# THE EFFECT OF PRETREATMENT METHODS ON METHANE YIELD AND NUTRIENT SOLUBILIZATION DURING ANAEROBIC DIGESTION OF MICROALGAE

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Master of Science in Civil and Environmental Engineering

by

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# ABSTRACT

## The Effect of Pretreatment Methods on Methane Yield and Nutrient Solubilization During Anaerobic Digestion of Microalgae

Alexander Scott Hill

Microalgal biomass is a candidate feedstock for biofuel production. To improve the sustainability of algae biofuel production, following biofuel recovery, the biomass nutrients should be recycled for additional algae growth. Anaerobic digestion of algae or oil-extracted algae is a means of recovering carbon and other nutrients, while offsetting algae production electricity demand. The major limiting factor in microalgae digestion is the low biodegradability of the cell walls. In the present study, various pretreatment technologies were tested at bench scale for their ability to improve raw, non-lipid-extracted algae biodegradability, which was assessed in terms of methane yield, volatile solids destruction, and solubilization of N, P, and K. The microalgae were harvested by sedimentation from outdoor wastewater-fed raceways ponds operated in coastal southern California. Four pretreatment methods (sonication, high-pressure homogenization, autoclaving, and boiling) were used on the algae slurries, each followed by batch anaerobic digestion (40 days at 35$^{o}$C). Biomass sonication for 10 minutes showed the highest methane yield of 0.315 L $CH_4$/ g $VS_{IN}$, which is a 28% increase over the untreated control. Conversely, autoclaved algae slurry inhibited methane production (0.200 vs. 0.228 L $CH_4$/ g $VS_{IN}$ for the treatment and control). A preliminary energy balance indicated that none of the pretreatments led to a net increase in energy conversion to biomethane. However, pretreatment did increase the initial N and P solubilization rates, but, after digestion, the ultimate N and P solubilization was nearly the same among the treatments and controls. After 40 days of digestion, solubilization of N, P, and K

reached, respectively, 50-60% of average total Kjeldahl N, 40-50% of average total P, and 80-90% of average total K. Descriptive first-order models of solubilization were developed. Overall, certain pretreatments marginally improved methane yield and nutrient solubilization rate, which cast doubt on the efficacy of, or even the need for, algae biomass pretreatment prior to anaerobic digestion.

Keywords: batch anaerobic digestion, pretreatment, solubilization, digestate, NPK, lysis, sonication, high pressure homogenization, heat treatment, L $CH_4$/g $VS_{IN}$

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# 1 Introduction

Human health and well-being are beginning to be threatened by excessive demand for resources like water, clean air, and nutritious food. In 2014, the global population is around 7.2 billion (Census Bureau, 2014), with a projected population of 9.6 billion by 2050 (United Nations, 2013). Underpinning the remarkable growth is the imminent need to develop and promote sustainable food, energy, and water systems that will meet the demands of our future as a whole. The wastewater treatment and energy production sectors are resource-based industries that are fervently seeking out more efficient technologies.

California has a long history of supporting renewable energy production, and, in 2011, took another step towards advancing renewable energy production when Governor Brown signed a legislative bill mandating that California utilities provide 33% of their total energy needs using renewables (CEC, California Renewable Energy Program and Overview, 2013). A regulatory mandate like this is paramount especially when "water related energy use" consumes a staggering 20% of the entire state's electricity (CEC, Managing an uncertain future-Climate Change adaptations for California's water, 2008).

Recycling wastewater contributes to stabilizing water resources in drought-prone California, but recycling has required energy-intensive treatment to reach reuse standards. The inherent energy content of wastewater has been recaptured at some major wastewater treatment plants through anaerobic digestion of sludge with biogas-fired power generation, but the power use by conventional mechanical treatment plants usually exceeds the on-site power generating capacity of sludge digestion

The energy balance of wastewater treatment can be improved by using treatment ponds, which can have low energy-intensity. Furthermore, if microalgae are cultivated in the treatment ponds, the biomass can be used to produce biofuels. Biogas from anaerobic digestion of algae slurry is the most developed process algae biofuel process, but production of liquid transportation fuels is the topic of extensive research around the world (NRC, 2012). Thus, if algae wastewater treatment plants with anaerobic digestion can be successfully implemented, both wastewater treatment and sustainable energy production could be advanced, making a contribution to improved management of our water and energy resources.

Ultimately, however, if algae biofuels are to make a noticeable contribution to national biofuels, algae farming must become an extensive endeavor, with consumption of water and fertilizer (Lundquist et al., 2010). In this scenario, wastewater would not be treated and discharged but rather consumed in evaporation and other losses at algae farms. To minimize such consumption, algae growth media must be recycled (NRC, 2012). Water would be recycled by harvesting the algae and returning the clarified water to the algae production ponds. Nutrients would be recycled from residual algae biomass following extraction of fuel precursors, as will be further described in the Background section.

The aim of the present research to identify the extent to which microalgae grown on wastewater can be used as both a biogas source, via methane generation from anaerobic digestion, as well as a nutrient source for the growth of additional algae. Specifically, the present study aims to address the following questions.

**Research Questions:**

1. What effect do different pretreatment technologies have on specific methane yield?
2. What effect do different pretreatment technologies have on the fraction of nitrogen, phosphorus, and potassium ultimately solubilized from microalgae during digestion?
3. What model and rate constants can describe solubilization for the different pretreatments?

# 2 Background

The following section describes the historical experiments as well as pertinent background information that provide context for this study.

## 2.1 Anaerobic Digestion

Anaerobic digestion is a widely used and relatively well-understood process that could ameliorate some of the looming resource shortages, especially when paired with microalgal cultivation on an inexpensive feedstock like wastewater (Woertz et al., 2014). Anaerobic digestion is a biological process that involves bacterial degradation of organic matter into a renewable energy source, biomethane, and nutrient rich digestate. In a simplified biochemical pathway of anaerobic digestion (**Figure 1)**, carbon-rich organic matter is solubilized via hydrolysis, followed by extensive production of volatile fatty acids such as acetic acid and hydrogen in the processes of acidogenesis, acetogenesis, and dehydrogenation of fatty acids. The acetic acid and hydrogen produced can be consumed by methanogens to make methane and additional carbon dioxide (McCarty P. L., 1964).

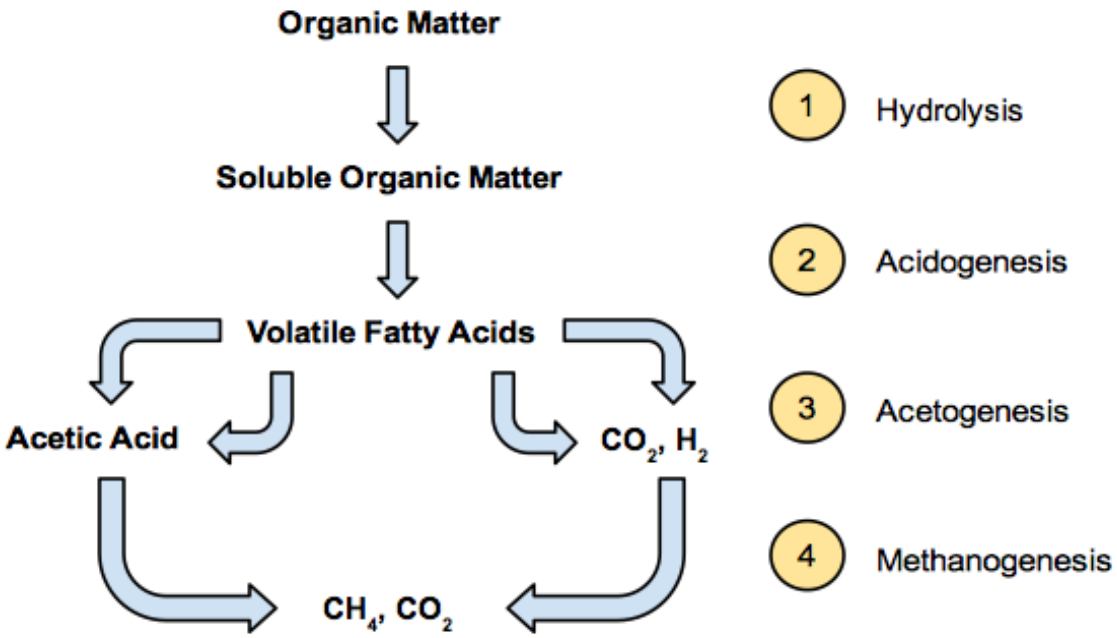


**Figure 1.** Anaerobic digestion occurs in four basic biochemical steps. The end products include methane, carbon dioxide, and digestate rich in soluble nitrogen, phosphorus and potassium.

### 2.2 Benefits of Microalgae for Biofuel Production

Microalgae are an appealing feedstock for biofuel development because of their potential for high biomass yield per area, high lipid content compared to other crops, and low competition for non-arable land (Marsolek et al., 2014) (Collet, 2011). These inherent properties of algae, on top of the fact that they can be used to remediate municipal wastewater, support the notion of cost effective, sustainable biofuel production. Researchers have projected that anaerobic digestion paired with microalgal wastewater treatment can be economical (Collet, 2011) (Ras et al., 2011) (Sialve B., 2009). Further bolstering this claim, a life cycle assessment analysis applied to "coupled microalgae and biogas production" determined that inexpensive harvesting techniques on top of fertilizer supplementation though digestate recycling can significantly improve the economic merits of this current study (Collet, 2011).

## 2.3 Historical Experiments Involving Anaerobic Digestion of Microalgae

The joint process of wastewater treatment using mixed algal species and subsequent anaerobic digestion of the cultivated biomass has long been a topic of research, and the first scientific publishing on the subject dates back to the seminal paper by Golueke, Oswald and Gotaas in 1957. The methane yield of their semi-continuous digester fed untreated, raw algae grown on wastewater was nearly 0.32 L $CH_4$/g $VS_{IN}$ (Golueke C. O., 1957). However, that yield is low in relation to the appreciably higher theoretical yield of 0.59-0.79 L $CH_4$/g $VS_{IN}$ for the same *Chlorella-Scenedesmus* mixture (Sialve B., 2009). The shortcomings of microalgal anaerobic digestion are especially apparent when compared to the typical yield for municipal wastewater sludge which is reported to be 0.6 L $CH_4$/g $VS_{IN}$ (Marsolek et al., 2014). The culmination of many experiments has ultimately led researchers to seek out a way to improve algal biomass degradation and methane yields.

## 2.4 Anaerobic Digestion Enhancement

Pretreatment of waste activated sludge has been a successful practice for a handful of municipal water treatment facilities, and the increase in cumulative methane production can be as high as 76% for sludge treated at 170°C for 30 minutes (Valo et al., 2004). Many of these same technologies listed in **Table 1** have been exploited in their ability to improve the biodegradability of microalgae-fed anaerobic digesters.

**Table 1**. Partial list of various pretreatment technologies that have been applied to increase biogas yields of anaerobic digestion of microalgae.

| Pretreatment type | Example |
|---|---|
| Mechanical | Grinding<br>Milling<br>Homogenization<br>Sonication<br>Maceration<br>Liquid shear |
| Thermal | Hydrothermal<br>Drying<br>Steam |
| Chemical | Acid or Alkali hydrolysis<br>Ozone<br>Hydrogen peroxide |
| Biological | Enzymatic |
| Irradiation | Gamma-ray<br>Electron-beam<br>Microwave |
| Electrical | Electro-Fenton |
| Combination | Thermo-chemical |

In the literature, hydrolysis has frequently been identified as the rate-limiting step in anaerobic digestion (Bohutskyi, 2014). Pretreatment of the algal biomass before digestion is meant to hydrolyze the large macromolecular structures that comprise the algae cell wall and increase both the rate and overall extent of their biodegradability (Sialve B., 2009). Thermochemical pretreatments have been tested, with the maximum increase of 33% in methane production resulting from the algae being heated for 8 h at 100°C. However, the "heat treated" methane specific gas yield was still only 0.30 L $CH_4$/g $VS_{IN}$ as compared to the 0.26 L $CH_4$/g $VS_{IN}$ of the untreated control (Chen, 1998). Building on that progress, numerous attempts have been made to achieve better methane yields by

using various pretreatments. Despite improvements, the ultimate yields vary widely amongst research groups: values range from 0.1-0.5 L $CH_4$/g $VS_{IN}$ (Sialve B., 2009), (Marsolek et al., 2014). One study in particular evaluated the effect of thermal, ultrasonic and alkali pretreatments on the methane production of the same species of algae. Their efforts revealed that, despite all of the pretreatments stimulating substantial soluble COD release and implied cell lysis, the resultant methane production and net energy gain was not favorable (Cho et al., 2013). Several other studies have shown similar results, indicating that the cost of pretreatment did not substantiate the ultimate improvements in energy gain from methane production (Marsolek et al., 2014), (Alzate et al., 2012), (Cho et al., 2013). However, if the cost of nutrient addition for algal cultivation is offset by recycling nutrient-rich digestate, the economics of algae biofuels would improve.

### 2.5 Nutrient Recycling

The increasing cost of fertilizers adds motivation to this study. For example, phosphorus is a mined resource that is not only being depleted, but the quality is diminishing while the cost of production is increasing (Cordell et al., 2009). Even though algal cells are comprised of nominally 0.5-1% phosphorus and 8% nitrogen, the cost of supplying those nutrients in the required amount for optimal growth can be substantial (Lundquist et al., 2010). The possibility of recycling nutrient rich digestate from oil-extracted residual biomass, to supplement further rounds of algae cultivation, increases the sustainability of algae biofuels as mentioned previously. One such coupled biofuels configuration can be seen in **Figure 2**.

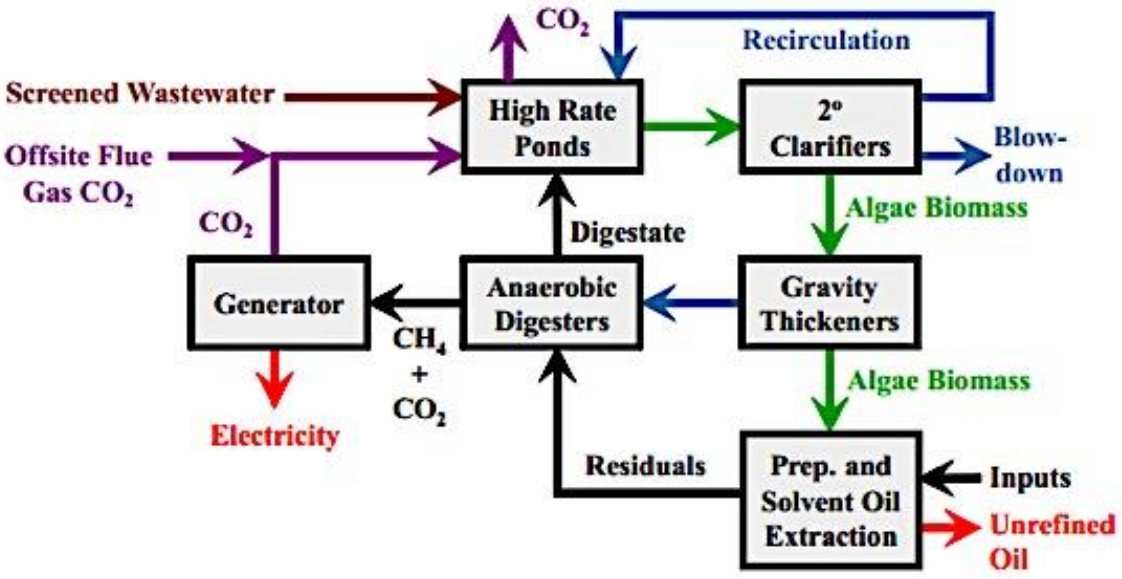


**Figure 2.** Hypothetical algae biofuels production process flow. Both raw algae slurry and residual oil-extracted biomass are feedstock for anaerobic digestion. Source: (Lundquist et al. 2010)

Thus, another goal of algae pretreatment is to hydrolyze digester feedstock algae and release the nitrogen, phosphorus and potassium locked inside the tough cell walls, thereby promoting resolubilization of those nutrients for subsequent rounds of algae cultivation. Coupled biofuel production and nutrient recycling of microalgae has garnered significant attention and funding in the last two decades and has been reported in numerous studies (Bjornsson, 2013) (Collet, 2011) (Ras et al., 2011). In fact, it has been suggested that nutrient recycling can offset the need for algal fertilizer costs by ten-fold (Collet, 2011).

The hypothetical methane yield and TAN ratio on a volatile solids basis was calculated and reported in **Table 2**. Both of the hypothetical values will be cited as benchmark values in the present study.

**Table 2.** The methane yield and TAN concentrations were normalized by initial volatile solids. Source: (Sialve B., 2009)

| Species | Proteins (%) | Lipids (%) | Carbohydrates (%) | $CH_4$ (L $CH_4$/g $VS_{IN}$) | TAN (mg TAN/g $VS_{IN}$) |
|---|---|---|---|---|---|
| *Euglena gracilis* | 39-61 | 14-20 | 14-18 | 0.53-0.8 | 54.3-84.9 |
| *Chlamydomonas reinhardtii* | 48 | 21 | 17 | 0.69 | 44.7 |
| *Chlorella pyrenoidosa* | 57 | 2 | 26 | 0.80 | 53.1 |
| *Chlorella vulgaris* | 51-58 | 14-22 | 12-17 | 0.63-0.79 | 47.5-54.0 |
| *Dunaliella salina* | 57 | 6 | 32 | 0.68 | 53.1 |
| *Spirulina maxima* | 60-71 | 6-7 | 13-16 | 0.63-0.74 | 55.9-66.1 |
| *Spirulina platensis* | 46-63 | 4-9 | 8-14 | 0.47-0.69 | 42.8-58.7 |
| *Scenedesmus obliquus* | 50-56 | 12-14 | 10-17 | 0.59-0.69 | 42.2-46.6 |

### 2.6 Variables Affecting Anaerobic Digestion of Microalgae

Despite the increase in methane production from pretreated microalgae, the recalcitrant compounds inherent in algal cell walls pose a challenge for anaerobic digestion. Specific recalcitrant molecules present in some algal species include polyaromatics, hetero-polysaccharides, algaenan, sporopollenin, silica, uronic acid and lignine (Alzate et al., 2012). For example, it was discovered that for *Chlorella vulgaris,* 50% of the biomass did not degrade even at a digestion period of 200 days (Ras et al., 2011). However, to some extent, the genera of algae are the determining factor in its overall biodegradability because different strains of algae contain different resistant molecules (Mussgnug et al., 2010) (Foree, 1970).

In addition to strain-specific biogas production, operational parameters like organic loading rate, hydraulic residence time, temperature of digestion, substrate to inoculum ratio (S/I), lipid content, C:N ratio, etc., all play a major role in the efficacy of anaerobic digestion. For example, research conducted by Yen and Brune optimized the C:N ratio using supplementary carbon from waste paper, and they saw a doubling of the methane production from 0.57 L $CH_4$/g $VS_{IN}$ to 1.2 L $CH_4$/g $VS_{IN}$ (Yen, 2007). The C:N ratio is also cause for concern in regards to digestion of microalgae because the high protein content of the cells can result in ammonia toxicity at high organic loading rates (Sialve B., 2009). Methanogenic bacteria are noted to have adapted to higher ammonia concentrations in some studies. However, they are typically adversely affected at concentrations above 3000 mg/L (McCarty P. L., 1964).

Some pretreatment technologies might actually decrease the overall biodegradability of microalgae. The Maillard reaction is the complex, non-enzymatic browning of organic matter that occurs under high heat conditions. The products that form as a result of the reaction are found to reduce the nutritive value of the biomass as well as cause toxic byproduct formation (Ledl, 1990), which may have occurred in the present study.

### 2.7 Rationale of the Present Study

Renewable biomethane and mineralized fertilizer production from anaerobically digested microalgae is a long sought after technology, but knowledge gaps still exist despite extensive previous research. In the present, study polycultures of wild type algae grown on municipal wastewater were subjected to several types of pretreatment and subsequently anaerobically digested in mesophilic batch digesters. This research aimed to quantify the effects of pretreatment on biomethane yield and the rate and extent of

nitrogen, phosphorus, and potassium resolubilization during digestion. A better understanding of these parameters will aid in the development of sustainable algal technologies of the future.

The following Methods chapter describes the materials and procedures used to quantify the above parameters.

# 3 Methods

This chapter describes the materials and methods used in the present research, including both the pilot plant, which was the source of algae feedstock, and the laboratory anaerobic digestion methods. Note that the "algae" biomass referred to throughout this thesis is actually a polyculture of various genera of algae and bacteria, presumably containing some detritus.

## 3.1 Overview of Experiments

The pilot plant process under development to test algae digestion consists of growing algae polycultures in raceway ponds followed by sedimentation harvesting of biomass in tube settler tanks. The growth medium has been primary clarifier effluent ("sewage") from a municipal wastewater treatment plant. The settler subnatant slurry or "slurry" is to be pretreated to promote more complete digestion followed by anaerobic digestion. The research described in this thesis involves laboratory-scale pretreatment methods of algae grown and harvested at the pilot scale. A conceptual process flow of the pilot scale cultivation and digestion (including pretreatment) is described in **Figure 3**.

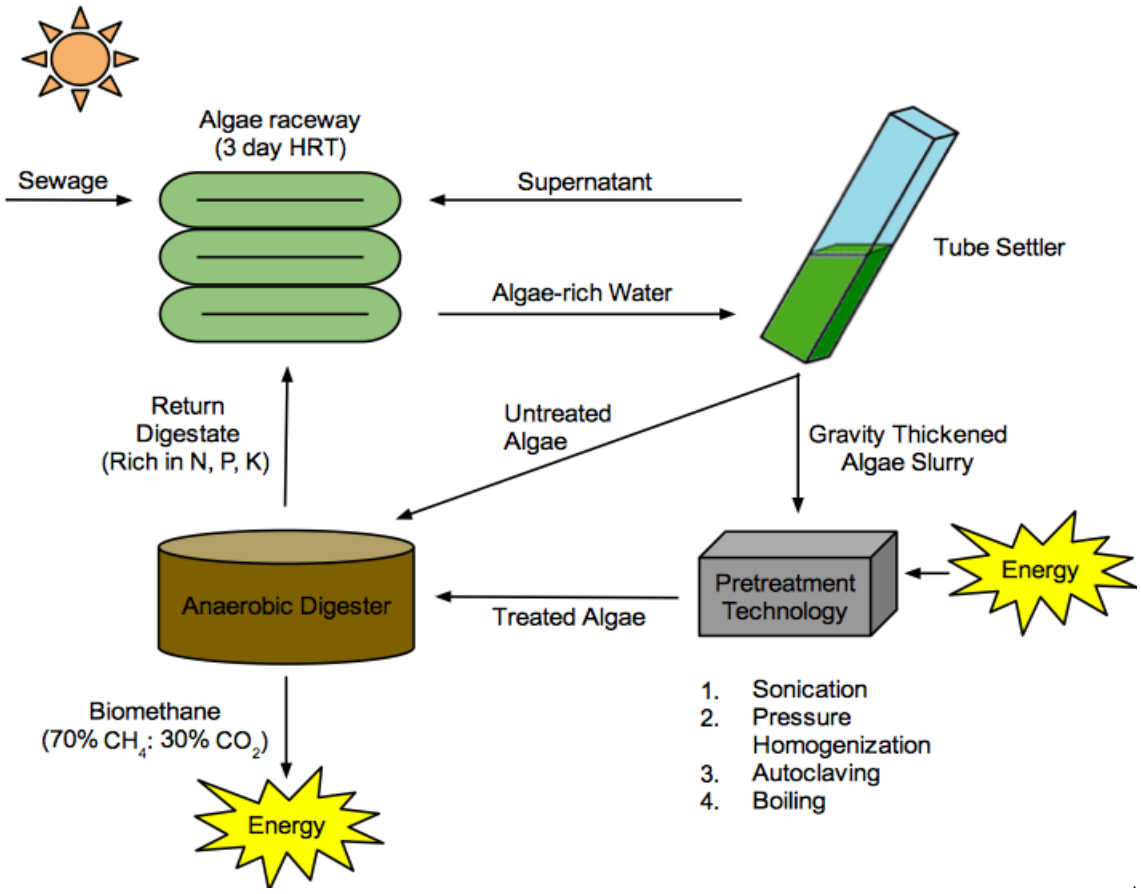


**Figure 3.** The basic conceptual process flow of the pilot scale facility, including some energy inputs and outputs. The list beneath the "pretreatment technology" box indicates the four different pretreatment technologies that were tested at laboratory-scale in the present study. (Note: instead of the laboratory digestate being returned to the algae raceways, as depicted, it was passed along to other researchers interested in aerobic degradation Chang (2014) and microalgal regrowth allelopathy Boggess (2014).)

Five serum bottle, batch-mode digestion experiments are reported herein, which used four different laboratory-scale pretreatment methods: sonication, high pressure homogenization through a small orifice, autoclaving, and boiling (**Table 3**). Sonication was seen as a benchmark cell disruption technique that could not be scaled-up easily at the pilot plant. The other three pretreatment were considered scalable at the Cal Poly pilot plant. For example, Cal Poly has an autoclave with a capacity of several cubic meters, which was available for autoclave or boiling pretreatments.

