Spring 4-6-2017

REACTOR DESIGN AND OPERATION VARIABLES TO IMPROVE MIXED ALGAL BIOMASS PRODUCTIVITY AND SOLIDS SEPARATION: AN EXAMINATION OF OPERATION PARAMETERS, DENSITY, AND SETTLEABILITY

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by

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THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Civil Engineering

The University of New Mexico

Albuquerque, New Mexico

May 2017

ACKNOWLEDGEMENTS

I would like to thank Dr. Schuler, my advisor and committee chair, for his guidance and assistance in completing this study, and for his detailed teaching in both the classroom and in professional writing.

I also wish to thank committee members, Dr. Bixby and Dr. Cerrato, for their continued advice and recommendations throughout the duration of this study.

I want to express immense gratitude to New Mexico EPSCoR and UNM'S Center for Water and the Environment for funding this project and for covering tuition expenses in my masters program.

Lastly, I must thank my family, my friends, and lab peers for their seemingly limitless understanding and support of me and my aspirations.

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B.S., CIVIL ENGINEERING, THE UNIVERSTITY OF NEW MEXICO, 2014

ABSTRACT

Algae have been identified as a source of renewable, clean energy, with much research being devoted to identifying high energy yield algae, energy extraction methods, and reactor design with mono-cultures of algae, but little research has been done on reactor design and operational conditions with mixed cultures of algae to improve productivity and solids separation. The objective of this study was to determine if the settling characteristics of mixed algal cultures can be enriched or improved through manipulation of reactor design characteristics by incorporating cyclic settling or floating phases into operation, timing of light/dark cycles, and/or control of the solids and hydraulic residence time. Photobioreactors constructed of cast acrylic were operated as sequencing batch reactors. Each reactor was run with identical feed, light/dark cycle, and working volume. Biomass measurements were taken regularly to measure growth and productivity. Density measurements were taken to observe operation conditions effects on solids separation. The sludge volume index (SVI) was used to assess the degree of solids separation in each reactor. Selection for algal biomass with good settling or flotation

characteristics was assessed in experiment 1. In experiment 2 the effects of the light/dark cycle on settling and density were investigated. In experiment 3 the solids separation time, hydraulic retention time, and solids retention time were explored in regards to solids separation and biomass productivity. Experiment 4, the final experiment, investigated the effects of low and high carbon environments on solids separation, SVI, and biomass productivity. Results from these studies suggest that the light/dark cycle does not influence density or solids separation, low carbon environments are not an underlying mechanism for solids separation, and that good settling systems are more dependent on the species of algae, morphology and size of the settling flocs. Results from this study could be applied for practical purposes in solids separation.

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Chapter 1 Introduction

Energy is a critical resource for human development, but an increasing rate of its consumption is no longer compatible with the biophysical limits of the earth (Brown et al., 2014). In 2012, fossil fuels accounted for 78% of our energy use (Annual Energy Review, 2016), and those fuels are limited resources. Renewable energy sources such as wind, hydropower, and solar energy are increasingly being used to meet demands for electrical energy, but their potential for liquid fuel production is limited. This is a significant shortcoming, as liquid fuels are largely used to meet energy needs for transportation, which accounted for 28% of the total energy usage in the U.S. in 2012 (Annual Energy Review, 2016).

Algae have been identified as a potential renewable energy source due to their rapid growth rates, ability to accumulate energy rich lipids for conversion to biodiesel (Rajkumar, Yaakob, & Takriff, 2014), and their potential for conversion of total biomass to biofuels through processes such as hydrothermal liquefaction. Much algal biofuel research has focused on biodiesel production from algal monocultures, while less is known about mixed culture systems. One major challenge to commercial algal biofuel production is high energy use in solids separation and energy extraction (Lardon, Hélias, Sialve, Steyer, & Bernard, 2009). Little is known about how reactor design and operation affects biomass characteristics, and in particular solids separation.

a. Research Objective

The objective of this study was to determine if the settling characteristics of mixed algal cultures can be improved through manipulation of reactor design characteristics,

including incorporating cyclic settling or floating phases into operation, timing of light/dark cycles, and/or control of the solids and hydraulic residence time.

The experimental approach was to grow mixed algal cultures in bench-scale photobioreactors and operate them under different conditions to assess solids separation. This was achieved by running the reactors over four experiments; the specifics are addressed in Chapter 2, section C. By gaining a better understanding of the factors of algal biomass settling and by developing cultivation methods to improve settling, the algal biofuel production process should become more sustainable, cost effective and commercially viable.

Chapter 2 Background

a. Algal Biofuel Process Overview

i. Cultivation

Engineered systems for growing algae can be categorized as closed systems (such as tubular reactors) or open systems (such as raceway ponds). Design aspects that need to be addressed when designing photobioreactors include light distribution, aeration and mass transfer, and energy demand. Aeration is a major point of energy loss because pumping air, which often enriched with CO_2 is an energy intensive process. Improvement of efficiency could be achieved through larger inner surface areas and improved light distribution via microstructures (Posten, 2009). Other important parameters for cultivation include nutrient supply and pH control.

Tubular reactors come in different configurations: vertical, horizontal, helical, and alpha shaped (Figure 1). Each type of reactor has different considerations within the design phase. Vertical tube reactors (VTR) are low cost and easy to operate. Horizontal reactors allow for large working volumes. Helical reactors require a small land area for large working volumes but the pumps in the design hamper bio-production and these types of closed reactors cannot meet all the efficiency requirements needed to operate on a large scale (Carvalho, Meireles, & Malcata, 2006). Such requirements include proper degassing of oxygen, CO_2 addition, and proper light intensity. Improper degassing of oxygen has led to crashes in commercial scale devices, but there are no clear methods of addressing this issue, so further study is needed. Carbon addition in general is expensive but could be mitigated through devices that supply it in a non-continuous manner. Light intensity in large scale reactors could be an issue; light supplied by the sun is cheap but

cannot be controlled and light supplied artificially is expensive but controllable. Also, Carvalho et al. (2006) recommends future studies to select single cultures that have intrinsic characteristics that are desirable for biofuel production including a tendency to clump or settle and specific growth rates.

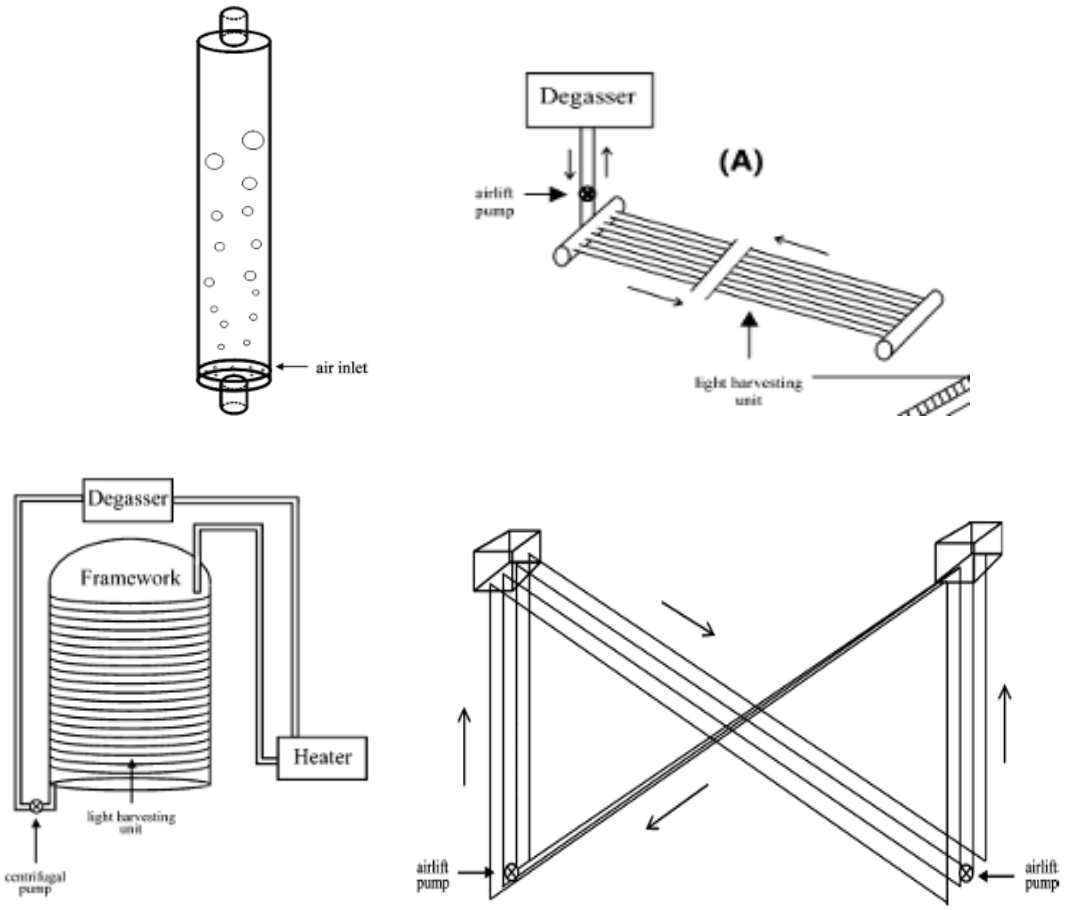

Figure 1: Types of tubular reactors. Clockwise, top left to right: vertical, horizontal, helical and alpha shaped (Carvalho et al., 2006). Each reactor type has its advantages and disadvantages.

Open systems are commonly designed using a racetrack configuration (Figure 2). An economic study (Amanor-Boadu, Pfromm, & Nelson, 2014) on a model racetrack system at steady-state demonstrated that it could produce 42.53 million gallons of biodiesel at

peak production per year based on 3289 hectares of production area. The authors indicate that further government support would be needed for algal biofuels to become a significant contributor to the biofuel supply because of a lack of existing policy support and the established advantage of the fossil fuel industry.

Open and closed systems that included mixed cultures grown in three different bioreactor systems: a raceway pond, a VTR and polybags, were compared in one study to evaluate potential for mass production of algae consortia (Chinnasamy, Bhatnagar, Claxton, & Das, 2010). The study was operated batch wise in a greenhouse from March 13, 2009 to May 15, 2009, with one raceway pond, three tubular reactors and three polybags. Light penetration was weakly correlated with biomass production, while temperature and pH were positively correlated with biomass production, and depth was negatively correlated with biomass production (Table 1). This study also found that mixed cultures in polybags had the potential to produce 13,144 kWhr, but the cost needs to be considerably lower to be viable (goal of 10 $/m^2 versus a calculated 26 $/m^2). The polybag system had the greatest biomass productivity (0.070 g/L/day) as compared to the raceway pond (0.057 g/L/day) and the VTR (0.044 g/L/day).

Table 1: Comparisons of parameters for a raceway pond, a vertical tubular reactor, and a polybag reactor operated by Chinnasamy et al. (2010). Volumes: raceway pond 950 L, VTR 100 L, and polybags 20 L. *P* values are statistical probabilities of correlation.

Parameter		Light	Temperature	pH	Depth[b]	Biomass
Light	r^2		0.548	0.370	−0.401	0.454
	P		0.065[a]	0.236[a]	0.196[a]	0.138[a]
Temperature	r^2			0.677	−0.862	0.790
	P			0.0156	0.0003	0.0022
pH	r^2				−0.609	0.926
	P				0.0357	0.00002
Depth	r^2					−0.836
	P					0.0007

a. No significant correlation between two variables if $P > 0.050$.
b. Light penetration depth perpendicular to largest surface area for raceways, VTR, and polybags.

Park, Craggs, and Shilton (2013), demonstrated the use of biomass recycling with a single loop raceway High Rate Algal Pond (HRAP) to select for a dominant species of algae with good harvesting characteristics (Figure 2). The HRAP used settled wastewater with a 1:1 dilution of tap water, and a hydraulic residence time (HRT) of 8 and 4 days in winter and summer respectively. The mean cell retention time for the recycling HRAP was between 4 and 9 days and between 4 and 13 days for the control HRAP, over the course of the experiment. CO_2 addition was used to maintain a pH below 8. The algae that settled in the bottom of the first settling cone were recycled back to the HRAP every 24 hours, while the control had no recycle.

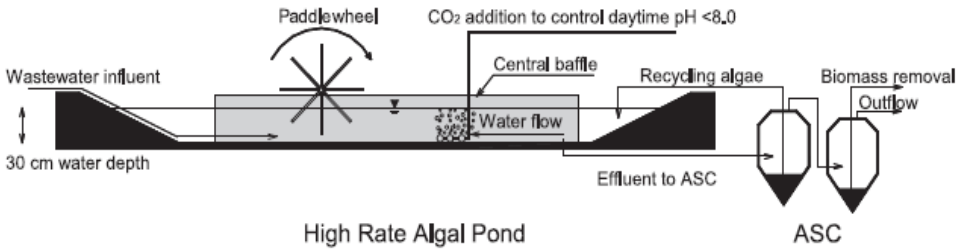

Figure 2: Single loop raceway HRAP (Park et al., 2013). A common method for growing on an industrial scale. However, this type of system is vulnerable to predation and introduction of foreign objects.

The authors conclude that using a recycle system selected for a species of algae (*Pediastrum boryanum*), which dominated 90% by biovolume of the total community. The use of biomass recycling resulted in a 66% increase in net biomass energy as compared to no recycling. Both the recycling HRAP and the control had similar productivities (between 3 and 12 $g/m^2/day$), but recycling the algae resulted in more biomass energy. This occurred via better biomass harvest efficiency, which is the percentage of biomass (volatile suspended solids or VSS) removed in an algal settling cone (ASC). The relative dominance and species of algae in the reactors were determined by biovolume and microscope images. However, this system was not free from issues, as it was dominated by one species of algae which showed vulnerability to zooplankton grazing, resulting in crashes. This was one of the few studies of algae to determine if reactor design parameters affect settling.

ii. Harvesting Methods

Harvesting of algae is a critical and problematic step in the production of algal biofuels. Harvesting is commonly performed with or without the addition of chemical or biological coagulants by settling, centrifugation, and biofilm scraping.

ii a. Fundamentals of settling

Reactor operation and design can be employed to improve solids separation. Biomass settling is a major concern in water and wastewater treatment, and much research has been devoted to understanding the factors affecting it. Settling of biomass is a complex process, and has been described as occurring in four categories: Type I, Type II, Type III, and Type IV (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). Type I settling takes place when particles settle without influencing other particles. Type II characterizes flocculent settling, where particles can adhere to one another. Type III covers zone settling, where particles form a blanket that sweeps and overtakes other particles, as found in many sedimentation basins. Type IV involves compressed settling, where water is displaced from pores in the settled sludge as more particles settle and compress.

Increases in algae settling velocity were correlated with volume and morphology but not density (Choi, Lee, Kwon, & Cho, 2016). However, Anderson and Sweeney (1977) reported that changes in algal settling velocity were controlled by short term physiological (via ion channels) changes and not morphology changes.

ii b. Activated sludge

The biological process known as activated sludge is commonly used in wastewater treatment trains for removal of nitrogen, phosphorus, and organic constituents. The principles of activated sludge can be applied to algal remediation set-ups. Activated sludge includes one or more bioreactors (aeration tank in Figure 3), a settling basin (secondary clarifier), recycling of settled biosolids to the bioreactor, and wasting of a portion of settled biomass. Process design must take into consideration solids retention

time, sludge production, settling characteristics of biosolids, oxygen requirements and nutrient requirements (i.e. the food to micro-organism ratio).

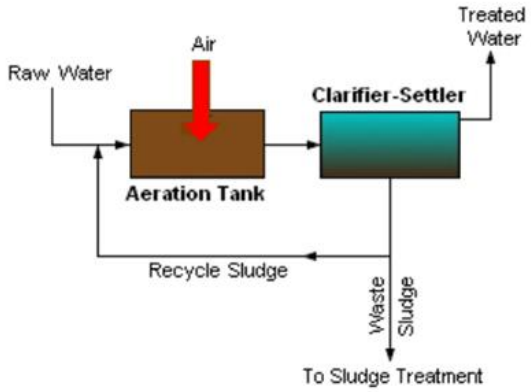

Figure 3: Basic schematic for activated sludge systems (Tchobanoglous, Burton, & Stensel, 2003). The principles of activated sludge systems can be applied to algal systems to improve remediation.

Activated sludge is considered a cost-effective, environmentally acceptable, and mature technology for the removal of wastewater constituents (Tchobanoglous et al., 2003). The settleability of biomass is greatly enhanced by including settling in the process stream, with recycle of biomass to the bioreactor, as this selects for bacteria that form well-settling flocs, while poorly settling biomass is washed out of the system. Nevertheless, settling is often problematic in activated sludge systems and much research has been devoted to improving settling rates.

ii c. Coagulation and Flocculation

The treatment method known as coagulation and flocculation is widely used in drinking water and wastewater treatment plants to improve removal of solids by settling. Coagulation is the process in which the surfaces of charged particles are neutralized for processing by flocculation. Flocculation is the aggregation of the particles that are then

removed by sedimentation. Ferric chloride and alum are two commonly used coagulation reagents. However, major issues with these reagents usage include their costs, pH sensitivity, and their incorporation of large concentrations of metals in the sludge blanket (Lee, Robinson, & Chong, 2014). Use of organic flocculants is of interest because of their biodegradability and effectiveness (90% removal of solids after sedimentation), however existing options may be prohibitively expensive (Lee et al., 2014).

ii d. Bio-flocculation with bacteria

The use of bacteria to assist in flocculation of algae was assessed by Manheim and Nelson (2013). This study investigated the settleability of two species of algae, *Scenedesmus* sp. and *Calluna vulgaris*, with and without the bacteria *Burkholderia cepacia*. The algae *Scenedesmus* sp. and *Burkholderia cepacia* were grown in separate reactors. *Scenedesmus* sp. grown alone exhibited an improved 2-hour period settling over 15 days of batch growth (Figure 4). When *B. cepacia* was added to the *Scenedesmus* sp. cultures, settleability improved at earlier and later growth phases (Figure 5). Although, the authors did not include any analyses of statistical significance, as the error bars do not suggest any significance. By contrast, the settling of *Calluna vulgaris* with the same bacterium only improved slightly, suggesting that bacterial effects may be algae-specific.

