

Increasing Dissolved Phosphorus Concentration in Anaerobic Digesters Utilizing Wastewater Grown Algae

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Abstract

The sequestration, recovery and recycling of phosphorus from both environmental and industrial processes is of great importance due to its high demand in fertilizer production. Additionally, high concentrations of phosphorus from agriculture runoff cause eutrophication of lakes disrupting eco-systems. Microalgae has been identified as an ideal concentrating agent of phosphorus by capturing dissolved phosphorus for growth. The high in phosphorus microalgae must then be harvested and degraded for phosphorus recovery using methods such as anaerobic digestion.

This thesis looked to identify ideal conditions for dissolved phosphorus recovery from anaerobic digesters utilizing wastewater grown microalgae. Firstly, we identified stable substrate load rates (15g/L) and growing conditions (35°C, 10rpm) for our anaerobic bacterial inoculum of 500mL. We analyzed volatile solid removal and organic acid and biogas production to determine the level of digestion of the substrate. Secondly, we operated several digesters at different retention times, analyzing most notably pH and dissolved phosphorus concentrations.

The results of this thesis showed anaerobic digesters with lower retention times maintained a lower pH and higher levels of dissolved phosphorus. However the retention time must also be high enough to prevent washing out of anaerobic bacteria. Therefore, there exists an optimal retention time for dissolved phosphorus recovery which can be identified with further experimentation. Future work would also include optimization of a two-stage anaerobic digester process with both dissolved and precipitated phosphorus recovery points.

Advisor: Dr. Michael Betenbaugh
Reader: Dr. Chao Wang

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Chapter 1: Phosphorus Recovery from Anaerobically Digested Microalgae

High concentrations of both nitrogen and phosphorus are present in wastewater runoff of farmland due to currently used crop fertilization techniques. Nitrogen (N) and phosphorus (P) leeching back into water resources can cause chemical and biological imbalance by being toxic to the native ecosystem and providing an abundance of food to unwanted species.¹ Agriculture production uses fertilizers allowing for increased crop growth. About 20% of the nitrogen and phosphorus used in fertilizer is lost due to water runoff, which increases the cost of agricultural production significantly.²

The eutrophication of naturally occurring bodies of water due to wastewater runoff from agriculture land poses a considerable threat to the environment. Rainstorms of >20mm/hr for >9min have the largest impact on agriculture runoff with irrigation not contributing significantly making it impossible to limit wastewater runoff.³ There have been attempts to sequester and recover high nitrogen and phosphorus due to wastewater runoff from large bodies of water. Nitrogen and phosphorus differ in their transport efficiency. Nitrogen is capable of traveling long distances by remaining soluble in moving bodies of water. Phosphorus will absorb into clay quickly and remain close to where it was originally introduced into a body of water.⁴ Early attempts on mitigating eutrophication looked at preventing only the input of nitrogen due to its mobility.¹ A study by Schindler et al. in 2008 looked at the effects on phytoplankton over 30 years of regulating nitrogen input of a lake. They hypothesized that eliminating the limiting nutrient nitrogen in phytoplankton growth would prevent the utilization of the other nutrients in wastewater runoff. Starting from a high ratio of 12:1 N:P they reduced nitrogen input over time and studied the change in composition and overall biomass of

phytoplankton. As N:P ratio decreased total phytoplankton biomass did not decrease but the composition became dominated by nitrogen fixing cyanobacteria. They concluded that reducing nitrogen input into lakes would not decrease eutrophication, as species capable of nitrogen fixation would continue to provide a source of nitrogen. Additionally nitrogen removal from wastewater runoff allows P concentrations to build up and move downstream causing further eutrophication and nitrogen fixing cyanobacteria growth.⁵ In order to prevent eutrophication both nitrogen and phosphorus would need to be removed from wastewater runoff.

Phosphorus Recovery:

Nitrogen and phosphorus in the wastewater exist in both organic and inorganic forms. While the nitrogen and phosphorus in wastewater streams exist in high quantities it is still a challenge to separate them out of aqueous form efficiently.⁶ Work has been done to protect fragile coastal ecosystems from high phosphorus and nitrogen concentrations.⁷ Vegetative shelters have been built along the problem points of agriculture and animal farm wastewater runoff to prevent nutrient rich fresh water from reaching the ocean. The plants in the shelters utilize the nutrients for growth while preventing eutrophication of ecosystems downstream. Microalgae can also act as a mediator as it is capable of utilizing both inorganic and organic forms of N/P for growth essentially concentrating and removing the two elements from the water it is grown in.⁸

Additionally phosphorus has been deemed a non-renewable chemical. Dana Cordell, PhD in Water and Environmental Studies predicted a shortage of industrial grade phosphorus by the year 2035 in her 2010 thesis.⁹ Currently, majority of phosphorus is mined from shale deposits and according to an estimate US phosphorus reservoirs will be depleted in next 35 years.⁴ Concerns related to the finite nature of

phosphorus, its depletion, environmental pollution and increased demand for fertilizer make the algal sequestration an important and sustainable nutrient recovery process.⁹

Anaerobic Digestion:

In order to recycle the phosphorus sequestered by microalgae grown on wastewater, the microalgae must first be broken down into smaller biomolecules. One of the better-developed methods of biomass breakdown is anaerobic digestion.

A flora of bacteria found in both mammalian digestion tracts and sewage systems are responsible for the process known as anaerobic digestion. Anaerobic bacteria break down organic, carbon containing polymers producing most notably methane, CO₂ and inorganic nitrogen and phosphorus. Conventional applications of anaerobic digestion involve a batch process of substrate digestion utilizing acid and methane forming microorganisms in one single bioreactor.¹⁰

Phase Breakdown of Anaerobic Digestion:

The diagram pictured below (figure 1) shows the pathway of organic material through the different phases of anaerobic digestion. The process is broken up into two different phases and then further broken down into four main digestion steps.

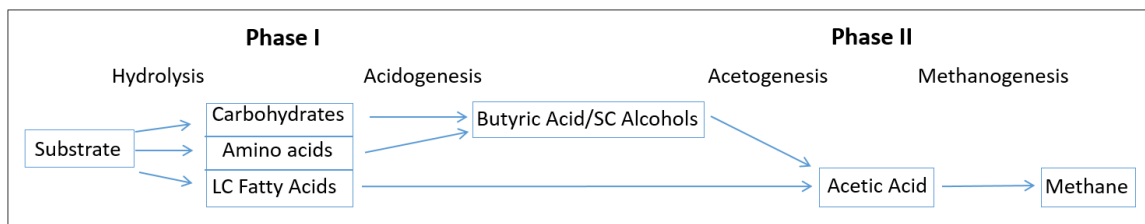


Figure 1: Basic phase breakdown of anaerobic digestion depicting the major organic carbon molecules and their pathway through the process.

Hydrolysis:

The hydrolysis phase takes large, insoluble biomolecules of a substrate and breaks them down into smaller components of monomers and oligomers.¹¹ These biomolecules include proteins, carbohydrates, fatty acids and nucleic acids.¹² The composition of substrate used for digestion can affect the rate of hydrolysis breakdown.¹³ Many of the energy crops used contain long hydrocarbon compounds like lignocellulose. Lignocellulose consists of lignin, cellulose and hemicellulose and makes up majority of plant cell walls including microalgae.¹⁴ Degradation of lignocellulose must occur before any saccharolytic or proteolytic bacterial enzymes can begin to further digest the substrate. Hydrolysis, therefore, is known as the rate-limiting step of the anaerobic digestion process due to the energy and time required for the initial breakdown of plant cell walls.¹⁵ Substrate added to an anaerobic digester usually comprises of a slurry of varying biomass concentration most of which is simply too large for anaerobic microbes to digest. Initial breakdown of the biomass begins with bacteria secreted enzymes.¹⁶ For example, saccharolytic bacteria secrete saccharolytic enzymes, which break down polysaccharides. The final products of the hydrolysis step are organic polymers and monomers, which can be broken down further by more niche bacteria.