**Table 3.** Overview of all five experiments and their respective logistical information.

| Experiment Number | Pretreatment Technology | Start Date | End Date | Overall Duration (Days) | Nutrient Sampling Days | Total Number of Serum Bottle Digesters |
|---|---|---|---|---|---|---|
| 1 | Sonication (Biogas Determination) | 6/5/2013 | 8/6/2013 | 62 | 0, 62 | 19 |
| 2 | Sonication (Nutrient Solubilization) | 7/9/2013 | 8/20/2013 | 42 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 42 | 39 |
| 3 | High Pressure Homogenization | 9/16/2013 | 10/25/2013 | 39 | 0, 4, 10, 21, 39 | 30 |
| 4 | Autoclaving | 11/14/2013 | 12/27/2013 | 43 | 0, 2, 5, 10, 20, 43 | 19 |
| 5 | Boiling | 1/23/2014 | 3/7/2014 | 43 | 0, 2, 5, 10, 20, 43 | 28 |

### *3.1.1 Collection and Storage of Algae*

During this study, all algae samples were collected from the Alpha set of raceway ponds (RWs) that operate at a hydraulic residence time of 3 days at the pilot scale algae field station (AFS). The RWs are located at the City of San Luis Obispo (California) Water Reclamation Facility (SLOWRF) (**Figure 4**). Primary clarifier wastewater effluent was the feedstock for algal growth.

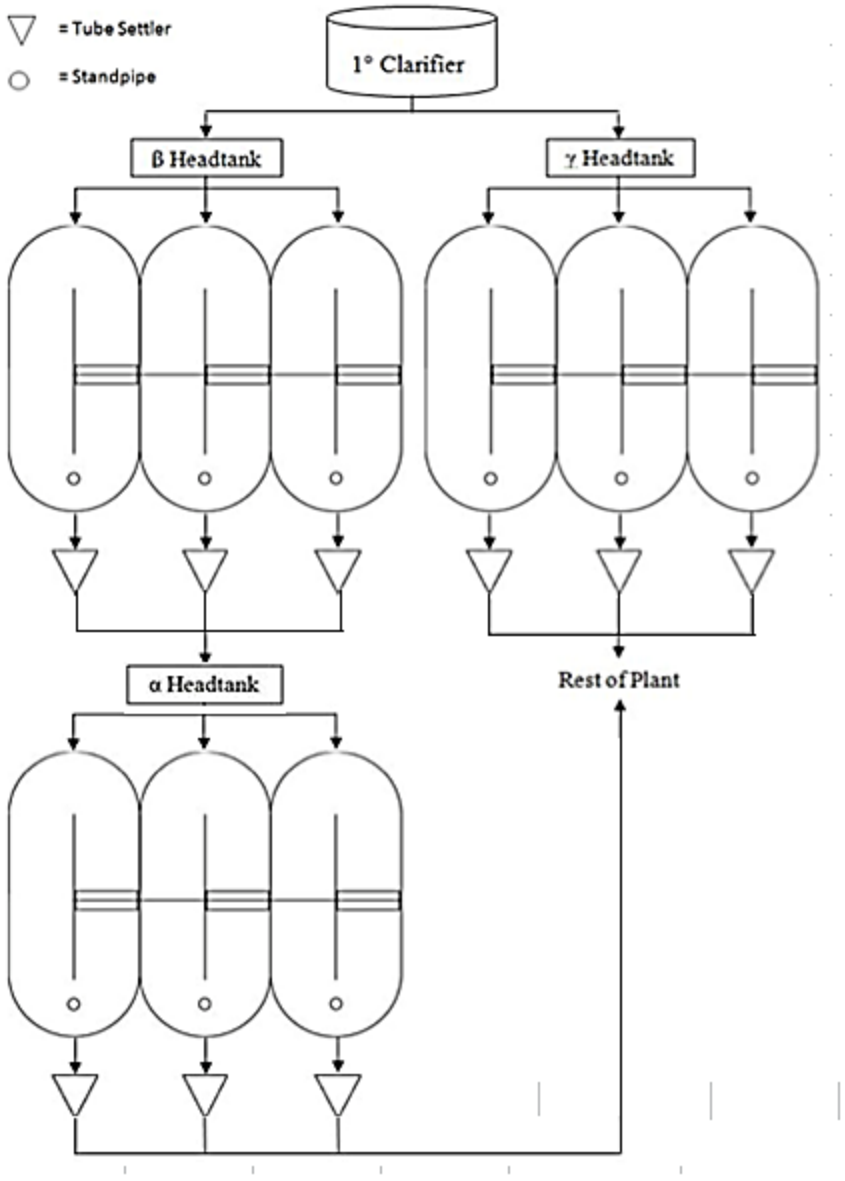


**Figure 4.** Overview of the AFS RW ponds. The Beta pond set received primary clarifier effluent, and the pond effluent was passed through a tube settler before being fed into a head tank which distributed the water into the Alpha pond set (image: (Ripley, 2013)). Alpha and Beta both had a hydraulic residence time of 3 days. The Gamma pond set was not used in the present research. Alpha and Gamma tube settler effluent was returned to the main SLOWRF wastewater flow ("Rest of Plant").

The algal populations were always a diverse mix of genera. Microscopy was regularly performed on the raceway samples in an effort to record the ecological changes in microalgal populations (**Figure 5** and **Figure 6**).

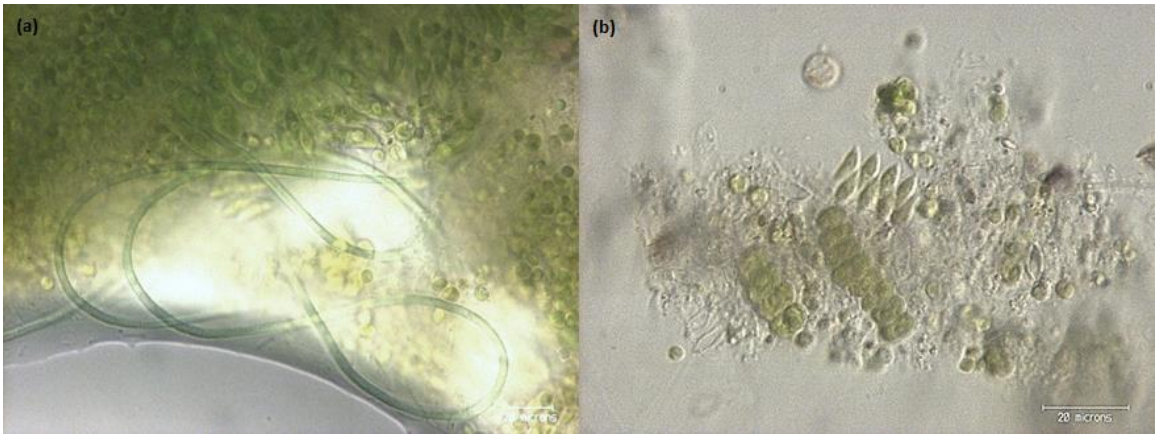


**Figure 5.** Example of micrographs taken of the mixed culture (June 9, 2013 at a magnification of 1000X). (a) *Oscillatora* sp. can be identified as the rope-like structure (b) Algal colony containing multiple genera including *Scenedesmus* and *Chlorella*. The first sonication experiment used algae that were collected on June 5, 2013.

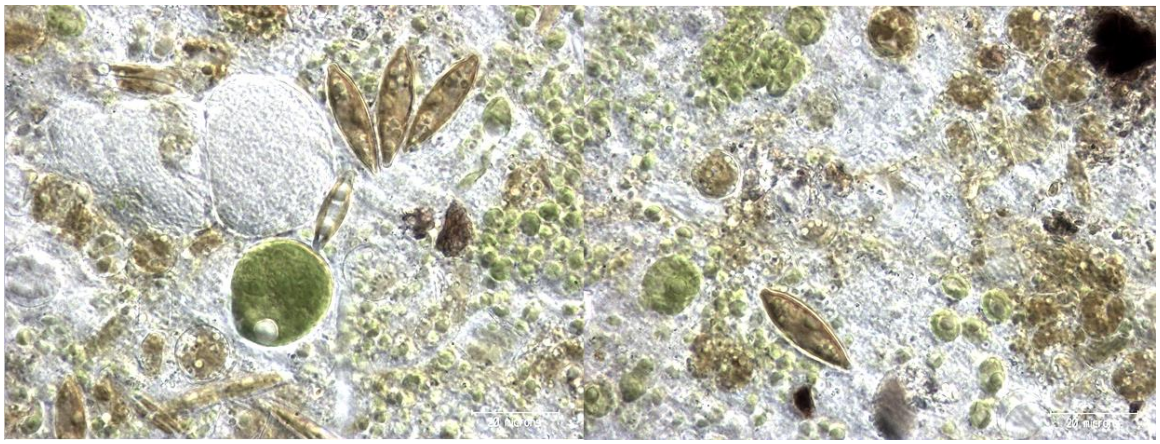

**Figure 6.** Stitched micrographs of Alpha pond water on January 15, 2014 at a magnification of 1000X. The level of biodiversity is clearly seen by the large number of species. The boiling experiment used algae that were collected on January 23, 2014.

Gravity separation of the algae was achieved with the use of a single tube settler (**Figure 7**) for each pond. No chemical flocculants were added. The entire separation process occurred naturally, and an explanation of bioflocculation can be found in Ripley (2013).

**Figure 7.** Each pond was connected to a 123 L tube settler packed with nine 3" PVC tubes. The apparatus is positioned at a 60° angle of repose. The influent lines can be seen entering the vessel approximately 1/3 from the bottom. Image source: (Ripley, 2013).

Algae biomass was collected from the bottom draw-off valves of the tube settlers and stored in 1000-mL screw top HDPE bottles (Nalgene). The collected biomass was transported to the laboratory within 20 minutes of collection and placed in a refrigerator at 3.5 ºC to minimize degradation. Typical tube settler algal sludge concentrations ranged from 15-30 g/L.

### *3.1.2 Collection and Storage of Digester Inoculum*

Municipal sludge digester effluent was used as seed in the batch digestion experiments. The digester effluent came from the SLOWRF (design flow 5.2 MGD). The facility operates a series of three anaerobic digesters at a temperature of 32°C, and a total hydraulic residence time of 60 days. Effluent from Digester 3 was collected from a draw off valve, and stored in 1000-mL screw top HDPE bottles (Nalgene), while they were transported to the University laboratory. The period between collection and storage was

roughly 20 minutes. The inoculum was kept anaerobic by tightly sealing the container, which was stored in an incubator at 20°C until the digesters were assembled.

The following subsections describe the algae biomass pretreatment methods, which were the main variable in the experiments conducted for the present thesis.

### *3.1.3 Sonication*

Sonication was conducted on volumes of approximately 45 mL of harvested algae slurry with an approximate total solids concentration of 30 g/L for the first experiment and roughly 75 g/L for the second sonication experiment. For the first experiment, algae were collected from all three alpha tube settlers on June 5, 2013. For the second experiment, algae-laden water was pumped directly from Alpha pond 3 raceway (**Figure 4**) and thickened using a continuous centrifuge (US Centrifuge Model M212) on July 9, 2013. For both experiments, the algae slurry was placed into individual 50-mL Falcon centrifuge tubes and run in small batches to increase the surface area contact of the sonifier tip and the sample volume. The analog Branson Sonifier 250 (Danbury, Connecticut) was run at an output of 8 using the ½" tapped horn.

A dual thermometer (Fisher Scientific, #4137) with a thermistor attachment was used to monitor the temperature rise in the algae slurry throughout sonication. A plot of temperature rise against sonication duration was generated and can be found in **Appendix A.** The algae slurry consistently reached a final temperature of 100°C after 10 minutes of treatment. After the sonication run was complete, the centrifuge tubes were capped and placed in the freezer for a period of 5 minutes before being transferred to the refrigerator at 3.5ºC. This was intended to cease any additional cell lysis induced by the residual heat,

and maintain consistency among the samples that were run in the beginning and end of the treatment. Once all of the sonication runs were complete, the treated algae slurry was blended in a 4-L graduated cylinder and the mixture was diluted to the target percent solids based on the %TS value taken before treatment began. A previous experiment showed that the %TS did not change throughout the treatment process.

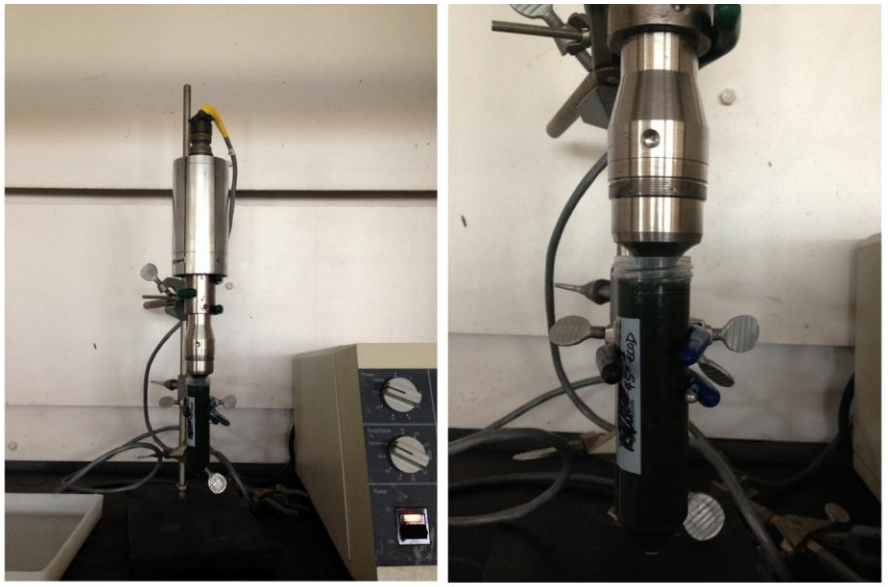

**Figure 8.** An analog Branson Sonifier 250 equipped with a 1/2" tapped tip was used to lyse algae slurry at a starting concentration between 3-7.5 % TS.

### *3.1.4 High Pressure Homogenization*

High pressure homogenization was the second pretreatment tested. After the algae slurry was harvested from all three Alpha set tube settlers on September 16, 2013, all of the material was sieved using a metal screen with 1-mm openings to remove particulate debris that was shown to clog the nominal 100-µm ceramic interaction chamber in a preliminary run. The debris that caused equipment failure can be seen in **Figure 9**.

After the algae slurry was screened, it was split into two fractions; one that was to become the untreated mixture and the other that was to become the treated. The algae were treated using a Microfluidics pneumatic M-110L cell homogenizer (Newton, Mass.) equipped with a 400-mL glass feed hopper (**Figures 10 and 11**). A maximum of 3% total solids algae slurry was forced through the 100-µm interaction chamber in a single pass. Air was used as the pressurized gas achieving a maximum pressure of 20,000 psi and a maximum liquid flow rate of 400 mL/min. The maximum temperature rise during the treatment was 40°C

**Figure 9.** A metal 1-mm screen was used to remove particulates that were clogging the 100-µm Microfluidics interaction chamber. Objects that were removed mostly consisted of ostracods and bloodworms, as seen in the image on the right. Each square in the grid is ~1 mm in size.

**Figure 10.** Front view of the Microfluidics pneumatic M-110L cell homogenizer. The unit is pneumatically powered and has a large piston that compressed air up to 20,000 psi. Untreated samples were loaded into the glass hopper on the left, and treated samples exited the downspout in the front, and were collected in a beaker.

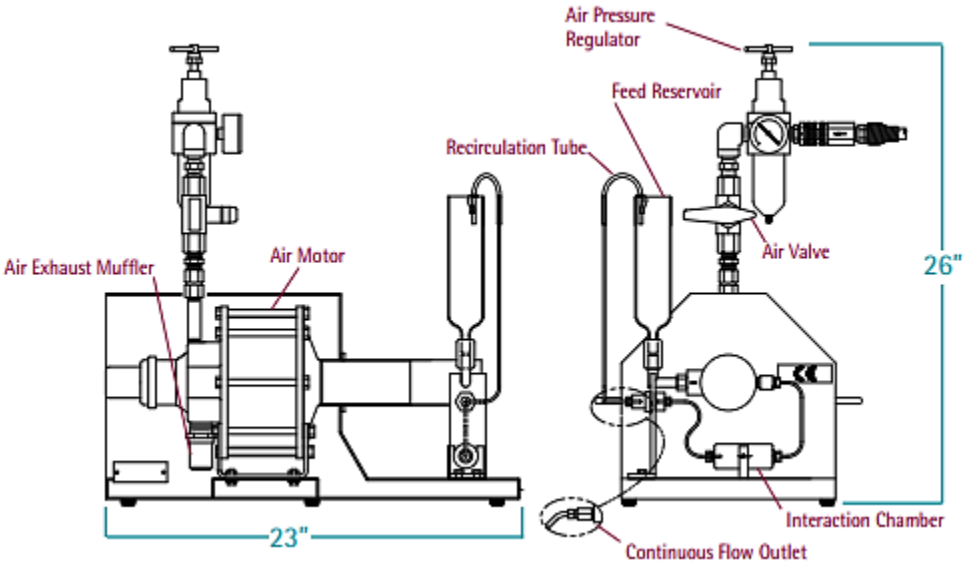


**Figure 11.** Schematic of the Microfluidics M-110L. Image source: (Microfluidics, 2008)

### *3.1.5 Autoclaving*

Algae slurry was harvested from the Alpha tube settlers on November 14, 2013 and split into two process streams: untreated and treated. The untreated algae slurry was stored in the refrigerator at 3.5ºC while the remainder of the algae biomass was treated. Two 4-L Erlenmeyer flasks were filled with 1.2 L of biomass at a total solids concentration of 4%. The flasks were covered with aluminum foil and then loaded into a Lancer Medical Services Autoclave (Serial No. 218718) that was run on the liquid sterilization program which consists of a cycle temperature of 121°C and a gage pressure of 15 psi for a duration of 27 minutes (**Figure 12**). The device took approximately 10 minutes before the desired temperature of 121$^{o}$C was reached.

**Figure 12.** Flask spacing was approximately 8 cm at their bases. Considerably more biomass could be treated using the autoclave.

Once the cycle had completed, the flasks were carefully removed and allowed to cool on the laboratory bench in a container filled with tap water to facilitate faster cooling. Before diluting the samples to the target 10 g/L TS organic load of the digesters, the slurry was well-mixed with a glass stir rod to ensure sample homogenization.

### *3.1.6 Boiling*

Boiling was seen as a way to waste heat that could potentially be available at full-scale algae processing facilities. Algae slurry was harvested on January 23, 2014 from the Alpha tube settlers and split into two process streams: one fated for treatment and the other for an untreated control. A volume of 800 mL of the algae slurry was placed in a capped 1000-mL HDPE Nalgene bottle in the refrigerator at 3.5°C, while a total of 1200 mL of algae was poured into a 4-L Erlenmeyer flask along with a large 5-cm magnetic stir bar. The flask was placed on a hot plate stirrer (Corning PC-351) and heated on high for 1.5 hours (**Figure 13**). The temperature of the mixture was recorded using a dual thermometer (Fisher Scientific #4137) with a thermistor attachment. The mixture reached 100°C after 45 minutes of heating. At this point, an 800-mL aliquot of the sample was removed and set aside to be a sample that was run as a thermal pretreatment designated "just boiled," or "0-BAS" meaning "0 minutes Boiled Algae + Seed." The remainder of the algal slurry continued to receive heating for 30 minutes. This mixture was labeled "30-BAS," or "30 minute Boiled Algae + Seed." The nomenclature can be found in **Table 5**.


**Figure 13.** A 4-L Erlenmeyer flask was filled with 1200 mL of tube settler harvested algae and stirred with 5-cm long magnetic stir bar. A combination hot plate stirrer was used to heat the algae to 100°C and hold it constant for a period of 30 minutes.

## 3.2 Digester Setup

Experimental setup consisted of collecting fresh algae slurry from the Alpha set tube settlers and fresh seed from Digester 3 at the SLOWRF on the day of the experiment. Each experiment involved a mixture of treated algae and seed, untreated algae and seed control, and seed only digesters (**Figure 14**). Digestion was conducted in serum bottles of either 125 mL or 1.2 L working volume depending on the experiment.

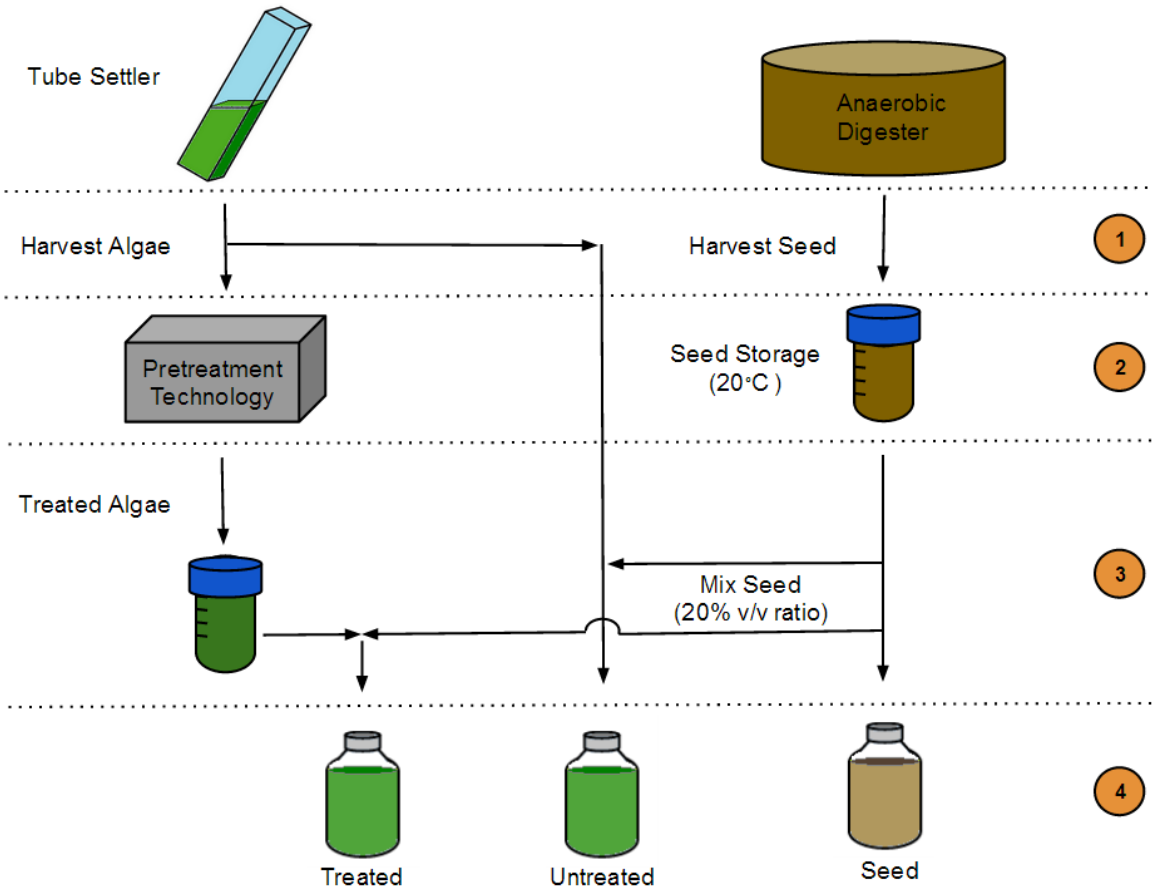


**Figure 14.** Process flow of digester setup. Analytical samples were pulled from each stage of setup (orange markers: 1, 2, 3, and 4) for further analysis detailed in **Table 4**.

After the samples were collected and brought back to the laboratory the tests outlined in **Table 4** were conducted and/or preserved in order to characterize each component. The thickened algae slurry was then subjected to the respective pretreatment technology. Once pretreatment was complete, both the treated and untreated algae slurry were diluted to the proper percent solids using **Equation 1.**

**Equation 1.** Dilution

$$C_1V_1 = C_2V_2$$

C is the solids concentration (g/L), and V is the sample volume (L).

The diluted algae slurry was mixed with 20% v/v Digester 3 seed. The new mixture that contained algae and seed was then subjected to all of the analytical tests listed in **Table 4**. For the standard 160-mL serum bottle digesters, 100 mL of algae slurry and 25 mL of seed was combined to yield a total working volume of 125 mL and a headspace of 35 mL. After the algae/seed mixtures were combined and placed in the glass bottles, the digester headspace was purged with pure nitrogen gas for a period of 30 seconds to create an anaerobic environment. Then the gas was shut off and the digesters were quickly capped with self-healing 20-mm Teflon-faced butyl septa (Sigma Aldrich #27201). All of the digesters were placed in a gravity convection incubator (Precision, Chicago, Ill.) that maintained a constant mesophilic temperature of 35± 2°C for the duration of the experiment. All five experiments were run in batch mode for the duration listed in **Table 3**.