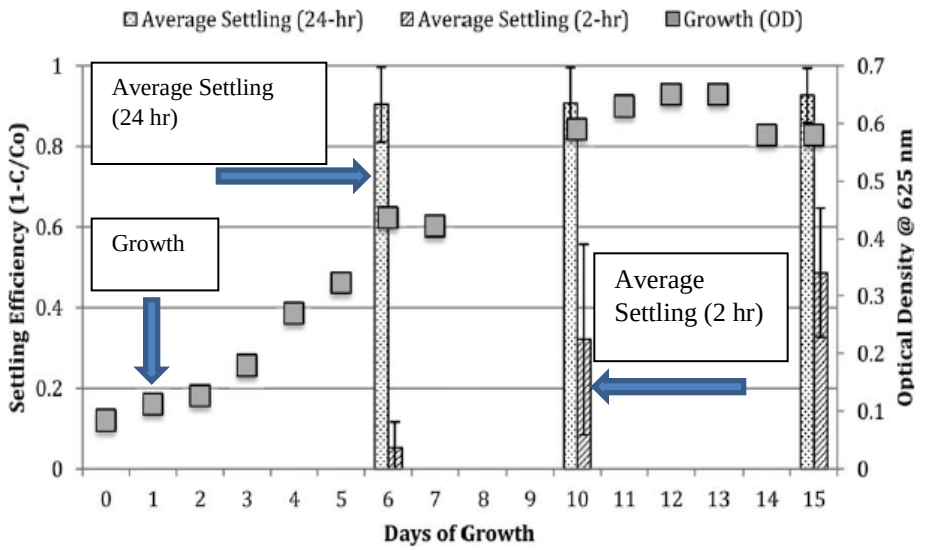

Figure 4: Settling efficiency of *Scenedesmus sp.* over time. C is average cell concentration and C_o is unsettled cell counts. *Scenedesmus sp.* showed improvement in 2-hour settling efficiency (Manheim et al., 2013).

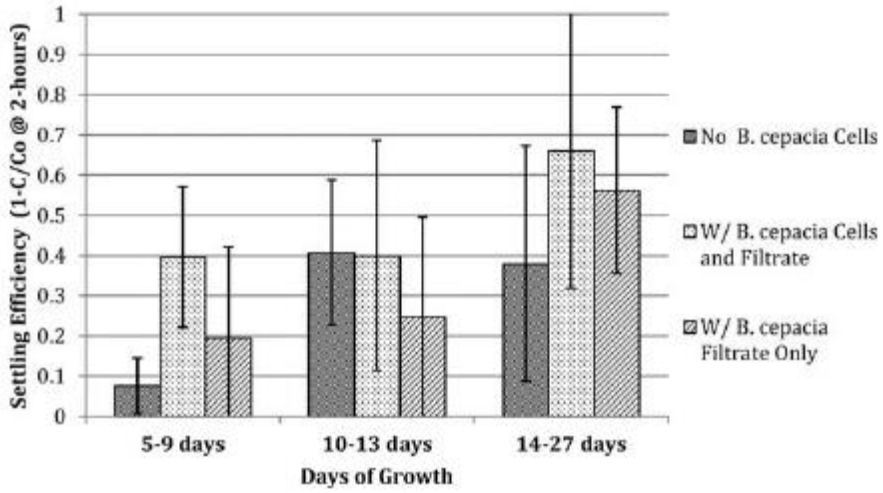

Figure 5: Settling efficiency of *Scenedesmus sp.* with bacteria *B. cepacia* in 2-hour settling tests. The large error bars are not discussed by the authors (Manheim et al., 2013).

The authors conclude that more research needs to be conducted on physical factors controlling settling like algal species, HRT's, varying bioflocculant dosage and type, and mixing times.

iii. Extraction of oil and conversion to biofuel

The first step in oil extraction is dewatering which is accomplished by expeller pressing, except with hydrothermal liquefaction (HTL), followed by extraction. Extraction methods include HTL, ultrasound subjugation, and solvent mixing. HTL extracts oils by exposing the biomass to high temperature and pressures, upwards of 360°C and 10 MPa respectively, followed by hydrotreating. HTL is of interest because this technology includes whole use of algae, no dewatering, and it is not necessary to promote or extract lipids, which are all considered advantages. Ultrasonic techniques use soundwaves and solvents, and reportedly have higher oil yields than conventional methods (upwards of 500%) (Suali & Sarbatly, 2012).

Extracted lipids can be converted to biodiesel through the chemical process of transesterification (Figure 6). Other methods include pyrolysis, liquefaction, gasification and hydrogenation (Suali & Sarbatly, 2012).

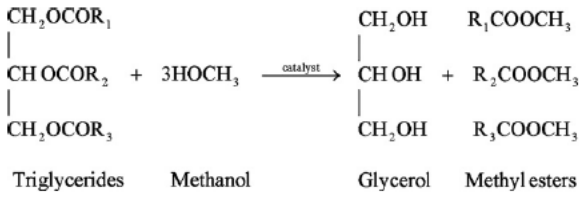

Figure 6: Transesterification of triglycerides. The methanol attacks the carbonyl group on the triglyceride, replacing the R-group with a hydroxide group (Suali & Sarbatly, 2012).

Algal Metabolism

b. Algal Metabolism

i. Lipids as an Energy Source

Triglycerides are lipids that are commonly used as an energy storage by plants and mammals. The energy content of triglycerides is approximately 9 kcal/g. They are

formed by the condensation of glycerol with three long carbon chain fatty acids (carboxylic acids). The fatty acids in a triglyceride are known as fatty acid residues and control the structure, physical properties, and energy storage of the triglyceride (Wade, 2006). The energy inherent in lipids is of vital importance to the cultivation of algae as an energy source, and lipids convertibility to biodiesel provides advantages over other biofuels such as corn-based ethanol (Lardon et al., 2009).

i. Photosynthesis

Biofuel research and production has focused on algae because of their ability to harness light energy for the synthesis of energy rich compounds like lipids and carbohydrates (Lardon et al., 2009). Photosynthesis is the process where plants use light energy to convert CO_2 to carbohydrates. Algae use oxygenic photosynthesis, which uses water as the reductant. The net chemical reaction for photosynthesis can be found in the below equation, where *A* is either oxygen or sulfur depending on the specific photosynthetic agent (McMurry & Begley, 2005).

$$\boldsymbol{CO_2 + 2H_2A + light \rightarrow (CH_2O)_n + 2A + H_2O}$$

Equation 1: Net reaction for photosynthesis (McMurry & Begley, 2005).

Oxygenic photosynthesis is endergonic in nature because the product glucose has higher free energy than the chemical reactants. Photosynthesis is localized in the chloroplasts, and is divided into two systems: photosystem 1 (PSI) and photosystem 2 (PSII). PSI is the site of light capture reactions and PSII is the site of carbon reduction (fixation of CO_2) reactions.

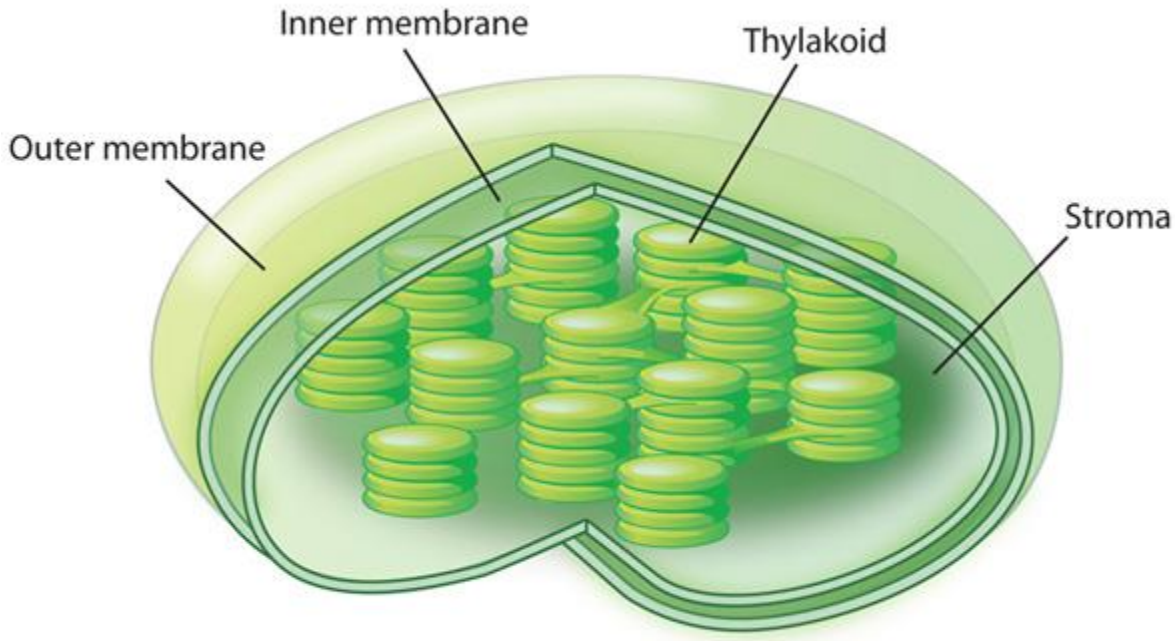

Figure 7: Chloroplast; the center of light reactions in the algal cell. The double membrane ensures tight regulation of the inside of the structure. The thylakoid is where the light reactions take place and the stroma is the open space within the organelle. (Structure of a chloroplast, 2010)

PSII splits water and electrons are transferred via an electron-transport train to PSI. This transfer of electrons provides the power to pump hydrogens for chemismotic ATP synthesis. PSI is devoted to $NADP^+$ reduction, and in PSII the electrons flow from water to $NADP^+$ resulting in water oxidation. ATP, NADPH, and molecular oxygen are produced.

Key enzymes in the Calvin cycle are coordinated with the output of photosynthesis, and are light activated, which serves to regulate CO_2 fixation. Changes in pH, light energy, and Mg^{2+}, contribute to the efficiency of the Calvin cycle (McMurry & Begley, 2005). Overall, CO_2 and a five carbon acceptor (Rbiulose-1,5 bisphosphate) are input to the

Calvin cycle and produce two 3-phosphogylcerates, which are eventually reduced to glucose.

ii. Lipid synthesis

Lipids are synthesized in the stroma of plastids, and are synthesized by the two-carbon addition of acetyl-CoA. The acetate units are activated by the formation of malonyl-CoA. Two carbon units are added via the decarboxylation of malonyl-CoA until the chain is at least 16-carbon-units long (Figure 8). The length and structure of the lipids are of importance to biofuel production, because these physical properties can affect the performance and efficiency of biodiesel (Knothe, Krahl, & Gerpen, 2010).

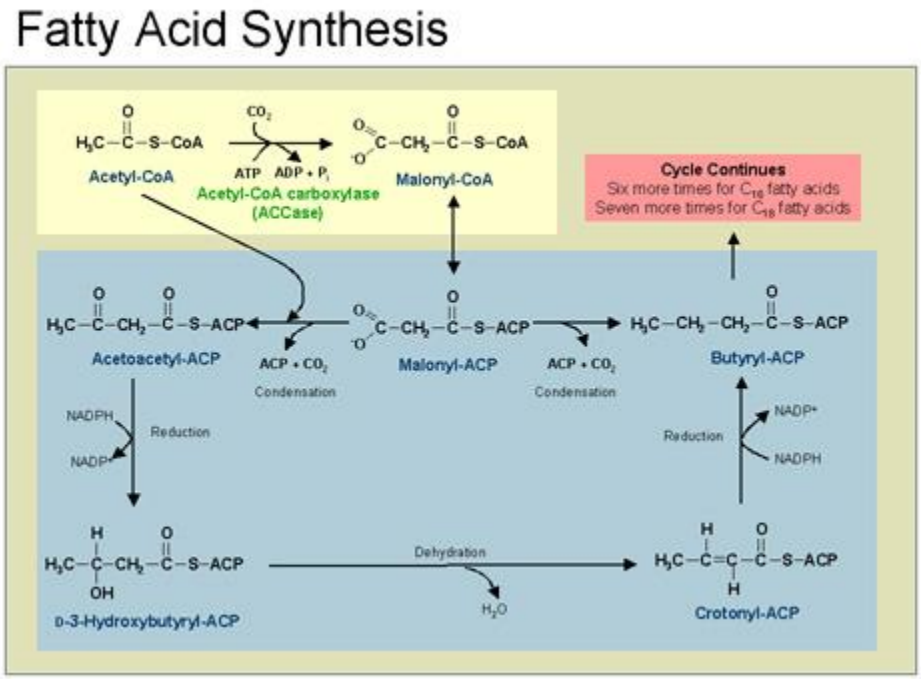

Figure 8: Fatty acid biosynthesis. Acetyl-CoA is the starting block for fatty acid synthesis, two carbons are added on for each cycle (McMurry & Begley, 2005).

iii. Algal buoyancy

There are three general factors that control algal density (or buoyancy): size and chemical composition of cell walls (e.g. silicon in diatoms), physiological mechanisms, and selective ion regulation. Anderson and Sweeney (1977) concludes that short-term regulation of buoyancy in the diatom *Ditylum brightwelli* is by ion channels and that selective incorporation of ions affects the density of the cell. Increases in settling rate did not correlate to increases in lipid content, thus ruling out lipid accumulation as a mechanism of density control. Ion channel regulation suggests that sedimentation rates are determined by physiological changes and not morphological changes.

c. Hypothesis and Research Objectives

As described above, little is known about how bioreactor design and operation can affect algal solids separation by sedimentation, a critical unit process in biofuels production. Because these parameters are known to greatly affect biomass settleability in biological wastewater treatment systems through their effects on floc formation, filamentous growth, and biomass density, it is hypothesized that bioreactor design and operation can be manipulated to improve algal settleability.

The overall objective of this study was to test this hypothesis of bioreactor design and operation to provide both fundamental and applied information useful to future research and practical application for algal biofuels. The experimental approach was to run three benchtop (1L) algal bioreactors operated under a variety of operational conditions.

The specific objectives were as follows:

Experiment 1: To determine whether selection pressures applied to algal sequencing batch photobioreactors (SBPRs) (incorporation of floating or settling phases) can be used to select for algae with improved solids separation characteristics (floatability or settleability, respectively).

Experiment 2: To investigate the light/dark cycle effects on settleability and algal biomass density.

Experiment 3: To determine whether varying hydraulic residence times and/or solids separation duration (the duration of the settling phase) affected algal biomass settleability.

Experiment 4: To determine the effects of carbon addition, in the form of sodium bicarbonate, and long solids retention time on biomass settling.

Chapter 3 Methods

a. Experimental Systems

Three bench-scale sequencing batch photobioreactors (SBPRs) were operated to grow algae under varying conditions over four experiments. The overall system configuration is shown in Figure 9. The general reactor specifications are shown in Table 2. Reactors 1 and 2 (R1 and R2) were operated as sequencing batch reactors (SBRs), with cycle phases described in Table 5. Operation as SBRs allowed the inclusion of settling or flotation phase, where mixing was stopped prior to withdrawal of effluent.

Each of the three reactors were placed in an opaque plastic container (height 15 inches and diameter 12.5 inches), completely lined with foil and covered with a lid.

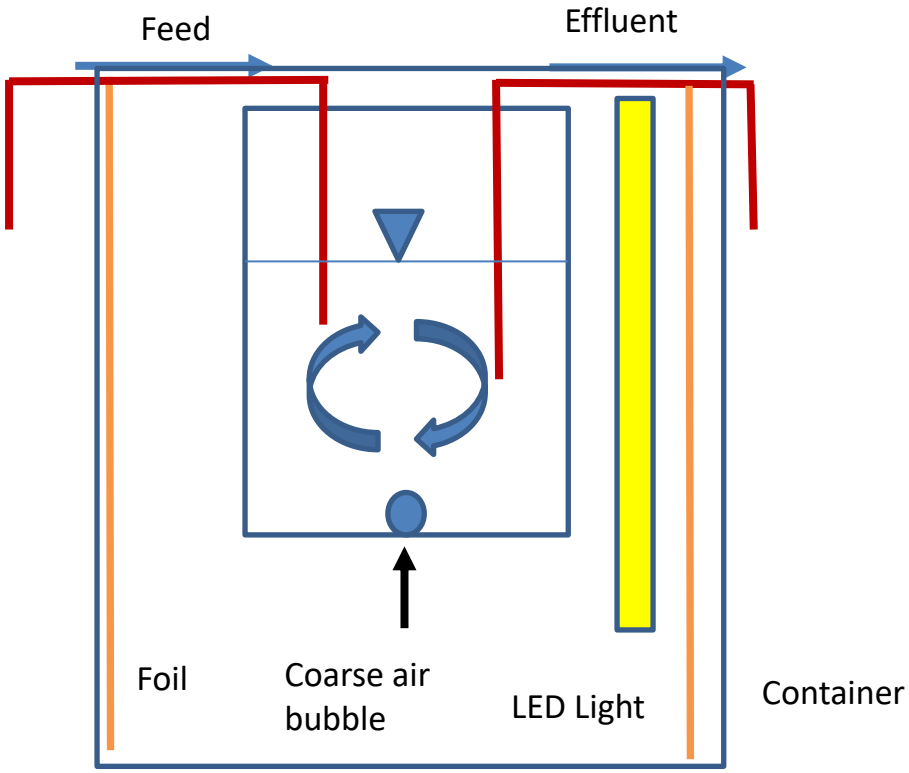

Figure 9: System configuration. The set-up included feed and effluent lines, LED light, reactor, container, air bubble mixer, and foil lining the container.