Acidogenesis:

Acidogenic bacteria break down the organic acids, polymers and monomers produced by hydrolytic bacteria. The acidogenesis process involves the widest variety of microorganisms and reactions compared to the other 3 digestion phases. These bacteria produce long chain organic fatty acids and alcohols.¹⁷

Hydrolysis and acidogenesis make up what is designated as the first phase of anaerobic digestion. Both bacterial groups produce large organic acids and operate most

efficiently in low pH conditions. It is important to note that the species of bacteria present will depend entirely on reactor conditions and the substrate used.¹⁸

Acetogenesis:

Acidogenesis and acetogenesis are responsible for producing the soluble nitrogen and phosphorus during the breakdown of the substrate. Nitrogen and phosphorus present in phospholipids, proteins and nucleic acids are taken and converted to inorganic phosphate and ammonia.^{19 20} Acetogenesis also contributes to organic acid formation and lower pH but is grouped into phase II with methanogens because of a symbiotic relationship between methanogens and acetogens. Anaerobic oxidation of short chain hydrocarbons to acetic acid produces hydrogen gas along with carbon dioxide. The rate of acetic acid formation is dependent on the concentration of hydrogen gas. At high concentrations of hydrogen gas acetic acid production slows down.²¹

Methanogenesis:

Methanogens utilize the hydrogen gas produced in acetogenesis to breakdown acetic acid and release methane. In this way acetic acid formation can continue as long as there are methanogens present.²¹ Most methanogens have a long doubling time of between 10-12 days and are also the most susceptible to oxygen poisoning. Many of the operating constraints of anaerobic digesters are chosen specifically for methanogen viability.¹⁸

Anaerobic Digester Parameters:

Temperature is an important parameter and can affect efficiency and composition of bacteria in an anaerobic digester. There are two ideal temperature points for anaerobic bacteria. Bacteria that operate well at 37°C are known as mesophilic bacteria while those that operate well at 55°C are known as thermophilic bacteria.²¹ In

general thermophilic bacteria operate more efficiently than mesophilic bacteria but are more susceptible to instability. The choice between operating temperatures largely depends on the conditions of the incoming substrate and the availability of a heat source.¹⁹

Agitation is important for slurry homogenization, bacterial distribution and temperature uniformity. Hydrolytic bacteria that utilize extra-cellular enzymes especially benefit from mixing. The agitation however should be either slow (<20rpm) or sporadic to accommodate the slow growing methanogenic bacteria.²²

A suitable load rate for an anaerobic digester must be determined before any substrate can be added. Load rate means the amount of total solids and volatile solids of substrate that are added into a reactor.²¹ Volatile solids consist of the organic material that can be digested by the bacteria.²³ If too high of a load rate is used it becomes difficult for acetogenic and methanogenic bacteria to digest the large quantity of substrate. This can result in a buildup of organic acids leading to a drop in pH and reactor failure.²⁰ Typical load rates will depend on whether the reactor is established or not. An established reactor will already have a robust microbial environment suitable for digestion of its specific substrate. In these cases a high load rate can be used. This commonly means between 15-20kg/m³ but load rates as high as 25kg/m³ have been reported. For digesters without an established specific microbial colony a common load rate is between 0.5-5kg/m³.²¹

Retention time is the amount of time substrate remains in digester. Depending on the desired level of digestion or difficulty of digesting the substrate the retention time can be changed accordingly.¹³ The shortest possible retention times that still involve

complete digestion of the substrate to methane are around 10 days as this is approximately the doubling time for most methanogens. Retention times of shorter than 10 days pose the risk of potential washing out of digesting bacteria.²¹

The substrate chosen for methane production dictates the amount of time each phase of anaerobic digestion will be active. As mentioned before energy crops require a long hydrolysis period for initial breakdown of cell walls. Conversely, more basic substrates such as vegetable oil or starches would have little to no hydrolysis phase as both substrates can already be digested by acidogenic bacteria. Other problems can occur depending on the chemical composition of a substrate besides organic carbon compounds. For example, using manure as a substrate introduces high levels of ammonia to a reactor, which can cause inhibition of the anaerobic digestion process.²⁴ Ammonia inhibition is also common in digesters using substrate high in protein content as the eventual degradation leads to the release of nitrogen as ammonia.²¹

Among many other substrates, algal biomass has also been used for biogas production by anaerobic digestion. Reports from previous studies proved that *Chlorella vulgaris*, *Phaeodactylum tricornutum*, *Scenedesmus obliquus*, and *Spirulina maxima* produced reasonable amount of biogas. Algae being a potential aspirant for wastewater treatment tend to accumulate a large quantity of nutrients especially nitrogen and phosphorus.⁸ Therefore algal biomass grown on wastewater recycle significant amount of nutrients when digested anaerobically.

Chapter 2: Nutrient Analysis of Two-phase Anaerobic Digestion of the Microalgae *Chlorella vulgaris*

Two Phase Anaerobic Digestion:

One of the major difficulties with anaerobic digestion is the determination of the reactor conditions for optimal bacterial growth. Methanogens require a neutral to slightly basic pH for optimal activity and growth, while hydrolytic and acidogenic operate in a more acidic environment. Additionally, when dealing with substrate high in lignocellulose such as energy crops and certain algae species, the long duration of the hydrolysis and acidogenesis stages can cause acidification of a digester killing off acetogens and methanogens.

To maximize efficiency of both the acidic bacteria and methanogens, scientists Pohland and Ghosh proposed the idea of separating acid and methane production phases for the digestion of maize.²⁵ This allows for ideal conditions for both phase I and phase II bacteria to be maintained independently producing enhanced yields of end products and a greater stability of the entire process.¹³ By controlling the retention time of digesters, it is possible to create a primarily phase I environment in which hydrolytic and acidogenic bacteria are the dominant digesting bacteria.²¹

Purpose of Experiments:

We decided to analyze the resulting products of anaerobically digested algae, which had previously been grown on wastewater. Algae grown on wastewater are high in both phosphorus and nitrogen meaning the concentration of the two elements would be higher post-digestion than with other commonly used substrates. By varying the retention time of the algae being digested we looked to identify under what conditions phosphorus concentrations would be highest in the supernatant of digested effluent.

High phosphorus levels in the supernatant could potentially lead to higher recovery rates using established phosphorus recovery methods.

Methods:

Anaerobic digester setup:

Four 5L bioreactors were filled with 1.5L of 15g/L algae slurry after which nitrogen gas was sparged into the reactors until establishing 0% dissolved oxygen. Dissolved oxygen readings can be seen in appendix B. The reactors were then placed in a 35°C hot room and their impellers activated at 10rpm for 2hr to allow for the slurry to heat up. 0.5L of activated sludge obtained from Backwater Wastewater Treatment Plant was then added to each reactor by means of a peristaltic pump to prevent oxygen contamination. For the following 21day cycle, each reactor was maintained at a different retention time. Retention times of 4, 6.6, 10 and 21 days were kept by controlling the rate of algae slurry influent and digested effluent. A parts list for the anaerobic digester can be seen in appendix E.

Retention Time Maintenance

Individual containers of algae slurry at 15g/L were prepared with volume being dictated by the desired retention time for each reactor. The same volume was removed for each specific reactor using a peristaltic pump. The algae slurries prepared were then sparged with nitrogen until reaching a DO level of 0% and then added to each specific reactor.