**Table 4.** Analytical tests and the corresponding stage during experimental setup at which point the samples were pulled. The orange markers relate to when the samples were pulled as seen in **Figure 14**.

| Analytical Test | Raw, untreated algae | Digester Seed Only | Treated Algae | Untreated Algae + Seed | Treated Algae + Seed | Required Sample Size | Sample Storage |
|---|---|---|---|---|---|---|---|
| TS/VS | X | X | X | X | X | 15 mL | None |
| pH/Alkalinity | | X | X | X | X | 20 mL | None |
| tCOD | X | X | X | X | X | 10 mL | $H_2SO_4$ |
| sCOD | X | X | X | X | X | 30 mL | G4, $H_2SO_4$ |
| TKN | | X | X | X | X | 25 mL | $H_2SO_4$ |
| TAN | | X | X | X | X | 20 mL | $H_2SO_4$ |
| Total P | | X | X | X | X | 15 mL | Frozen |
| DRP | | X | X | X | X | 10 mL | G4, 0.45 µm, $H_2SO_4$ |
| Total Potassium | | X | | X | X | 100 mL | $HNO_3$ |
| Soluble Potassium | | X | | X | X | 100 mL | G4, 0.45 µm, $HNO_3$ |
| | 1 | 2 | 3 | 4 | | | |

**Figure 15.** Assembled triplicate digesters sitting in the 35±2°C incubator. All of the digesters pictured are 160-mL serum bottles, but in some experiments, custom 2-L digesters with septa were used to have enough digestate for experiments on aerobic degradation Chang (2014) and allelopathy of regrowth Boggess (2014).

### *3.2.1 Experimental Overview and Sample Identification*

Five separate digestion experiments were conducted over the course of this study. Each experiment had its own unique set of sample identification that is detailed in the following table.

**Table 5.** Overview of all five experiments and their respective sample identification. TS in experiment 3 refers to "total solids."

| | Sample | Number of Serum Bottles | Serum bottle Volume (mL) | Algae Volume (mL) | Seed Volume (mL) | Sample ID |
|---|---|---|---|---|---|---|
| Experiment 1 - Sonication | Unsonicated Algae + Seed | 2 | 160 | 100 | 25 | UAS |
| | 1 min Sonicated Algae + Seed | 3 | 160 | 100 | 25 | 1" SAS |
| | 2 min Sonicated Algae + Seed | 3 | 160 | 100 | 25 | 2" SAS |
| | 5 min Sonicated Algae + Seed | 3 | 160 | 100 | 25 | 5" SAS |
| | 10 min Sonicated Algae + Seed | 3 | 160 | 100 | 25 | 10" SAS |
| | 45 min Sonicated Algae + Seed | 3 | 160 | 100 | 25 | 45" SAS |
| | Seed only | 2 | 160 | -- | 125 | Seed |
| Experiment 2 - Sonication | 10 min Sonicated Algae + Seed | 35 | 160 | 100 | 25 | SAS |
| | Unsonicated Algae + Seed | 4 | 2000 | 800 | 200 | UAS |
| | Seed Only | 4 | 2000 | -- | 1000 | Seed |
| Experiment 3 - High Pressured Homogenization | 3% TS Unlysed Algae + Seed | 2<br>4 | 2000<br>160 | 800<br>100 | 200<br>25 | 3% UAS |
| | 3% TS Lysed Algae + Seed | 2<br>4 | 2000<br>160 | 800<br>100 | 200<br>25 | 3% LAS |
| | 2% TS Lysed Algae + Seed | 6 | 160 | 100 | 25 | 2% LAS |
| | 1% TS Lysed Algae + Seed | 2<br>4 | 2000<br>160 | 800<br>100 | 200<br>25 | 1% LAS |
| | Seed Only | 2<br>4 | 2000<br>160 | --<br>-- | 1000<br>25 | Seed |
| Experiment 4 - Autoclaving | Untreated Algae + Seed | 8 | 160 | 100 | 25 | UAS |
| | Autoclaved Algae + Seed | 8 | 160 | 100 | 25 | AAS |
| | Seed Only | 3 | 160 | -- | 125 | Seed |
| Experiment 5 - Boiling | Untreated Algae + Seed | 2<br>6 | 2000<br>160 | 800<br>100 | 200<br>25 | UAS |
| | Heated to Boiling Algae + Seed | 8 | 160 | 100 | 25 | 0 min BAS |
| | Boiled 30 minutes Algae + Seed | 2<br>6 | 2000<br>160 | 800<br>100 | 200<br>25 | 30 min BAS |
| | Seed Only | 4 | 160 | -- | 125 | Seed |

## 3.3 Analytical Procedures

This section highlights the laboratory procedures that were routinely conducted throughout the course of this study.

**Table 6.** Analytical procedures that were performed in order to track digester health, biogas production, and nutrient release. The third party laboratory used for the potassium testing was the U.C. Davis Analytical Laboratory.

| Analytical Test | Required Sample Size | Materials and Analysis of Methods |
|---|---|---|
| **Total Solids/Volatile Solids** | 15 mL | Modified gravimetric method APHA 2540B. and 2540E. |
| **Biogas Volume and Composition** | 1 mL | Inverted cylinder water displacement. GC-TCD by ARI (Torrance California) |
| **pH/Alkalinity** | 20 mL | Oakton pH electrode. Manual Acid titration (APHA 2320B) |
| **Total COD (tCOD)** | 10 mL | CHEMetrics 0-1500 ppm USEPA Approved Vials, two hour digestion at 150°C (CHEMetrics method; APHA 5220D) |
| **Soluble COD (sCOD)** | 30 mL | Vacuum filtered through G4 filter (1.2 µm). Digestion same as total COD |
| **Total Kjeldahl Nitrogen (TKN)** | 25 mL | Labconco 18 burner Kjeldahl apparatus. (Macro-Kjeldahl, APHA 4500-$N_{org}$ B) |
| **Total Ammonia Nitrogen (TAN)** | 20 mL | Orion 9512 Ammonia Selective Electrode, (APHA 4500-$NH_3$ D) |
| **Total Phosphorus (TP)** | 15 mL | Sulfuric Acid-Nitric Acid Digestion (APHA 4500-P B.) followed by Vanadomolybdophosphoric Acid Colorimetric Method (APHA 4500-P C.) |
| **Dissolved Reactive Phosphorus (DRP)** | 10 mL | Vacuum filtered through 0.45 µm nitrocellulose filter. Ascorbic acid method (APHA 4500-P E) |
| **Total Potassium** | 100 mL | Analyzed by third party laboratory |
| **Soluble Potassium** | 100 mL | Analyzed by third party laboratory |

### *3.3.1 Overview of Sample Day Breakdown*

In order to gain insight into how the different nutrient fractions were changing throughout the course of digestion, some serum bottles were sacrificed for the various tests depicted in **Figure 16**.

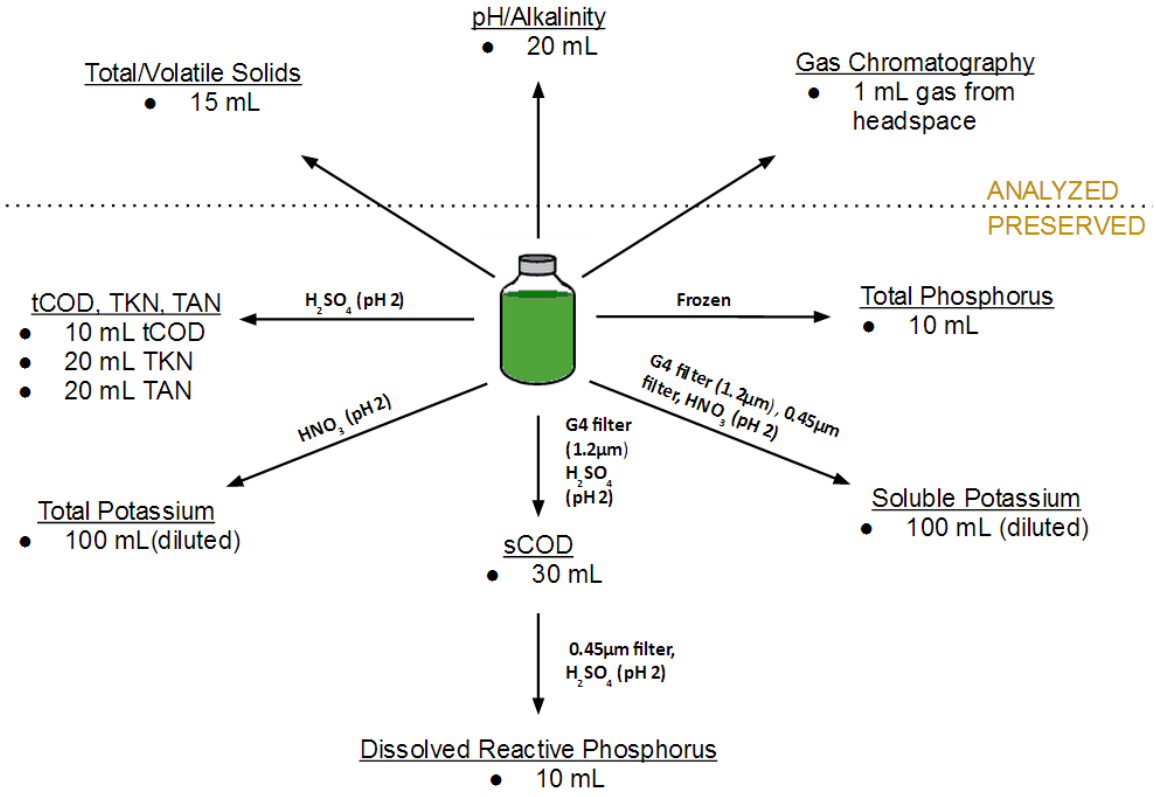


**Figure 16.** The bottle in the center represents a digester that was sacrificed on a sample breakdown day. The total 125 mL volume was used to fulfill sampling requirements. All of the analytical tests pictured were run on the initial and final days of the experiment, while only select tests were run on intermediate-day samples. Tests above the dotted line were analyzed on the day of the breakdown, and those that fall below the line were preserved as indicated, and ran at a later date.

Triplicate measurements were not logistically realistic for all of the nutrient tests; instead an alternating duplicate sampling acted as an ongoing quality control (QC) step. For example, on Day 2 of digestion, two serum bottles of the untreated control would be opened in addition to a single bottle of the other mixtures. All of the tests in **Figure 16** would be conducted on each sample bottle. On the following breakdown day, two serum bottles of the treated mixture would be opened and subjected to all of the analytical tests along with the other sample mixtures. This strategy ensured consistent behavior among

pretreatment mixtures as well as consistency of laboratory procedures that were conducted on experimental duplicates.

### *3.3.2 Solids Concentration*

All solids samples were measured as a mass per volume basis and always run in triplicate. The reported values are the average of the triplicate set and were never more than 10% different from each other. In sampling, 3 mL of well-mixed sample was collected using a 3-mL syringe and expelled into an aluminum fluted weighing dish (Fisherbrand, No. 08-732-100). Total solids were measured using a modified version of the Standard Method 2540B (APHA 2005). Total solids were determined by drying the sample in an oven at 105°C, and volatile solids were determined by ashing for 15 minutes at 550°C, according to the Standard Method 2540E (APHA 2005).

### *3.3.3 Biogas Volume and Composition*

The digesters were removed from the incubator and shaken before they were allowed to equilibrate and cool to room temperature. Each digester's biogas volume was measured by using an inverted graduated cylinder placed inside a larger graduated cylinder filled with tap water. The temperature of the gas was measured using a Fisher Scientific thermistor attached to the interior graduated cylinder. With this method, it was confirmed that the biogas temperature came to equilibrium with the room temperature after sitting on the bench top for 10 minutes. This equilibration period was assumed to be consistent, considering that both the incubator and laboratory temperature remained constant. For every biogas measurement of every experiment, the 10-minute equilibration period was consistently used. The interior cylinder was attached to a 1/4" clear vinyl tubing adapted to a Cole Palmer luer lock and a 22G x 1 in hypodermic needle (Exel International, Los

Angeles, Calif.). The needle was used to pierce the self-healing 20-mm Teflon-faced butyl septa (Sigma Aldrich #27201), and the gas volume was recorded once the gas volume increase was less than a 1 mL in 30 seconds.

Biogas composition was determined using a gas chromatograph (GC) (SRI 8610, Torrance, Calif.) equipped with a thermal conductivity detector and a six-foot concentric packed column that contained an inner and outer column (Alltech CTR I, Deerfield Ill.). Ultra high-purity argon was used as the carrier gas with a flow rate of 0.91 mL/min and a running temperature of 45°C. Before conducting GC on samples, the instrument was powered up, permitted to reach 45°C and purged for ten minutes using pure argon. Then, a 1-mL calibration sample of air was injected, which was expected to yield percentages of roughly 78% nitrogen gas and 21% oxygen. If the air sample did not return accurate readouts, a second air sample was injected. At no point was the second air sample out of range. Digester gas sampling involved injecting a 1-mL sample and allowing the instrument to run for 22 minutes. In the event that the cumulative percent total of biogas sample was greater than 110%, a two-point calibration curve was created using two 80:20 mixtures of $CO_2$ and $CH_4$. A tedlar bag was filled with 80 mL of $CO_2$ and 20 mL of $CH_4$, and a 1 mL sample of that mixture was injected into the GC. Next, the opposite mixture was made (20 mL of $CO_2$ and 80 mL of $CH_4$) and that sample was injected. Finally, the digester gas compositions were corrected using the correctly calibrated values.

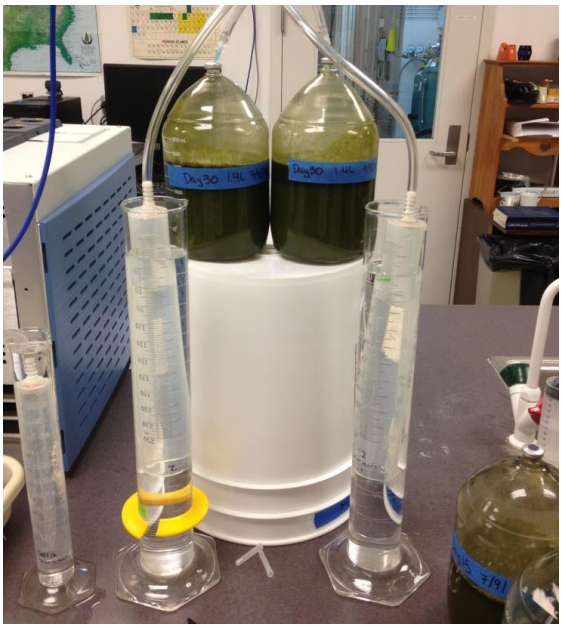

**Figure 17.** Setup for measuring biogas yields of the 2-L digesters. Graduated cylinders (250 mL) were adapted with silicone and ¼ inch barbed fittings and inverted in 1000-mL graduated cylinders to measure the volumetric gas production.

Methane is the main energy source of anaerobic digestion, so biogas composition was routinely analyzed. The measured methane percentage from the GC was recorded on the day of analysis and multiplied by the biogas volume to determine the daily methane volume. The cumulative sum of the methane volume for each mixture was divided by the respective initial volatile solids concentration to get the final specific methane yield (**Equation 2.**)

**Equation 2.** Specific Methane Yield

$$Specific\ Methane\ Yield\ \left(\frac{L\ CH_4}{gVS_{IN}}\right) = \frac{Cumulative\ methane\ [\frac{(L\ CH_4)}{L\ Digester\ volume}]}{Initial\ Volatile\ Solids\ \left(\frac{g}{L}\right)}$$

### *3.3.4 Chemical Oxygen Demand (COD) Determination*

COD was regularly performed in order to quantify the degree of cell disruption, as well as the level of oxidizable substrate available for the anaerobic bacteria. Both total and soluble COD were tested throughout the course of all five experiments. CHEMetrics 0-

1500-ppm vials were used per the APHA 5220 D methods. A five-point calibration curve was created by diluting 6,000 mg/L potassium hydrogen phthalate stock solution to make 180, 360, 540, and 720 mg/L standards. A blank was also included in each batch. Samples for total COD were typically diluted 1:100, while samples for soluble COD were diluted between 8:100-20:100. The setup that was used to filter soluble samples can be seen in **Figure 18**.

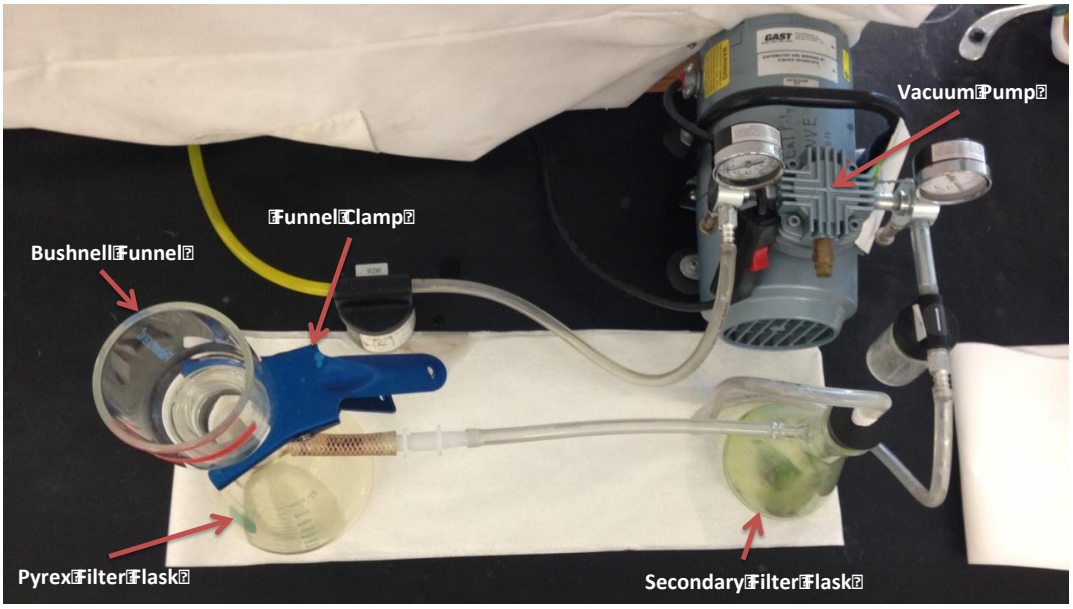


**Figure 18.** Overhead view of the filtering apparatus that was used to prepare soluble COD samples and dissolved reactive phosphorus samples. All sCOD samples were centrifuged and filtered through a 1.2-µm pore-size glass fiber filter (Fisher G4).

In addition to running the required samples, two splits and two spikes were run in order to ensure adequate QC. Splits within ± 10% of the original sample concentration, and spikes within ± 15% were acceptable values. Spikes were calculated using **Equation 3**.

**Equation 3.** Spike Calculation

$$CV_{\text{sample}} + \text{CV}_{\text{standard}} = \text{CV}_{\text{spike}}$$

Once the diluted samples were pipetted into their respective CHEMetrics vials, they were loaded into a heating block and digested for a period of 2 hours at 150ºC. After cooling, the vials were loaded into a Hach DR 890 colorimeter, and the percent transmittance was recorded.

### *3.3.5 pH/Alkalinity Determination*

To monitor the health of the digesters, pH and alkalinity were tested each time a digester was sacrificed for sampling: 15 mL of raw digester sludge was measured in a graduated cylinder and placed into a 25-mL beaker with a stir bar. That beaker was placed on a magnetic stir plate while the pH of this mixture was measured using a pH/ion analyzer (Corning Model 355). After the value was recorded, alkalinity as $CaCO_3$ was measured per the acid titration method (APHA 4230D). $H_2SO_4$ (0.2 N) was used to titrate the sample to pH 4.5. Thorough mixing during the acid addition proved to be a critical step in obtaining accurate values because samples with high alkalinity would foam excessively and prevent the subsequent acid additions from intermixing with the rest of the sample. A vigorous stir bar speed and supplementary mixing using the pH probe appeared to avoid error in the titration volume due to inadequate mixing.

### *3.3.6 Nitrogen Determination*

#### *3.3.6.1 Total Ammonia Nitrogen (TAN)*

Samples for total ammonia nitrogen determination were acidified to pH 2 and stored in the refrigerator at 3.5ºC. On the day of analysis, samples were removed from the refrigerator and set on the bench to allow for the samples to come to room temperature. While the sample was equilibrating, a five-point calibration curve was created for

concentrations of 1, 10, 100, 1000, and 2500 mg/L-N. The stock standard that was used was 2500 ppm as $NH_3$. R-squared values were typically 0.98-1.0, and a split and spike were run to ensure passing QC. Splits within ± 10% of the original sample concentration, and spikes within ± 15% were acceptable values. Spike calculations were conducted using **Equation 3**. Samples that were tested were diluted up to 1:100 for the concentrated samples (digester seed), but generally 4:25 dilution was conducted, consisting of 4 mL of sample diluted into a 25-mL volumetric flask using de-ionized water to reach the fill line. The diluted sample was poured into a 25-mL beaker with a stir bar, and place on a magnetic stir plate. Concentrated alkaline reagent (Orion 951011) was used to adjust the pH above 11, converting all ammonia species to $NH_3$. Next, an ammonia selective electrode (Orion 9512) was used to measure ammonia concentration of the sample according to APHA 4500-$NH_3$ D.

#### *3.3.6.2 Total Kjeldahl Nitrogen (TKN)*

During digester breakdown days, 10 mL of raw digestate was acidified to pH 2 and stored in a 50-mL centrifuge Falcon tube in a refrigerator at 3.5°C. TKN runs always consisted of the following QC solutions in addition to the samples: a 20-mg/L and a 50-mg/L as N standard, a blank (DI water), a split, and a spike. The split and spike had to be within ±10% and ±15%, respectively, for the analytical batch to be accepted. The spike was calculated using **Equation 3**.

On testing day, the acidified sample was well mixed before 1 mL of sample was pulled using a 1-mL volumetric syringe. This aliquot was then diluted with 299 mL of DI water and analyzed in a modified version of the Macro Kjeldahl analysis outlined in APHA 4500-$N_{org}$ B. The equipment used for the digestion was a Labconco 18-burner Kjeldahl

apparatus (Cat. No. 2117803). Manual titration of the distillate was conducted using 0.02 N $H_2SO_4$ to turn the color of the solution back to the original purple color of the boric acid mixed indicator solution.

**Figure 19.** The distillation step of TKN analyses. Distillate (250 mL) was collected in Erlenmeyer flasks and manually titrated using 0.02 N $H_2SO_4$.

**Equation 4.** TKN Determination

$$TKN\ \left(\frac{mg}{L}\right) = \ [vol.of\ titrant\ (mL) - vol.of\ titrant\ used\ for\ blank\ (mL)]$$

$$* \left(\frac{1L}{1000mL}\right) * (Normality\ of\ titrant(N)) * \left(\frac{molNH^3}{molH^+}\right) * \left(\frac{17gNH^3}{molNH^3}\right)$$

$$* \left(\frac{14gN}{17gNH^3}\right) * \frac{\left(\frac{1000mg}{g}\right)}{\left[vol\ sample\ (mL) * \left(\frac{1L}{1000mL}\right)\right]}$$

### *3.3.7 Phosphorus Determination*

Phosphorus was determined as dissolved reactive phosphorus and as total phosphorus. The difference was particulate phosphorus, which could be in particles such as biomass and precipitates.

#### *3.3.7.1 Dissolved Reactive Phosphorus*

Samples slated for dissolved reactive phosphorus (DRP) analysis were first centrifuged to separate the solid and liquid constituents: 30 mL of each sample was placed in a 50-mL Falcon tube and centrifuged at 20°C at 11,000 RPM for three minutes (Sorvall Legend XTR). The supernatant was then filtered using an acid-washed glass filtration setup (**Figure 18**)**.** Each sample was first passed through a Fisher Scientific G4 filter (1.2-µm nominal pore size), followed by filtration through a Fisher Scientific 0.45-µm nitrocellulose filter. The collected filtrate usually amounted to approximately 10 mL, which was acidified with high purity, concentrated $H_2SO_4$ and stored at 3.5°C in acid-washed glass vials. The samples were stored for up to one week before being analyzed using a modified version of the ascorbic acid method (APHA 4500-P E).

A five-point calibration curve was created using a DI blank and 326.1-ppm as P stock solution diluted to 0.25, 0.5, 0.75 and 1.25 mg/L. Digester samples were diluted between 1:100-2:100 using a calibrated micropipette to pull the concentrated sample and dilute it using deionized water, in an acid-washed 50-mL volumetric flask. All samples, including standards, were mixed with digestion reagent and allowed to react for 15 minutes at ambient temperature before absorbance was recorded at 880 nm using a spectrophotometer (Shimadzu UV-1700 PharmaSpec UV-VIS Model #36853). A split and a spike were analyzed, with passing recoveries set within ± 10% and ± 15%, respectively.

#### *3.3.7.2 Total Phosphorus*

During sample breakdown days, 10 mL of raw digestate was frozen in an acid-washed Pyrex vial and sealed with a screw-cap fitted with a Teflon insert. On testing day, the

samples were slowly thawed in a water bath. Freezing caused significant flocculation of the algae, so the samples were homogenized using a touch mixer (Fischer Scientific #12-811-10). A 1-mL aliquot was quickly pulled from the re-suspended sample and diluted in a 25-mL volumetric flask. This sample was then run using the sulfuric-nitric acid digestion (APHA 4500-P B), followed by the vanadomolybdophosphoric acid colorimetric finish (APHA 4500-P C). Sample absorbance was read using the same spectrophotometer used in DRP analysis. Splits within ± 10% of the original sample concentration, and spikes within ± 15% were acceptable QC sample values.