Light was provided by an LED light strip (Build My LED, Austin, TX) oriented vertically in each container as shown in Figure 9. These warm LED strip lights were inserted into each reactor via two pre-cut holes and a zip tie was inserted to keep each light up and parallel to the reactor. The LED light photosynthetic photon flux was 90 μmol/s. Aeration and mixing were provided by three air pumps, influent and effluent were provided by three Cole Parmer Masterflex pumps (Model 7520-25, Vernon Hills, IL) with two Masterflex pump heads (Model 7016-18, Vernon Hills, IL). All pumps and lights were connected between two Chrontrol program controllers (XT Table Top, Chrontrol Corporation, San Diego, CA) to accurately control each reactor's on/off cycle.

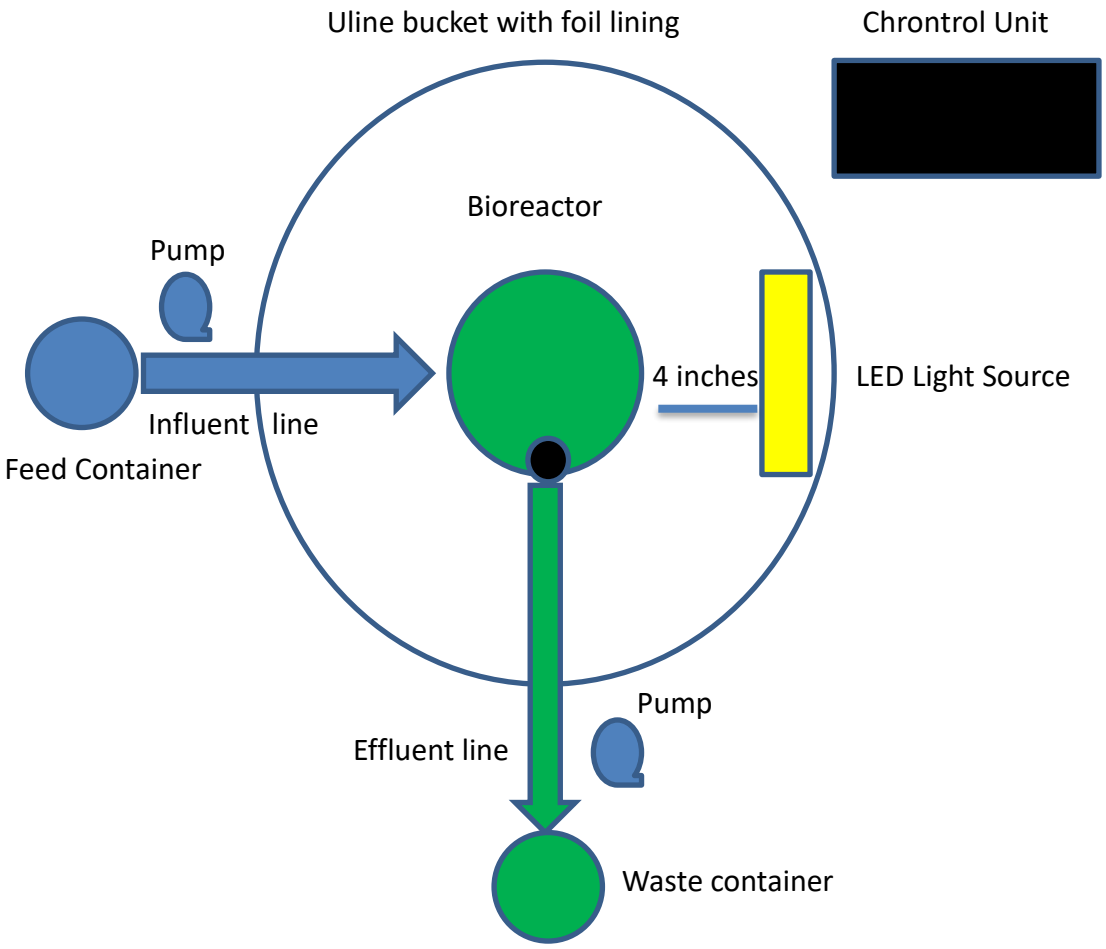

Figure 10: Bioreactor plan view. The bioreactor was 4 inches from the light source. The Chrontrol unit was programmed to turn on/off pumps, lights and air mixers.

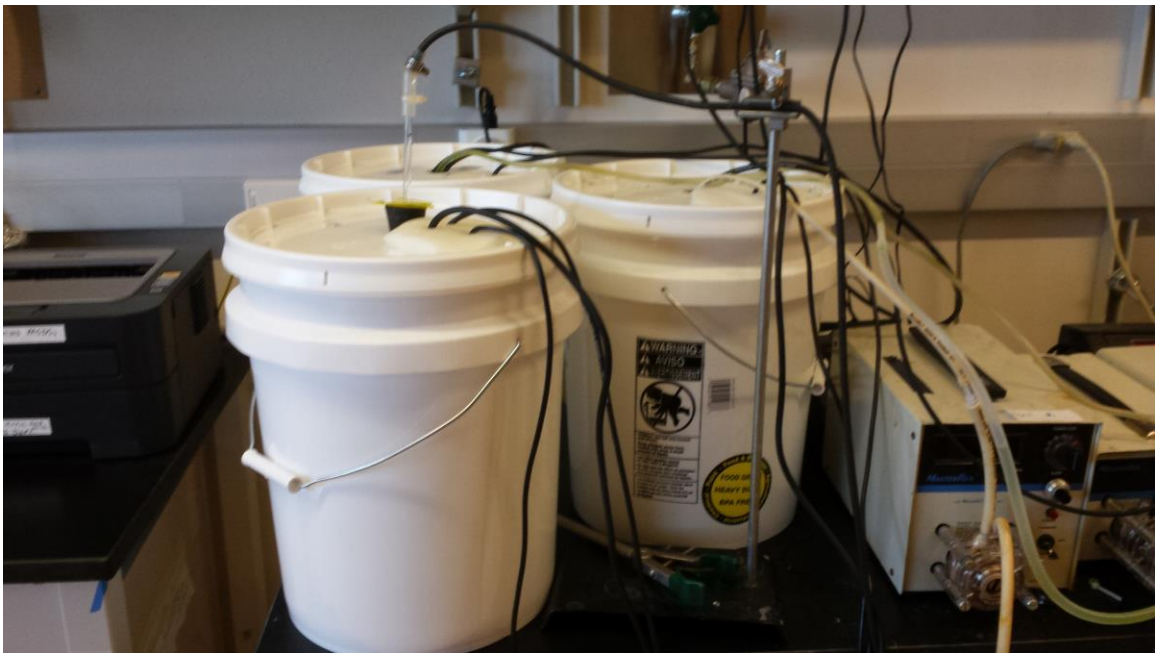

Figure 11: Photobioreactors. Photograph showing container, effluent/influent lines and pumps.

Table 2: Reactor specifications. Information about timing of the sequencing batch reactors cycle phases in each experiment is shown in Table 5. HRT is hydraulic retention time and SRT is solids retention time.

Parameter	R1	R2	R3 (control)
Abbreviations			
Exp. 1	R1-1	R2-1	RC-1
Exp. 2	R1-2	R2-2	RC-2
Exp. 3	R1-3	R2-3	RC-3
Working Volume (L)	1	1	1
Inner Diameter (cm)	10.2	10.2	10.2
Outer Diameter (cm)	10.8	10.8	10.8
Height (cm)	30.5	30.5	30.5
Liquid height (cm)	15	15	15
Influent Rate (mL/day)	500	500	200
HRT (days)			

Exp. 1	2	2	5
Exp. 2	2	2	5
Exp. 3	2	2	5
Exp. 4	2	2	5
SRT (days)			
Exp. 1	43	N/A	N/A
Exp. 2	43	N/A	N/A
Exp. 3	10	10	5
Exp. 4	23	23	5
Light/dark cycle			
Exp. 1	12h/12h	12h/12h	12h/12h
Exp. 2	12h/12h	12h/12h	12h/12h
Exp. 3	12h/12h	12h/12h	12h/12h
Exp. 4	24h/0h	24h/0h	24h/0h

The photobioreactors (Figure 11) were constructed of clear cast acrylic tubes and square sheets (Port Plastics, Albuquerque, NM). The dimensions of the reactors are shown in Table 2. The square sheets were cut to cover the bottom of each reactor and glued with acrylic glue. A section of glass tubing 2 cm in diameter was glued to the side of each reactor with the lower end at a depth of 500 cm for experiments requiring top withdrawal (R1 all experiments, and R2 experiments 2-4). This tubing was connected to the effluent line, so that 50% of the reactor working volume as supernatant was withdrawn each cycle, as described below. For R2 experiment 1, the glass tubing was extended to the bottom of the reactor to allow for withdrawal of 50% of the settled reactor volume. In the control reactor (R3) the tubing was lowered to 250 cm below the surface to remove 20% reactor volume each day (5 Day HRT).

a. Synthetic feed

The synthetic feed (growth media) chosen for all experiments was Bold's basal medium (BBM), adapted from Nichols and Bold (1965), except for experiment 4, where sodium bicarbonate was added to the feed of R2 and the R3, as described below. Sodium bicarbonate was added because it is a carbon source for algae. To make the feed (BBM), 10 mL of each of 10 stock solutions (Table 3) were diluted to 1L for R1 and R2, and 25 mL of each was used for the control R3 to maintain the same nutrient loading rate, as this reactor had a lower influent flow rate (Table 2). The net concentrations are shown in Table 4. R1, R2, and R3 had HRTs of 2, 2, and 5 days (Table 2), and so the feed to R3 was 2.5 x higher than the feeds to R1 and R2 to provide the same loading to all three reactors. The feed mixture was transferred to a 2-liter bottle and stirred on a stir plate for approximately 10 minutes to mix all chemicals. In experiment 4 only, sodium bicarbonate was added after all stock solutions were diluted. The synthetic feed was stored in 2-liter glass bottles and was not autoclaved. The effluent container was a 50-liter HDPE carboy.

Table 3: Components of BBM

Stock solution (SL)	Component	Concentration in stock solution ($g \cdot 100\ ml^{-1}$)
SL1	$NaNO_3$	2.5
SL2	$MgSO_4 \cdot 7H_2O$	0.75
SL3	NaCl	0.25

SL4	K_2HPO_4	0.75
SL5	KH_2PO_4	1.75
SL6	$CaCl_2 \cdot 2H_2O$	0.25
SL7	$ZnSO_4 \cdot 7H_2O$ $MnCl_2 \cdot 4H_2O$ MoO_3 $CuSO_4 \cdot 5H_2O$ $Co(NO_3) \cdot 6H_2O$	8.82 1.44 0.71 1.57 0.49
SL8	H_3BO_3	1.14
SL9	EDTA Na_2 KOH	1.14 3.1
SL10	$FeSO_4 \cdot 7H_2O$ H_2SO_4 conc.	4.98 g 1 L^{-1} 1 ml

Table 4: Net synthetic feed for R1 and R2. The concentrations in the R3 synthetic feed were 2.5 x greater (200 mL vs. 500 ml in R1 and R2), to provide the same loading to R3 as R1 and R2.

Chemical	Concentration (M)	Concentration (mg/L)
$NaNO_3$	$2.94 \cdot 10^{-3}$	0.249

$MgSO_4 \cdot 7H_2O$	$3.04 \cdot 10^{-4}$	0.037
NaCl	$4.28 \cdot 10^{-4}$	0.025
K_2HPO_4	$4.31 \cdot 10^{-4}$	0.075
KH_2PO_4	$1.29 \cdot 10^{-3}$	0.176
$CaCl_2 \cdot 2H_2O$	$1.7 \cdot 10^{-4}$	0.019
$ZnSO_4 \cdot 7H_2O$	$3.07 \cdot 10^{-5}$	0.008
$MnCl_2 \cdot 4H_2O$	$7.28 \cdot 10^{-6}$	0.00144
MoO_3	$4.93 \cdot 10^{-6}$	0.0007
$CuSO_4 \cdot 5H_2O$	$6.29 \cdot 10^{-6}$	0.0016
$Co(NO_3) \cdot 6H_2O$	$1.68 \cdot 10^{-6}$	0.0003
H_3BO_3	$1.85 \cdot 10^{-4}$	0.0114
EDTA Na_2	$1.71 \cdot 10^{-4}$	0.049
KOH	$5.53 \cdot 10^{-4}$	0.031
$FeSO_4 \cdot 7H_2O$	$1.79 \cdot 10^{-5}$	0.005
H_2SO_4 conc.	7	
$NaHCO_3$ (Experiment 4 only; R2 and R3)	R1: 0 R2: 0.0119 and 0.0357	0 1 and 3.5

	R3: 0.02975 and 0.0893	2.5 and 7.5

b. Reactor inoculation

Before startup, 300 mL of water was collected from the University of New Mexico Duck Pond and inoculated in 300 mL of BBM and allowed to grow on a shaker table for one week. After the incubation period was over, 200 mL was distributed to each of the reactors and filled with BBM to a total volume of 1 L.

c. Reactor operation

The experimental approach was to operate three lab scale photobioreactors in four experiments, which occurred sequentially. The differences in operational parameters are summarized in Table 5. In all experiments, R1 and R2 were operated as SBRs, with cyclic settling or flotation phases, and R3 was a control system operated as a continuous flow system. In experiments 1 thru 3, the reactors were run on a light/dark cycle of 12 hour/12 hour, but in experiment 4 the light/dark cycle was 24 hour/0 hour. Total suspended solids, volatile suspended solids, settled volume, and sludge volume index (SVI) were measured by the methods described below.

i. Experiment 1: Operation of sequencing batch photobioreactos with selection for settling or floating algal biomass

The objective of experiment 1 was to test the hypothesis that including a settling or flotation phase in the operation of a photobioreactor system can improve solids separation by selecting for biomass with improved algal settleability or floatability, respectively. The three reactors were operated with a settling phase (R1), a floatation phase (R2), and with continuous mixing (R3). R1 and R2 were operated as SBRs on a 24-hour cycle,

with programmed events for no mixing (settling or flotation phases), withdrawal of effluent, filling with synthetic feed, and resumption of mixing, on the schedules presented in Table 5. R1 and R2 were aerated for 23 hours a day, while the control was aerated for 24 hours a day. R1 and R2 had a 1-hour settling/floatation phase (no aeration), after which the draw phase began and the effluent pumps were turned on to remove half of each reactor volume. The top half of R1 was removed to preferentially waste poor settlers in the effluent, while for R2 the wasting occurred from the bottom half to preferentially waste poor floaters in the effluent. After the draw phase was complete, 500 mL of synthetic feed was added via the peristaltic pumps. The HRT of R1 and R2 was 2 days. The SRT was not formally calculated, however sampling would indicate a long SRT of about 42 days for R1. The reactors were scrubbed bi-weekly to detach algae that had grown on the sides. R2 had to occasionally be manually wasted due to insufficient biomass removal thru the effluent line.

As a continuous mixing system R3 had an HRT and SRT that were equal and set at 5 days. While the HRT of R3 was greater than the HRT of 2 in R1 and R2, the nutrient loading was made equal by adjusting the synthetic feed concentration (see description of synthetic feed above).

ii. Experiment 2: The effects of the light/dark cycle on the sludge volume index and density

The objective of experiment 2 was to determine the effects of the light/dark cycle on the SVI and biomass density. Anderson and Sweeney (1977) showed that algal density and lipid production are related; this experiment set out to explore other parameters of interest (SVI and settleability) and their relationship to the light/dark cycle. The operational

schedule for all reactors is shown in Table 5. All reactors were run as they were in experiment 1 (R1 and R2 on a 24-hour cycle with a mixing phase, settling phase, withdrawal phase, and inflow phase using a programmable Chrontrol unit, and R3 with continuous mixing and no settling phase) with the following changes. The effluent line was changed in R2 to waste from the bottom of the reactor, R1 had its setting phase (no aeration) at the end of its light phase, and R2 had its settling phase at the end of the dark phase. Each reactor was run on a 12 hour/12 hour light/dark cycle, with R1 being wasted at the end of the light phase and R2 being wasted at the end of the dark phase.

iii. Experiment 3: Evaluation of HRT and solids separation times effects on algal settleability

The objective of experiment 3 was to determine whether varying HRT and/or solids separation duration (the duration of the settling phase) affected algal biomass settleability. R1 and R2 were run with a solids separation phase, while R3 (the control) was run as a continuous mixed system. The HRT for R1 and R2 was run as in experiment 1 for half of the experiment and then lowered to 1 day for the latter half (Phase 2), with a SRT of 10 days. The first half of the experiment was run with one settling phase with a separation phase of 10 minutes for R1 and 1 hour for R2, for the latter half (Phase 2) there were two settling phases (12 hours apart) with the same separation times. The top half of R1 and R2 were wasted to select for settling algae, then filled with 500 mL of fresh feed. Each reactor was run on a 12 hour/12 hour light/dark cycle.