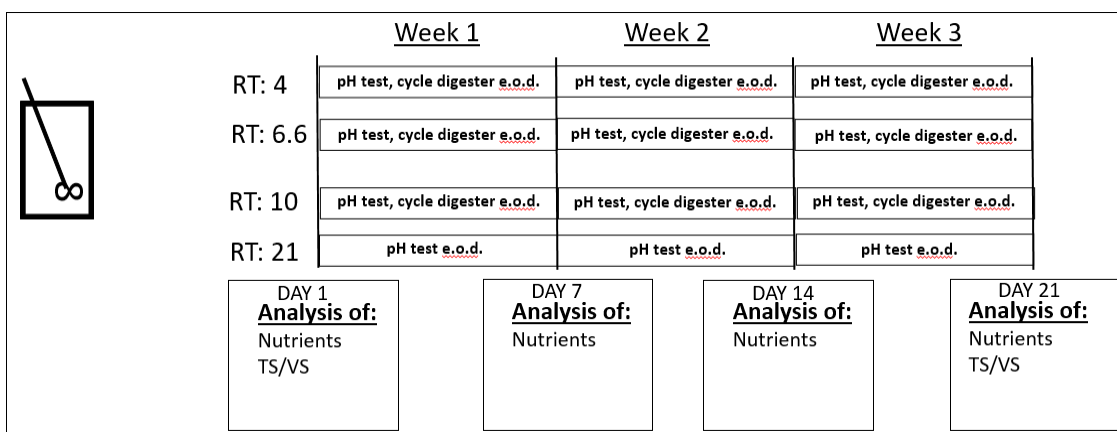


Figure 2: Process diagram of anaerobic digester setup depicting when and what samples were to be taken during the experimental runtime

pH and Biogas Measurements:

pH tests were performed every other day using the effluent removed from each reactor. In the case of the batch reactor (RT: 21 days) a 20mL sample was used for pH analysis Biogas readings were taken using a 60mL syringe attached to a port on the bioreactors.

Volatile Solids and Nutrient Analyses:

Samples were taken for both supernatant and volatile solids analysis. Supernatant samples were obtained by centrifugation of the effluent and then filtration of the remaining supernatant through 0.2um filters. High Range HACH Chemical Testing Kits were used on the supernatant to identify concentrations of dissolved nutrients. Details for these kits can be found in appendix A. The kits use a colorimetric change to show varying concentrations with each compound having its own test. The tests were performed on a weekly basis for each reactor during the 21day runtime. Standard curves for each chemical tested for can be seen in appendix C. A percent volatile solids reading was obtained for each reactor. Foil weighing boats were pre-treated at 550°C for 2hr to remove any impurities and then weighed after cooling. 1mL of digested effluent was put into each foil weighing boat and placed for 1hr into an 110°C

oven for water removal. Once dried, the foil boats were weighed again and then placed into the 550°C oven for 2hr. The remaining mass and foil boat were weighed once more before being discarded.

Results and Discussion-Run 1:

We began the first set of digesters with varying retention times to identify trends for digesters operating in primarily phase I of anaerobic digestion. The lowest retention time of 4 days looked at the extreme case of phase I anaerobic digestion having a high likelihood of methanogenic organisms washing out due to their slow doubling time. We used a batch reactor as a control to show differences in total digestion between phase I and standard digestion.

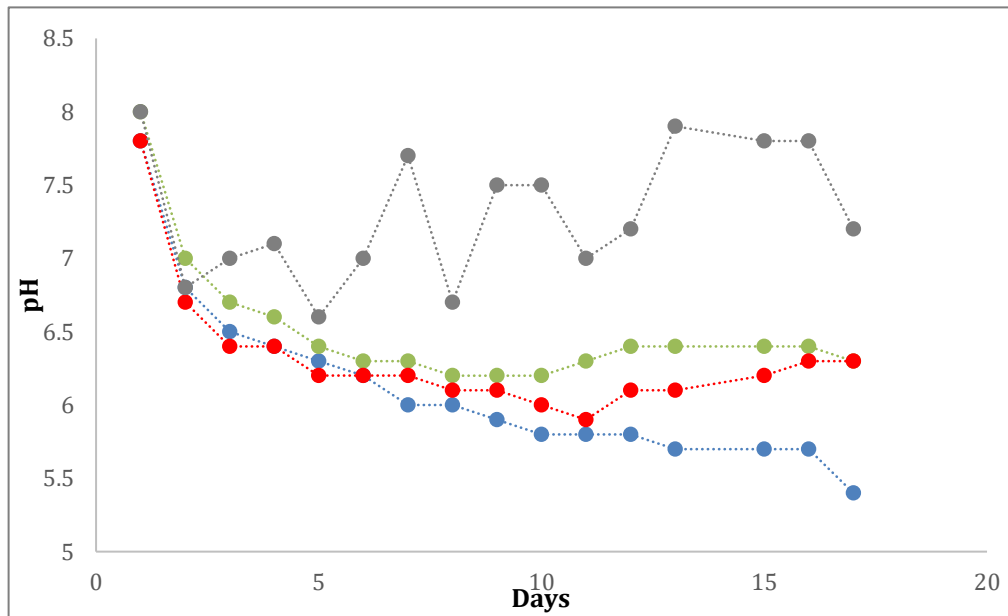


Figure 3: pH readings for anaerobic digesters run at different retention times
Blue: RT=4(days), Red: RT=6.6, Green: RT=10, Grey: Batch

pH readings for the anaerobic digesters show a decreasing trend with shorter retention times. All reactors exhibited a significant drop during the initial week to below a pH of 7 at which point the reactor of 21 days began to increase.

Retention times of 6.6 and 10 days remained consistent after the first week while retention time of 4 days continued to drop during the final week. Retention time of 21 days also showed much more sporadic pH readings when compared to the other three reactors. This was most likely caused by effluent becoming stuck in the sampling port. The stuck effluent would then be used as the next day's sample for pH readings. We remedied this in future runs by flushing the sample port after every removal of effluent.

The pH readings obtained were expected. The first drop in pH for all the reactors denotes the hydrolytic and acidogenic breakdown of the algae substrate. The further decrease in pH of the continuous reactors shows a lack of continued breakdown of organic acids by phase II bacteria. Finally, we concluded that the drop in pH of the 4 day RT reactor to a pH of 5.5 during the final week of operation was due to a complete washout of acetogenic and methanogenic bacteria causing an acidification of the reactor.

To confirm our theories on pH, GC-MS organic chemical analyses were performed on the supernatant of each digester with the help of Dr. Michael Guarnieri's lab. The concentration of organic compounds in the supernatant helps to determine the degree of digestion of the substrate.