### *3.3.8 Potassium Determination*

Both total and soluble potassium samples were measured for the boiling experiment. Sample preparation for the soluble fraction mimicked DRP sample preparation. The only variance was that the sample was stored in a non acid-washed 50-mL centrifuge Falcon tube and acidified to pH 2 using concentrated nitric acid. Because the filtering process reduces the sample volume, most of the soluble samples had to be diluted 10:50 to meet the minimum required volume. Additionally, total potassium was pulled directly from the sacrificed serum bottle, placed in a 50-mL Falcon tube and acidified to pH 2 using nitric acid. Samples were sent to UC Davis via 2-day ground UPS shipping where the Analytical Laboratory in the College of Agricultural and Environmental Sciences analyzed them. Potassium was analyzed using a nitric acid/hydrogen peroxide microwave digestion followed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The method has a range of measurement between 0.1 ppm to 100 ppm, and generally has a maximum split difference of 8% between sample duplicates.

# 4 Results and Discussion

## 4.1 Effect of Pretreatment on Methane Yield and Nutrient Solubilization

Four pretreatments meant to disrupt algae cell walls, exposing their contents to biodegradation, were evaluated. An overview of each technology is listed in **Table 7**.

**Table 7.** Summary of pretreatment methods and means of cell disruption

| Method of Disruption | Sonication | High pressure Homogenization | Autoclaving | Boiling |
|---|---|---|---|---|
| **Means of disruption** | Cavitation and temperature rise | Liquid shear and pressure drop | Temperature and pressure rise | Temperature rise |
| **Duration of treatment** | 1-45 min | 7 min | 27 min | 30 min |
| **Max temperature rise** | 100°C | 40°C | 121°C | 100°C |

The temperature rise of the sonicated algae slurry can be seen in **Appendix A**. Pretreatment had visible effects on the biomass. The filtrate of untreated samples was nearly clear, whereas filtrate of treated samples was highly colored (**Figure 20**, **Figure 28**). This trend was conserved throughout all pretreatments and is discussed in further detail in the section *Neutral to Negative Effects of High Heat on Biodegradability*.

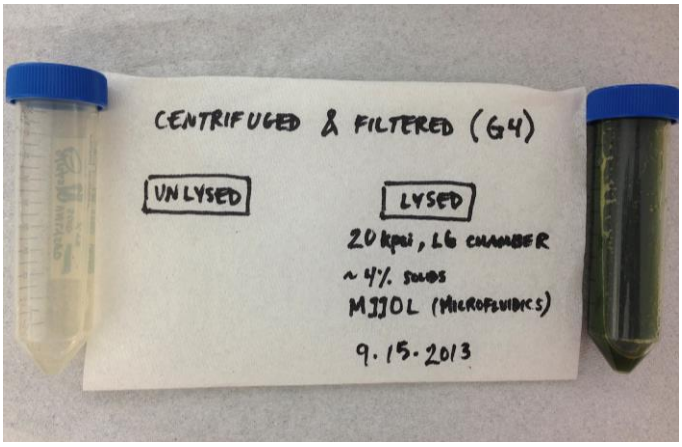


**Figure 20.** A comparison of the soluble COD sample for the Microfluidics unlysed and the lysed algae after a single pass through 100-µm interaction chamber at 20,000 psi. Both samples have been passed through a G4 filter (1.2µm pore size). The color difference is hypothesized to be chlorophyll release due to cell lysis.

### *4.1.1 Degree of Cell Disruption*

The two main metrics used to quantitatively and qualitatively measure cell disruption, without resorting to time-consuming methane potential testing, were COD solubilization and microscopy.

For the first experiment, algae slurry was sonicated between 0 and 45 minutes, which correlated to a maximum temperature rise of 100°C, and immediate sCOD release was found to increase with the duration of sonication (**Figure 21.** ).

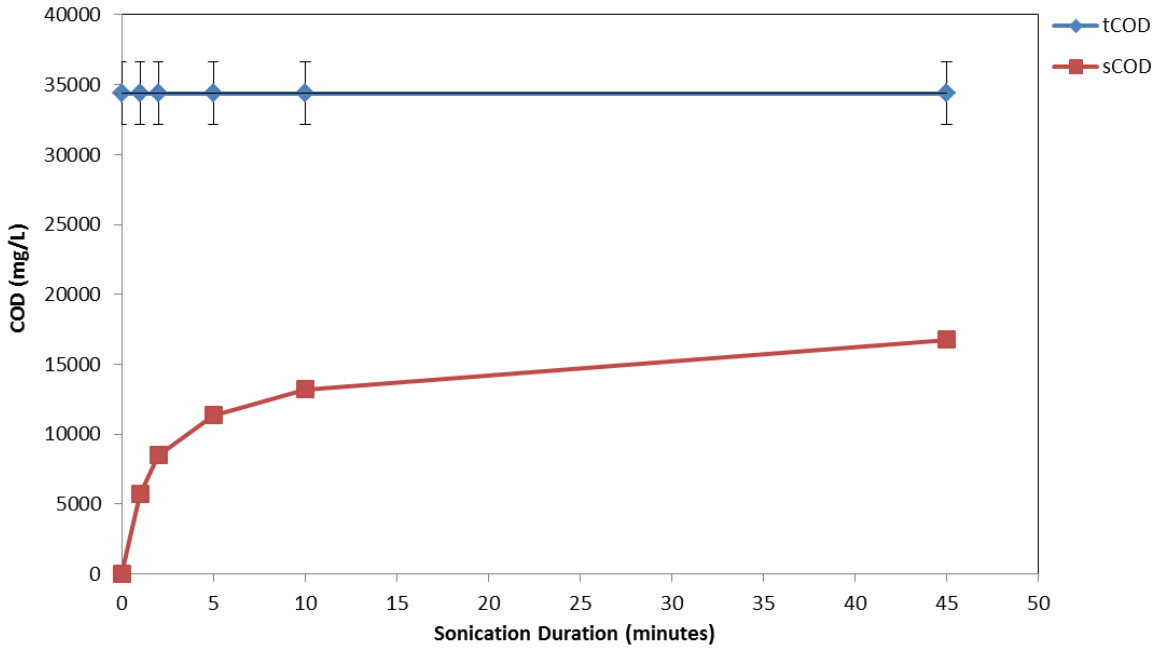


**Figure 21.** COD solubilization increased with increasing sonication duration. As indicated, sCOD release occurred most rapidly within the first minute of sonication. sCOD for the unsonicated sample (at the graph origin) was not detectible or a 99.37% transmittance during COD analysis. All samples were sonicated at a total solids concentration of 24.4 g/L. The error bars on the tCOD values represent the standard deviation from the average of all six samples, while sCOD points are from single values.

The pattern of rapid sCOD release in the first minutes of both sonication trials was constant, however the rate of sCOD release was not tested for the other pretreatments. Instead, the ultimate COD solubilization extent was recorded for all four pretreatments. 45 minutes of sonication proved to be the most effective pretreatment technology for releasing sCOD (**Table 8**).

**Table 8.** Comparison of the degree of cell disruption by various pretreatments, based on soluble COD release. All untreated samples had a sCOD/tCOD ratio of 3-5% (not shown).

| Sample | sCOD (mg/L) | tCOD (mg/L) | % of total (sCOD/tCOD) | TSS at time of disruption (g/L) |
|---|---|---|---|---|
| 45 Min Sonicated Algae | 16754 | 34375 | 48.7% | 24.4 |
| 10 Min Sonicated Algae | 13213 | 34375 | 38.4% | 24.4 |
| High Pressure Lysed Algae | 11297 | 39301 | 28.7% | 28.9 |
| Autoclaved Algae | 12368 | 57630 | 21.5% | 41.1 |
| 30 min Boiled Algae | 8390 | 39171 | 21.4% | 29.7 |

At a microscopic level, cell breakage was observed in samples treated by sonication and homogenization. However, cellular debris could be found in untreated samples, as well as intact cells in extensively treated samples. Microscopy was found to be an ineffective way of identifying the degree of cell disruption (**Figure 22** and **Figure 23**), and. the heat-treated samples (autoclave and boiling) were not observed under the microscope.

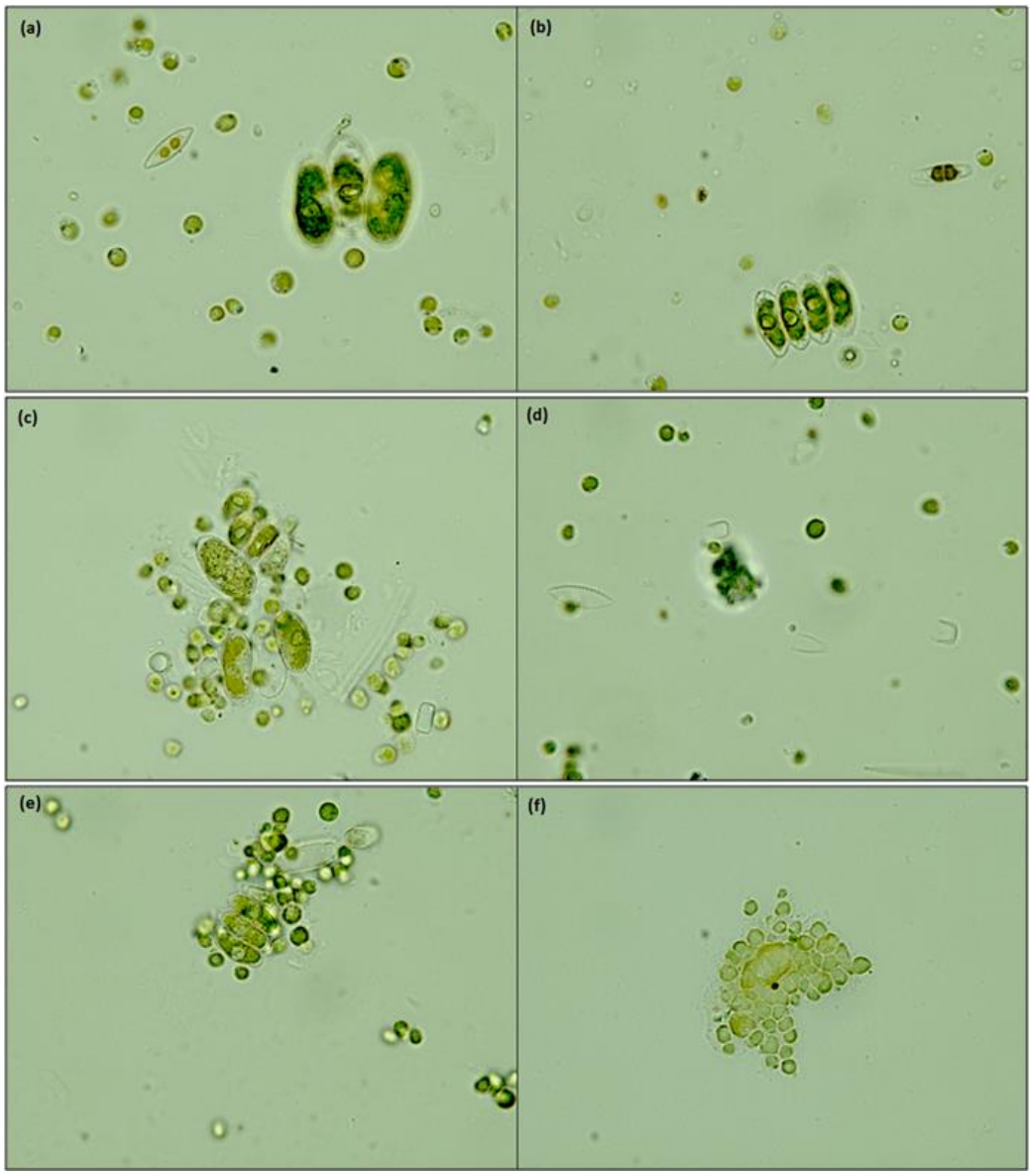


**Figure 22.** Micrographs taken on June 5, 2013 at 1000X magnification of sonicated algae. (a) unsonicated, (b) 1 minute sonicated, (c) 2 minutes sonicated, (d) 5 minutes sonicated, (e) 10 minutes sonicated, (f) 45 minutes sonicated. Cellular debris can be seen in (c), (d), and (e) however, intact whole cells are still seen after 45 minutes of sonication.

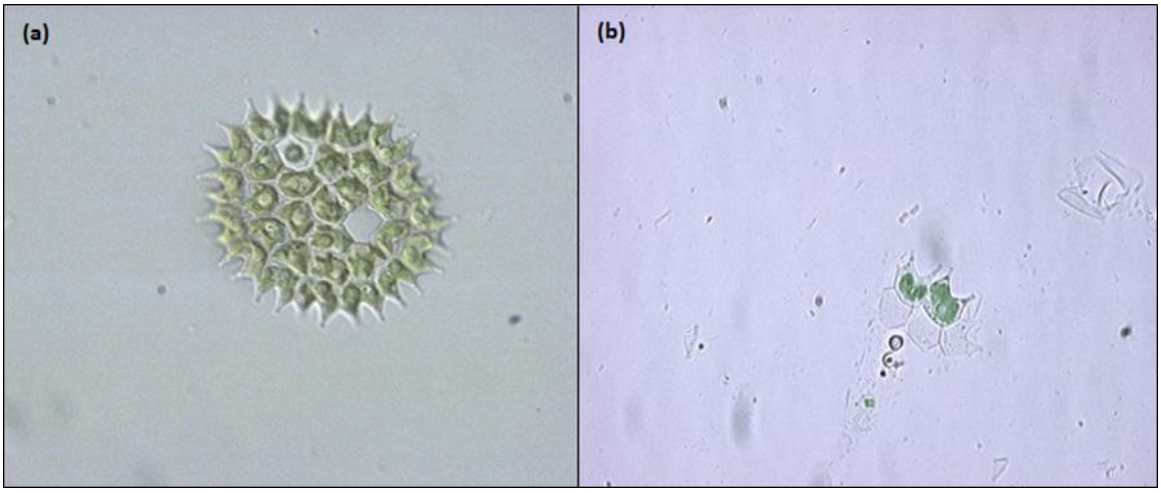


**Figure 23.** (a) untreated *Pediastrum* sp. (b) fragmented cellular debris after a single pass through Microfluidics high pressure homogenizer (M-110L, 100 µm nozzle at 20,000 psi). The algal cell appears to be *Pediastrum* sp. Both images captured at 1000X magnification on September 16, 2013.

### *4.1.2 Specific Methane Yield*

Surprisingly, a greater COD solubilization did not equate to a greater methane yield. For example, the algae that had been pretreated using the autoclave had an increase in COD solubilization of 15% over the untreated mixture. Interestingly, the specific methane yields were 200 mL $CH_4$/g $VS_{IN}$ for the autoclaved mixture and 228 mL $CH_4$/g $VS_{IN}$ for the untreated control (**Table 9**). This result was seen in other similar studies, noting "there is a lack of correlation between the solubilization degree and the methane enhancement potential," (Alzate et al., 2012). A third research group supports the notion that sCOD increase isn't proportional to increased gas production (Cho et al., 2013).

To gain insight into the relationship between sCOD consumption and methane production, both variables were plotted for the second sonication experiment. The analysis was performed solely on this experiment because of the availability of extensive sCOD data. The resulting plot can be seen in **Figure 24**.

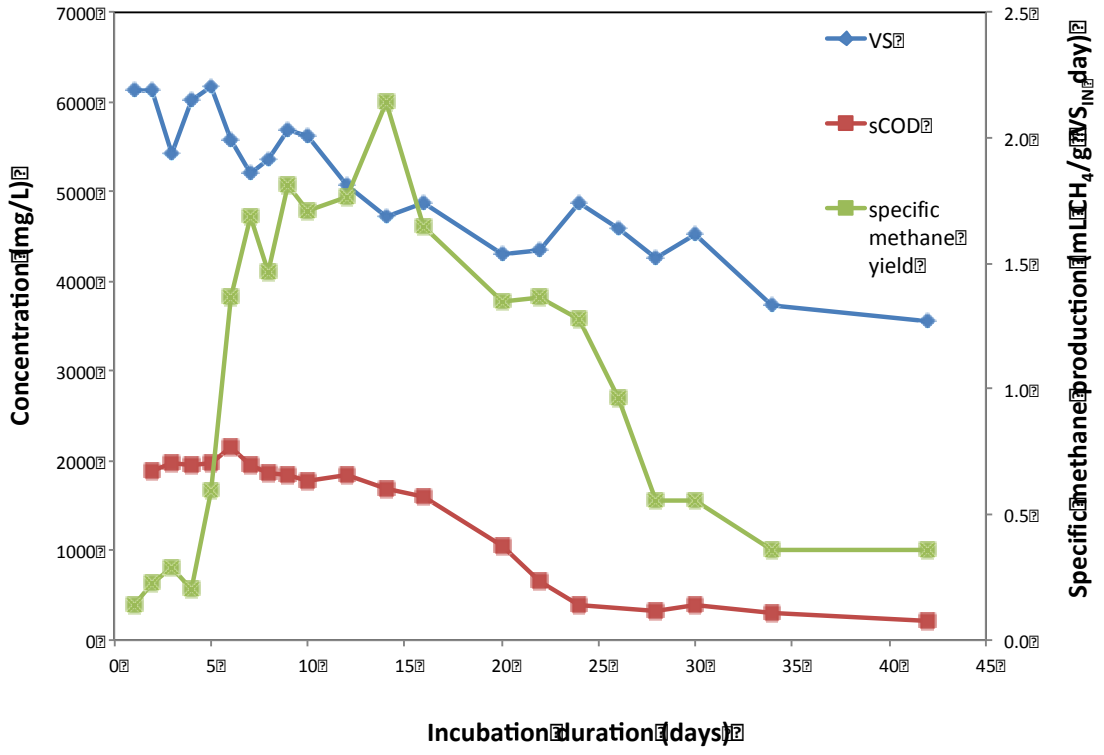


**Figure 24.** This graph details the degradation rates versus the specific methane yield of the 10-minute sonication experiment throughout the course of the 42-day batch digestion. Strangely, within the first 12 days sCOD remained relatively unchanged, while methane production increased rapidly. However, on day 14 that trend shifts and marks the period where the substrate (sCOD) begins decreasing and limiting the methane production.

One of the major objectives of this study was to determine the effect different pretreatment methods have on the specific methane yields. GC was regularly performed on the digesters during each experiment except for experiment 3 (high pressure homogenization), which was designed to test the effect of digester organic load on nutrient solubilization rates. Due to time constraints for sample analysis, GC was not run as frequently as the other experiments, so specific methane yield values were extrapolated from data collected from the other experiments. To determine the specific methane yield for homogenization digesters, the average "% of total biogas that is methane" of all four other experiments was calculated. It was determined that the "% of

total biogas that is methane" value remained relatively constant for the algae mixtures regardless of pretreatment technology applied (**Table 9**). The overall methane content for the other four experiments was calculated to be 61 ± 2% for the algae mixtures and 50 ± 5% for the seed-only digesters. Therefore, the specific methane yield was calculated for the homogenized digesters by multiplying the biogas volume (known) by the methane content estimation factor and applying the new cumulative methane value to **Equation 2**.

Additionally, the Day 0 VS concentration for the untreated mixture in Experiment 2 was not recorded, so it was retroactively calculated using the VS concentration from Day 6, and the VS % reduction (**Equation 9.**) for the sonicated mixture. It was assumed that the volatile solids reduction was equivalent between the sonicated and unsonicated mixtures, as was confirmed in the Experiment 3 comparison of 3% TS treated (3% LAS) and untreated mixtures (3% UAS) (**Table 13**). The methane correction factor was also applied to the untreated mixture biogas volume as described above.

The specific methane yields for all four pretreatments (sonication, homogenization, autoclaving and boiling) are shown in **Table 9**, and are consistent with values recorded in literature: 0.1-0.5 L $CH_4$/ g $VS_{IN}$ (Sialve B., 2009), (Marsolek et al., 2014).

**Table 9.** Comparison of specific methane yields of all five experiments. Values highlighted in yellow were extrapolated from data (see explanation below). Sample coding is explained by the blue headers. UAS means untreated sample. The culture volumes were either 125 mL or 1.2 L and used to calculate the specific methane yield. 1st line example calculation: 0.200 L $CH_4$ / (7.3 g/L slurry * 0.125 L slurry) = 0.218 L $CH_4$/g VS

| Sample Name | Cumulative Biogas (mL) | % of Total Biogas That is Methane (%) | Cumulative Methane (mL) | Day 0 VS (g/L) | Specific Methane Yield (L $CH_4$/g $VS_{in}$) |
|---|---|---|---|---|---|
| **Experiment 1: Sonication** | | | | | |
| 1 min SAS | 338 | 59 | 200 | 7.3 | 0.218 |
| 2 min SAS | 298 | 60 | 178 | 7.5 | 0.190 |
| 5 min SAS | 317 | 61 | 193 | 7.4 | 0.208 |
| 10 min SAS | 412 | 62 | 253 | 7.3 | 0.276 |
| 45 min SAS | 421 | 61 | 258 | 8.3 | 0.249 |
| UAS | 310 | 60 | 185 | 7.3 | 0.203 |
| Seed | 181 | 45 | 81 | 11.9 | 0.054 |
| **Experiment 2: Sonication** | | | | | |
| 10 min SAS | 406 | 59 | 240 | 6.1 | 0.315 |
| UAS | 3227 | 61 | 1954 | 8.0 | 0.245 |
| **Experiment 3: High Pressure Homogenization** | | | | | |
| 3% UAS | 1017 | 61 | 616 | 22.7 | 0.217 |
| 3% LAS | 1107 | 61 | 670 | 21.6 | 0.249 |
| 2% LAS | 780 | 61 | 472 | 16.0 | 0.236 |
| 1% LAS | 478 | 61 | 289 | 9.8 | 0.235 |
| Seed | 435 | 50 | 218 | 22.2 | 0.079 |
| **Experiment 4: Autoclaving** | | | | | |
| UAS | 541 | 58 | 316 | 11.1 | 0.228 |
| AAS | 482 | 59 | 282 | 12.3 | 0.200 |
| Seed | 610 | 53 | 321 | 22.0 | 0.117 |
| **Experiment 5: Boiling** | | | | | |
| UAS | 473 | 62 | 294 | 12.0 | 0.197 |
| 0 min BAS | 530 | 63 | 333 | 13.0 | 0.205 |
| 30 min BAS | 551 | 63 | 345 | 12.2 | 0.227 |
| Seed | 173 | 53 | 92 | 21.2 | 0.035 |

A summary of the percent difference between the specific methane yield of the treated and the untreated biomass is shown in **Table 10**. The effectiveness of each pretreatment method at improving methane yield is reflected in the positive or negative effect on methane yield.

**Table 10.** Overview of the specific methane yield between all five experiments. Methane content was extrapolated for the highlighted valued as mentioned previously.

| Experiment | Sample | Specific Methane Yield ($L\ CH_4/g\ VS_{in}$) | Methane Yield Percent Increase |
|---|---|---|---|
| 1 Sonication | untreated (UAS) | 0.203 | -- |
| | 10 min sonicated (SAS) | 0.276 | 36% |
| 2 Sonication | untreated (UAS) | 0.245 | -- |
| | 10 min sonicated (SAS) | 0.315 | 29% |
| 3 Homogenization | 3% TS untreated (UAS) | 0.217 | -- |
| | 3% TS treated (LAS) | 0.249 | 15% |
| 4 Autoclaving | untreated (UAS) | 0.228 | -- |
| | autoclaved (AAS) | 0.200 | -12% |
| 5 Boiling | untreated (UAS) | 0.197 | -- |
| | 30 min boiled (30-BAS) | 0.227 | 15% |

### *4.1.3 Net Energy Balance*

In the following section, the net energy required to generate the specific methane volume was calculated. The input energy ($E_{in}$) was calculated for the pretreatment device only. For example, centrifugation of the algae slurry for Experiment 2 was not taken into account. **Equation 5** and **Equation 6** were adapted from Cho et al. (2013) in order to quantify the subsequent energy values listed in **Table 11**. Input energy (**Equation 5**) was estimated for each pretreatment device. Electrical efficiency was assumed to be 100% in the calculations that used the following equations.

**Equation 5.** Input Energy

$$E_{in}\left(\frac{kJ}{g\ VS_{initial}}\right) = \frac{[Power\ (Watts) * Time\ (seconds)]}{1000\ \left(\frac{W}{kW}\right) * volatile\ solids\ (g\ VS_{initial}) * effective\ volume\ (L)}$$

**Equation 6.** Output Energy

$$E_{out}\left(\frac{kJ}{g\ VS_{initial}}\right) = \frac{35.8\frac{kJ}{L\ CH_4} * Y_{CH_4}\left(\frac{mL}{g\ VS_{initial}}\right) * R}{1000(\frac{mL}{L})}$$

R is the percent recovery of produced methane, assumed to be 100%.