The control was run as a continuous mixed system with a 5-day HRT and SRT. Throughout the experiment total suspended solids, volatile suspended solids, settled volume, and SVI were measured by the methods described below.

iv. Experiment 4: Addition of sodium bicarbonate and long SRT effect on settling algae.

The objective of experiment 4 was to investigate the effect of inorganic carbon addition and long SRT (approximately 23 days) on algal settleability and productivity. The reactors were run as in experiment 3, with the following changes. Fresh inoculum from the University New Mexico Duck Pond was added to the experiment. For the first 10 days, 1 g/L of sodium bicarbonate was added to R2 after which it was increased to 3 g/L. To achieve the same loading in R3, 2.5 g/L were added initially and then increased to 7.5 g/L. R1 was run with no addition of sodium bicarbonate. The light/dark cycle for all reactors was 24 hour/0 hour to test the limits of settleability at maximum productivity.

Table 5: 24-hour cycles in experiments 1-4. R3 was continuously aerated.

	Event		
Daily cycle	R1	R2	R3 (control)
Experiment 1			
Start: 0800	Begin settling phase, aeration off.	Begin floatation phase, aeration off	No event
0900	Begin draw phase, withdraw 50% volume from **top** half reactor	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 20% volume
0910	Begin feed phase, refill reactor	Begin feed phase, refill reactor	Begin feed phase, refill reactor
0915	Begin mix phase, turn on aeration	Begin mix phase, turn on aeration	No event
2000	Begin dark phase, lights off	Begin dark phase, lights off	Begin dark phase, lights off
Re-start: 0800	Begin light phase, lights on	Begin light phase, lights on	Begin light phase, lights on

Experiment 2			
Start: 0800	Begin settling phase, aeration off	Begin floatation phase, aeration off	No event
0900	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 20% volume
0910	Begin feed phase, refill reactor	Begin feed phase, refill reactor	Begin feed phase, refill reactor
0915	Begin mix phase, aeration on	Begin mix phase, aeration on	No event
2000	Begin dark phase, lights off	Begin light phase, lights on	Begin dark phase, lights off
Re-start 0800	Begin light phase, lights on	Begin dark phase, lights off	Begin light phase, lights on
Experiment 3			
Start: 0800	Begin settling phase, aeration off	Begin floatation phase, aeration off	No event
0900	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 20% volume.
0910	Begin feed phase, refill reactor	Begin feed phase, refill reactor	Begin feed phase, refill reactor
0915	Begin mix phase, aeration on	Begin mix phase, aeration on	No event
2000	Begin dark phase, lights off	Begin dark phase, lights off	Begin dark phase, lights off
Re-start 0800	Begin light phase, lights on	Begin light phase, lights on	Begin light phase, lights on
Experiment 4			
Start: 0800	Begin settling phase, aeration off	Begin floatation phase, aeration off	No event
0900	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 50% volume from **bottom** half reactor	Begin draw phase, withdraw 20% volume.
0910	Begin feed phase, refill reactor	Begin feed phase, refill reactor	Begin feed phase, refill reactor
0915	Begin mix phase, aeration on	Begin mix phase, aeration on	No event
2000	Begin dark phase, lights off	Begin dark phase, lights off	Begin dark phase, lights off
Re-start 0800	Begin light phase, lights on	Begin light phase, lights on	Begin light phase, lights on

d. Analytical Methods

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured using standard methods 2450B and 2540E as outlined in *Standard Methods for the Examination of Water and Wastewater* (2012). For each measurement, a total of 15 mL was taken three times from each reactor. The samples were taken from the mixed reactor expect in experiment 1 where it was taken from the settled biomass in R1 and from the top half of R1. VSS were measured by igniting the biomass at 550^{o} C for 10 minutes. The biomass productivity was evaluated using Equation 6.

The sludge volume index (SVI) was determined via a commonly used method (Dick & Vesilind, 1969). The SVI is a measure of how well the biomass is settling and is measured in units of mL/g. Samples of 1 L were added to a 1-liter graduated cylinder and mixed by inversion of the cylinder. The samples were allowed to sit undisturbed for 30 minutes and the volumes occupied by the settled biomass were recorded. The SVI was calculated using Equation 2. The settleability was calculated by measuring the suspended solids at the end of the SVI test (Equation 3).

Total carbohydrates were measured by a method described in Sluiter et al. (2005). Well-mixed samples of 25 mL were freeze-dried on a Labconco Freeze Dry System (Freezone 4.5, Kansas City, MO) and the total carbohydrates were determined by light absorption on a Hach spectrophotometer (DR 2800, Hach, Loveland, CO). The reaction of MBTH working solution (MBTH and Dithiothreitol) with the extracted carbohydrates allowed for light absorption in the visible light range (620 nm), and quantification of total carbohydrates. Density was measured using a method described in Schuler and Jang (2007). This method relies on observation of whether a sample settles or floats in a series

of dilutions of a high-density solution (Percoll, GE Healthcare Life Sciences, Marlborough, MA). Phase contrast micropscpy was taken on a Olympus BX51, mounted with a Olympus DP71 camera (BX51 and DP71, Olympus, Parkway Center Valley, PA).

Mixed algal culture populations were genetically characterized using Illumina next-generation sequencing (Illumina, Inc., San Diego, CA). All analyses were performed by the MR DNA company (Shallowater, TX) under the following conditions: a single step 30 cycle PCR with 16S rRNA general primers 515f (GTGYCAGCMGCCGCGGTAA) /806rB(GGACTACNVGGGTWTCTAAT) with a HotStarTaq *Plus* Master Mix Kit (Qiagen, Germantown, MD) operated at 94° C for 3 minutes, followed by 28 cycles (5 cycle used on PCR products) of 94° C for 30 seconds, 53° C for 40 seconds and 72° C for 1 minute, after which a final elongation step at 72° C for 5 minutes was performed. Sequencing was performed on an Ion Torrent PGM following the manufacturer's guidelines. Sequence data were processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX). Sequences were depleted of barcodes and primers, then sequences < 150bp were removed. Sequences were then denoised, operational taxonomic units generated and chimeras removed. Operational taxonomic units were defined by clustering at 3% divergence (97% similarity). These primers were chosen because of their ability to generally target biological organisms (Caporaso et al., 2012) but also to target chloroplasts (Parada, Needham, & Fuhrman, 2016).

$$TSS \text{ or } TSS_{sus} = \frac{X_d}{V} \quad \text{(Equation 1)}$$

TSS_{sus} = Total suspended solids in supernatant at the end of the SVI test (mg/L)

TSS = total suspended solids of mixed sample (mg/L)

X_d = Biomass dried at 105 ^{o}C for 1 hour (mg)

V = volume of sample (L)

$$VSS = \frac{X_v}{V} \quad \text{(Equation 2)}$$

VSS = Volatile suspended solids (mg/L)

X_v = Volatile solids at 550^{o}C for 15 minutes (mg)

V = Volume (L)

$$SVI = 1000\left(\frac{V_s}{TSS}\right) \quad \text{(Equation 3)}$$

SVI= Sludge volume index (mL/g)

V_s = Volume of sludge settled (mL)

TSS = Total suspended solids of reactor (mg/L)

$$SVI = 1000\left(\frac{V_s}{TSS}\right) * settleability/100 \quad \text{(Equation 4)}$$

SVI= Sludge Volume Index (mL/g)

V_s = Volume of sludge settled (mL)

TSS = Total Suspended Solids of reactor (mg/L)

$$Settleability = 100\left(1 - \frac{TSS_{sus}}{TSS}\right) \quad \text{(Equation 5)}$$

Settleability = percent of biomass that has settled into a sludge blanket

TSS_{sus} = Total suspended solids at the end of the SVI test (mg/L)

TSS = total suspended solids of reactor (mg/L)

$$Productivity = \frac{(X_R)Q_R+(X_S)Qs}{\frac{A_S}{10}}$$ (Equation 6)

Productivity = biomass produced per day per liter ($g/m^2/day$)

X_R = biomass in reactor (mg/L)

X_S = suspended biomass/effluent (mg)

A_S= Surface area of reactor (cm^2)

Q_R = flow rate of biomass leaving reactor (L/day)

Q_S = flow rate of biomass leaving via effluent (L/day)

Chapter 4 Results and Discussion

a. Experiment 1: Operation of sequencing batch photobioreactos with selection for settling or floating algal biomass

Experiment 1 operated three sequencing batch photobioreactos (SBPRs) to select for settling or floating algal biomass. Reactor 1 (R1) had a settling phase followed by wasting the top half of the reactor, reactor 2 (R2) had a flotation phase, and the control (R3) was continuously mixed. All were run as mixed cultures for 120 days. The sludge volume index (SVI) was not evaluated in any reactor. The solids retention time (SRT) was approximately 42 days based on sampling (an average of 15 mL/day) during stationary growth (no increase or decrease in growth) in R1.

i. Biomass concentrations

The biomass (as total suspended solids (TSS)) in all reactors is shown in Figure 12, 13, and 14. The approximate stationary phase measurements are shown in Table 6 and *t*-tests for variables of interest are shown in Table 7. A selection pressure for a settling algal biomass was successful in R1, while R2 did not select for floating algal biomass.

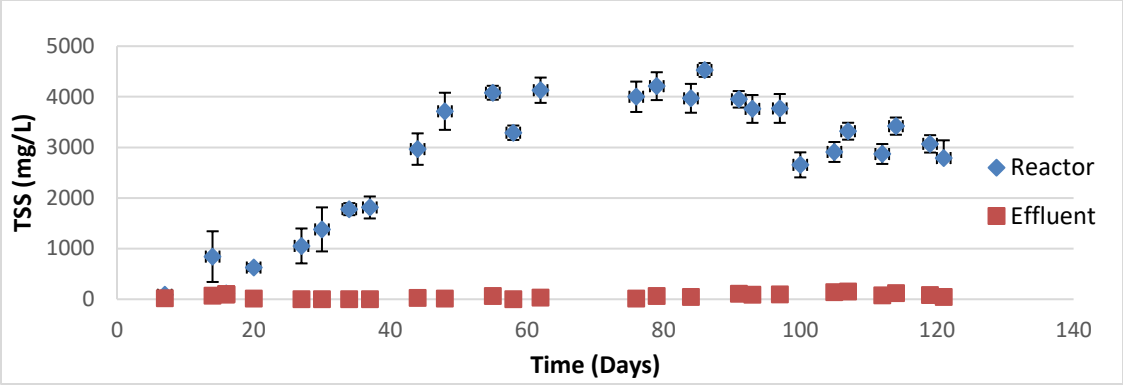

Figure 12: R1TSS with error bars (reactor with wasting from the top after no-mixing phase to select for settling algae). Biomass concentrations were measured from the bottom of the reactor. Reactor TSS values indicate that the majority of the biomass was staying in the reactor when compared to the effluent TSS. Experiment 1.

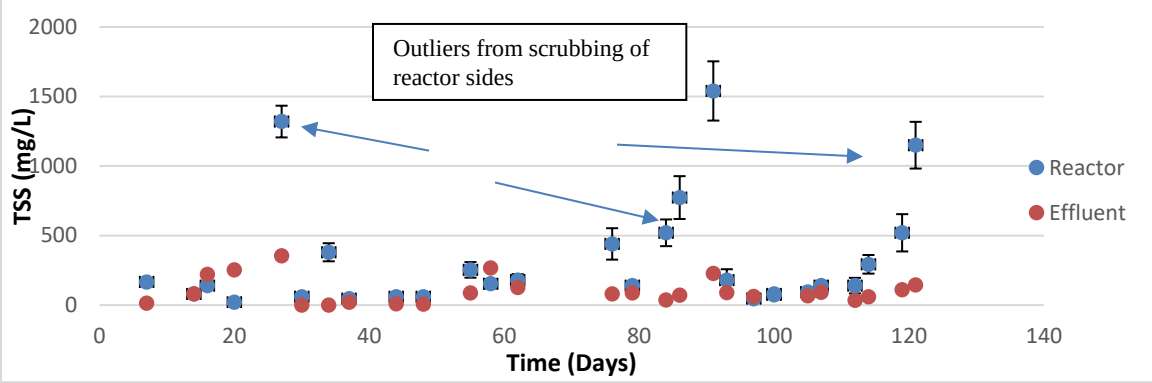

Figure 13: R2 TSS with error bars (reactor wasting from bottom after no-mixing to select for floating algae). Biomass concentrations were measured from the top of the reactor. Selection for floating algae was not achieved. Experiment 1.

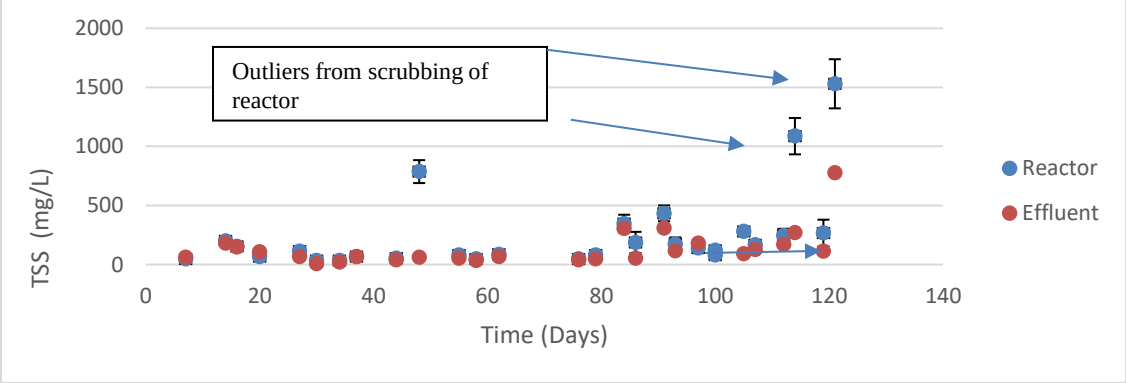

Figure 14: R3 TSS with error bars (control reactor operated with continuous mixing, with no settling or floating phases). Biomass was low due to high rate of removal (HRT=SRT=5 days). Experiment 1.

Table 6: Approximate stationary phase in reactors. Experiment 1.

	R1	R2	R3
Start of stationary phase	7/20/2015 (48 days)	7/2/2015 (30 days)	6/29/2015 (27 days)
End of stationary phase	End of experiment	End of experiment	End of experiment

Table 7: t-Test for variables of interest on 7/20/2016 for R1 and R2 and 7/16/2016 for R3. *P* values less than 0.050 indicate significant differences in the measurements. Three replicants for each day and reactor. Experiment 1.

P value	R1 Reactor TSS (7/20)	R2 Reactor TSS (7/20)	R3 Reactor TSS (7/16)
R1 Reactor TSS (7/20)		0.003	0.003
R2 Reactor TSS (7/20)	0.008		0.200
R3 Reactor TSS (7/16)	0.003	0.200	
R1 Effluent TSS (7/20)	0.003		
R2 Effluent TSS (7/20)		0.420	
R3 Effluent TSS (7/16)			0.120

The operation of R1 to select for a settling biomass was achieved as seen by the high biomass concentrations (Figure 12). The biomass in R1, increased steadily over the first 48 days of the experiment, and reached an approximately stationary value of 3969 ± 411 mg TSS/L in days 48-97 (Table 1), followed by a small decease to 2961 ± 302 mg TSS/L

from days 100 to 121 (Figure 12). Notably, the effluent TSS remained low throughout the experiment. This demonstrated that the biomass was settling well, as the reactor TSS concentrations were larger than the effluent concentrations. These findings agree with Valigore, Gostomski, Wareham, and O'Sullivan (2012), who report achieving a maximum TSS of approximately 850 mg/L.

In contrast, the biomass in R2, with wasting of the bottom half of the reactor to select for floatable algal biomass, remained at relatively low levels throughout the experiment. The reactor biomass concentrations were similar to its effluent concentrations. These measurements suggest that floatable algal biomass was not enriched by wasting from the bottom half of the reactor after the no-mixing phase. In this case, the algal biomass concentrations remained relatively low because the biomass was wasted from the reactor at a high rate in the effluent. The similar effluent and reactor TSS concentrations indicate that the SRT was approximately equal to the HRT of 2 days, which was apparently low enough to maintain the biomass at a low concentration (120 $\pm$ 70 mg TSS/L, day 7 to end) relative to R1. The outliers seen in Figure 13 can be attributed to scrubbing and release of algae on the walls of the reactor and growth of algae on the bottom of reactor before manually wasting.

R3 (the control reactor operated as a continuously mixed sequencing batch reactor) exhibited similar behaviors to R2. This result was expected, as the effluent was wasted directly from the mixed reactor, and so the SRT was equal to the HRT of 5 days. As in R2, it appears the biomass was removed from the system. The biomass growth in R3 stayed stagnant over the entire experiment and reached an approximate stationary value of 129 $\pm$ 87 mg TSS/L.

Correlations between parameters of interest were investigated using a *t*-test. Table 7 shows calculated *P* values ($P < 0.05$) using a two-tailed non-similar variance *t*-test between total suspended solids and effluent TSS. The reactor TSS in R2 and R3 has no significant differences. There was no relationship found between R1 and its effluent, but R2 and R3 had correlations between their respective reactor TSS and effluent TSS. The relationship between R2's reactor TSS and its effluent TSS suggests that dispersed growth (non-floc formation) was selected; this can also be seen in R3.

ii. Density

Density on select days was plotted for all reactors in Figure 15, Figure 16, and Figure 17. A *t*-test was conducted on the days plotted to determine if there were any significant change in density over 7 days. Biomass density did not elucidate the difference in performance between the reactors.