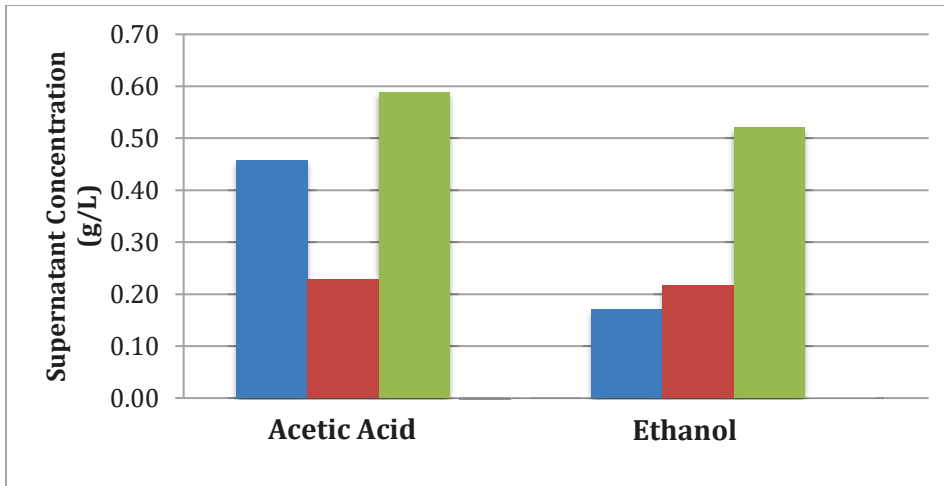


Figure 4: Metabolite analysis for samples taken from anaerobic digesters operating at different retention times after three weeks of operation in mg/L
 Blue: RT=4 (days), Red: RT=6.6, Green: RT=10, Grey: Batch

Figure 4 shows both acetic acid and methanol present in the continuous reactors while the batch reactor gave no readings for either compound. The lack of intermediate chemicals in the batch reactor supernatant shows complete phase II digestion of the algae substrate. The reactor with the RT of 10 days showed the highest acetic acid concentration indicating both high rates of phase I digestion and a low rate of phase II digestion. The higher concentration of acetic acid in the RT 4 reactor compared to the RT 6.6 reactor could be the result of bacterial washout of phase II methanogens and acetogens.

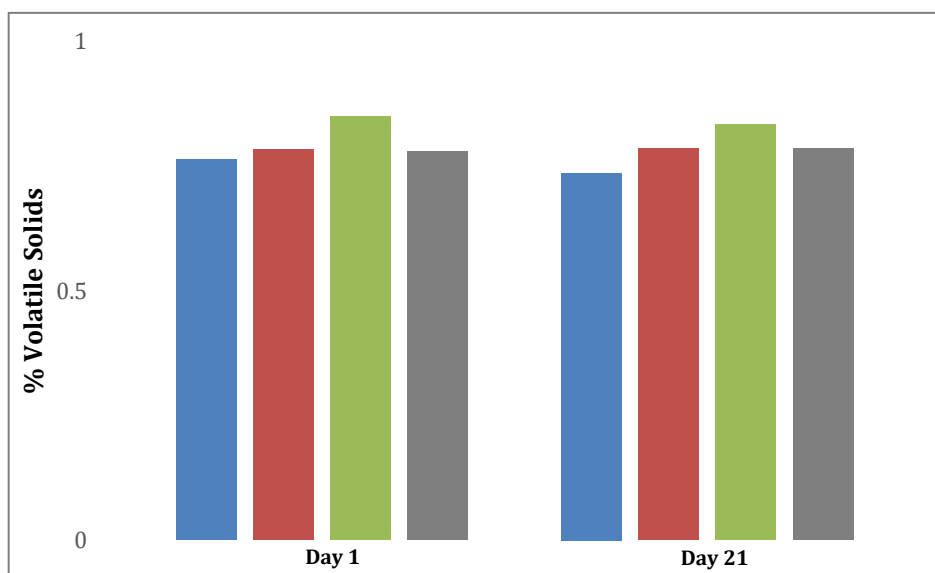


Figure 5: %Volatile Solids for samples taken per week from anaerobic digesters operating at different retention times.

Blue: RT=4 (days), Red: RT=6.6, Green: RT=10, Grey: Batch

In addition to the organic chemical analysis we also looked at volatile solid content of digester effluent. Volatile solid analyses help to show the degree of phase II digestion. Figure 5 shows no significant difference between day 1 samples and day 21 samples from any of the digesters. We determined after the trial that the sample ports had been clogged with particulate making it difficult to get an accurate reading on the solid content of the digesters. In future runs we expanded the sampling tube to prevent particulate buildup.

Lastly, we looked at the concentrations of phosphorus and nitrogen compounds in the supernatant. Figure 6 shows the total nitrogen, ammonia, total phosphorus and reactive phosphorus concentrations at the end of the three week runtime.

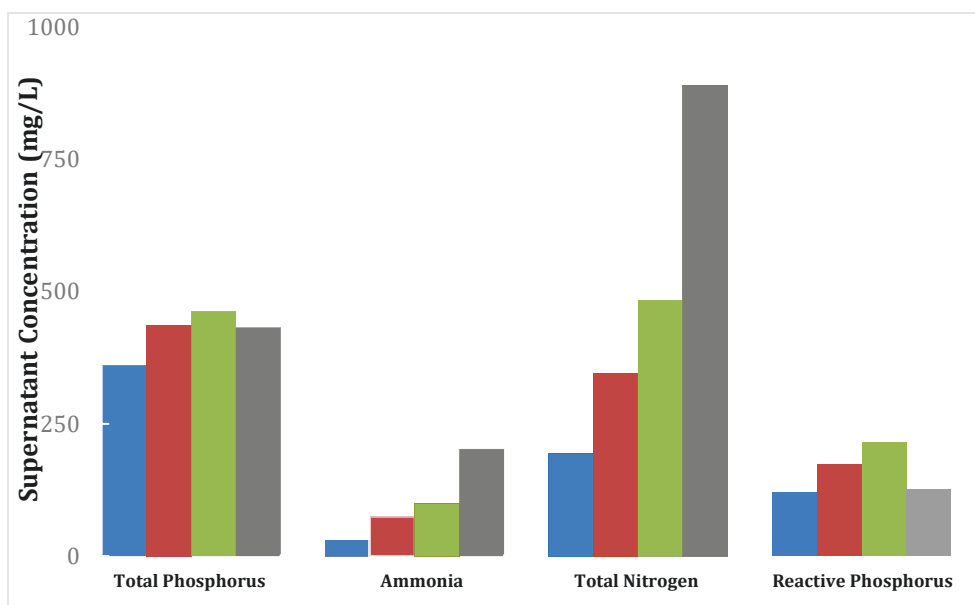


Figure 6: Nutrient analysis on the third week of runtime in mg/L for the supernatant of anaerobic digesters operated at different retention times

Blue: RT=4 (days), Red: RT=6.6, Green: RT=10, Grey: Batch

The most notable difference between the reactors was their total nitrogen concentration. With an increase in retention time came an increase of total nitrogen up to the batch reactor which had a total nitrogen concentration of 890mg/L. Ammonia values followed a similar trend to total nitrogen but at much lower concentrations; well below the 1500mg/L that results in ammonia poisoning of anaerobic bacteria. Both the ammonia and total nitrogen concentrations successfully show increased digestion occurring at longer retention times.

Phosphorus levels also showed an increase in concentration with increasing retention time up to 462mg/L of retention time 10 days. However, the batch reactor had both total and reactive phosphorus concentrations lower than that of reactors with retention times of 10 and 6.6 days. While the batch reactor showed the highest dissolved nitrogen concentration and therefore highest overall digestion it had the second lowest phosphorus concentration.

Changes to Materials and Methods for Run 2:

Having found evidence that a batch system did not show ideal conditions for phosphorus recovery we decided to repeat experiments on reactors of retention time of 10 days and the batch reactor. For the next run we looked to further confirm favorable conditions for algae digestion and to show higher phosphorus concentrations in a first phase dominant anaerobic digester. For the second run we made notable changes to our effluent sampling protocol. Firstly, we widened the sampling port to prevent particulate build-up in the reactor tubes, which had caused sporadic and inaccurate readings during our first run. Secondly, we decided to take initial volatile solids readings for the individual components (sludge and substrate) within the reactor. This allowed us to better show that substrate digestion had occurred at the end of the run. Lastly, we performed a nutrient analysis of the reactors on day 1 to account for phosphorus and nitrogen concentrations contributed by the sludge. The sewage sludge providing the anaerobic bacteria is non-homogeneous slurry leading to the possibility of a slight disproportionality in initial phosphorus and nitrogen concentrations.