The lower heating value of methane is 35.8 kJ/ L $CH_4$ (Metcalf & Eddy, 2003). Power consumption by the Lancer autoclave was calculated using **Equation 7**. The power factor (PF) was assumed to be 0.85.

**Equation 7.** Autoclave Power

$$W_{applied} = 3^{1/2} * I * V * 0.85$$

$W_{applied}$ is the power of the device (W)
I is current (amps)
V is voltage (volts)
0.85 is the power factor (PF)

Fluid horsepower of the high-pressure homogenizer was calculated using **Equation 8** and the flow rate of 400 mL/min (0.105 gal/min), a pressure of 19,985 psi and a run duration of 420 seconds.

**Equation 8.** Homogenizer Power

$$Power\ (hp) = \frac{Pressure\ (psi) * Flow\left(\frac{gal}{min}\right)}{1714}$$

The unit conversion factor 1714 was used to convert to horsepower (hp).

The net energy input for this particular study, given the conditions of each pretreatment technology, is outlined in **Table 11**

.

**Table 11.** Preliminary net energy calculation of each pretreatment technology in this particular study. Input energy is for the pretreatment device, output energy is the calculated energy from methane production and net energy is the combination of both terms (output minus input).

| Sample | Cumulative specific methane yield (mL $CH_4$/g $VS_{IN}$) | Volume during diusruption (L) | VS at time of disruption (g/L) | VS after dilution (g/L) | Input Energy (kJ/g $VS_{IN}$) | Output Energy (kJ/g $VS_{IN}$) | Net Energy Production (kJ/g $VS_{IN}$) |
|---|---|---|---|---|---|---|---|
| 10 min Sonication | 315 | 0.045 | 61.8 | 8.6 | 7.50 | 11.28 | 3.78 |
| Untreated | 245 | N/A | N/A | 7.3 | N/A | 8.77 | 8.77 |
| 3% TS High Pressure Homogenization | 249 | 2.8 | 23.0 | 22.2 | 2.92 | 8.91 | 6.00 |
| 3% TS Untreated | 217 | N/A | N/A | 22.7 | N/A | 7.77 | 7.77 |
| Autoclaved | 200 | 2.4 | 34.4 | 12.3 | 18.57 | 7.16 | -11.41 |
| Untreated | 228 | N/A | N/A | 11.1 | N/A | 8.16 | 8.16 |
| Boiled | 227 | 1.2 | 24.8 | 12.2 | 6.35 | 8.13 | 1.78 |
| Untreated | 197 | N/A | N/A | 12.0 | N/A | 7.05 | 7.05 |

In every case, the untreated mixture had more favorable net energy production values (**Table 11**). However, these net energy values are a function of many variables, none of which were optimized to be economical. For example, 2.4 L of algae was autoclaved in a unit that has the capacity to accommodate considerably more material. The autoclave chamber measured 60cm x 60cm x 97cm and had ample room for additional biomass (**Figure 12**). Consequently, if the volume of treated biomass were maximized to 30 L, the input energy drops from 18.57 kJ/g $VS_{IN}$ to 1.48 kJ/g $VS_{IN}$ and improves the net energy production from -11.41 kJ/g $VS_{IN}$ to 5.68 kJ/g $VS_{IN}$**.**

Another critical factor in determining the input energy was the solids concentration of the algae slurry during pretreatment, with thicker slurry generally requiring lower unit energy input as shown by Passos et al. (2013) when microalgae was thermally treated at 55, 75 and 95°C for 5, 10 and 15 hours. For example, in the second sonication experiment algae

were collected directly from the RWs and centrifuged to thicken the algae to 61.8 g VS/L (compared to the 20-30 g VS/L typical for the gravity thickened slurry such as from the tube settlers).

Additionally, the "freshness" of the collected algae may also have an impact on the output energy by affecting the $Y_{CH_4}\left(\frac{mL}{g\ VS_{initial}}\right)$ term in **Equation 6**. The fresh algae slurry from the RW ponds was presumed to contain more methanogenic substrate than slurry harvested and stored over the course of a day in the bottom of the tube settlers. Even a period of 24 hours in the tube settler can cause sCOD release into the environment that is consumed by bacteria and permanently lost as a substrate for the methanogenic bacteria to create methane (see **Appendix B**). This is presumably the reason why the digested algae that underwent 10 minutes of sonication in the second experiment outperformed the first 10 minute sonication experiment in terms of specific methane yield. Assuming that all other variables were constant throughout both experiments, the methane yield was 0.315 L $CH_4$/ g $VS_{IN}$ for the freshly harvested algae in the second experiment as compared to 0.276 L $CH_4$/ g $VS_{IN}$ of the first experiment.

Despite the numerous variables affecting the energy balance, it should be noted that similar results were found by (Cho et al., 2013) and are detailed in **Table 12** below.

**Table 12.** Summary of energy balance. Source: (Cho et al., 2013).

| Pre-treatment method | Non pretreatment | Thermal treatment | | | Ultrasonic treatment | | |
|---|---|---|---|---|---|---|---|
| | | 50 °C | 80 °C | 120 °C | 30 s | 90 s | 180 s |
| *Solubilization* | | | | | | | |
| TCOD (mg $L^{-1}$) | 12,327 | 12,327 | 12,327 | 12,327 | 12,327 | 12,327 | 12,327 |
| SCOD (mg $L^{-1}$) | 735 | 904 | 2693 | 4080 | 973 | 1639 | 2282 |
| Solubiliztion (%) | 0 | 1.5 | 16.9 | 28.9 | 2.1 | 7.8 | 13.4 |
| *Energy balances* | | | | | | | |
| Device or reagent | – | Water bath | Water bath | Autoclave | Sonicator | Sonicator | Sonicator |
| Power ($W = J\ s^{-1}$) | – | 1000 | 1000 | 2000 | 130 | 130 | 130 |
| Reaction time (s) | – | 1800 | 1800 | 1800 | 30 | 90 | 180 |
| Methane productivity (mL $CH_4$ g $VS^{-1}$) | 336 | 351 | 384 | 405 | 356 | 368 | 385 |
| Input energy (kJ g $VS^{-1}$) | – | 18 | 18 | 8 | 39 | 117 | 234 |
| Output energy (kJ g $VS^{-1}$) | 11.9 | 12.4 | 13.6 | 14.3 | 12.6 | 13.0 | 13.6 |
| Net energy production (kJ g $VS^{-1}$) | 11.9 | −5.6 | −4.4 | 6.3 | −26.4 | −104.0 | −220.4 |

The algae used in the Cho et al. (2013) study were a mixture of 70% *Chlorella* (wt/wt) and 30% *Scenedesmus* (wt/wt), cultivated in cylindrical photobioreactors and grown on a modified Bold's Basal media. The fact that pure cultures of algae were used and treated immediately after harvest may be one of the reasons the methane yield are consistently higher than those of the current study. Additionally, the low input energy for the autoclave in **Table 12** may be due to a larger treatment volume than used in the current study. The high input energy for ultrasonic pretreatment in **Table 12** is partly due to the low solids concentration of algae biomass that was treated; a mere 10 g VS/L.

### 4.1.4 *Effect of Organic Loading on Degradation Rate*

One of the primary goals of the high-pressure homogenization experiment was to identify the effect of the organic load on digester performance. In this section the percent before the sample name refers to total solids. For example, the sample identification 2% LAS actually means, "2% total solids lysed algae + seed." Volatile solids degradation was plotted for the three different organic loads and the results are shown in **Figure 25** and **Table 13**.

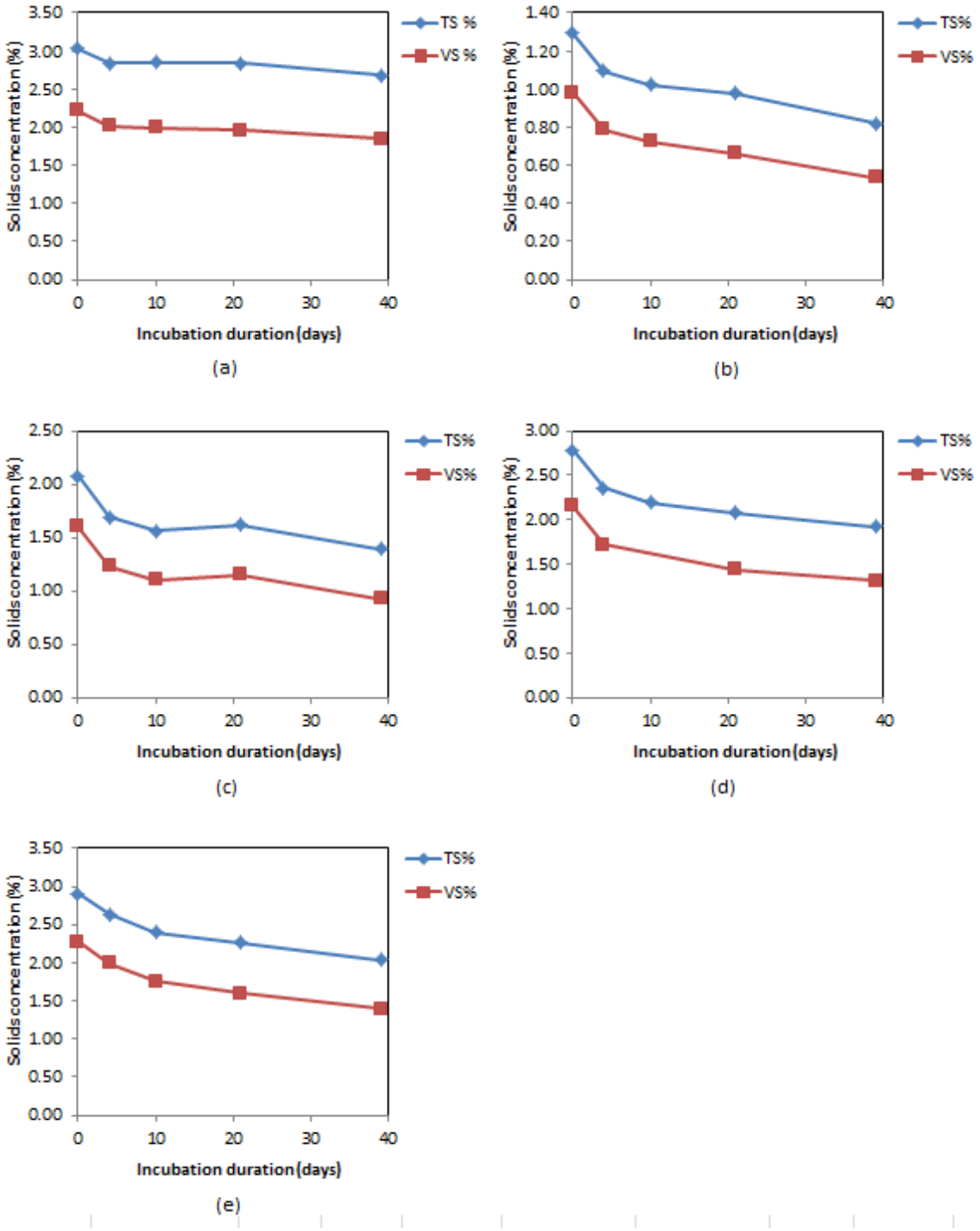


**Figure 25.** Solids degradation in batch digesters with different initial solids concentrations (i.e., organic load). Homogenized algae ("LAS") were used in this experiment. (a) Seed, (b) 1% TS LAS, (c) 2% TS LAS, (d) 3% TS LAS, (e) 3% TS UAS (untreated).

Volatile solids destruction was an important parameter that was examined in the homogenization experiment. VS destruction was quantified using **Equation 9**.

**Equation 9.** VS Destruction

$$Volatile\ Solids\ \%\ Destruction = \left(\frac{\left[VS_{initial} - VS_{final}\right]}{VS_{initial}}\right) * 100$$

**Table 13.** Volatile solids percent destruction over the course of 39 days of digestion. The standard deviation was calculated by comparing triplicate VS measurements on Day 0 and Day 39, for each mixture. Percent destruction was calculated using **Equation 9.** The percent destruction for the 3% TS treated and 3% TS untreated control is within one standard deviation of each other.

| Sample | Day 0 VS% | Day 39 VS% | % Destruction | % Destruction Standard Deviation |
|---|---|---|---|---|
| Seed | 2.22 | 1.85 | 16.67 | 0.91 |
| 1% Lysed Algae + Seed | 0.98 | 0.53 | 45.92 | 0.97 |
| 2% Lysed Algae + Seed | 1.60 | 0.93 | 41.88 | 0.92 |
| 3% Lysed Algae + Seed | 2.16 | 1.31 | 39.35 | 1.80 |
| 3% Unlysed Algae + Seed | 2.27 | 1.40 | 38.33 | 1.21 |

The volatile solids destruction for all three loading rates was consistent with literature values of 20-60% destruction (Bohutskyi, 2014). The samples with higher initial VS concentrations exhibited slightly less overall VS destruction. This may be due to the nature of the batch-mode digestion setup favoring a lower initial %VS because the seed fraction was added on a 20% v/v basis regardless of initial VS concentration of the algae. That would mean more methanogenic bacteria were present from the beginning of digestion and were able to metabolize the substrate to a greater degree.

### *4.1.5 Neutral to Negative Effects of High Heat on Biodegradability*

The visible effects of pretreating the algae slurry were recorded. Autoclaving and boiling both altered the coloration of the slurry. The vibrant, rich green color of untreated algae changed to a dull brown after pretreatment (**Figure 26**, **Figure 27**). The change in color may be due to the conversion of chlorophyll to pheophytin. Additionally, boiling increased the coloration of the filtered sCOD samples, implying cell lysis (**Figure 28**).

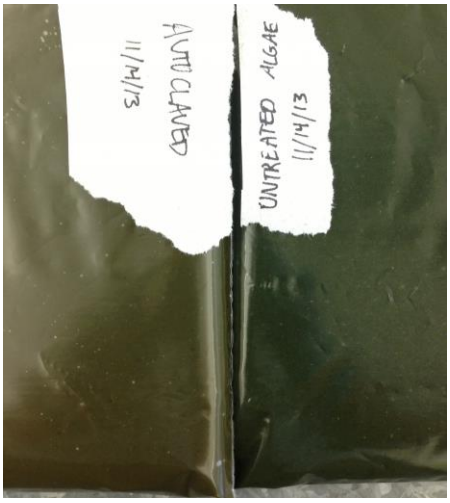


**Figure 26.** The high heat and pressure of the autoclave had a visible effect on the algae. Left: autoclaved, Right: untreated. The coloration changed from green to brown, and the texture from ketchup-like consistency to more gelatinous, slimy pudding. This may be indicative of chemical reactions occurring, possibly including formation of toxic or inhibitory compounds.

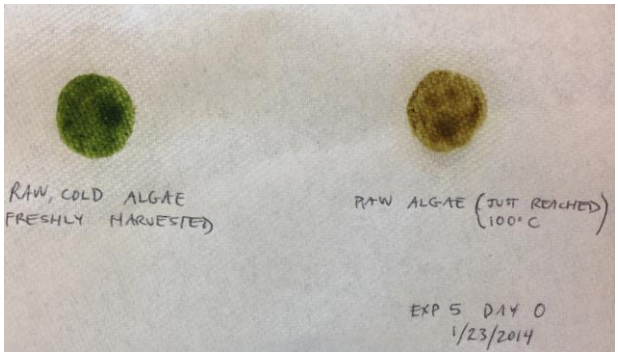


**Figure 27.** As seen previously in the autoclaving pretreatment, boiling the algae also altered the natural vibrant green coloration to a dull brown. Left: raw untreated algae. Right: heat treated for 45 minutes, just reaching 100°C

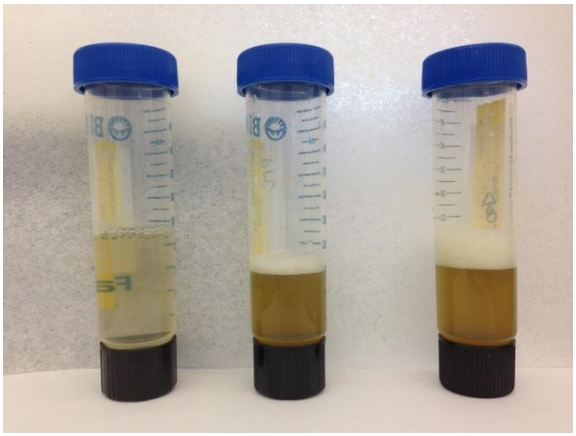

**Figure 28.** Comparison of unacidified soluble COD samples for all three boiling times. Left to right: untreated, 0 min boiled, 30 min boiled. All samples were passed through a G4 filter by this point (1.2 μm pore size). The gelatinous foam layer increased in the sample with increased heat treatment. This may be caused by the formation of alternate forms of proteinaceous compounds.

Throughout the present study, it was noted that boiling, autoclaving, and extended sonication had a minimal or even adverse effect on cumulative methane production compared to the untreated control (**Table 9**). This result is supported by other research that concluded thermal pretreatment of *Chorella* sp. and *Spirulina maxima* had no effect or a negative effect on methane production (Bohutskyi, 2014). Another research group discovered that a range of thermal pretreatment at 50°C, 100°C and 150°C had no effect on ultimate methane productivity (Samson, 1983). A reoccurring explanation for a decrease in methane yield induced by heat treatment may be the formation of recalcitrant, inhibitory compounds (Alzate et al., 2012). Furthermore, in a study that evaluated waste-grown algae as a potential animal feed, it was determined that the control group of rats fed autoclaved algae suffered the greatest weight loss due to a low feed conversion ratio (g gain/ g consumed), at roughly half that of dried algae (Cook, 1962). That result substantiates the notion that some pretreatment technologies involving high heat and/or pressure may negatively impact the biomass nutrient content that would diminish the efficacy of anaerobic bacterial conversion to methane.

### *4.1.6 Nitrogen Solubilization*

In all cases except sonication, additional pretreatment led to higher TAN solubilization. Sonication had the greatest ultimate TAN solubilization of 94% for the untreated and 86% solubilization for 10 minutes of treatment. The TAN solubilization for each successive pretreatment method (sonication, homogenization, autoclaving, boiling) is reported in the following bar graphs (**Figure 29-32**), and then summarized in the tables directly following (**Table 14-17**). Similar to (Foree, 1970), sonication did not change the small soluble fraction of nitrogen (TAN) of the algae therefore, TAN release due to pretreatment alone was omitted from the following graphs under the assumption that TAN release was low for the other pretreatments too. Only two TKN values for each mixture were measured (initial and final day of digestion), so those two values were averaged and the percent difference between the average value and the difference between the two measured values was calculated (**Table 19**). None of the experimental mixtures had a percent difference more than 14%, except for in the sonication experiment, which presumably had some analytical sampling error. Additionally, in all of the following bar graphs the 100% maximum on the y- axis represents the average TKN value from the initial and final days of digestion. The homogenization experiment had TAN probe issues and as a result does not have a Day 0 data point for any of the mixtures (**Figure 30**). Organic nitrogen was calculated using **Equation 10.**

**Equation 10.** Nitrogen Balance

$$TKN - TAN = Organic\ N$$

The sonication experiment saw the greatest nitrogen solubilization out of all four experiments; greater than 80% nitrogen solubilization (**Figure 29**).

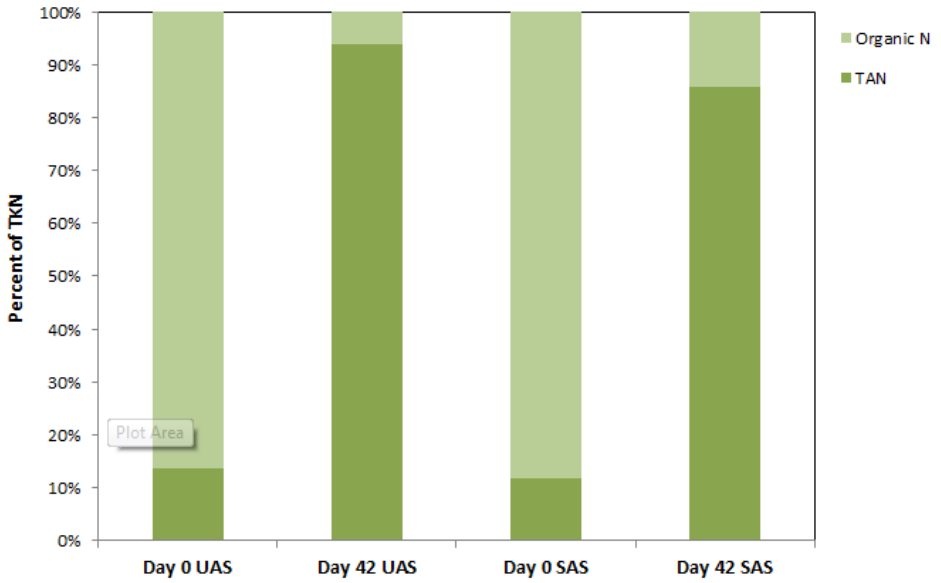


**Figure 29.** *Sonication*: overview of nitrogen release during 42 days of digestion. All TAN values represent a single value generated from a single digester. On the final day of digestion, the ammonia concentration seemed excessively high. This percent solubilization was by far the highest of all of the experiments and requires confirmation. UAS is untreated and 10" SAS is 10 minutes of sonication.

The starting TAN concentration of both mixtures in the sonication experiment was the lowest of all of the experiments. This was the only experiment in which the seed was diluted from ~30 mg/L TS to ~10 mg/L TS, and that might have dropped the initial TAN levels. The initial and final nitrogen concentrations were recorded (**Table 14**).

**Table 14.** *Sonication*: summary of nitrogen constituents on the initial and final day of digestion. Both of the algae digester samples achieved greater than 85% nitrogen solubilization. The ammonia selective electrode may have caused significant drift in the samples run later in the run, artificially increasing the concentration of the final samples.

| | Sample | TAN (mg/L) | Organic N (mg/L) | TKN (mg/L) | % of TKN that is TAN |
|---|---|---|---|---|---|
| Exp 2 - Sonication | Day 0 UAS | 72 | 451 | 523 | 14% |
| | Day 42 UAS | 490 | 32 | 523 | 94% |
| | Day 0 10" SAS | 72 | 545 | 617 | 12% |
| | Day 42 10" SAS | 529 | 88 | 617 | 86% |
| | Day 42 Seed | 431 | 222 | 653 | 66% |

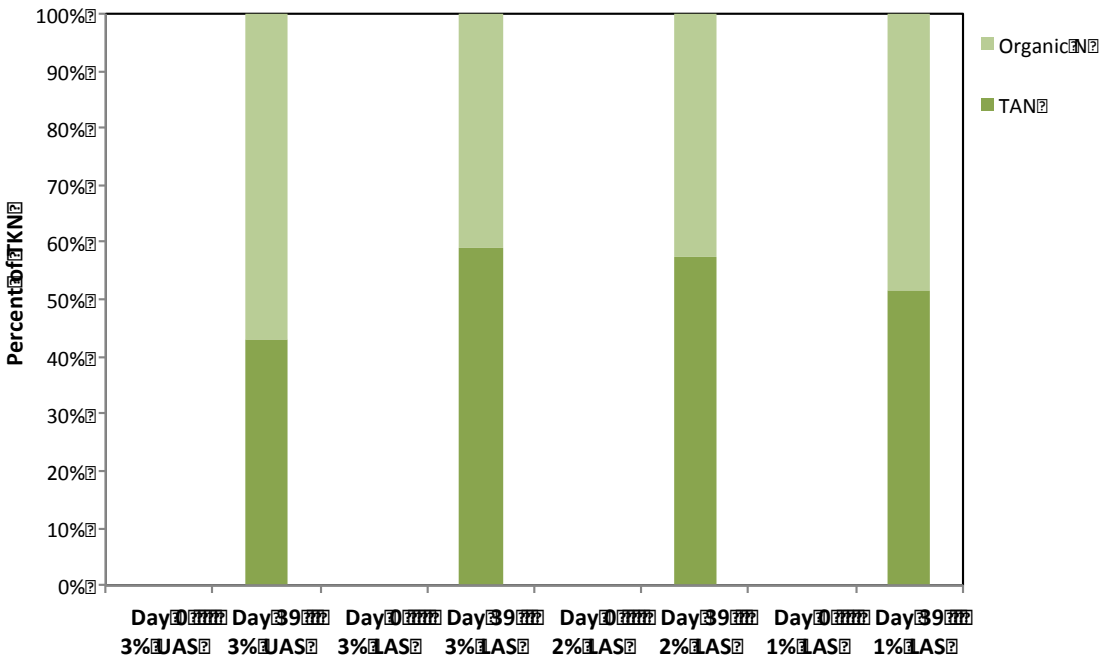


**Figure 30.** *High pressure homogenization*: overview of nitrogen release during 39 days of digestion. All TAN values represent a single value generated from a single digester. The percentage in the x-axis label is a reference to the approximate percent total solids of the mixture. Initial days are not shown due to unreliable analytical results. QC did not pass, and all sample volume was exhausted. All of the treated mixtures outperformed the 3% untreated algae control in terms of ultimate percent solubilization.