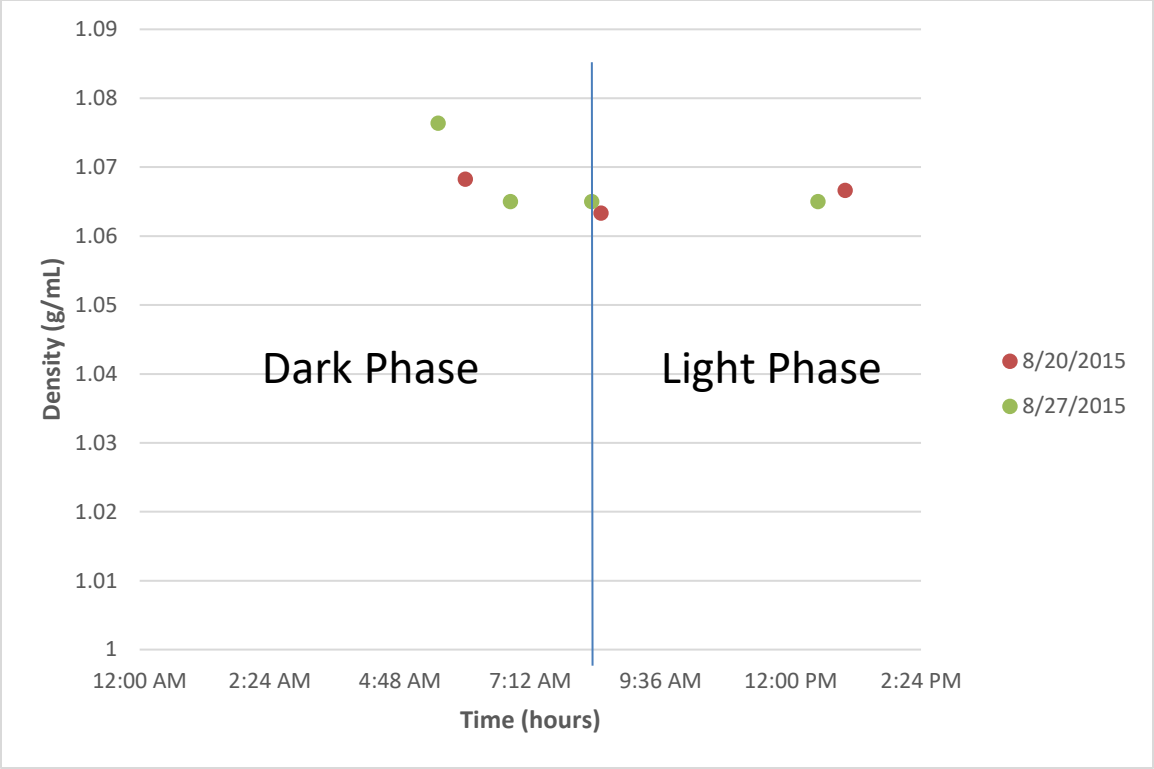

Figure 15: Biomass density in R1 (wasting from top to select for settling algae). There was no change in density of the biomass over 7 days. Density ranged from 1.076 to 1.065 g/mL. Experiment 1.

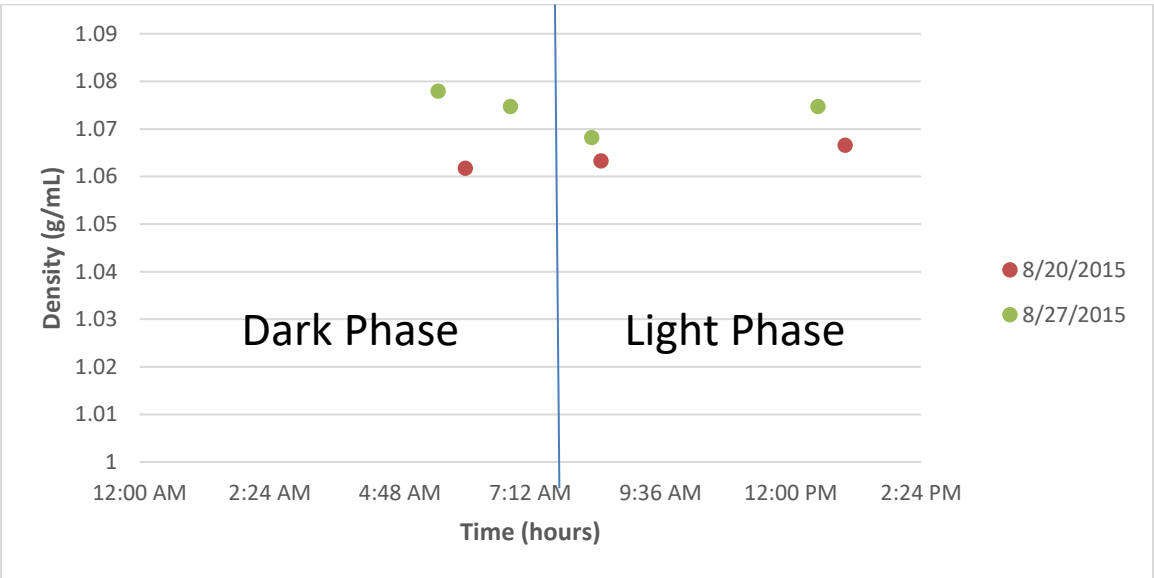

Figure 16: Biomass density in R2 (wasting from bottom of reactor to select for floating algae). There was no change in density of the biomass over 7 days. The density ranged from 1.078 to 1.064 g/mL. Experiment 1.

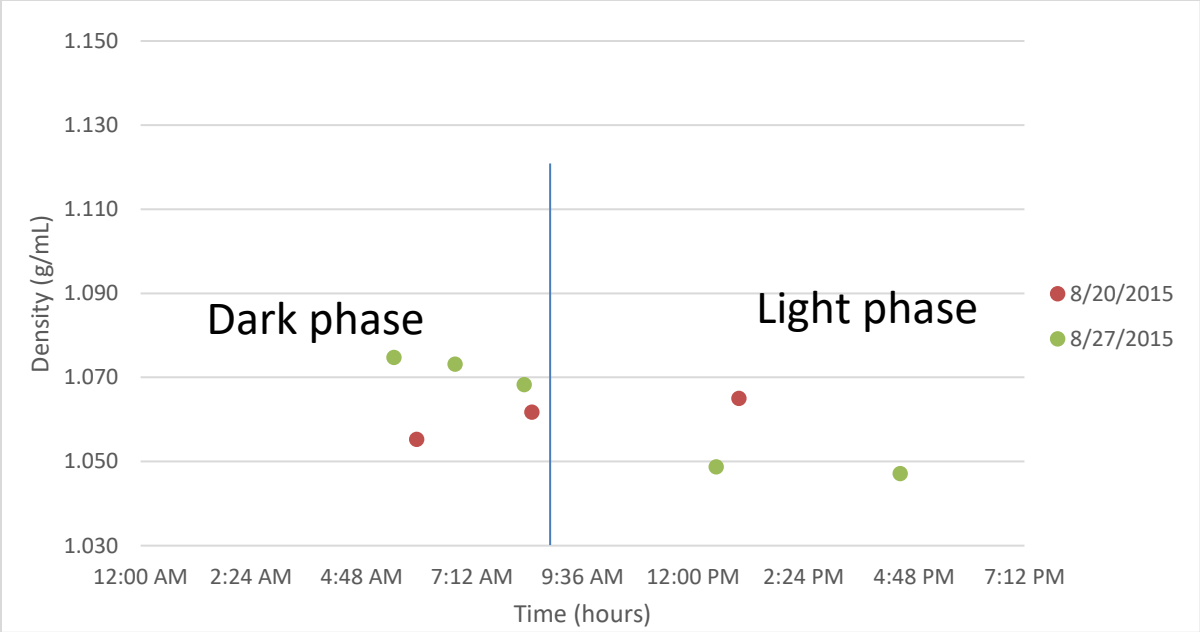

Figure 17: Biomass density in R3 (control). There was no change in density of the biomass over 7 days. The density ranged from 1.075 to 1.047 g/mL. Experiment 1.

Table 8: Density *t*-test on Day 79 and Day 86 of August, 2015. *P*-values indicate measurements on both days were similar, suggesting no change in density over 7 days. Experiment 1.

P- Value	R1 Day 79	R2 Day 79	R3 Day 79
R1 Day 86	0.4		
R2 Day 86		0.1	
R3 Day 86			0.1

Table 9: Average density values with standard deviations in dark and light phase in all reactors. Values indicate a small change in density in the light/dark cycle and overall. Experiment 1.

Reactor	Light phase Average ± Standard deviation (mg/L)	Dark phase Average ± Standard deviation (mg/L)	All measurements Average ± Standard deviation (mg/L)
R1	1.06 ± 0.010	1.059 ± 0.008	1.059 ± 0.009
R2	1.071 ± 0.015	1.074 ± 0.012	1.073 ± 0.012

R3	1.070 ± 0.018	1.076 ± 0.012	1.073 ± 0.018

The density in all three reactors were similar, with no change over a period of 7 days (Table 8 and Table 9). In R1 the density ranged from 1.076 g/mL to 1.065 g/mL, with a maximum density of 1.068 g/mL and a minimum of 1.063 g/mL on Day 79. On Day 86 the maximum was 1.076 g/mL and the minimum 1.065 g/mL. In comparison to water (1.0 g/mL), these densities are higher. In R2 the density ranged from 1.061 g/mL to 1.078 g/mL, with a maximum density of 1.066 g/mL and a minimum of 1.061 g/mL on the 20th. On the 86, the maximum was 1.078 g/mL and the minimum 1.068 g/mL. In R3 the density ranged from 1.047 g/mL to 1.075 g/mL, with a maximum density of 1.065 g/mL and a minimum of 1.055 g/mL on the 20th. On the Day 86, the maximum was 1.075 g/mL and the minimum 1.047 g/mL. Biomass density in R3 (control). There was no change in density of the biomass over 7 days. The density ranged from 1.075 to 1.047 g/mL. Experiment 1. Table 8 indicates that the densities on Days 79 and 86 were very similar, which suggests that there was no major change in density over the seven days. Because of this, differences in biomass density were unlikely to explain the observed differences in settling among R1, R2 and R3.

iii. Phase contrast microscopy

Figure 18 and Figure 19 show the phase contrast microscopy images of R1, R2, and R3. Phase contrast microscopy images indicate the presence of filamentous biomass in R1 and dispersed growth in R2 and R3.

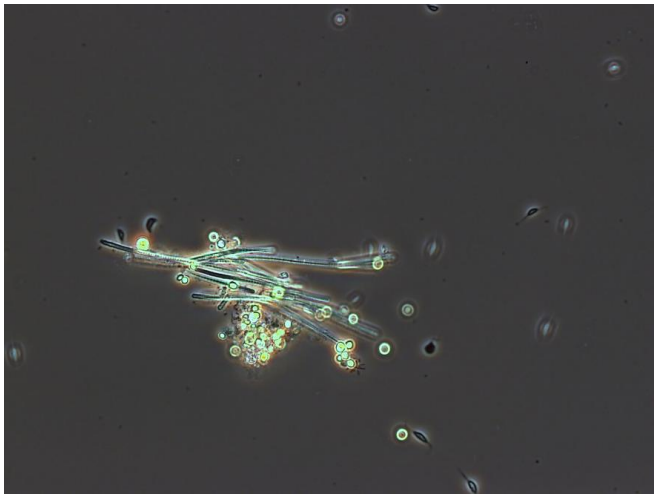

Figure 18: Phase contrast microscopy image of inoculum. Magnification of 400x. Experiment 1.

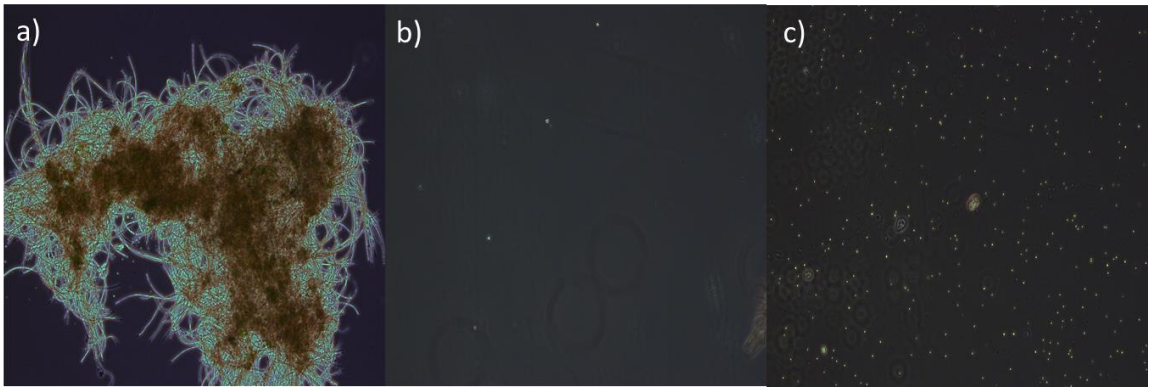

Figure 19: Phase contrast microscopy images of R1 (a), R2 (b), and R3 (c). Magnification of 100x. Taken on day 37. Presences of filamentous biomass in R1 only. Experiment 1.

Before startup of the reactors the inoculum had the presence of filamentous and spherical organisms (Figure 18). After start up (Figure 19) R1 had large filamentous flocs present, while in R2 and R3 there were only spherical and dispersed organisms present.

R1 (selection for settling algal biomass) achieved the highest biomass concentrations of the three reactors, while R2 (selection for flotation algal biomass) and R3 (control) achieved similar, low biomass concentrations. The density values of the biomass in each

reactor do not explain the observed differences between the reactors. The following experiment was aimed at exploring the relationship between biomass density and settling in the light/dark phase.

b. Experiment 2: The effects of the light/dark cycle on the sludge volume index and density

Experiment 2 explored the effects of the light/dark cycle on sludge volume index (SVI) and density. The results indicate that the light/dark cycle did not influence the settling performance nor did it have any impact on the biomass density. R1 and R2 were operated with a settling phase (R1 during the light phase and R2 during the dark phase) and R3 with no settling (a control system with continuous mixing). All were run as mixed cultures for 90 days. R1 and R2 were run with identical HRTs (2 days), settling times (1 hour), light/dark cycles (12 hour/12 hour) but the settling phase took place in different light conditions. R3 (control) was run with a 5 day SRT and HRT, with a 12 hour/12 hour light/dark cycle. To begin the experiment, the contents of the three reactors post experiment 1 were mixed together and then this was divided equally between the reactors.

i. Biomass concentrations

The biomass concentrations (as total suspended solids or TSS) for all reactors are shown in Figure 20. R1 and R2 experienced biomass loss throughout the experiment, reaching concentrations equivalent to the control in the last 26 days of operation.

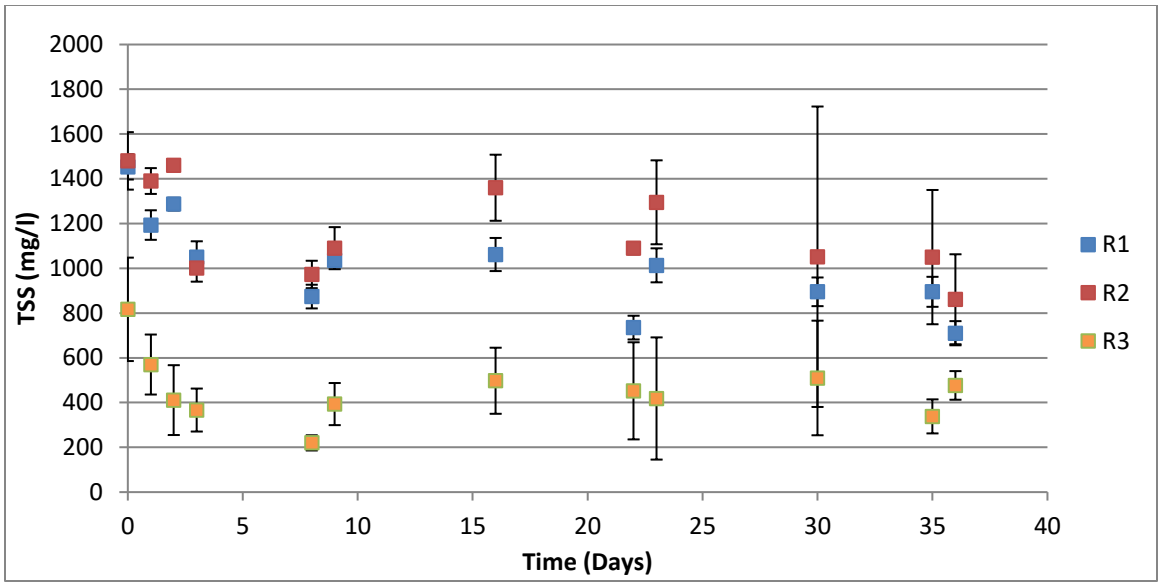

Figure 20: TSS in all three reactors. TSS indicates loss of biomass in all reactors over the experiment. Between days 44 and 62, TSS in R1 and R2 was larger than expected and could be due to error in measurement. Experiment 2.