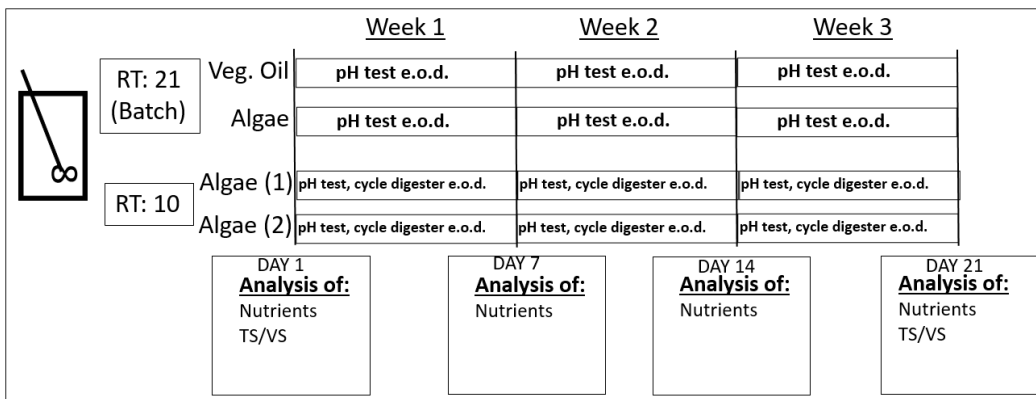


Figure 7: Process diagram of anaerobic digester setup depicting when and what samples were to be taken during the experimental runtime

In addition to the algae bioreactors we also ran another anaerobic digester but using vegetable oil as a substrate. Vegetable oil consists of highly concentrated long chain organic fatty acids. Using vegetable oil as a substrate essentially skips the rate-limiting step of hydrolysis, allowing for higher efficiencies of substrate digestion. This reactor was run as a batch reactor and was used to show anaerobic digestion efficiencies between algae and a less phase I intensive substrate.

Results and Discussion-Run 2:

Figure 8 shows the percent volatile solids readings for sludge, algae and vegetable oil. Both substrates show high percent volatile solids with vegetable oil being slightly higher by about 6%. Sludge shows a much lower reading down at 59%. This is to be expected as the sludge obtained from the wastewater treatment center has already undergone digestion and should be close to 50% volatile solids.

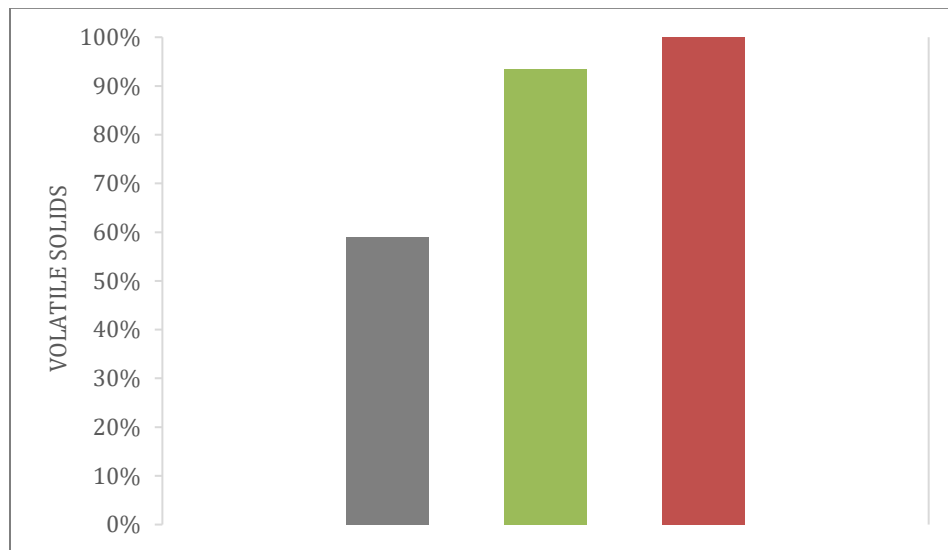
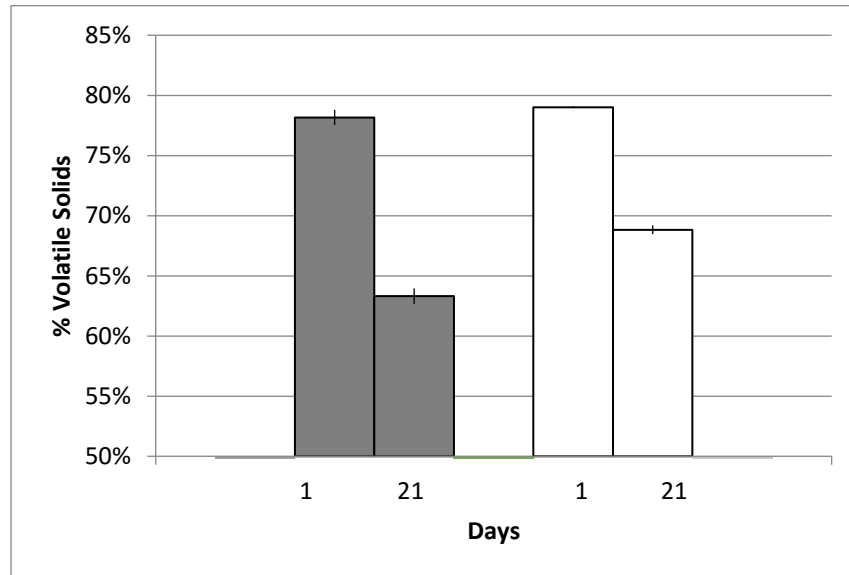


Figure 8: Percent volatile solid readings for activated sludge, algae and vegetable oil

We used the volatile solids readings to calculate substrate concentrations for each reactor so that every reactor would start with the same percent volatile solids. This meant a substrate concentration of 14.04g/L for the vegetable oil reactor and a concentration of 15g/L for the algae reactors. We also took day 1 volatile solids readings

for each reactor after mixing. Figure 9 shows percent volatile solids readings for day 1 and day 21 for both the batch and retention time of ten reactors. Both the batch and 10 day RT reactors started with close to the same volatile solids percentage being 78.1% and 79.0% respectively.



**Figure 9: % Volatile Solid readings for anaerobic digesters for days 1 and 21
Batch (Left), RT=10 days (Right)**

After 21 days of runtime the batch reactors had a much lower volatile solids reading than the 10day RT reactors. The vegetable oil reactor had a starting percent volatile solid of 71.1% and on day 21 had the lowest percent volatile solid, which was 51.8%.

The volatile solids readings confirm the organic acid analysis from the first test showing more digestion occurring in the batch reactor. However, both reactors with algae as the substrate had lower percent volatile solids than the vegetable oil which is expected due to the short phase 1 digestion of LC fatty acids. Further confirmation of less digestion in the continuous reactors comes from the biogas readings collected over the course of the run. Figure 10 shows biogas readings for the batch reactor and two biological replicates of the continuous 10day RT setup. The batch reactor (with the

exception of day 5) shows significantly higher biogas production than the continuous reactor.