**Table 15.** *High pressure homogenization*: summary of nitrogen constituents on the final day of digestion for each digester organic load. As would be expected, the lysed 3% algae mixture had the highest TAN concentration and the highest fraction of nitrogen in the soluble ammonia form. Values are presented for the final day of digestion only (initial day values omitted due to electrode malfunction). The 3% UAS and 3% LAS had an ultimate nitrogen solubilization of 43% and 59% respectively.

| | Sample | TAN (mg/L) | Organic N (mg/L) | TKN (mg/L) | % of TKN that is TAN |
|---|---|---|---|---|---|
| Exp 3 - High Pressure Homogenization | Day 0 3% UAS | -- | -- | 2044 | -- |
| | Day 39 3% UAS | 876 | 1169 | 2044 | 43% |
| | Day 0 3% LAS | -- | -- | 1960 | -- |
| | Day 39 3% LAS | 1158 | 802 | 1960 | 59% |
| | Day 0 2% LAS | -- | -- | 1452 | -- |
| | Day 39 2% LAS | 835 | 617 | 1452 | 58% |
| | Day 0 1% LAS | -- | -- | 989 | -- |
| | Day 39 1% LAS | 512 | 477 | 989 | 52% |
| | Day 39 Seed | 1651 | 1289 | 2940 | 56% |

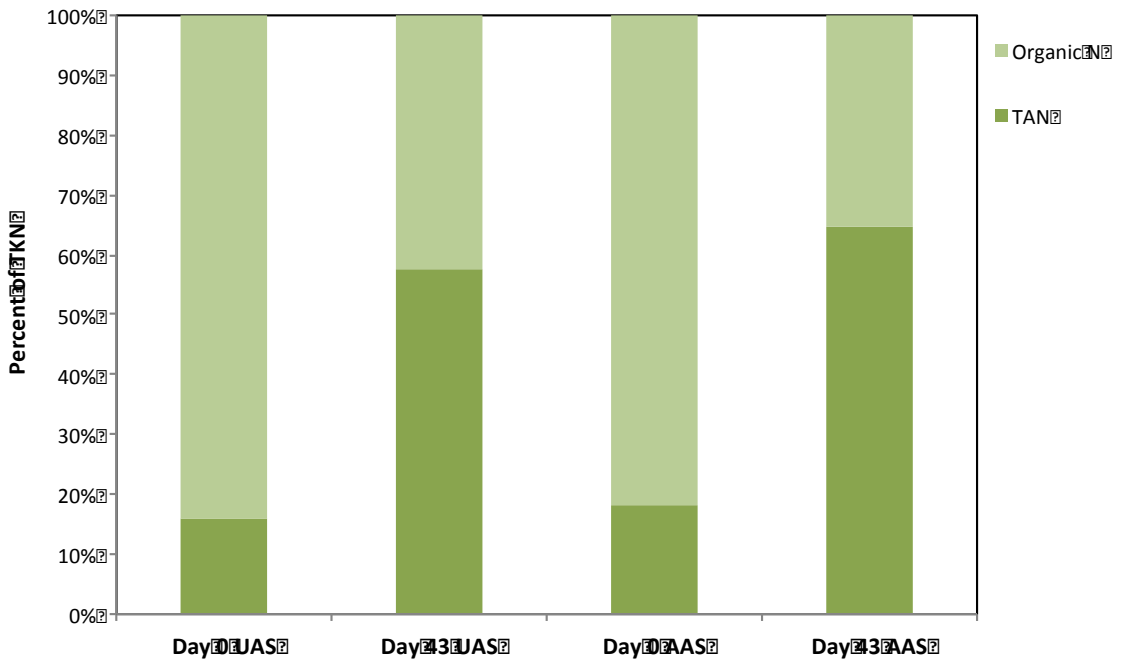


**Figure 31.** *Autoclaved*: overview of nitrogen release during 43 days of digestion. All TAN values represent a single value generated from a single digester. The autoclaved digestate (AAS) had a slightly greater percent nitrogen solubilization than the untreated control (UAS).

**Table 16.** *Autoclaved*: summary of nitrogen constituents on the initial and final day of digestion. Comparison between the treatment (AAS), control (UAS), and seed batch digestions. The untreated control and autoclaved mixtures had ultimate nitrogen solubilizations of 58% and 65% respectively.

| | Sample | TAN (mg/L) | Organic N (mg/L) | TKN (mg/L) | % of TKN that is TAN |
|---|---|---|---|---|---|
| Exp 4 - Autoclaved | Day 0 UAS | 242 | 1270 | 1512 | 16% |
| | Day 43 UAS | 870 | 642 | 1512 | 58% |
| | Day 0 AAS | 268 | 1209 | 1477 | 18% |
| | Day 43 AAS | 954 | 523 | 1477 | 65% |
| | Day 43 Seed | 1889 | 1233 | 3122 | 61% |

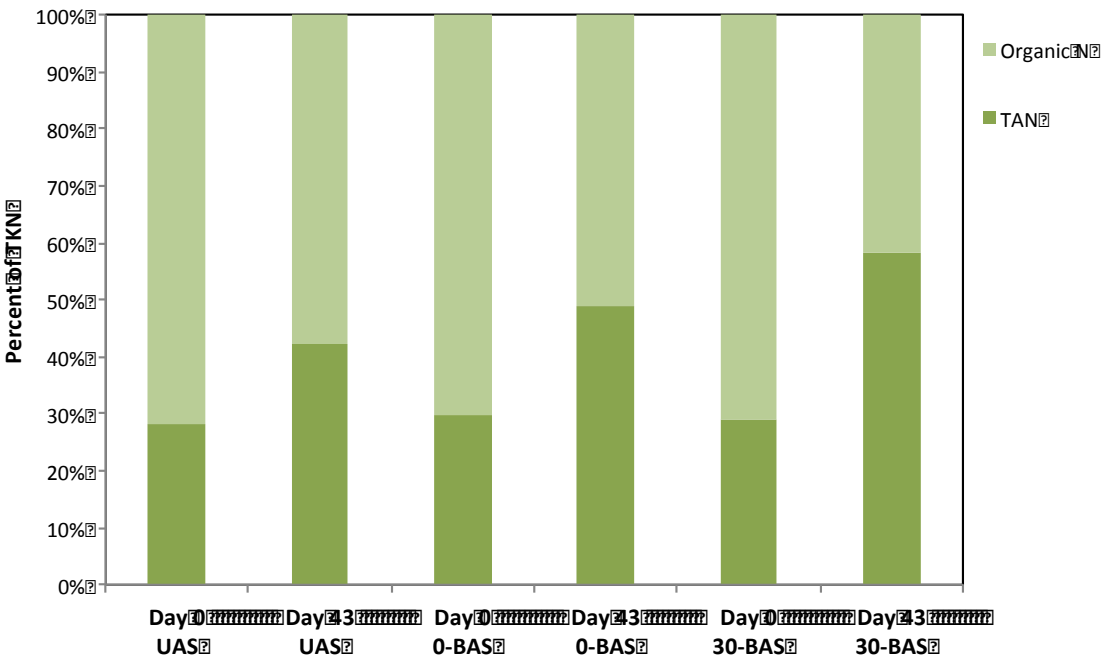


**Figure 32.** *Boiled*: overview of nitrogen release during 42 days of digestion. All TAN values represent a single value generated from a single digester. The samples shown are untreated (UAS), 0 minutes boiled (0-BAS) and thirty minutes boiled (30-BAS). The sample that received the most heat treatment (30-BAS) yielded the greatest ultimate nitrogen solubilization.

**Table 17.** *Boiled*: summary of nitrogen constituents on the initial and final day of digestion. Comparison of nitrogen fractions in the two treatment durations as well as the untreated control. The ultimate nitrogen solubilization for the untreated (UAS) was 42% while maximum pretreatment (30-BAS) reached 58%.

| | Sample | TAN (mg/L) | Organic N (mg/L) | TKN (mg/L) | % of TKN that is TAN |
|---|---|---|---|---|---|
| Exp 5 - Boiled | Day 0 UAS | 396 | 1004 | 1400 | 28% |
| | Day 43 UAS | 594 | 806 | 1400 | 42% |
| | Day 0 0-BAS | 409 | 970 | 1379 | 30% |
| | Day 43 0-BAS | 675 | 704 | 1379 | 49% |
| | Day 0 30-BAS | 388 | 956 | 1344 | 29% |
| | Day 43 30-BAS | 785 | 559 | 1344 | 58% |
| | Day 43 Seed | 1476 | 1562 | 3038 | 49% |

TAN data from all four experiments were normalized by the initial VS concentration

**(Table 18)** and compared to values from a similar study **(Table 2).**

**Table 18.** Overview of TAN yield from VS degradation on the final day of digestion. Untreated algae + seed for the boiled experiment did not have enough sample volume to produce a TAN concentration for the final day of digestion, so Day 20 TAN is presented.

| Experiment | Sample | Day of Digestion | TAN (mg TAN/ g $VS_{IN}$) |
|---|---|---|---|
| Sonication | Untreated Algae + Seed | 42 | 60 |
| | 10 min Sonicated Algae + Seed | 42 | 86 |
| High Pressure Homogenization | 3% TS Untreated Algae + Seed | 39 | 39 |
| | 3% TS Lysed Algae + Seed | 39 | 54 |
| | 2% TS Lysed Algae + Seed | 39 | 52 |
| | 1% TS Lysed Algae + Seed | 39 | 52 |
| Autoclaved | Untreated Algae + Seed | 43 | 79 |
| | Autoclaved Algae + Seed | 43 | 85 |
| Boiled | Untreated Algae + Seed | 20 | 50 |
| | 30 min Boiled Algae + Seed | 43 | 65 |

The experimental range of TAN values measured throughout all of the experiments in the present study is consistent with the theoretical TAN yields in Section 2 (**Table 2**). However, the slightly elevated TAN concentrations (60-80 mg TAN/ g $VS_{IN}$) in this study, are most likely due to the addition of anaerobic digester seed, which had a TAN concentration of approximately 1500-2000 mg/L. An additional nitrogen mass balance was conducted on each of the digester mixtures in each study. Due to the batch nature of the experiments, TKN should remain constant from the initial and final days; mass $TKN_{in}$ = mass $TKN_{out}$. Confirmation of the accuracy of the nitrogen mass balance performed in this study is detailed in **Table 19** below.

**Table 19.** Mass balance of nitrogen on initial and final days. Summary of the initial and final TKN values of each batch digester in all four experiments that analyzed nutrient release.

| | Sample | Initial day TKN concentration (mg/L) | Final day TKN concentration (mg/L) | Average TKN concentration (mg/L) | Difference between initial and final (mg/L) | Percent difference (%) |
|---|---|---|---|---|---|---|
| Exp 2- Sonication | SAS | 652 | 543 | 598 | 109 | 18% |
| | UAS | 554 | 493 | 524 | 62 | 12% |
| | Seed Only (diluted) | 717 | 588 | 652 | 129 | 20% |
| Exp 3-High Pressure Homogenization | 3% UAS | 2044 | 2044 | 2044 | 0 | 0% |
| | 3% LAS | 2100 | 1820 | 1960 | 280 | 14% |
| | 2% LAS | 1447 | 1456 | 1452 | 9 | 1% |
| | 1% LAS | 941 | 1036 | 989 | 95 | 10% |
| | Seed Only | 3136 | 2744 | 2940 | 392 | 13% |
| Exp 4- Autoclaving | UAS Bottle 7 | 1484 | 1456 | 1512 | 42 | 3% |
| | UAS Bottle 8 | -- | 1596 | -- | -- | -- |
| | AAS Bottle 7 | 1428 | 1596 | 1477 | 65 | 4% |
| | AAS Bottle 8 | -- | 1456 | -- | -- | -- |
| | AAS Bottle 8 (SPLIT) | -- | 1428 | -- | -- | -- |
| | Seed Only | 3136 | 3108 | 3122 | 28 | 1% |
| Exp 5- Boiling | UAS | 1456 | 1344 | 1400 | 112 | 8% |
| | 0-BAS | 1372 | 1386 | 1379 | 14 | 1% |
| | 30-BAS | 1316 | 1372 | 1344 | 56 | 4% |
| | Seed Only | 3080 | 2996 | 3038 | 84 | 3% |

### *4.1.7 Phosphorus Solubilization*

DRP solubilization in all four pretreatments was similar and within the range of approximately 40-50%, except for the homogenization pretreatment, due to phosphorus precipitation. The DRP solubilization for each successive pretreatment method (sonication, homogenization, autoclaving, boiling) is reported in the following bar graphs (**Figure 33-36**), and then summarized in the tables directly following the respective graph (**Table 20-23**). Only two TP samples were measured (initial and final day of digestion), so those two values were averaged and the percent difference between the average value, and the difference between the two measured values was calculated (**Table 24**). None of

the mixtures had a percent difference greater than 12% for the high pressure homogenization, autoclave or boiling experiments. However, the sonication experiment appears to have incurred some sort of experimental irregularities resulting in initial and final TP values ranging from 36-54% difference. A margin of error that large indicates some sort of major analytical disruption or sampling error. The 100% maximum on the y-axis of the following bar graphs (**Figure 33-36**) represents the average TP value from the initial and final days of digestion. Particulate phosphorus was calculated using **Equation 11**.

**Equation 11.** Phosphorus Balance

$$TP - DRP = Particulate\ P$$

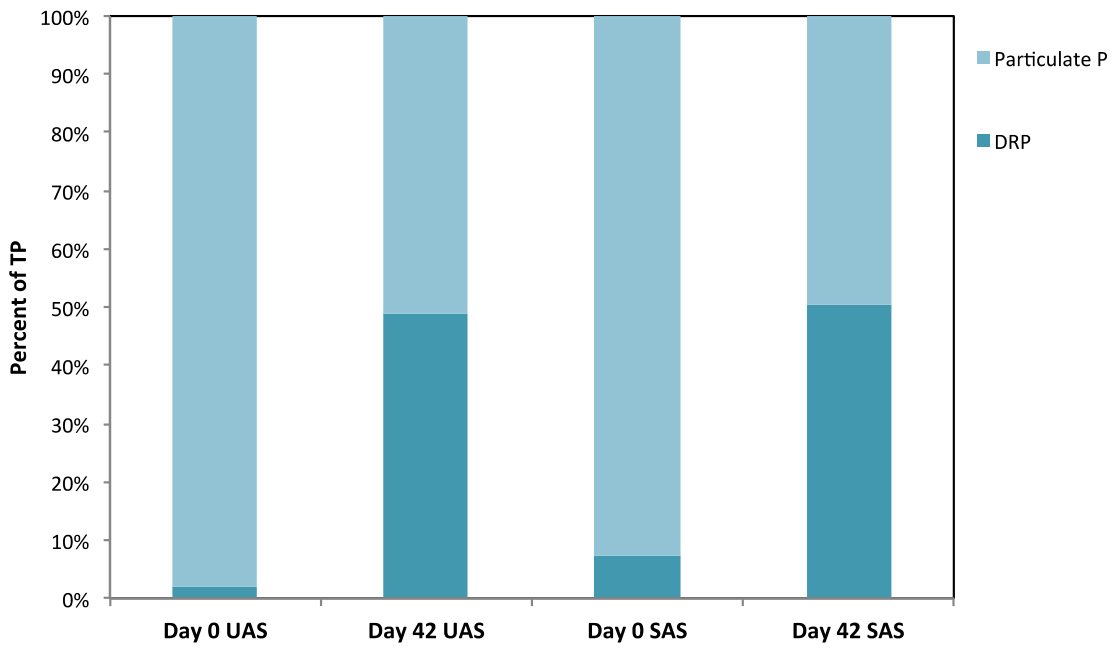


**Figure 33.** *Sonication*: overview of phosphorus release during 42 days of digestion. All DRP values represent a single value generated from a single digester. UAS correlates to untreated control and SAS to sonicated. Phosphorus solubilization was very similar in both the treated and untreated control.

**Table 20.** *Sonication*: summary of phosphorus constituents on the initial and final day of digestion. The untreated (UAS) and treated (SAS) had similar ultimate solubilization, 49% and 50% respectively.

| | Sample | DRP (mg/L) | Particulate P (mg/L) | TP (mg/L) | % of TP that is DRP |
|---|---|---|---|---|---|
| Exp 2 - Sonication | Day 0 UAS | 2 | 118 | 121 | 2% |
| | Day 42 UAS | 59 | 61 | 121 | 49% |
| | Day 0 SAS | 11 | 146 | 158 | 7% |
| | Day 42 SAS | 79 | 78 | 158 | 50% |
| | Day 42 Seed | 31 | 178 | 209 | 15% |

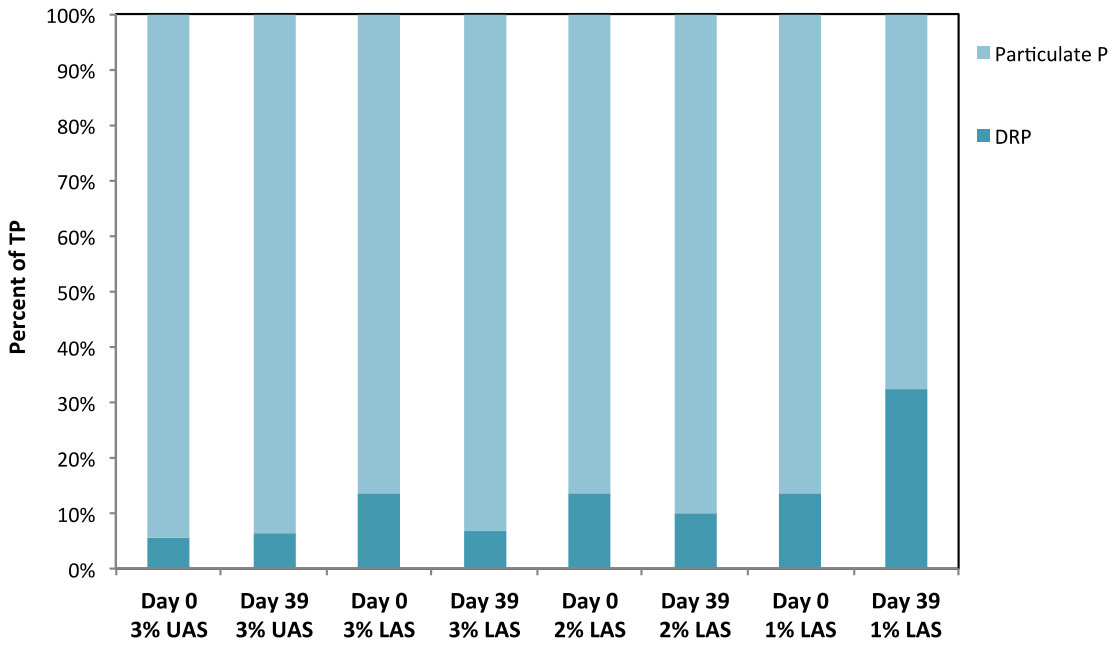


**Figure 34.** *High pressure homogenization*: overview of phosphorus release during 39 days of digestion. All DRP values represent a single value generated from a single digester. UAS means to untreated and LAS means lysed (homogenized). The percentage in the x-axis is a reference to the approximate percent total solids of the mixture.

The overall phosphorus solubilization in the homogenization experiment was low compared to other technologies because of the phosphorus precipitation that occurred.

**Table 21.** *High pressure homogenization*: summary of phosphorus constituents on the initial and final day of digestion. Low solubilization percentages reflect speculative precipitation. Even the 1% LAS had a low ultimate phosphorus solubilization of only 33%.

| | Sample | DRP (mg/L) | Particulate P (mg/L) | TP (mg/L) | % of TP that is DRP |
|---|---|---|---|---|---|
| Exp 3 - High Pressure Homogenization | Day 0 3% UAS | 31 | 536 | 567 | 5% |
| | Day 39 3% UAS | 35 | 532 | 567 | 6% |
| | Day 0 3% LAS | 74 | 478 | 552 | 13% |
| | Day 39 3% LAS | 38 | 514 | 552 | 7% |
| | Day 0 2% LAS | 59 | 382 | 441 | 13% |
| | Day 39 2% LAS | 43 | 398 | 441 | 10% |
| | Day 0 1% LAS | 36 | 228 | 264 | 14% |
| | Day 39 1% LAS | 86 | 178 | 264 | 33% |
| | Day 39 Seed | 35 | 673 | 708 | 5% |

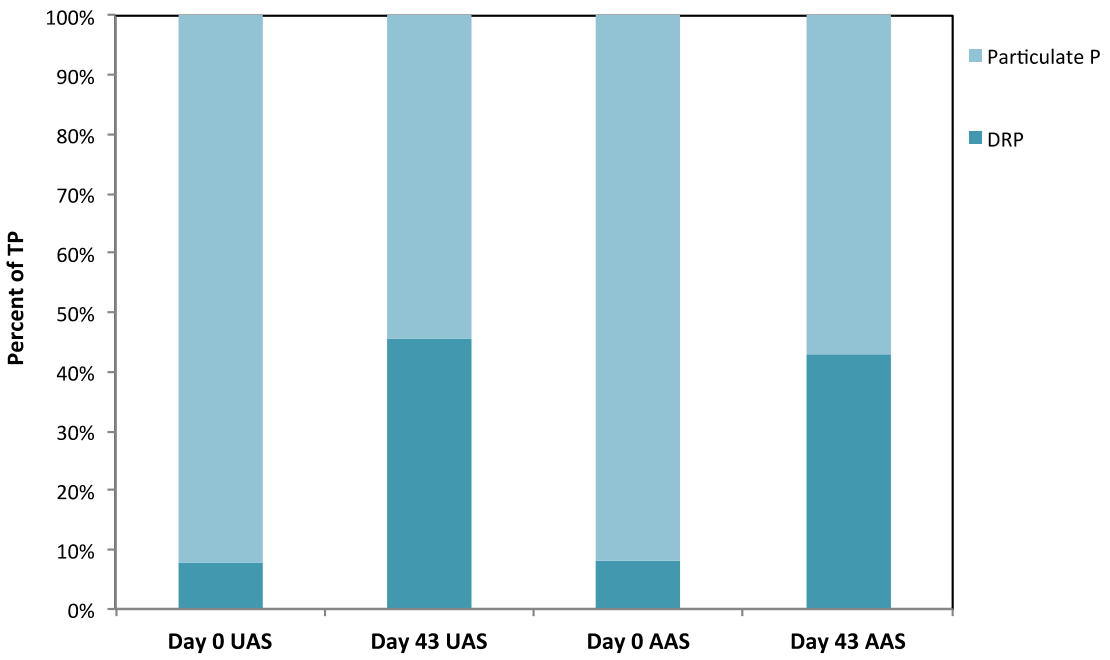


**Figure 35.** *Autoclaved*: overview of phosphorus release during 43 days of digestion. All DRP values represent a single value generated from a single digester. UAS correlates to untreated control and AAS to autoclaved algae. The autoclaved mixture actually had a final DRP concentration that was less than the untreated control, albeit slight.

**Table 22** *Autoclaved*: summary of phosphorus constituents on the initial and final day of digestion. Untreated (UAS) and treated (AAS) performed similarly in ultimate phosphorus solubilization, 46% and 43% respectively.

| | Sample | DRP (mg/L) | Particulate P (mg/L) | TP (mg/L) | % of TP that is DRP |
|---|---|---|---|---|---|
| Exp 4 - Autoclaved | Day 0 UAS | 24 | 275 | 299 | 8% |
| | Day 43 UAS | 136 | 163 | 299 | 46% |
| | Day 0 AAS | 26 | 295 | 321 | 8% |
| | Day 43 AAS | 137 | 184 | 321 | 43% |
| | Day 43 Seed | 69 | 747 | 816 | 9% |

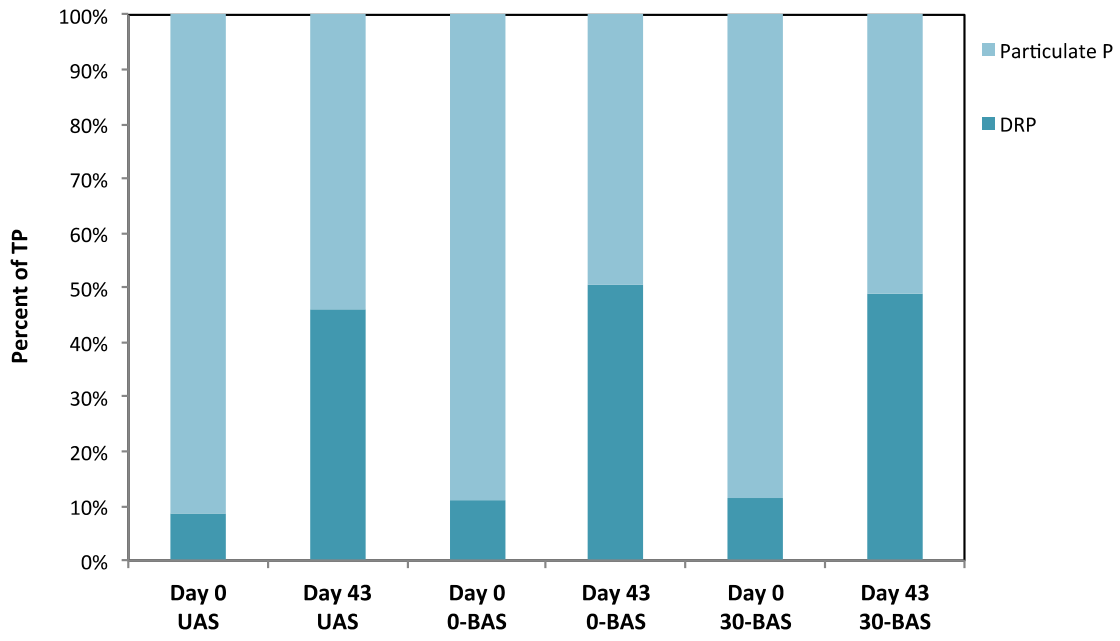


**Figure 36.** *Boiled*: overview of phosphorus release during the 43 days of digestion. All DRP values represent a single value generated from a single digester. UAS correlates to untreated control, 0-BAS to just boiled, and 30-BAS to 30 minutes at 100°C.