R1 and R2 lost biomass during the experiment while R3 remained at a constant concentration for most of the experiment. R1 (with settling in the light phase) had decreased growth during the 36 days of operation. It began with an initial value of 1,453 ± 60 mg TSS/L and then finished at a value of 710 ± 21 mg TSS/L. R2 (with settling in the dark phase) had decreased growth during the 36 days of operation. Starting with an initial value of 1480 ± 128 mg TSS/L, it ended with a final value of 861 ± 146 mg TSS/L. Neither reactor achieved a stationary phase. R3 (the control) had decreased growth during the first 9 days of operation, and then reached a stationary phase for the rest of the experiment (491 ± 100 mg TSS/L).

ii. Settled volume, sludge volume index and density

The settled volume, SVI, and density are shown in Figures 21 thru 27. R1 and R2 had better performance in settled volume and SVI compared to R3. These parameters were

not influenced by the light/dark cycle. Similarly, the density of the biomass in all three reactors was not influenced by the light/dark cycle, and it did not change during the experiment.

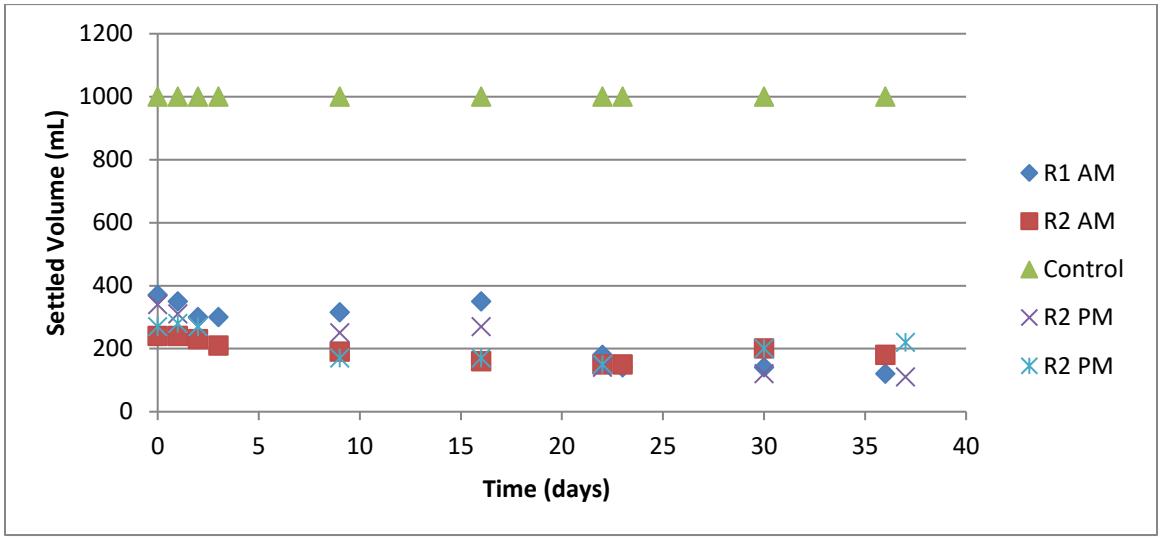

Figure 21: Settled volume of all three reactors. R1 and R2 had consistently lower settled volume than R3. The settled volume during the light/dark phase was nearly identical in R1 and R2. Experiment 2.

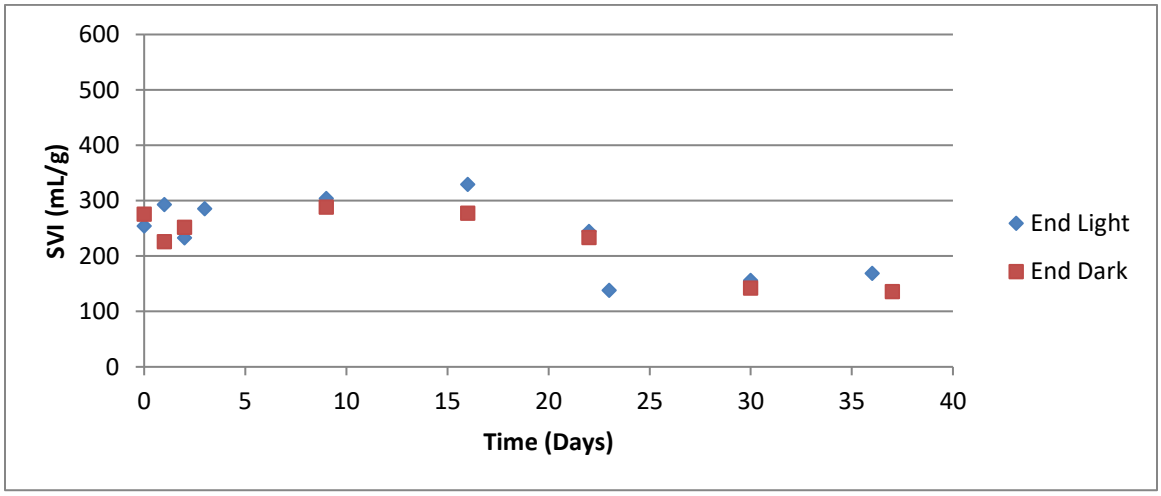

Figure 22: SVI for R1 at end of the light and dark phases. The SVI in R1 during the light and dark phases were nearly identical. Error in TSS measurements resulted in propagation in error in SVI measurements. Experiment 2.

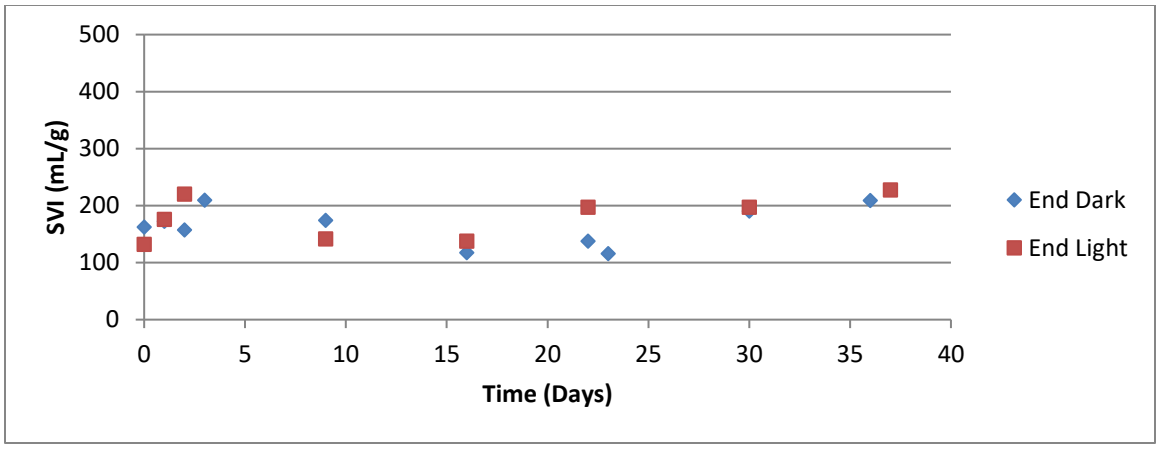

Figure 23: SVI for R2 at the end of the light and dark phases. The SVI in R2 during the light and dark phases were nearly identical. Error in TSS measurements resulted in propagation in error in SVI measurements. Experiment 2.

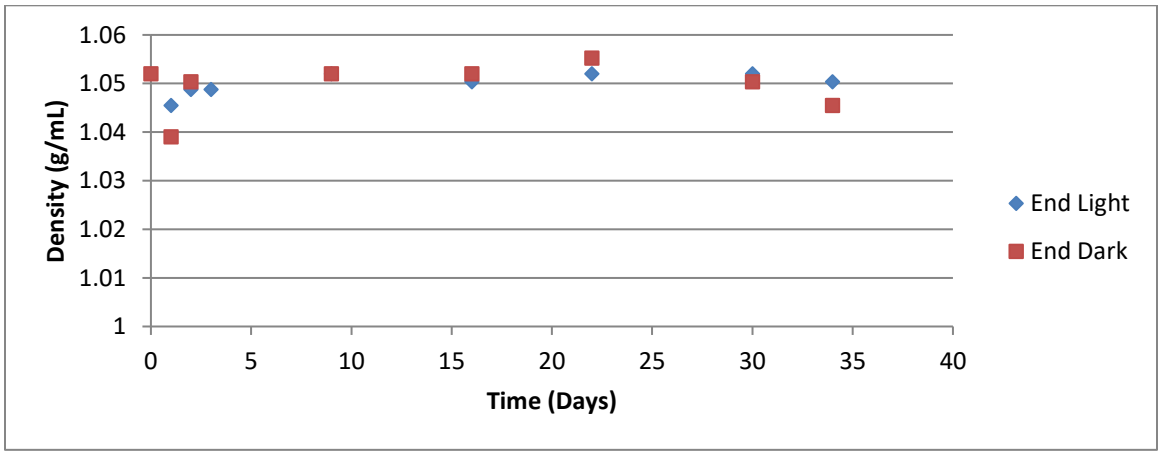

Figure 24: Density of R1 at the end of the light and dark phases. Density did not change over the course of the experiment. Density in the light and dark phases were nearly identical. Experiment 2.

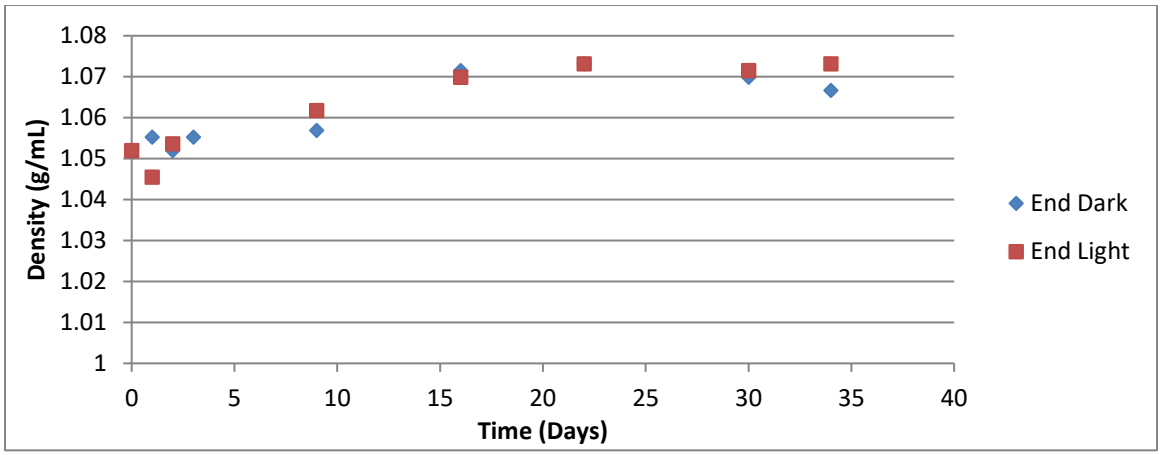

Figure 25: Density of R2 at the end of the light and dark phases. Density did not change over the course of the experiment. Density in the light and dark phases were nearly identical. Experiment 2.

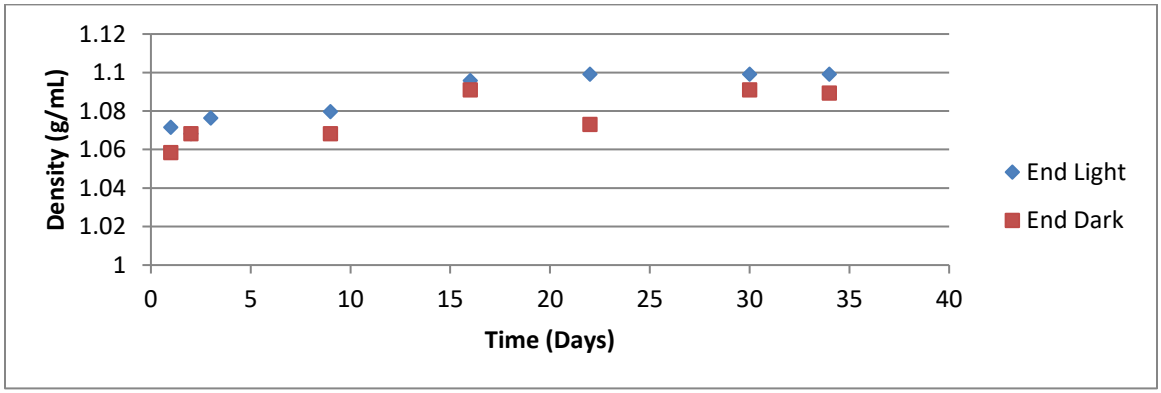

Figure 26: Density of R3 at the end of the light and dark phases. Density did not change over the course of the experiment. Density in the light and dark phases were nearly identical. Experiment 2.

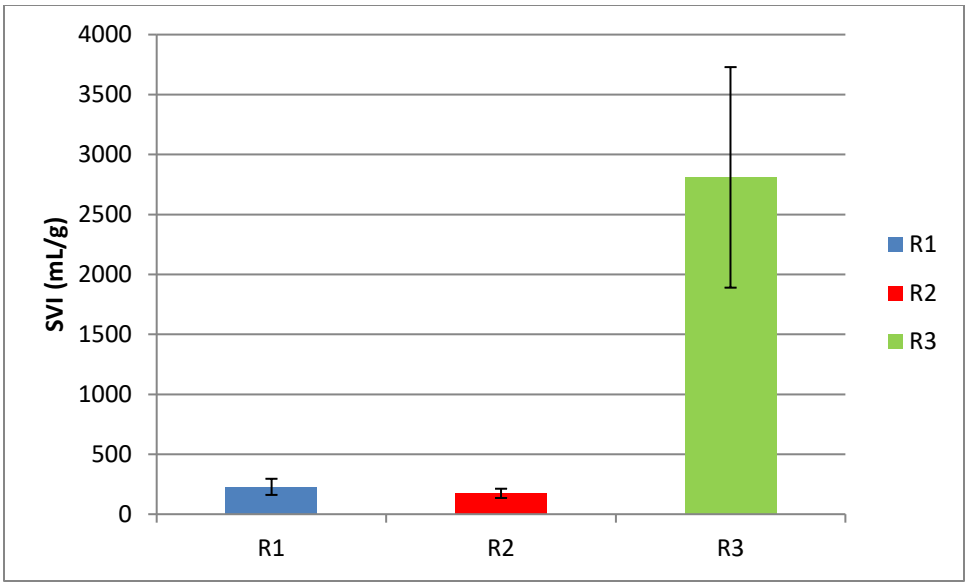

Figure 27: SVI of all three reactors. R1 and R2 had better settling than R3. Experiment 2.

Table 10: *t*-test of SVI and density during dark and light phases. There was a high correlation between settling in the light phase and settling in the dark phase and between density in the light phase and dark phase. Experiment 2.

P-Value	R1 SVI, light phase	R2 SVI, light phase	R1 density, light phase	R2 density, light phase	R3 density, light phase
R1 SVI, dark phase	0.800				
R2 SVI, dark phase		0.400			
R1 density, dark phase			0.500		
R2 density, dark phase				0.760	
R3 density, dark phase					0.200

Table 11: Ratios between SVI in light and dark phases for R1 and R2 (1/14/2015 to 2/17/2015). These ratios indicate the similarity between the SVI in the light phase and the dark phase. Experiment 2.

	R1	R2
Ratio	1.05	1.14
SVI (g/mL), light phase	233 ± 72	187 ± 39
SVI (g/mL), dark phase	222 ± 67	164 ± 36

Biomass settleability is expressed here as both settled volume (the biomass volume occupied in a 1-liter graduated cylinder after 30 minutes of settling, expressed in mL) and as SVI (the settled volume divided by the mass of biomass in the 1-liter settled sample, expressed as mL/g). Because SVI is normalized to biomass, it is considered a better indicator of settleability. However, it does not work well for samples with extremely poor settling since such samples approach the max value for the numerator (1000 mL) but the denominator can vary, giving a false appearance of variable settleability. Figure 21 shows that R1 and R2 had similar settled volumes, and that R3 did not have any settled biomass. R1 began with a settled volume of 370 mL, which decreased to 120 mL by the end of the experiment. R2 began with a settled volume of 240 mL, which decreased to 180 mL by the end of the experiment. R3 began and ended the experiment with a settled volume of 1000 mL. Because the R3 biomass did not settle at all throughout experiment 2, the settled volume was always 1000 mL, and so the settled volume was used to measure its settleability. SVI for R1 started at 476 mL/g, and then finished at 136 mL/g. The SVI of R2 started at 131 mL/g, and then finished at 227 mL/g. Figure 27 shows that R1 had the best performance in settling, R2 the second and R3 the worst.

Table 10 indicates that there was a strong correlation between SVI and density in both the light phase. Table 11 shows that the ratio between the SVI in the light phase and the dark phase was not much larger than 1, supporting the *t*-test results in Table 10. By these metrics, R1 and R2 produced biomass with better settling than R3 throughout experiment 2 (Figure 27).

The density in all three reactors was not influenced by the light/dark cycle. In R1 the density ranged from 1.040 to 1.050 g/mL during the light phase and 1.040 to 1.060 g/mL during the dark phase. In R2 the density ranged from 1.050 to 1.070 g/mL during the light phase and 1.040 to 1.070 g/mL during the dark phase. In R3, the density ranged from 1.070 to 1.100 g/mL during the light phase and 1.060 to 1.090 g/mL during the dark phase. Table 10 indicates that there was a correlation between the density during the light phase and the dark phase, which was a finding in experiment 1. Interestingly R3 had the highest biomass density but no settling. This finding, along withTable 10, suggests that density did not play a role in the settling of the algal biomass.

