on supplying the right aeration rates in order to encourage simultaneous nitrification and denitrification in GSBRs. Subsequent attempts at this experiment must be quicker to monitor and record declining removal efficiencies in the reactor (like ammonium) as a result of operational changes. Furthermore, the ability to supply an aeration rate that meets several competing requirements remains very difficult. These include supplying enough air for COD and ammonium removal and shear needed for granule stability as well as low enough air for denitrification to still take place in the granules.

One area of work that would aid research efforts in sustainable wastewater treatment technologies for AguaClara would be the improvement of measurement procedures for contaminants like COD, ammonium, and nitrate. The time to analyze concentration profiles for an entire cycle remains intensive and takes up a large bulk of research time.

Another area of study that would have helped to shed light on performance in the reactor would have been microbiological assessments of the bacteria within the granules. DNA and RNA sequencing could be done to see what types of organisms were present including nitrifiers. More work must be done to begin understanding the microbial ecology of granules in a GSBR.

Other future work for the GSBR still includes investigating ways to improve the inoculation period as well as better understanding the influences on granulation. This was outlined as a goal at the start of the semester, however experiments were not implemented.

Lastly, pairing between the GSBR and UASB to monitor the treatment of effluent from the UASBs could be done in the future.

References

Use Citation Machine APA Format

[1] M. k. de Kreuk, J. j. Heijnen, and M. c. m. van Loosdrecht, “Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge,” Biotechnology and Bioengineering, vol. 90, no. 6, pp. 761–769, 2005.

[2] Water Utilities Technical Assistance Program. (2007). *New Mexico Wastewater Systems Operator Certification Study Manual*. Santa Fe, NM: New Mexico Environmental Department Surface Water Quality Bureau. Retrieved from https://www.env.nm.gov/swqb/FOT/WastewaterStudyManual/13.pdf

[3] World Health Organization. (2015). *Methaemoglobinaemia*. Retrieved from http://www.who.int/water_sanitation_health/diseases/methaemoglob/en/

[4] Water Utilities Technical Assistance Program. (2007). *New Mexico Wastewater Systems Operator Certification Study Manual*. Santa Fe, NM: New Mexico Environmental Department Surface Water Quality Bureau. Retrieved from https://www.env.nm.gov/swqb/FOT/WastewaterStudyManual/13.pdf

[5] Cervantes, Francisco J. (Ed.). (2009). *Environmental technologies to treat nitrogen pollution*. London, UK: IWA Publishing.

[6] Y. Liu, J.H. Tay, The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge, Water Res. 36 (avr. (7)) (2002) 1653–1665).

[7] M. K. de Kreuk and M. C. M. van Loosdrecht, “Selection of slow growing organisms as a means for improving aerobic granular sludge stability,” Water Sci. Technol., vol. 49, no. 11–12, pp. 9–17, 2004.

[8] Moghaddam, S. Sadri & Moghaddam, M.R. Alavi (2015). Cultivation of aerobic granules under different pre-anaerobic reaction times in sequencing batch reactors. *Separation and Purification Technology*, 142, pp.149–154.

[9] Tay JH, Liu QS, Liu Y. 2002. Aerobic granulation in sequential sludge blanket reactor. Wat Sci Technol 46(4–5):13–18.

[10] M. k. de Kreuk, J. j. Heijnen, and M. c. m. van Loosdrecht, “Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge,” Biotechnology and Bioengineering, vol. 90, no. 6, pp. 761–769, 2005.

[11] Lochmatter, S., Gonzalez-Gil, G. & Holliger, C. (2013). Optimized aeration strategies for nitrogen and phosphorus removal with aerobic granular sludge. *Water Research*, 47(16), pp. 6187–6197.

[12] Gao, Ge, et al. (2014). *Wastewater Treatment*. Ithaca, NY.

[13] Dias, Maria Eduarda Simões and Mirelly Manica (2015). *Wastewater Treatment: Granular Sequential Batch Reactor - GSBR Team*. Ithaca, NY.

[14] Hodge, Matthew M. 2008. Wastewater Treatment in Las Vegas, Santa Barbara, Honduras: Thesis, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge.

[15] Mikelonis, A., A. Herrera, E. Adams and M. Hodge. 2010. "Honduran Imhoff Tanks: Potentials and Pitfalls." Journal of Water Management Modeling R236-22. doi: 10.14796/JWMM.R236-22.

[16] Secretaría de Recursos Naturales y Ambiente. (2005). Informe del Estado y Perspectivas del Ambiente: Geo Honduras 2005. Tegucigalpa, Honduras: Programa de las Naciones Unidas para el Medio Ambiente.

[17] National status of waste water generation & treatment, ENVIS Centre on Hygiene, Sanitation, Sewage Treatment Systems and Technology. Retrieved from http://www.sulabhenvis.nic.in/Database/STST_wastewater_2090.aspx

[18] Jun Li, Li-Bin Ding, Ang Cai, Guo-Xian Huang, and Harald Horn (2014) Aerobic Sludge Granulation in a Full-Scale Sequencing Batch Reactor. BioMed Research International Volume 2014 (2014), Article ID 268789. http://www.hindawi.com/journals/bmri/2014/268789/