**Table 23.** *Boiled***:** summary of phosphorus constituents on the initial and final day of digestion. All three algae digester mixtures had similar phosphorus solubilization values.

| | Sample | DRP (mg/L) | Particulate P (mg/L) | TP (mg/L) | % of TP that is DRP |
|---|---|---|---|---|---|
| Expt 5 - Boiled | Day 0 UAS | 21 | 225 | 246 | 9% |
| | Day 43 UAS | 113 | 133 | 246 | 46% |
| | Day 0, 0-BAS | 28 | 229 | 258 | 11% |
| | Day 43, 0-BAS | 130 | 128 | 258 | 51% |
| | Day 0, 30-BAS | 29 | 225 | 254 | 11% |
| | Day 43, 30-BAS | 124 | 130 | 254 | 49% |
| | Day 43 Seed | 44 | 543 | 587 | 8% |

A mass balance was conducted on the total phosphorus for each experiment. In theory, the initial and final total phosphorus concentrations should be equivalent; mass $TP_{in}$ = mass $TP_{out}$. The results are presented in **Table 24**.

**Table 24.** Summary of the initial and final TP values of each batch digester in the four main experiments. The final day TP values for the sonication experiment were actually samples taken from Day 22 of digestion, not Day 42; the actual final day.

| | Sample | Initial day TP concentration (mg/L) | Final day TP concentration (mg/L) | Average TP concentration (mg/L) | Difference between initial and final (mg/L) | Percent difference (%) |
|---|---|---|---|---|---|---|
| Exp 2- Sonication | SAS | 202 | 116 | 159 | 87 | 54% |
| | UAS | 98 | 148 | 123 | 50 | 41% |
| | Seed Only (diluted) | 240 | 168 | 204 | 72 | 36% |
| Exp 3-High Pressure Homogenization | 3% UAS | 594 | 540 | 567 | 54 | 10% |
| | 3% LAS | 558 | 546 | 552 | 12 | 2% |
| | 2% LAS | 468 | 414 | 441 | 54 | 12% |
| | 1% LAS | 252 | 276 | 264 | 24 | 9% |
| | Seed Only | 708 | 708 | 708 | 0 | 0% |
| Exp 4- Autoclaving | UAS Bottle 7 | 291 | 327 | 299 | 12 | 4% |
| | UAS Bottle 8 | -- | 279 | -- | -- | -- |
| | AAS Bottle 7 | 309 | 309 | 321 | 18 | 6% |
| | AAS Bottle 8 | -- | 345 | -- | -- | -- |
| | Seed Only | 822 | 810 | 816 | 12 | 1% |
| Exp 5- Boiling | UAS | 242 | 254 | 248 | 12 | 5% |
| | 0-BAS | 255 | 256 | 256 | 1 | 0% |
| | 30-BAS | 255 | 252 | 254 | 2 | 1% |
| | Seed Only | 550 | 625 | 587 | 75 | 13% |

The percent difference for the sonication experiment reflects a major incongruency in the data and is perceived to be experimental error due to analytical issues. Otherwise, the homogenization, autoclaved and boiled experiments conferred a reasonable percent difference; ranging from 0-13%.

Above all, it appears as if the rate and extent of soluble phosphorus release is similar regardless of the pretreatment technology applied. The untreated controls also appeared to have a similar behavior. In both cases, the autoclaved and boiled mixtures, as well as

the untreated controls of the same experiments, shared similar release patterns. The results are displayed in **Figure 37** and **Figure 38** below.

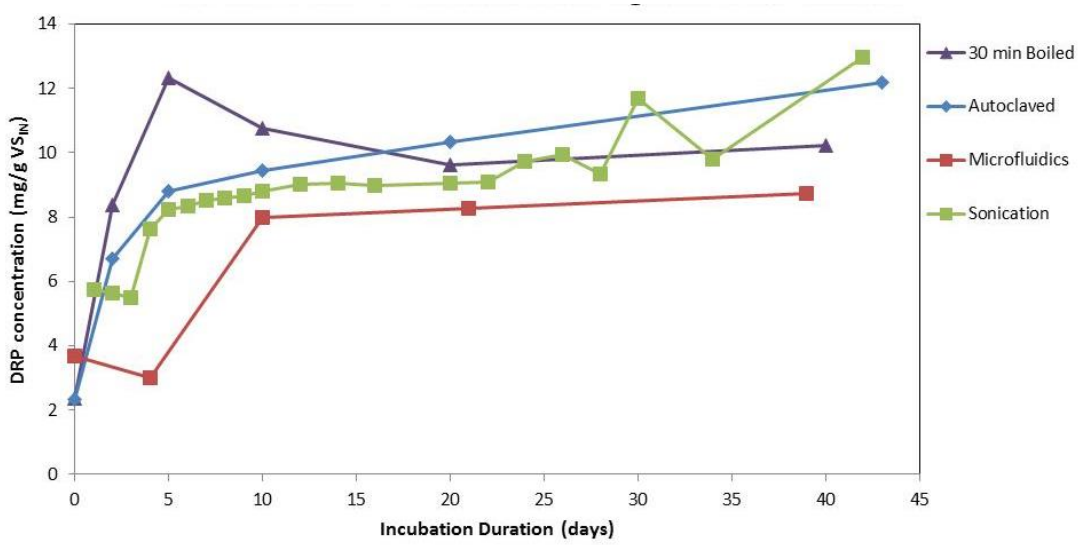


**Figure 37.** Normalized time series of DRP concentration for all four pretreated mixtures. Each day's DRP concentration was divided by the volatile solids concentration from Day 0 to normalize the phosphorus content per cell mass. As evidenced by the graph, the DRP solubilization for each pretreatment technology was similar if the outliers are neglected.

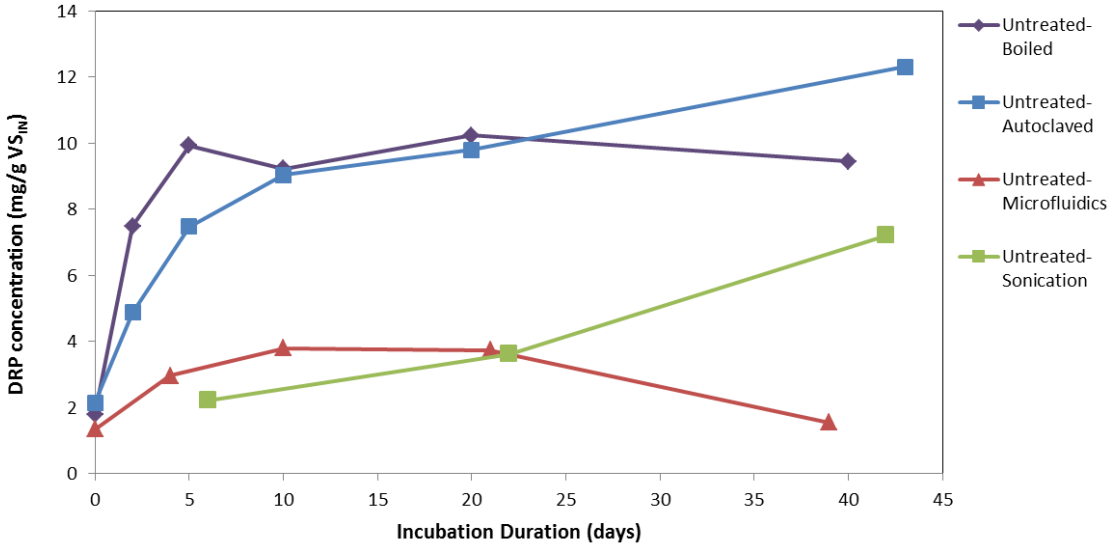


**Figure 38.** Normalized time series of DRP concentration for all untreated controls. Each day's DRP concentration was divided by the volatile solids concentration from Day 0 to normalize the phosphorus content per cell mass. The untreated mixture from the pressure homogenization was at 3% TS (not 1% TS like the others) and phosphorus precipitation was speculated.

### *4.1.7.1 Phosphorus Precipitation*

DRP measurements conducted on the high pressure homogenization experiment did not follow similar release patterns as the other experiments and after multiple rounds of testing the same samples, it was posited that precipitation had occurred. The diminishing DRP concentration can be seen in **Figure 39** below.

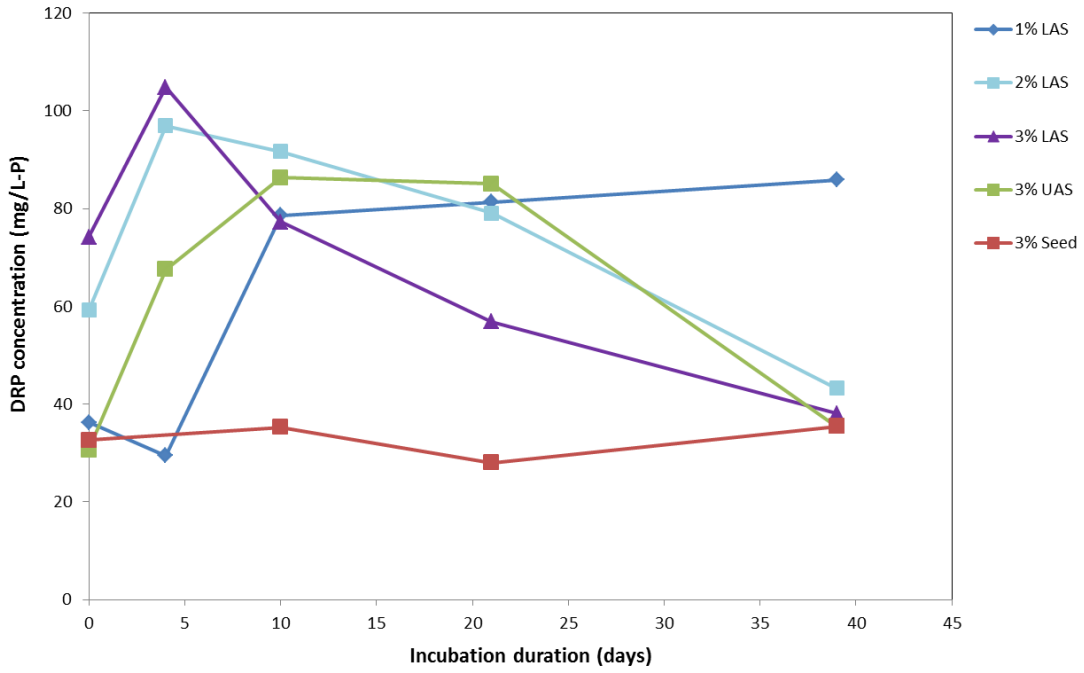


**Figure 39.** All lysed algae mixtures above 1% TS decreased in DRP concentration after 10 days of incubation. Contrary to patterns seen in previous experiments, this represented an anomalous result. The 1% lysed algae appeared to follow the same kinetics as previous experiments, except for the period between Day 0 and 5. The higher percent solids mixtures appeared to have undergone phosphorus precipitation.

In addition to having noticed a sharp decline in the DRP concentration in all samples above 1% TS, small visible clusters were noticed in the bottom of the digester bottles. These white clusters were never seen in any of the other experiments, so their presence was recorded. Additionally, these formations were not present in the beginning of the homogenization digestion and were speculated to have some amount of phosphorus

content “trapped” or “bound” up in the clusters. Images of the clusters can be seen in **Figure 40**.

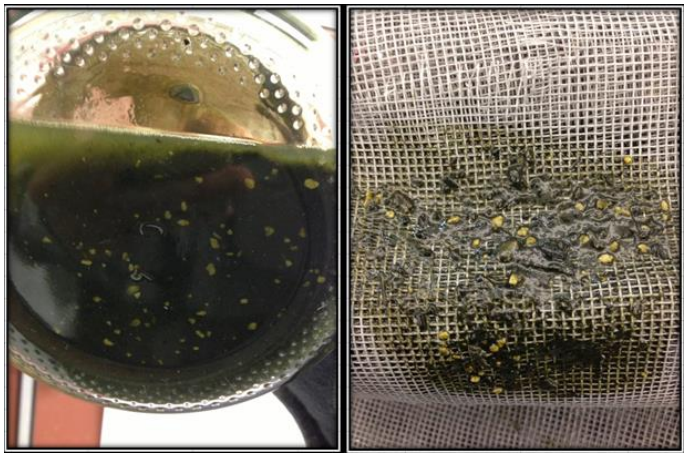

**Figure 40.** Settled white clusters present in the bottom of the 2-L bottle of the 2% TS mixture (left). Close up image of the formations (right). The digestate was sieved through a 1mm mesh metal screen and rinsed with DI water to clearly reveal the white formations.

The white clusters were selectively removed from the mesh and placed on a glass fiber filter to dry. At this point, the texture of the clusters was comparable to wet porcelain clay. After allowing the sample to air dry for several hours, the filter was then placed in a 50-mL vial, and stored overnight in the refrigerator at 3.5°C. The following day the clusters were examined using a dissecting microscope (Fostec, LR92240) and recorded (**Figure 41**).

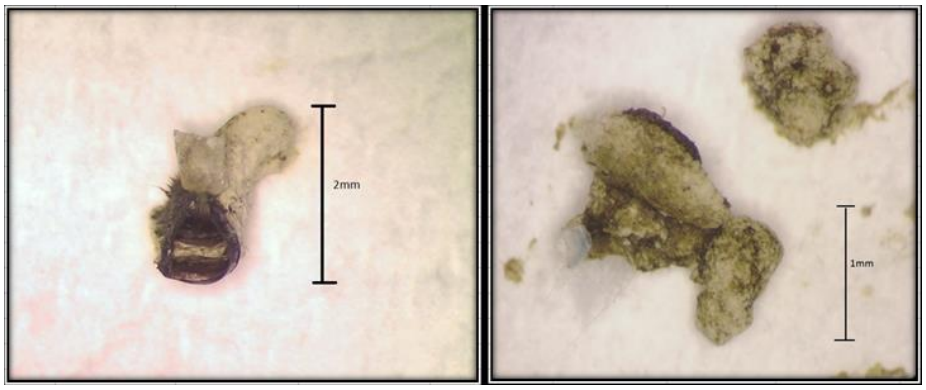


**Figure 41.** Upon closer examination, it was apparent that the white clusters were indeed crystals. The rectangle in the center of the photo on the left was the most translucent and clearly defined crystal of all of the samples examined.

In order to confirm the presence of phosphorus in the crystals, an ancillary experiment was conducted. First, 5.0 mg of refrigerator dried, crystals were diluted in 3 mL of DI water and 1 drop of 99% sulfuric acid. This solution was mixed for 5 minutes using a glass stir rod. After the crystals dissolved, small particulate matter appeared which resembled algal cell mass. Those clusters may have served as the nucleation site for phosphorus crystal formation. In order to remain consistent with other DRP tests, this 3 mL sample was filtered using a 0.45-µm syringe filter to remove the particulate matter. Next, two different volumes of were run in the ascorbic acid DRP test (APHA 4500-P E.). A calibrated micropipette was used to pull two different volumes of the acidified suspension and analyze the DRP concentration. Volumes of 0.5 mL and 0.1 mL were diluted into a 50-mL volumetric flask and filled using DI water. Although the actual percent of phosphorus in the crystals is still unknown, it is evident that the crystals themselves do contain a phosphorus component (**Table 25**).

**Table 25.** Comparison of two different dilutions of the precipitate suspensions. The average of the set was 33.0 mg/L and the standard deviation was 3.1 mg/L. The concentration in the right column is the actual concentration of resolubilized phosphorus in the undiluted 3 mL mixture. The crystals were not completely void of moisture so the wt/wt P content is unknown.

| Sample | Sample Volume (mL) | Dilution Factor | Concentration (mg/L) |
|---|---|---|---|
| Phosphorus Precipitate (small dilution) | 0.5 | 100 | 36 |
| Phosphorus Precipitate (large dilution) | 0.1 | 500 | 30 |

Precipitation of phosphorus from the high pressure homogenization experiment prevented the collection of phosphorus redissolution data and model creation for this treatment. However, the data that were collected can be used to further understand why precipitation occurred and how the controlling mechanism can be manipulated to take advantage of harvesting phosphorus at the end of anaerobic digestion (Keymer, 2013).

### *4.1.8 Potassium Solubilization*

Potassium analyses were conducted only on the boiled algae experiment and only on the initial and final samples, in order to indicate the ultimate release of potassium (**Table 26).**

**Table 26.** Overview of initial and final potassium values for Experiment 5 (Boiled).

| Sample | Soluble potassium | Particulate potassium | Total potassium | Average Total potassium | % difference (Initial and final total potassium) | % of total that is soluble |
|---|---|---|---|---|---|---|
| Day 0 UAS | 61 | 58 | 120 | 119 | 2% | 51% |
| Day 43 UAS | 98 | 21 | 118 | -- | -- | 82% |
| Day 0, 0-BAS | 115 | 10.5 | 130 | 125.5 | 7% | 92% |
| Day 43, 0-BAS | 109 | 16.5 | 121 | -- | -- | 87% |
| Day 0, 30-BAS | 107 | 15 | 124 | 122 | 3% | 88% |
| Day 43, 30-BAS | 109 | 13 | 120 | -- | -- | 89% |
| Day 0 Seed | 146 | 20 | 157 | 166 | 11% | 88% |
| Day 43 Seed | 145 | 21 | 175 | -- | -- | 87% |

The total values remained relatively unchanged from beginning to end, as expected. The most significant result is the apparent release of soluble potassium during boiling, as indicated by the lower soluble potassium in the untreated control (UAS) compared to the treated samples (0-BAS, 30-BAS). One hypothesis for the increase in soluble potassium of the treated samples was thickening of the biomass during heat treatment. This however, was not the case, and the biomass concentration remained at a steady 3% TS during the entire process.

Despite an initial difference in soluble potassium between the control and the treated samples, after 43 days of digestion, the concentration of soluble potassium nearly equalized between them. As with phosphorus, potassium release appears to be about equal in the long-run despite early differences apparently caused by pretreatment.

### 4.2 Descriptive Modeling

Mathematical models have been created to predict the outcome of anaerobic digestion of certain substrates, and simple empirical models of ammonia concentration in semi-continuous algae digesters exist (Spierling, 2011). However, the solubilization rate constants for nitrogen and phosphorus released from batch anaerobically digested microalgae have not been found in the literature. In an attempt to descriptively model batch nutrient solubilization, the following first order equation was assumed.

**Equation 12.** First Order Model

$$C = C_{initial} + S(1 - e^{-kt})$$

C = normalized concentration (mg DRP or TAN/g $VS_{initial}$) at time t

$C_{initial}$ = initial concentration at time t = 0 (mg DRP or TAN/g $VS_{initital}$)

S = difference between $C_{t=\infty}$ and $C_{initial}$ (mg DRP or TAN/g $VS_{inititial}$)

k = rate constant ($day^{-1}$)

t = time (days)

Plateau = maximum possible release at $C_{t=\infty}$ (mg DRP or TAN/g $VS_{inititial}$)

This equation describes pseudo-first order kinetics of the diminishing nitrogen and phosphorus solubilization rate as the digestion progresses and the concentration approaches the maximum release possible.

This equation was fitted to time series TAN and DRP data for sonication, autoclaved, and boiled pretreatments. Homogenization data for TAN and DRP were incomplete and omitted in the following models. In each case, the models were fitted to the data by minimizing the objective function, which was set as the residual sum of squares.

### *4.2.1 TAN Model Generation*

All of the following parameter fits were generated using Graphpad PRISM. The TAN concentrations measured during each experiment were normalized by dividing the measured TAN concentration by the initial volatile solids concentration. This normalization of TAN concentration on a cell mass basis allowed for model result comparisons between experiments with different initial organic concentrations (**Figure 42-44** and **Table 27**).

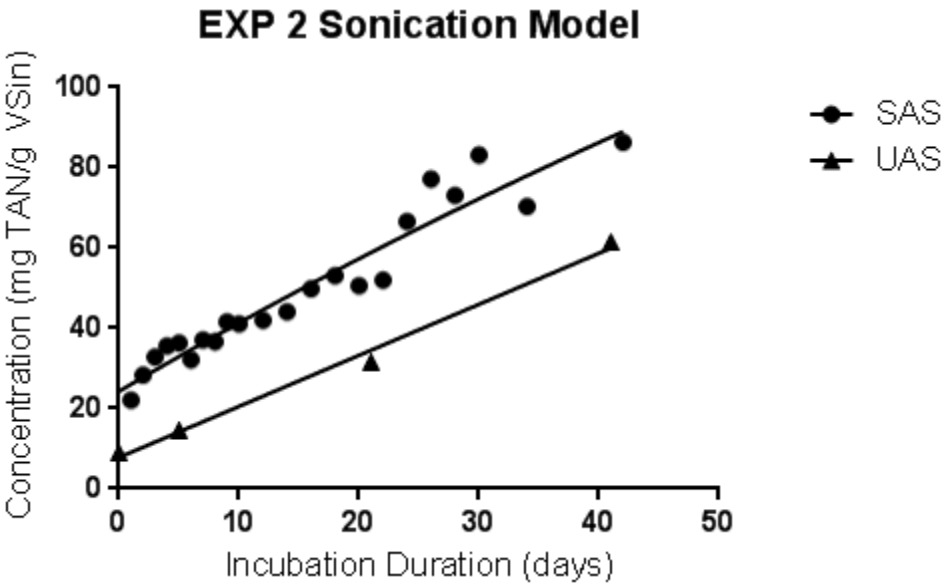


**Figure 42.** Model for TAN release in the sonication experiment. Both treated (SAS) and untreated (UAS) substrates appear to follow linear kinetics.

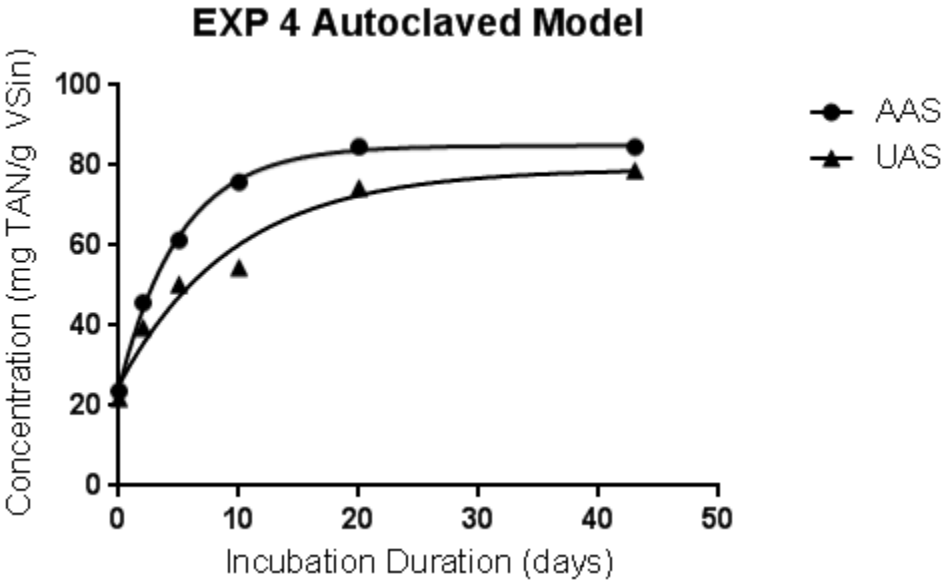


**Figure 43.** Model for TAN release in the autoclaved experiment. Both treated and untreated exhibit a similar release pattern.

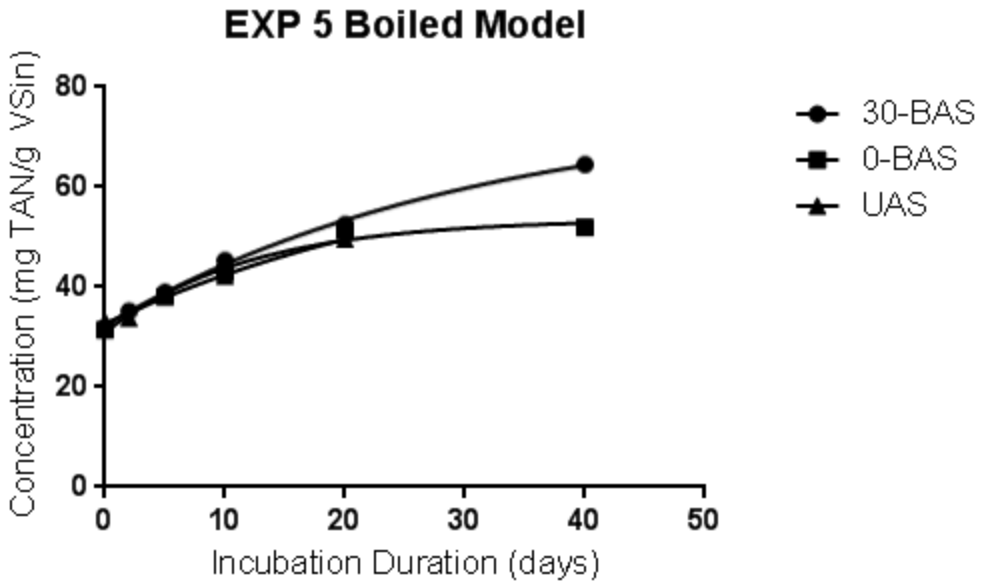


**Figure 44.** Model for TAN release in the boiled experiment. Similar to the autoclaved experiment, both treated and untreated mixtures share similar release patterns. The untreated sample (UAS) only extends to Day 20 due to the exhaustion of Day 43 sample volume.