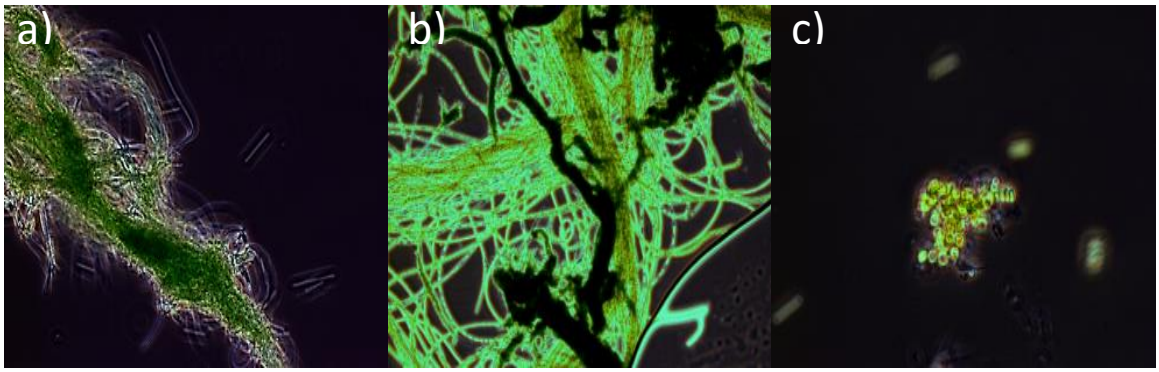

Figure 28: Phase contrast microscope images of R1(a), R2 (b), and R3 (c). Magnification of 400x. Taken on day 20. Presence of filamentous biomass in R1 and R2 and dispersed growth in R3. Experiment 2.

The phase contrast microscopy images (Figure 28) show that R1 and R2 had large filamentous biomass, while the R3 (the control) had small clusters and dispersed growth. The larger areas and volumes of the flocs in R1 and R2 along with the SVI results indicate that the presence of increased floc formation results in better settling. In addition, whether settling occurred in the light phase or the dark phase did not affect performance.

The following experiment attempted to evaluate HRT and solids separation time effects on algal settleability.

c. Experiment 3: Evaluation of hydraulic retention time and solids separation time effects on algal settleability

The objective of experiment 3 was to determine whether varying HRT and/or solids separation duration (the duration of the settling phase) affected algal biomass settleability. No clear conclusions are made because of inadequate sampling and lack of control over solids removal which resulted in large errors in measurements. As in experiment 2, R1 and R2 were run with a solids separation phase, while R3 (the control) was run as a continuously mixed system. The settling phase of R1 was 10 minutes and the settling phase of R2 was 1 hour, while the SRT was maintained at 10 days for both reactors throughout the experiment. For the first half of the experiment (Phase 1 was 31 days), R1 and R2 operated with several conditions similar to those of experiment 1; the HRT was 2 days, with one settling phase per day, followed by withdrawal of the top half of the reactor volume (0.5 L) and replacement with fresh feed. The HRT was decreased in the second half of the experiment (Phase 2) to increase the selection pressure for well-settling algae, as non-settling algae would more rapidly wash out of the system with a

shortened HRT. In the second half of the experiment (after day 31), R1 and R2 had a settling phase every 12 hours, followed by the same draw/fill procedure, which decreased the HRT to 1 day. Each reactor was run on a 12 hour/12 hour light/dark cycle, with settling after the light phase. The control was run with a 5-day HRT and SRT, as in experiments 1 and 2.

i. Biomass concentrations

The biomass (as total suspended solids or TSS) for all reactors is shown in Figure 29. All three reactors experienced loss of biomass due to a high rate of solids removal in the system. This loss of biomass coupled with inadequate measurements generated results that were difficult to analyze and draw conclusions from.

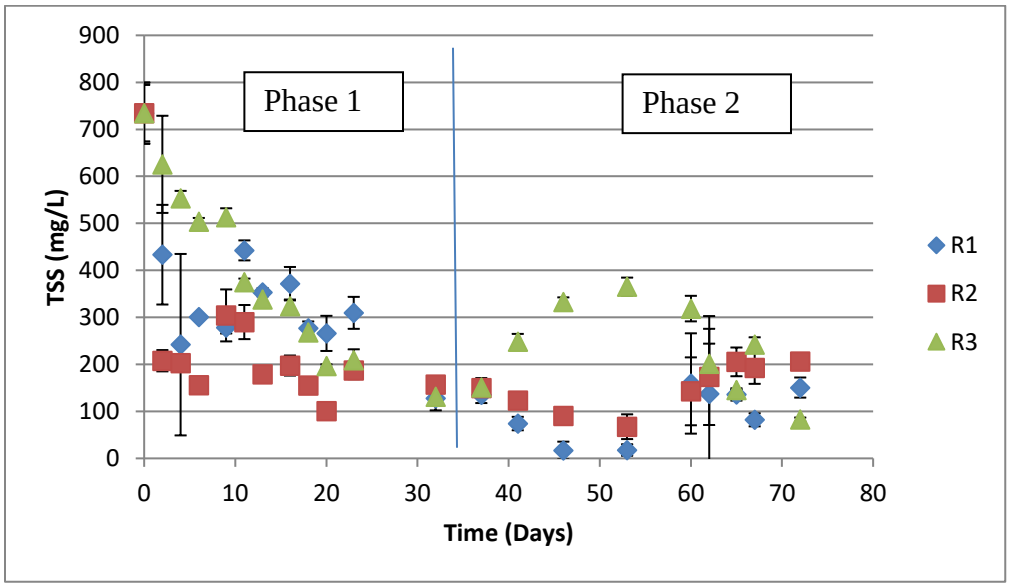

Figure 29: TSS for all three reactors. Biomass concentrations decreased in all reactors during Phase 1, with a small increase at the end of Phase 2. Loss of biomass in Phase 1 was the result of high biomass wasting (low SRT). In Phase 2 an increase in SRT allowed for growth. Experiment 3.

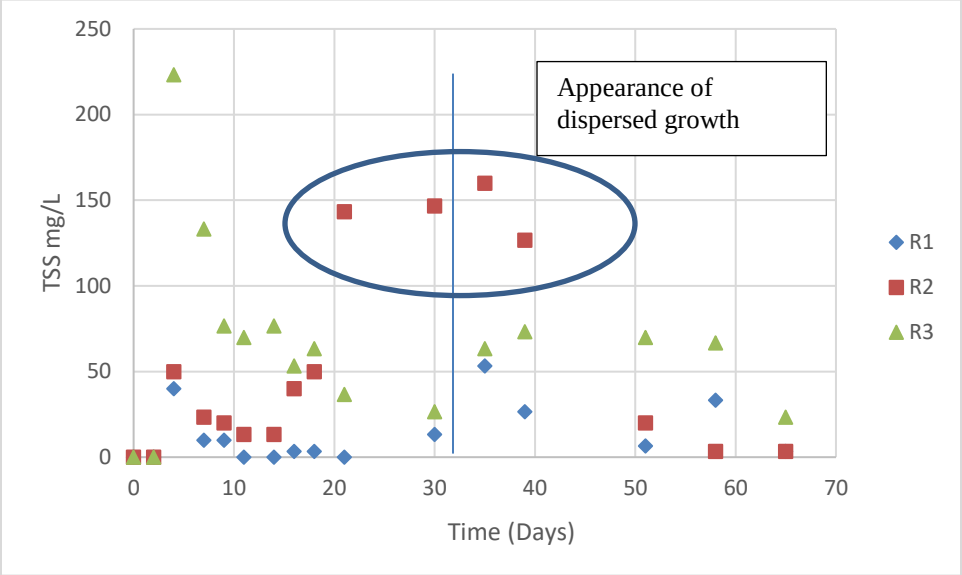

Figure 30: TSS of non-settling biomass (reactor effluent). The appearance of dispersed growth in R2 was the result of a high rate of biomass wasting. Experiment 3.

Table 12: Reactor TSS and effluent TSS *t*-test. No significance was found between reactor TSS and its effluent TSS. Experiment 3.

P-Value	R1 reactor TSS	R2 reactor TSS	R3 reactor TSS
R1 effluent TSS	0.00001		
R2 effluent TSS		0.00003	
R3 effluent TSS			0.000005

R1 experienced biomass loss throughout the experiment. The biomass concentrations in R1 decreased during the first 2 days of experiment 3, and then slowly decreased for the rest of Phase one (Figure 30). All reactors had an initial TSS value of 734 ± 65 mg TSS/L. In R1 the biomass decreased rapidly and was in the range of 304 to 100 mg TSS/L through the end of Phase 1. In Phase 2, when the HRT was decreased from 2 days

to 1 day, at the beginning the R1 biomass was largely washed out of the system decreasing to 67 mg TSS/L by day 45, and thereafter rebounded to 206 mg TSS/L by the end of the experiment. The washout of the R1 biomass coupled with the decreased HRT suggest that much of the biomass was not settling well in R1, since the settled portion of the biomass would not be affected by the shortened HRT. The average calculated SRT in R1 was 8 days during Phase 1, which could explain the higher biomass concentrations as compared to R2. While in Phase 2, R1 had an average SRT of 4 days from days 30 to 45 with an increase to an average of 6 days for the rest of the experiment. Biomass growth was observed during the last 13 days in the experiment, suggesting that the R1 biomass eventually adapted to the shorter HRT in Phase 2.

R2 also experienced loss of biomass throughout the experiment. Like R1, R2 TSS initially decreased in Phase 1, and thereafter was in the range 433 to 131 mg TSS/L until the end of Phase 1. In Phase 2, the R2 biomass initially decreased, but did not wash out of the system to the same degree as the R1 biomass (Figure 29). This may have been because R2 had a longer settling phase (1 h, as compared to 10 min), which would decrease the effect of the increased flow rate on the biomass. The average calculated SRT in R2 was 5 days during Phase 1, which explains the loss of biomass concentrations and washout. In Phase 2, R2 had an average SRT of 1 day for the first 10 days of operation followed by an increase to 7 days. The decrease in HRT in Phase 2 followed by an increase in SRT and reactor biomass would suggest that dispersed growth was being washed out and then followed by growth in settling biomass (Figure 29 and Figure 30). The relatively low SRT for the first 10 days of Phase 2 in R2 could be explained by the high effluent concentrations in the reactor during the first part of Phase 2. As in R1,

the R2 biomass partially rebounded after day 53; at the end of the experiment it was approximately 206 mg TSS/L.

The R3 (control) biomass also decreased during Phase 1 (734 mg TSS/L to 131 mg TSS/L), although it was a more gradual decline than in R1 and R2. This observation can be explained by the fact that the SRTs in R1 and R2 were actually much shorter than 10 days because of the losses in the effluent. In Phase 2, R3 had growth during the first 21 days of operation, reaching a maximum of 365 mg/L then decreased until the end of the experiment with a final value of 83 mg/L.

The high rate of biomass removal (low SRT) in R1 and R2 resulted in biomass loss and crash in TSS in Phase 1 (Figure 29) followed by a decrease in biomass during the first 20 days in Phase 2 and slight biomass recovery in R1 and R2 at the end of the experiment. This was partially alleviated in Phase 2 with the HRT being lowered to 1 day and allowing partial recovery of settling algae, as seen by the decrease in effluent TSS (Figures 29 and 30).

ii. Productivity

The productivity as calculated in equation 6 is the amount of biomass produced per surface area of the reactor per day (in $g/m^2/day$) and is shown for all three reactors in Figure 31: Productivity of all reactors.

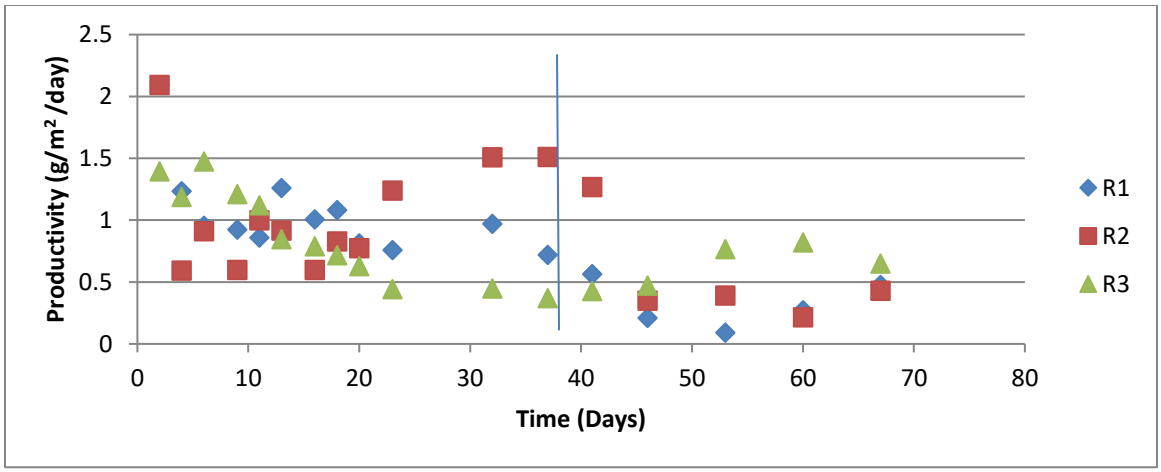

Figure 31: Productivity of all reactors. Decrease in productivity was the result of a loss in biomass in the reactors. R2 showed an increase in productivity from days 23 to 41. Experiment 3.

The productivity in R1 on day 2 was 2.09 g/m^2/day and decreased to 1.87 g/m^2/day on day 4 and then decreased to 0.75 g/m^2/day until the end of Phase 1. In Phase 2, R1 started at a productivity of 2.09 g/m^2/day and decreased to 0.01 g/m^2/day over 21 days, and then increased to 0.64 g/m^2/day by the end of the experiment. R2 had decreased productivity from day 2 to day 4 (2.09 g/m^2/day to 0.59 g/m^2/day) followed by a slight increase to end Phase 1 at 1.51 g/m^2/day. In Phase 2, R2 had an initial productivity of 1.27 g/m^2/day and then decreased to 0.43 g/m^2/day. The productivity in R3 decreased during Phase 1 from 1.39 g/m^2/day to 0.37 g/m^2/day and in Phase 2 started at 0.43 g/m^2/day and ended at 0.65 g/m^2/day. The decrease in productivity in R1 and R2 (Phase 1) was the result of a high rate of biomass removal due to an HRT of 2 days resulting in loss through the effluent. While in Phase 2 the productivity was low in both reactors due to the low biomass concentrations.

iii. Settled volume, sludge volume index, and settleability

The settled volume, SVI and settleability of all three reactors are shown in Figure 32, Figure 33, and Figure 34. All three reactors showed variable settling over the full 65 days, with dispersed growth in R2 for 20 days (days 21 thru 44). Dispersed growth in R2 coupled with settling biomass in R3 generated results that were difficult to draw any conclusions from.

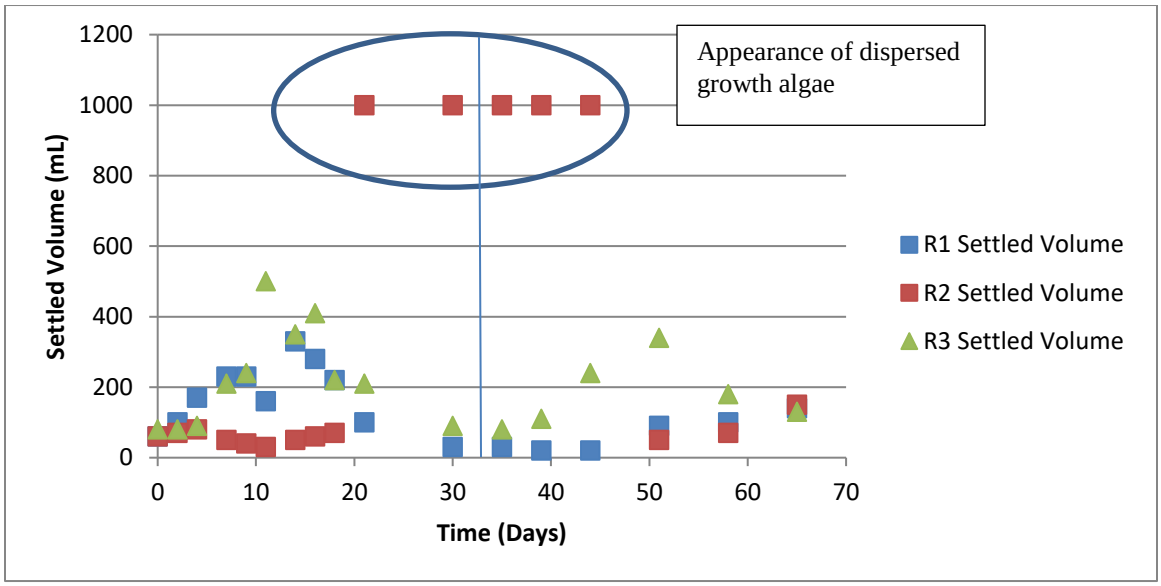

Figure 32: Settled biomass in all three reactors. Between days 20 and 50, R2 had a massive increase in settled volume indicating a loss of settling biomass and presence of dispersed growth. R3 settled volume indicates the presence of settling algae. Experiment 3.