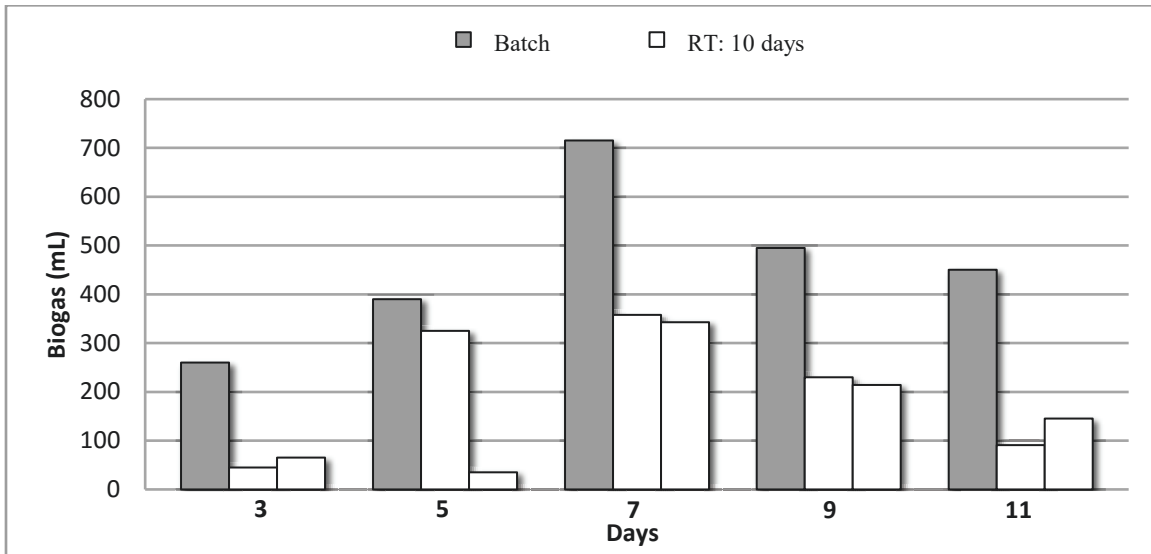


Figure 10: Biogas readings (mL) for anaerobic digesters taken every other day for the first week of runtime

Biogas production denotes phase 2 activity involving both acetogenic and methanogenic bacteria. While biogas production is a staple reading for most anaerobic digestion experiments, we struggled with obtaining accurate data due to our digester setup. Most of the reactor equipment used was modular meaning many potential sites for slight gas leaks.

Figure 11 shows the pH readings for both the batch and continuous reactors. All four reactors started near a pH of 7.7. The batch and continuous reactors diverged on day 3 with the continuous reactors dropping to a more acidic pH. Both batch reactors showed a decrease in pH between days 3 and 7 before rising to a pH of above 8 on day 15.

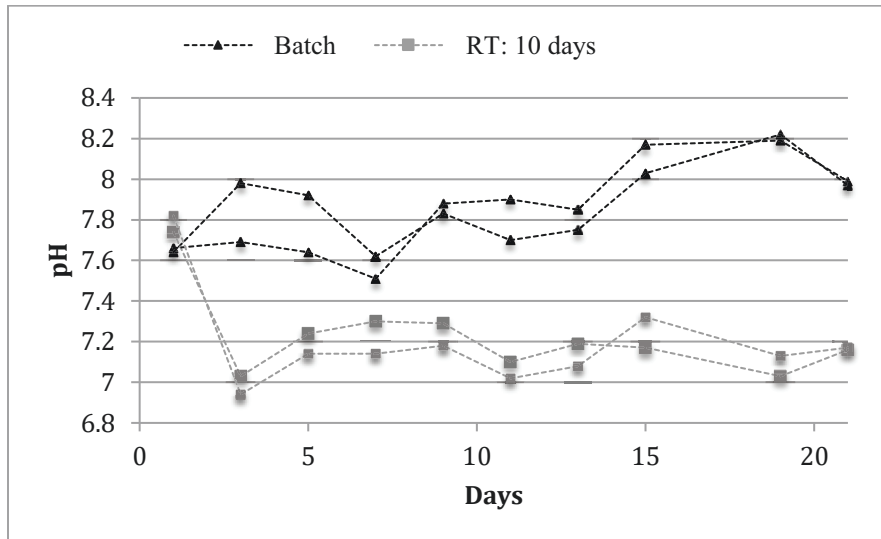


Figure 11: pH readings for anaerobic digesters run at different retention times.
Black: Batch, Grey: RT=10days

pH readings for run 2 gave similar results to what was seen in run 1 (figure 3). The continuous, 10day RT reactors had a more acidic environment denoting primarily phase I bacteria activity. The batch reactors while having a decline in pH early on in the run had a more basic pH as a result of continued digestion of substrate into phase II.

Figure 12 shows the net change in ammonia concentration of the supernatant of the effluent over the 21 day run time for the two batch and two continuous reactors. The net value of nutrients was taken in order to account for chemical compounds contained in the sludge used to inoculate the anaerobic digesters.

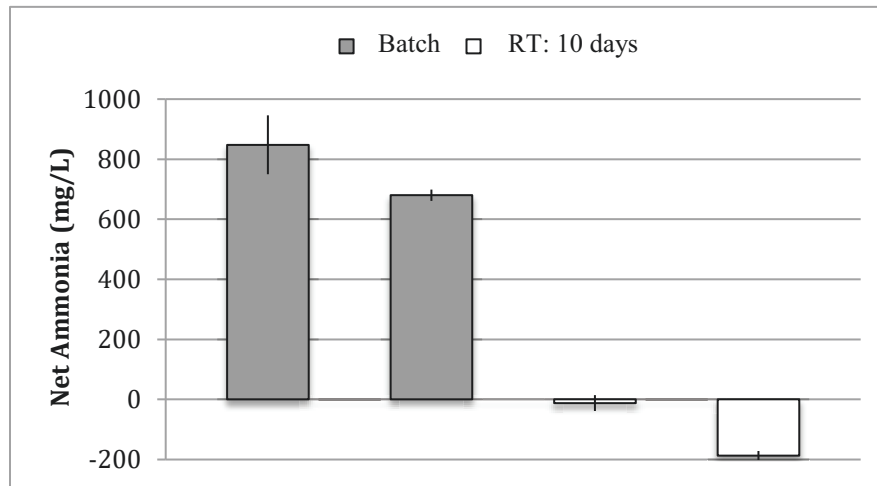


Figure 12: Net change in ammonia concentrations (mg/L) over 21 day run time accounting for sludge

Both batch reactors had increases of over 600mg/L in ammonia concentration of their supernatants. The first batch reactor had a high final total ammonia concentration being 1440mg/L. This value being close to 1500mg/L could have led to ammonia poisoning of phase II bacteria however this high ammonia concentration was not reached until the third and final week of runtime. Data for weekly ammonia concentrations can be found in appendix D. The continuous reactors actually showed a decrease in ammonia both being lower than that of the starting concentrations of the sludge. A high ammonia concentration for the batch reactors means greater overall digestion of the algae substrate. The lower ammonia concentration of the batch reactor means less digestion of substrate but effluent removal could also have contributed to the large difference in supernatant concentrations.

Figure 13 shows the net change in total phosphorus concentration of the supernatant of the effluent over the 21 day run time for the two batch and two continuous reactors. All reactors showed a net gain of total phosphorus in the supernatant with the highest being the first continuous reactor reaching 185mg/L.