**Table 27.** Overview of model parameters and outputs for TAN. All variables refer to **Equation 12**. No results are available from Experiment 3 due to ammonia electrode malfunctions.

| Experiment | Sample | $C_{initial}$ | Plateau | S | k | $R^2$ | Residual Sum of Squares |
|---|---|---|---|---|---|---|---|
| **2-Sonication** | Untreated Algae + Seed (UAS) | 7.87 | ~467226 | ~467218 | 2.72E-06 | 0.993 | 12.49 |
| | Sonicated Algae + Seed (SAS) | 24.34 | 293.3 | 269 | 0.0066 | 0.926 | 533.3 |
| **3- High Pressure Homogenization** | 3% TS Unntreated Algae + Seed (3%UAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 3% TS Lysed Algae + Seed (3%LAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 2% TS Lysed Algae + Seed (2%LAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 1% TS Lysed Algae + Seed (1%LAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| **4-Autoclaved** | Untreated Algae + Seed (UAS) | 25.22 | 79.17 | 53.96 | 0.105 | 0.968 | 73.7 |
| | Autoclaved Algae + Seed (AAS) | 24.41 | 84.95 | 60.54 | 0.197 | 0.998 | 5.541 |
| **5- Boiled** | Untreated Algae + Seed (UAS) | 32.69 | 68.61 | 35.91 | 0.032 | 0.988 | 2.287 |
| | 0 min Boiled Algae + Seed (0-BAS) | 31.0 | 53.65 | 22.64 | 0.084 | 0.980 | 6.204 |
| | 30 min Boiled Algae + Seed (30-BAS) | 32.3 | 76.18 | 43.87 | 0.033 | 0.998 | 1.598 |

The nutrient release information most relevant to nutrient recycling in algae production is the ultimate extent of nutrient resolubilization and the rate of resolubilization. The ultimate nitrogen resolubilization for the various pretreatments and digestion are provided

in the tables in Section 4.1.6 above. The first-order rate constants for nitrogen solubilization by the untreated controls (**Table 27)** ranged widely: 2.7E-06, 0.11, and 0.032 per day. For the pretreated algae, the range was 0.0066 to 0.20 per day, with the autoclaved having the highest rate constant. Thus, although most of the nitrogen kinetic results are consistent within experiments, across experiments, the results vary widely. These inconsistent results may be due to the different biomass and seed used in each experiment.

### *4.2.2 DRP Model Generation*

First-order kinetic parameters were determined for DRP release by following the same procedures as was used above for nitrogen (**Figures 45-47** and **Table 28**).

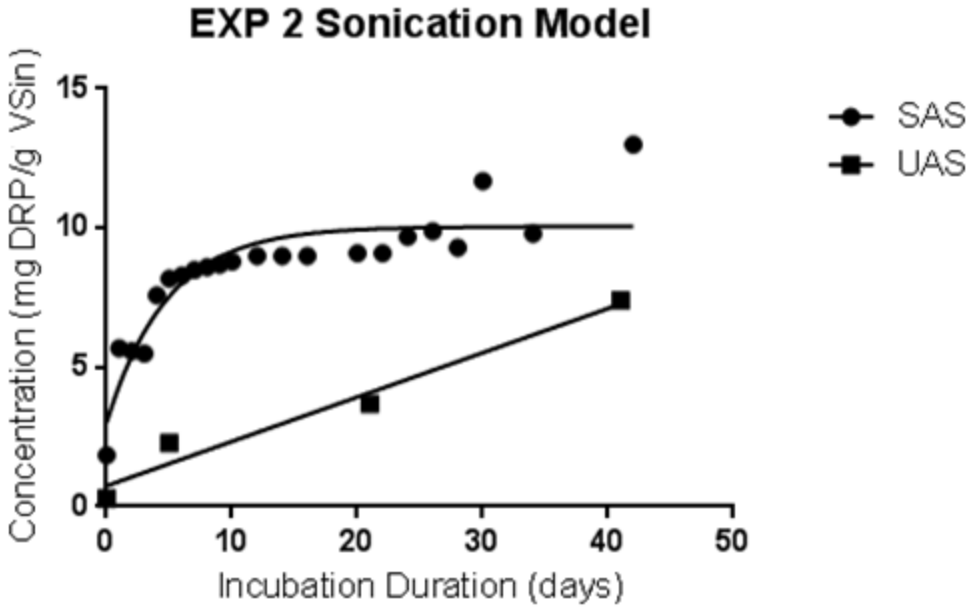


**Figure 45.** Model for DRP release in the sonication experiment. The release rates of the treated and untreated were dissimilar. The Day 30 and 42 points for the sonicated mixture appear to be outliers and their divergence from the normal release pattern is identified in the model's selective exclusion of them.

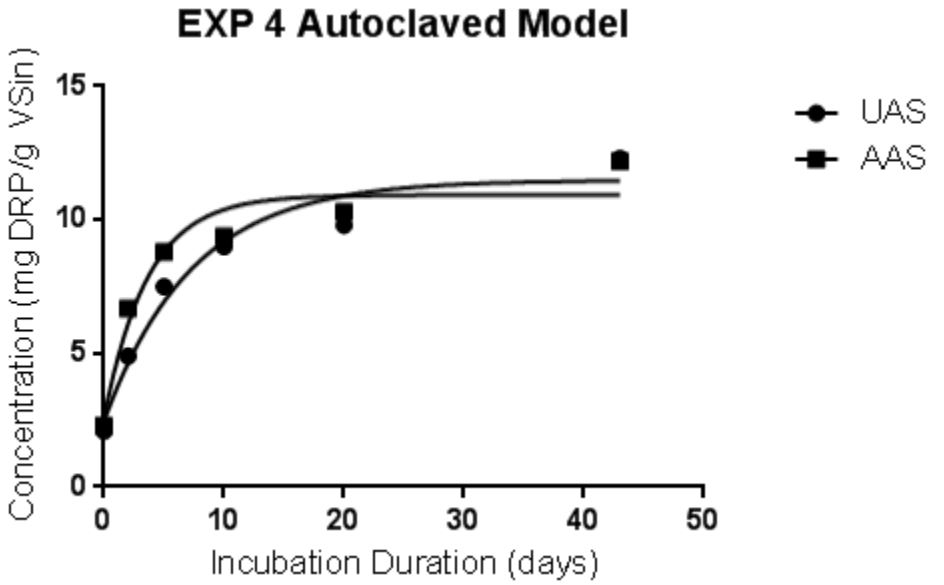


**Figure 46.** Model for DRP release for the autoclaved experiment. The untreated and autoclaved results mimic each other closely, as was seen in the TAN model for the same experiment (**Figure 43**).

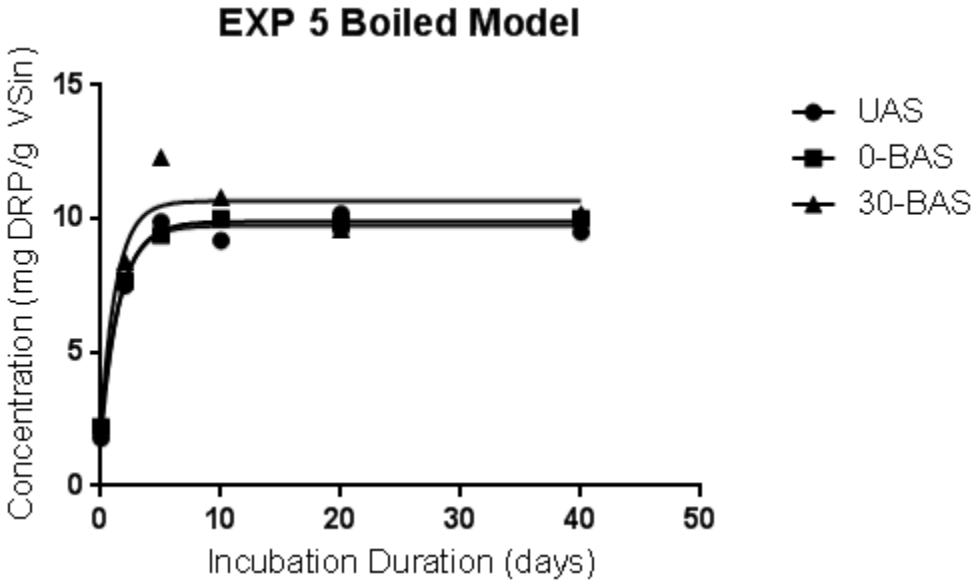


**Figure 47.** Model for DRP release for the boiled experiment. All three algae slurries closely match, as was seen in the TAN model for the same experiment (**Figure 44**).

**Table 28.** Overview of model parameters and outputs for DRP release, as determined by the Prism software. All variables refer to **Equation 12**. Limited results are shown for Experiment 3 due to phosphorus precipitation.

| Experiment | Sample | $C_{initial}$ | Plateau | S | k | $R^2$ | Residual Sum of Squares |
|---|---|---|---|---|---|---|---|
| **2-Sonication** | Untreated Algae + Seed (UAS) | 0.76 | ~1993 | ~1992 | ~8.003E-005 | 0.965 | 0.94 |
| | Sonicated Algae + Seed (SAS) | 3.03 | 10.06 | 7.03 | 0.207 | 0.816 | 19.88 |
| **3- High Pressure Homogenization** | 3% TS Unntreated Algae + Seed (3%UAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 3% TS Lysed Algae + Seed (3%LAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 2% TS Lysed Algae + Seed (2%LAS) | N/A | N/A | N/A | N/A | N/A | N/A |
| | 1% TS Lysed Algae + Seed (1%LAS) | 2.94 | 9.46 | 6.22 | 0.091 | 0.809 | 5.80 |
| **4-Autoclaved** | Untreated Algae + Seed (UAS) | 2.47 | 11.49 | 9.02 | 0.137 | 0.963 | 2.48 |
| | Autoclaved Algae + Seed (AAS) | 2.58 | 10.93 | 8.35 | 0.273 | 0.943 | 3.38 |
| **5- Boiled** | Untreated Algae + Seed (UAS) | 1.78 | 9.75 | 7.97 | 0.665 | 0.985 | 0.77 |
| | 0 min Boiled Algae + Seed (0-BAS) | 2.21 | 9.91 | 7.70 | 0.613 | 0.999 | 0.06 |
| | 30 min Boiled Algae + Seed (30-BAS) | 2.34 | 10.67 | 8.33 | 0.763 | 0.918 | 4.89 |

The ultimate phosphorus resolubilization for the various pretreatments and digestion are provided in the tables in Section 4.1.7 above. The first-order rate constants for phosphorus solubilization by the untreated controls (**Table 28)** ranged widely: 8.0E-05, 0.14, and 0.67 per day. For the pretreated algae, the range was 0.091 to 0.76 per day, with the 30-minute boiled having the highest rate constant. As with the nitrogen resolubilization, these inconsistent results may be due to the different biomass and seed used in each experiment.

# 5 Conclusions

The following section will attempt to answer the research questions posed in Section 1.

## 5.1 Pretreatment Effect on Specific Methane Yield

In most cases pretreatment led to a higher biogas production and therefore methane yield. A maximum percent increase in methane yield over the control for sonication, homogenization and boiling, measured 36%, 15% and 15% respectively. Autoclaving the algal biomass had a negative effect on the cumulative methane production as compared to the untreated control (-12%) and may be speculated to be due to toxic compound formation (Ledl, 1990).

After an adaptation and growth phase of approximately ten days, the daily methane content stabilized between 60-70% for each experiment, with the overall "average % methane of the total biogas" equal to 61± 2%. Sonication of the algae slurry for 10 minutes returned two different methane yields; the first trial was 0.276 L $CH_4$/ g $VS_{IN}$, while the second trial was 0.315 L $CH_4$/ g $VS_{IN}$. The difference is possibly due to the different harvesting methods and degrees of "freshness" of the algae. The experimental run that saw a higher methane yield was conducted using fresh algae (as compared to tube settler harvested), and it was shown that biomass spoilage can increase sCOD loss and result in a reduction in methane production potential (**Appendix B)**.

Overall, pretreatment did not appear to be energetically favorable due to the high amount of input energy required and net negative output energy compared to the control.

## 5.2 Pretreatment Effect on Nutrient Solubilization

One of the major goals of this research was to quantify the effect of four distinct pretreatment methods on the rate and extent of NPK solubilization. The result of those efforts is detailed in the following sections. In each case the seed was factored into the overall degree of solubilization, thereby creating a release for "algae + seed" as opposed to just algae alone.

### *5.2.1 Nitrogen Solubilization*

Every pretreatment technology examined in this study increased the final TAN concentration when compared to the untreated control. The range of ultimate TAN solubility was between 50-60% of total TKN for homogenization, autoclaving and boiling. The sonication experiment led to exceedingly high ultimate TAN values, which were much higher than the other pretreatments. The untreated slurry TAN solubilization was 94% of TKN, while the treated was 86% of TKN, both of which would need to be reconfirmed.

A nitrogen mass balance compared the initial and final TKN concentrations for each mixture within each experiment. Each experiment yielded a reasonably small percent difference between initial and final values except for the sonication experiment (**Table 19**).

### *5.2.2 Phosphorus Solubilization*

Pretreatment of the algal biomass led to an increase in the DRP release on the initial day, although the overall effectiveness of phosphorus solubilization varied across the different pretreatment technologies. Autoclaving performed the worst at improving the ultimate

phosphorus solubility and had a lower final "% of TP that was DRP" compared to the untreated; 46% and 43% respectively. In general ultimate phosphorus solubilization ranged between 40-50% of total TP. The pattern of release was similar in most cases except for the high pressure homogenization experiment because of the DRP losses due to precipitation.

### *5.2.3 Potassium Solubilization*

The amount of potassium data that were collected limits any major conclusions. The single most important finding was that the by the end of digestion, soluble potassium equalized regardless of whether the algae slurry was pretreated (boiled) or not.

## 5.3 Model Creation

The descriptive models that were generated seem to fit the data relatively well with R squared values between 0.81 and 0.99. Unfortunately, the kinetic rate constants were wide ranging for the untreated control across experiments, and cast doubt on the values. It would be expected that the rate constant for the untreated control would be similar across all four experiments; however, that was not the case. The k value for TAN solubilization of the control was 2.7E-06, 0.105 and 0.032 for sonication, autoclaving and boiling respectively. Similarly, the k value for DRP solubilization was 8E-005, 0.137 and 0.665 for the same mixtures respectively.

No model was created for either TAN or DRP solubilization in the high pressure homogenization experiment. A malfunctioning TAN electrode led to exhaustion of all of the sample volume, while phosphorus precipitation convoluted the DRP data.

### 5.4 Limitations of the Study

All of the experiments were run in batch mode, and these results are unlikely to represent semi-continuous or continuously-fed digesters (Qamaruz-Zaman, 2010), (Spierling, 2011). A bench scale or pilot-scale semi-continuous digester would be able to determine the optimal loading rate, solids retention time, C:N and other operational parameters that are involved in operating a successful anaerobic digester at pilot scale.

The small number of digesters that were sampled for each analytical test (n=1) prevented the possibility of any rigorous statistical analysis. The data that were collected were successfully related to other similar studies; however, the limited sample size precludes any autonomous conformation of the results. An expanded sampling plan would provide sufficient replicate values that would more accurately quantify each nutrient and how it changes throughout the course of digestion.

The algae biomass that was tested in this study was not oil-extracted as conceptualized in **Figure 2**. Therefore, the implementation of sustainable secondary biofuel production (biomethane) using residual biomass as anaerobic digester feedstock was not tested.

### 5.5 Future Research

Valuable information was gathered from this study, however, the need to expand on the findings is outline below.

The algae biomass that was used in the nutrient characterization tests was collected on 7/9/2013, 9/16/2013, 11/14/2013 and 1/23/2014. The duration from the first to last experiment coincides with seasonal fluctuations (daily temperature averages, solar insolation, etc.) that affect the raceway algae population ecology. In order to procure a

reliable comparison of pretreatment effects on algae, identical biomass should be used for the pretreatment mixtures, untreated controls, and digester inoculum. One possible way of achieving this is to use frozen inoculum and biomass in addition to a separate control feed such as dried *Spirulina* for each experiment. Additional controls between experiments will ensure that collected data is laterally commensurate across all experiments.

Furthermore, knowledge of the various strains of algae in the outdoor raceways at the time of sampling, as well as the biochemical composition of the cells, would aid in overall methane yield predictions. The environmental conditions in which algae grow have a large effect on synthesis of proteins, lipids and carbohydrates, which in different ratios, affect the biogas production, ammonia release, etc. A more comprehensive log of both the algae growth conditions and cellular nutrients would aid in better understanding the pretreatment effectiveness of solubilizing NPK fractions.

To further the understanding of how pretreatment affects different algae slurries that are comprised of different resistant genera, a standardized, benchmark pretreatment might be useful. Even though sCOD was not accurate at predicting the methane yield of the autoclaved experiment, it may still be the best predictor of algae biodegradability.

Inhibition of the digesters may have diminished methane production and the extent of nutrient solubilization. The autoclaving experiment seemed to have clear signs of inhibition, but there was a lack of established laboratory protocol to determine the presence of any inhibitory substances. For future research, it would be helpful to identify inhibitory compounds and quantify the extent that they affect digestion.

Phosphorus precipitation in the high pressure homogenization experiment was unexpected and unusual. In no other experiments did white formations appear in the digesters, however, it is possible that crystal formation did occur but were invisible to the naked eye. For future experiments, it would be helpful to know the exact conditions that promote phosphorus precipitation, to prevent soluble phosphorus losses and/or potentially recover and reuse precipitated phosphorus fractions.

One of the major goals of this research was to quantify the release rates and ultimate concentration of nitrogen, phosphorus and potassium. The need to use a third party laboratory limited the number of samples tested for potassium. More frequent sampling and the establishment of in-house quantification methods would be useful in generating more potassium data.

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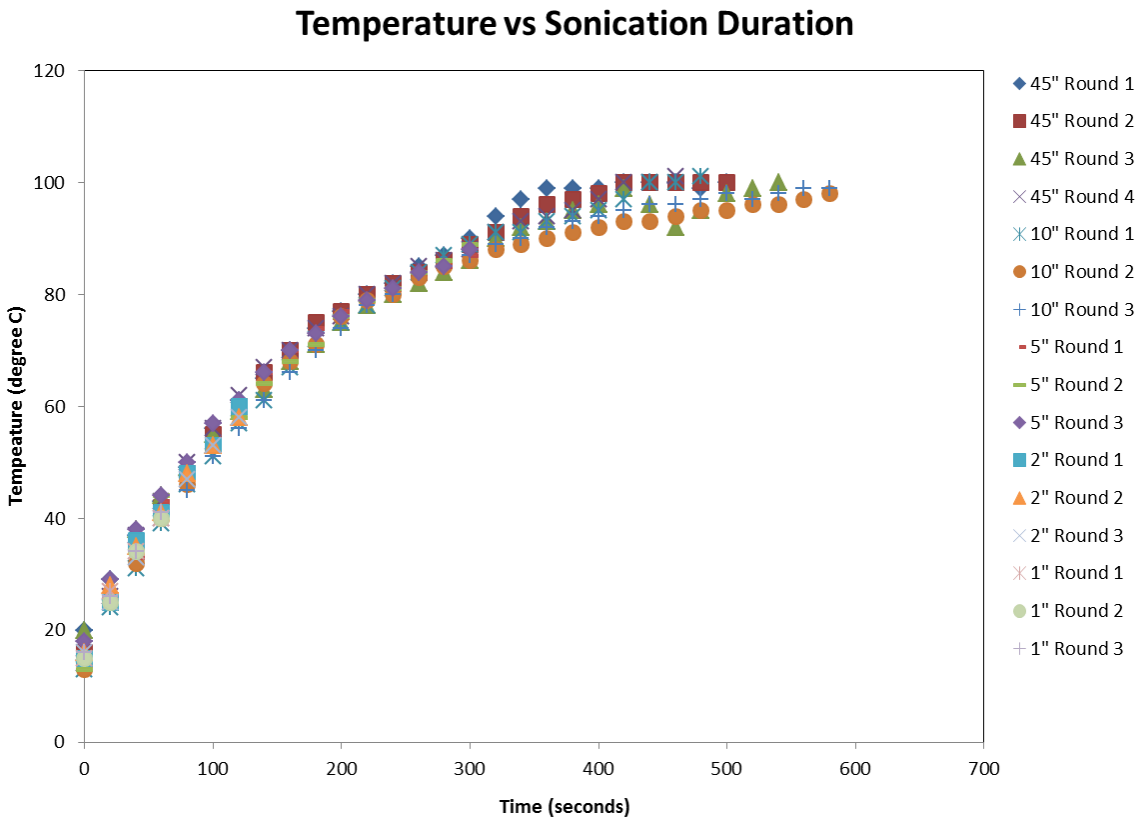
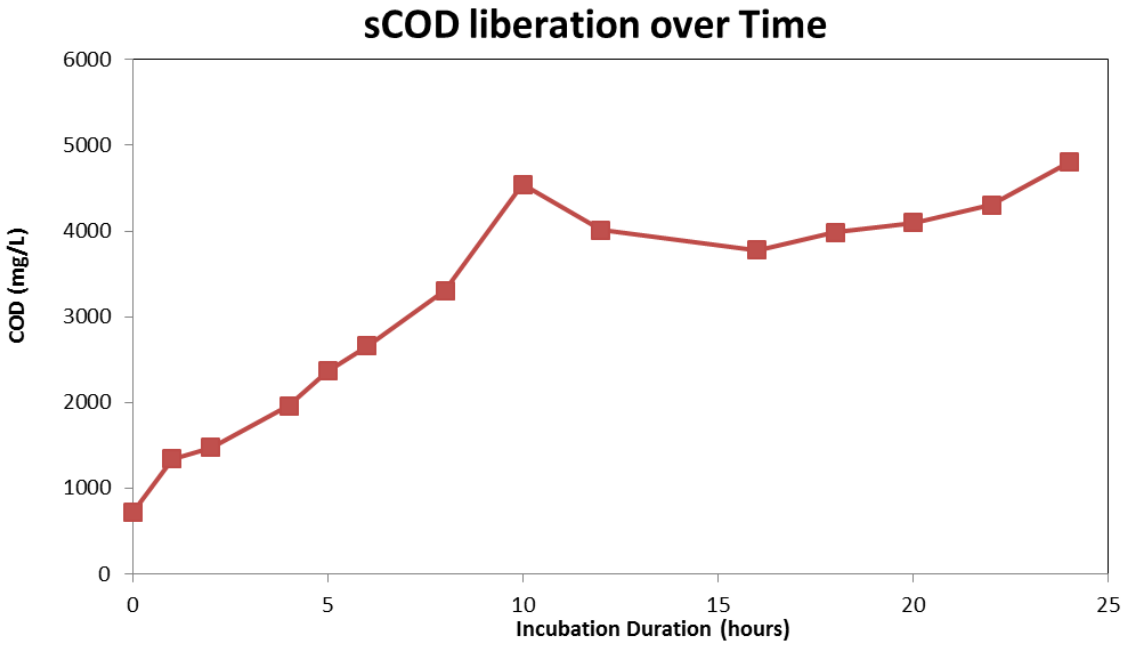
# 7 Appendices

## Appendix A Temperature Rise Sonication



## Appendix B COD Liberation Over Time

An auxiliary experiment was conducted on April 26, 2013 in order to determine the extent of soluble COD release when the algae slurry sat in the tube settlers between harvesting events. In order to ensure representative fresh algae were being tested, a continuous flow US centrifuge (US centrifuge Model M212) was used to harvest 3 L of actively photosynthesizing algae from pond 3 in the Alpha set. This slurry was diluted to the percent solids that typically come out of the tube settlers based on gravity separation (30 g/L), and placed in an insulated, double-walled stainless steel beaker. The beaker sat on the bench top at ambient temperatures and was unmixed. Over the course of a 24 hour period tCOD and sCOD samples were removed every 2 hours and preserved for testing later. The resultant sCOD time series data is shown below.



As illustrated in the graph above, the sCOD release increased from roughly 700mg/L up to 4,800 mg/L over the course of 24 hours. This amount of increase correlates to an increase of sCOD being 1% of the tCOD fraction to being just under 10%.