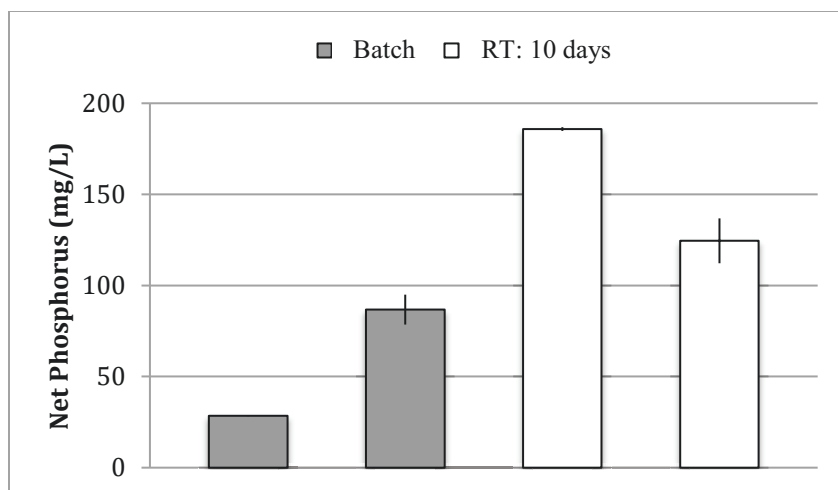


Figure 13: Net change in phosphorus concentrations (mg/L) over 21 day run time accounting for sludge

Conversely to the distribution of ammonia concentrations, the continuous anaerobic digesters showed the highest gain of total phosphorus in the supernatant despite both liquid exchange and less digestion occurring.

Conclusion:

In both the first run and second run of this experiment a primarily phase I (continuous) digester had a higher amount of phosphorus in the supernatant when compared to a standard batch anaerobic digester. However this only held true for digesters that operated at longer retention times due to both a lack of substrate digestion and supernatant dilution from liquid exchange. Therefore a phosphorus concentration in the supernatant vs. retention time distribution curve would have its highest point somewhere in the middle with less phosphorus seen at both lower and higher retention times. This highest concentration would be the result of adequate substrate digestion while mitigating the precipitation of phosphorus from a basic pH.

Future Steps:

While some current methods of phosphorus recovery rely on chemical precipitation, new, more economically and resource efficient options like zeolite nanoparticles are being explored. These methods look to capture the ortho-phosphorus contained in the supernatant before precipitation occurs due to high pH or chemical additives. Therefore identification of an anaerobic digester setup that allows for the most phosphorus in the supernatant is necessary.

Figure 14 shows a potential phosphorus recovery pathway for a two stage digester system. Pictured are two phosphorus recovery points: The first being a potential recovery site for ortho-phosphate in the supernatant recovered from a primarily phase I digestion unit. The second recovery point shown would involve chemical precipitation from a standard batch anaerobic digester.

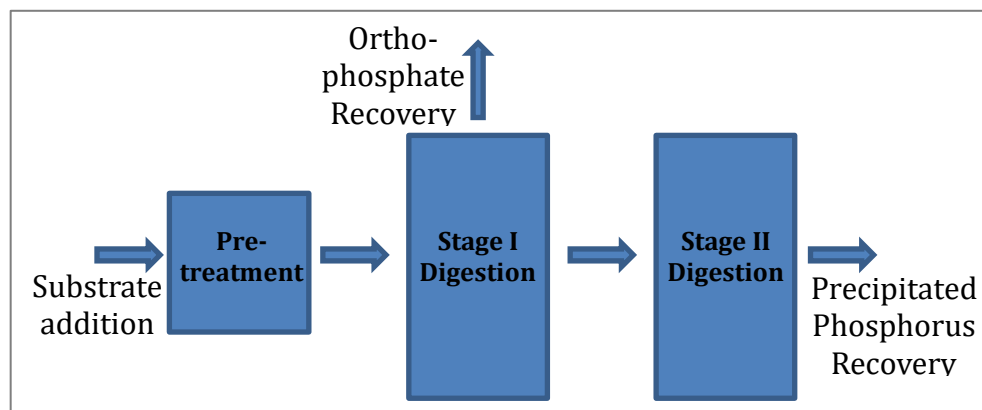


Figure 14: Potential phosphorus recovery process diagram depicting two separate phosphorus recovery streams

Appendix A: HACH Chemical Testing Kits

S. No.	Nutrient	Kit No.	Range	Manufacturer
1	Total Phosphorus	TNT 844	0.5-5.0 mg/L PO ₄ -P 1.5-15.0 mg/L PO ₄	HACH, Germany
2	Reactive phosphorus	TNT 846	1.6-30.0 mg/L PO ₄ -P 5.0-90.0 mg/L PO ₄	HACH, Germany
3	Total Nitrogen	TNT 826	1-16mg/L N	HACH, Germany
4	Ammonia	TNT 832	2-4mg/L NH ₃ -N	HACH, Germany
5	Nitrate	TNT 835	0.23-13.50mg/L NO ₃ -N 1.00-60.00 mg/L NO ₃	HACH, Germany
6	Nitrite	TNT 840	0.6-6.0 mg/L NO ₂ -N 2-20 mg/L NO ₂	HACH, Germany

Table 1: List of HACH Chemical Testing Kits used for nutrient analysis of anaerobic digesters

Appendix B: Dissolved Oxygen Readings

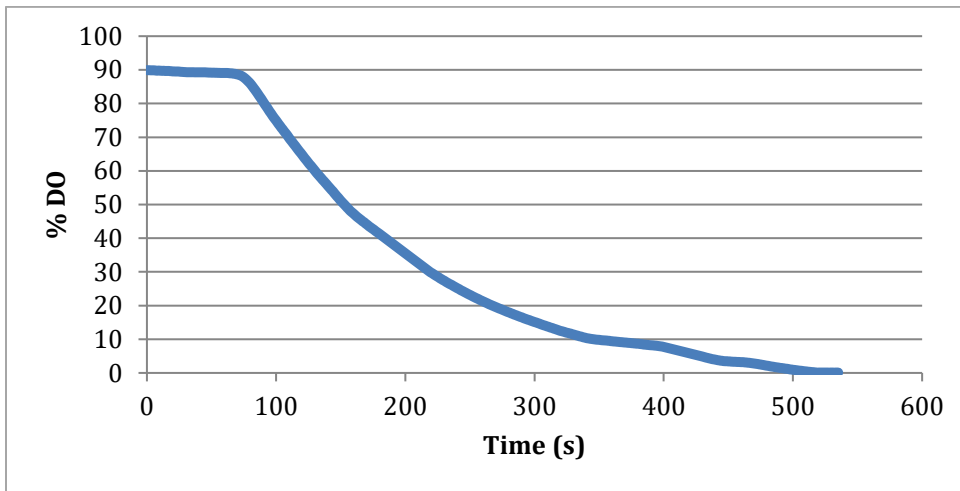


Figure 15: Dissolved oxygen readings for anaerobic digester being sparged with nitrogen

Appendix C: Standard Curves for HACH Chemical Testing Kits

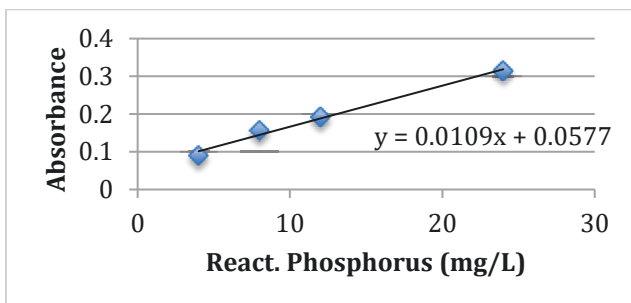


Figure 16: Standard Curve for reactive phosphorus HR HACH kit

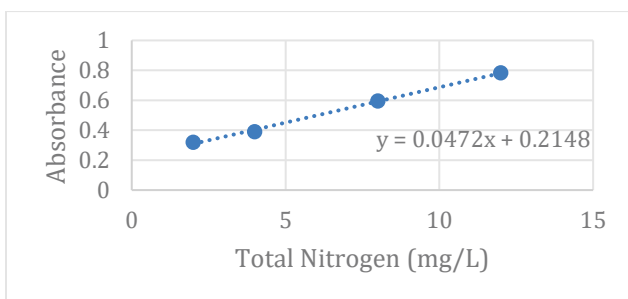


Figure 17: Standard Curve for total nitrogen HR HACH kit

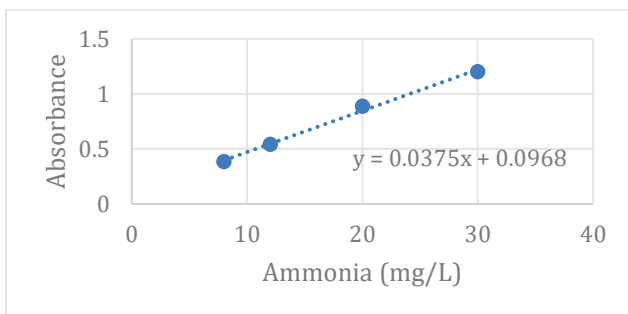


Figure 18: Standard Curve for ammonia HR HACH kit

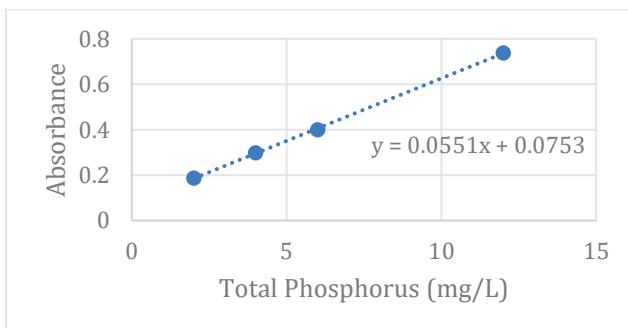


Figure 19: Standard Curve for total phosphorus HR HACH kit

Appendix D: Ammonia Readings for Run 2

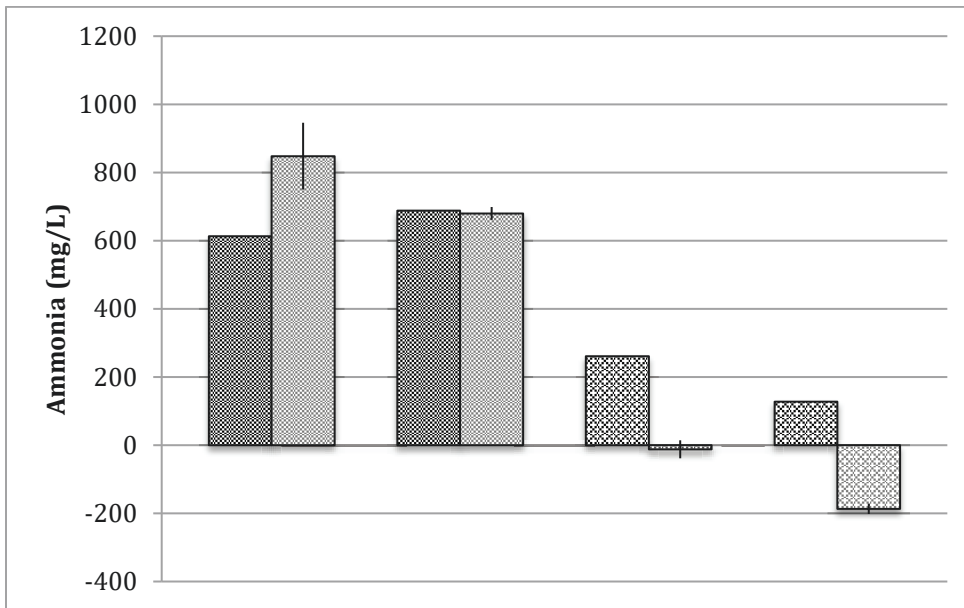


Figure 20: Net change in ammonia concentrations for days 14 and 21 for batch (left) and continuous (right) anaerobic digesters

Appendix E: List of Anaerobic Digester Equipment

Weighing balance: Mettler Toledo
Model: MS4036 (Switzerland)

Nitrogen gas from Airgas Company (US)

Overhead stirrers: Bellco Biotechnology
Serial No. : OHDD-859 (US)

Pump: Cole-Parmer
Model No. 7553-71 (US)

Motor: Cole-Parmer
Model No. 7553-02 (US)

Gravity Convention Oven
Model No: 17 (US)

Furnace
Lindberg/Blue
Thermo Fisher Scientific (US)
Couldn't get its model no as it's not moveable

Weighing balance for TS/VS
Mettler AE 100
No model no.
Switzerland

Heating equipment
Hach DRB 200
Serial # 10110Co871 (US)

Centrifuge 5810R
15 Amp version
Thermo Scientific (USA)

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Curriculum Vitae

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Objective: To obtain a full-time position where I can use my skills in Chemical and Biomolecular Engineering.

EDUCATION

Johns Hopkins University (Baltimore, MD)

Master in Chemical and Biomolecular Engineering

GPA: 3.3

Exp. Grad: Sept 2016

Bachelors of Engineering in Chemical and Biomolecular Engineering

GPA: 3.21

Graduated May 2015

EXPERIENCE

Johns Hopkins Chemical and Biomolecular Eng. Senior Lab

Baltimore, MD

Fall 2015-Present

Teaching Assistant/Lab Technician

- Responsible for maintenance of lab equipment: Bioreactor, distillation column, gas adsorption unit.
- Communicated essential lab techniques and how to safely and properly use lab equipment.
- Responsible for grading and compiling final grades of students

NexGen Algae

Columbia, MD

Summer 2014

Lab Technician

- Integral member of an 8 person start-up seeking to produce carotenoids for pharmaceutical use from microalgae.
- Ownership of maintaining strains of microalgae *Haematococcus pluvialis* in a 64L bioreactor.
- Succeeded in the scale up and maintenance of small scale cultures of *Haematococcus pluvialis* to 163 liter bioreactor for pharmaceutical development.

Betenbaugh Algae Lab at Johns Hopkins University

Baltimore, MD

Fall 2013 - Present

Research Assistant

- Oversaw the start-up, maintenance and analysis of several two-stage anaerobic digesters designed for nutrient recovery.
- Maintained and grew two strains of the algae *Chlorella Vulgaris* (UTEX 395, 265) to use as experimental data for a computer-based co-culture growth model

Capstone Project - The Green Program

Guanacaste, Costa Rica

Summer 2013

Capstone Project Leader

- Conducted experiential hands on site visitations to five commercial scale renewable energy facilities including geothermal, wind power, biomass, solar and hydroelectric plants.
- Led an interdisciplinary capstone project focused on harnessing the electric potential of piezoelectric crystals.
- Gained global perspective and fortified intercultural communication skills through activities and immersion experiences.

Hawaii Pathology Laboratory

Honolulu, HI

Summer 2012

Gene Research & Laboratory Experience

- Diagnostic laboratory; DNA extraction from blood samples to be used for DNA sequencing.
- Extensive research in pharmacogenetics by exploring the adverse effects of Methotrexate on psoriasis patients.

SKILLS

Computer: Software knowledge in Microsoft Office and MATLAB

Lab: Cell culturing, media preparation bioreactor setup, aseptic technique, UV-VIS spectrophotometry, distillation, microscopy, flow cytometry, hemocytometer technique.

ATHLETICS

College Varsity Wrestling

Baltimore, MD

Fall 2011-Spring 2015

- Four year starter for the Johns Hopkins wrestling team at weight classes 174, 184 and 197.
- Academic All-American and 4th place at 184 in the 2015 North Eastern Regional Championships.