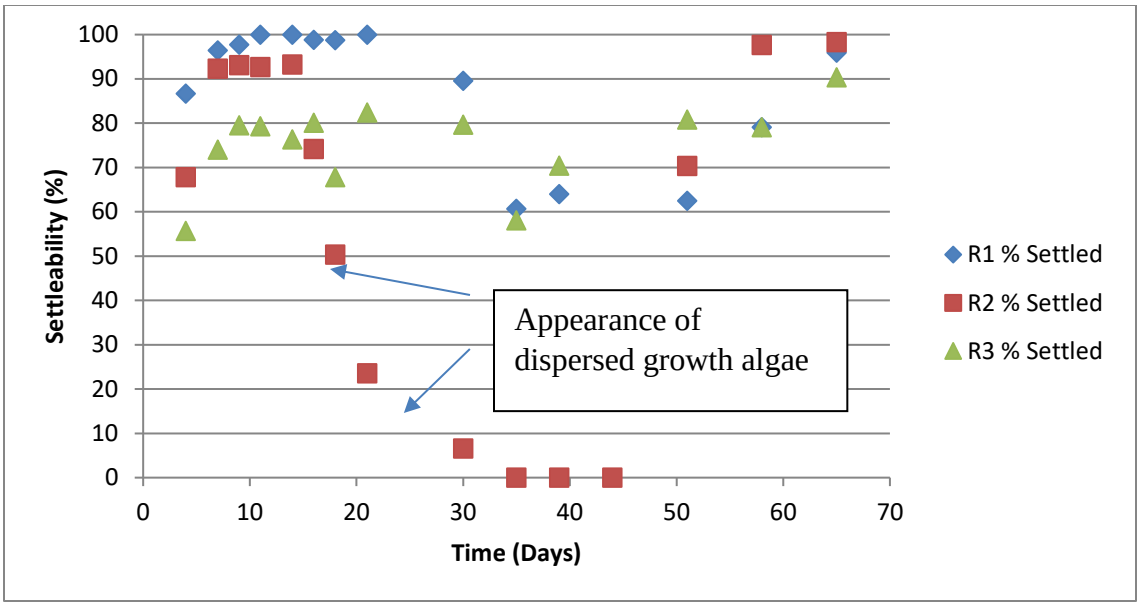

Figure 33: Settleability of all reactors as % of biomass settled (30 minutes of settling). R1 had high settleability for the first 20 days followed by a decrease, then recovered at the end of the experiment. The decrease in settleability in R2 was due to the appearance of dispersed growth and loss of settling biomass. R3 had settling biomass. Experiment 3.

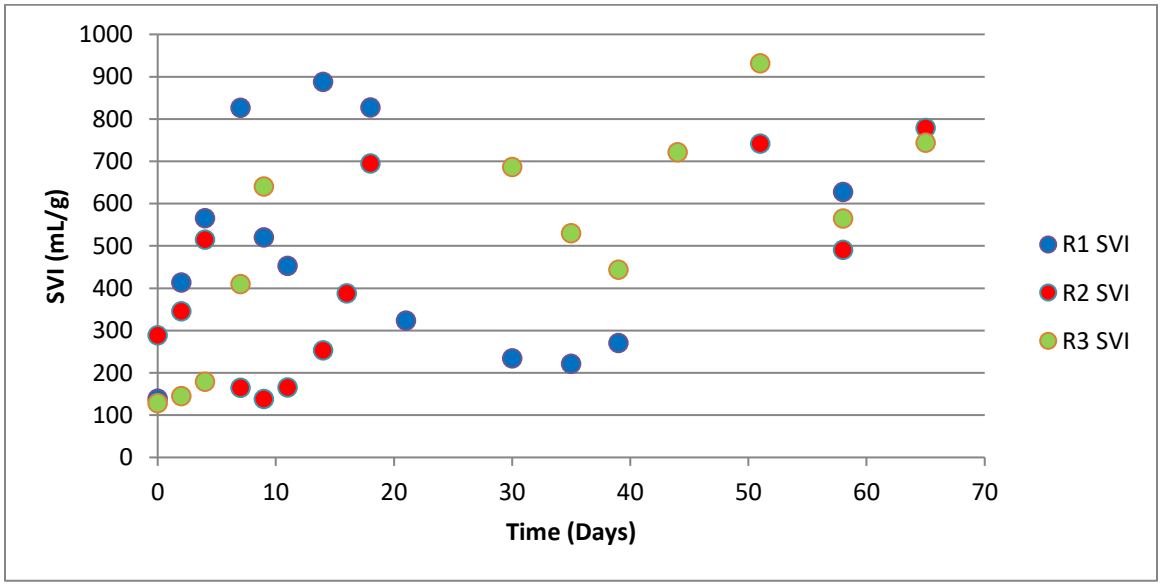

Figure 34: SVI of R1, R2, and R3. Values for R2 between days 20 and 50 are not shown because of their magnitude. SVI values indicate that R1 and R2 did not perform better than R3. Experiment 3.

The SVIs indicated poor settleability in all three reactors during Phase 1 of the experiment; all reactors experienced a dramatic increase in SVI values. R1 increased from 127 mL/g to 827 mL/g, R2 increased from 288 mL/g to 694 mL/g and R3 increased from 127 mL/g to 931 mL/g. In Phase 2 there were moderate decreases in SVI values, but they were still indicative of poor performance. R1 decreased from 827 mL/g to 234 mL/g, then increased to finish the experiment at 778 mL/g. R2 stayed at an average of 670 mL/g for Phase 2. R3 reached a constant value of approximately 600 mL/g for Phase 2. High biomass wasting during Phase 1 resulted in dispersed growth and poor SVI performance, in addition Figure 33 indicates that after approximately 10 days of operation, settleability decreased followed by an improved settleability in Phase 2 of all three reactors. The high rate of liquid removal in phase 2 (a 1-day HRT) resulted in removal of dispersed growth algae.

iv. Illumina sequencing

The Illumina sequencing using polymerase chain reaction for R1, R2, and R3 is shown in Figure 35. Presence of algae/bacteria was not consistent in the settling reactors (R1 and R2).

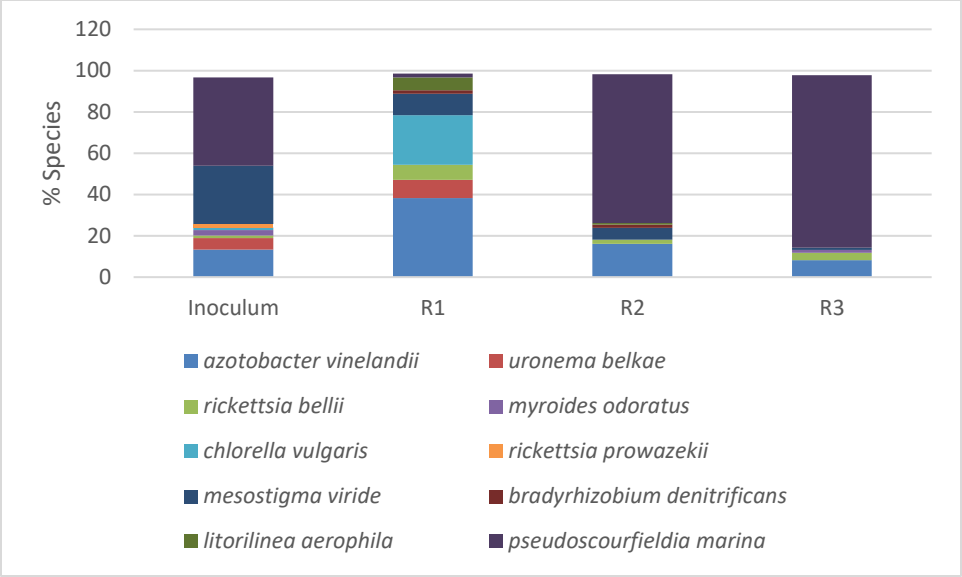

Figure 35: Illumina sequencing shown as species. Samples of R1, R2, and R3 taken on day 60. X-axis is source. Incoculum dominated by *pseudoscourfieldia marina* and *mesostigma viride.* R1 was dominated by *chlorella vulgaris* and *azotobacter vinelandii*. R2 and R3 dominated by *pseudoscourfieldia marina*. Experiment 3.

Figure 35 (showing the organism as percent of total genera) indicates that before startup, the dominant organism in the inoculum was *pseudoscourfieldia marina.* After two months of operation, the dominant species in R1 were the bacteria *azotobacter vinelandii* and the algae *chlorella vulgaris* (38% and 24% respectively), and in R2 and R3 the algae *pseudoscourfieldia marina*, (Figure 35). *Pseudoscrourfieldia marina* and *chlorella vulgaris* are genera of green algae and *azotobacter vinelandii* is a genus of spherical bacteria. These results indicate that after two months of operation, the conditions in R1 favored bacterial and algal growth, while in R2 and R3 the operation conditions favored green algal growth. *Azotobacter* could explain the improvement in settling at the end of the experiment in R1, as it has been identified in Patil et al. (2010) as a potential bioflocculant in wastewater treatment.

This study highlighted the importance of taking adequate samples and controlling the solids removal. Too low of an SRT resulted in high loss of biomass in all reactors. The operation conditions imposed on these reactors (a 2-day HRT and a 10-day SRT) resulted in conditions that could not be accurately measured to determine any differences in separation times for R1 and R2. The following experiment took these observations into consideration.

d. Experiment 4: Addition of sodium bicarbonate and long solids retention time effect on settling algae

R2 had the best overall performance in terms of biomass concentrations, productivity, SVI and settleability. The objective of experiment 4 was to investigate the effect of inorganic carbon addition and long SRT (approximately 22 days in R1 and R2) on algal settleability and productivity. The reactors were run as in experiment 3, with the following changes. Fresh inoculum from the University of New Mexico Duck Pond was used in all reactors. The R1 feed was operated as it was in experiment 2 with identical feed composition and a 60-minute settling time. R2 was operated identically to R1, except the feed included an addition of 1.0 g/L of sodium bicarbonate for the first 10 days which was then increased to 3.0 g/L for the remainder of the experiment. The sodium bicarbonate in the feed was increased to ensure there was adequate carbon for the biomass as it grew. The effects of carbon rich versus carbon deficient environments were assessed in terms of settling and settleability. The dark phase was eliminated in all reactors with 24 hours of illumination to test the limits of settleability at maximum light exposure. R3 was operated as it was in experiment 3, except for the elimination of the dark phase and the inclusion of 2.5 g/L of sodium bicarbonate for the first 10 days, and

7.5 g/L of sodium bicarbonate thereafter. In this manner, the sodium bicarbonate loading in R3 was identical to R2 (0.5 g and 1.5 g added per cycle).

i. Biomass concentrations

The biomass concentrations in the reactors and in the effluent are shown in Figure 36 and Figure 37. R2 had overall higher biomass concentrations than R1 and R3.

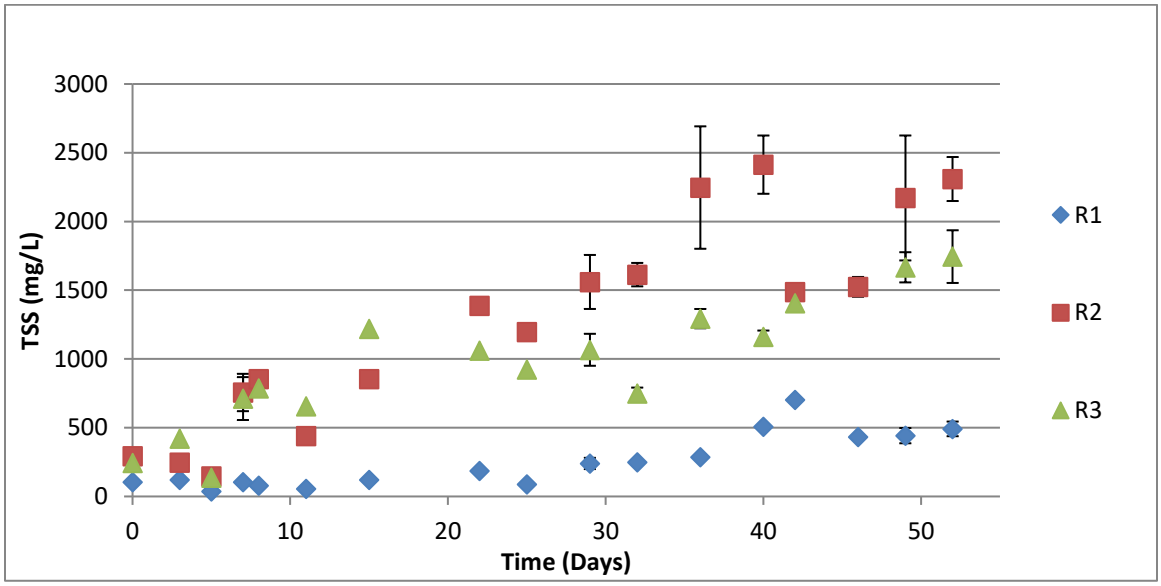

Figure 36: Biomass concentrations in R1, R2, and R3. R1 had little growth over the experiment. R2 had the most growth, followed by R3. On day 42, R2 had a massive loss of biomass followed by recovery. Experiment 4.

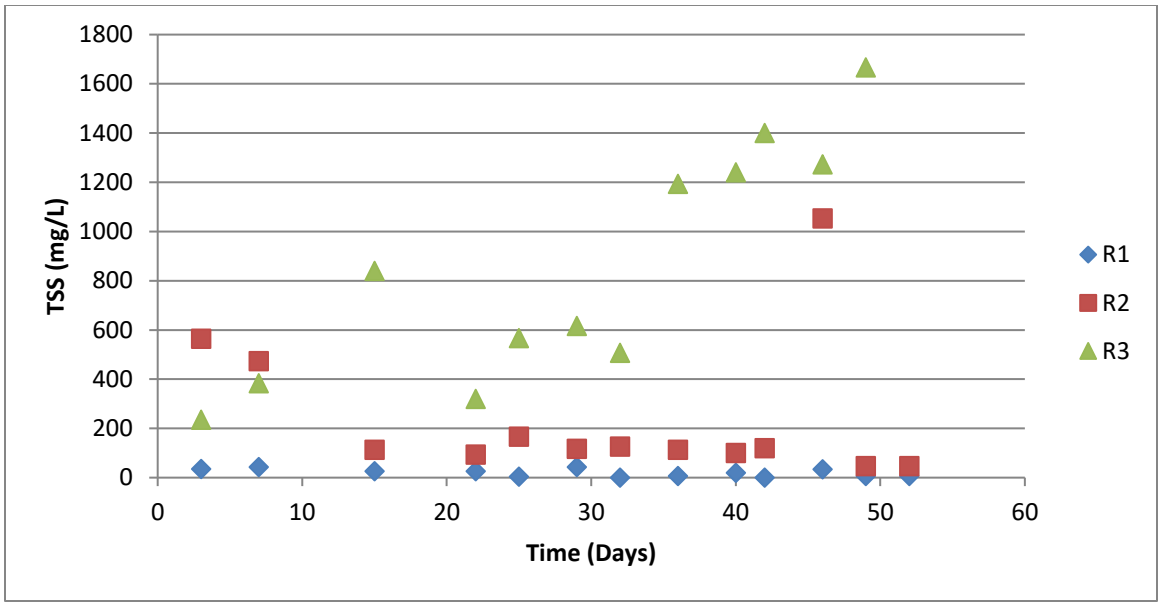

Figure 37: Effluent biomass concentrations in R1, R2, and R3. R1 and R2 had low biomass concentrations in the effluent. Experiment 4.

Table 13: *t*-test of reactor concentration and effluent concentrations. R3 reactor concentrations are not significantly different from its effluent. Experiment 4.

P-Value	R1 reactor TSS	R2 reactor TSS	R3 reactor TSS
R1 effluent TSS	0.000003		
R2 effluent TSS		0.000001	
R3 effluent TSS			0.9

The addition of sodium bicarbonate in R2 and R3 resulted in higher biomass concentrations. The R1 biomass concentration was low during experiment 4 (Figure 37), ranging from 104 mg TSS/L to 702 mg TSS/L. The increase towards the end of the

experiment may have been due to an increase in SRT. The SRT for the first 22 days was 7 ± 3 days then increased to 22 ± 8 days for the rest of the experiment. The added sodium bicarbonate in R2 greatly increased growth rates, with an increase in biomass concentration from 244 mg TSS/L to 2413 ± 211 mg TSS/L (initial measurement lacking standard deviation due to initial biomass concentration too small for multiple measurements) during the first 39 days of operation, and 1872 ± 428 mg TSS/L through the last 10 days of the experiment. The SRT for the first 32 days of operation was 10 days and then increased to 22 days for the rest of the experiment. R3 (control) biomass increased for the first 15 days of operation (from 244 ± 12 mg TSS/L to 1220 ± 25 mg TSS/L). The R3 biomass was 1241 ± 314 mg TSS/L through the last 37 days of the experiment. The SRT in R3 for the first 32 days of operation was approximately 9 days and then decreased to 4 days for the remainder for the experiment. Table 13 indicates that there was a similarity between R3 reactor TSS and its effluent TSS, suggesting that the SRT and HRT were similar, however it does not explain the findings in Figure 40. The higher SRT for the first 32 days could have resulted in some floc formation (Figure 44) which would falsely give the impression of a higher settleability. However, after the first 32 days, the SRT and HRT were identical, which resulted in dispersed growth allowing for an accurate calculation of settleability. The pH in R2 and R3 ranged from 7.5 after the feed phase then increased to 9.5 near the end of the 24-hour cycle. The pH in R1 remained high throughout the cycle (approximately 10), due to the lack of sodium bicarbonate.

i. Productivity

The productivity of all three reactors is shown in Figure 38. R2 had overall better productivity.

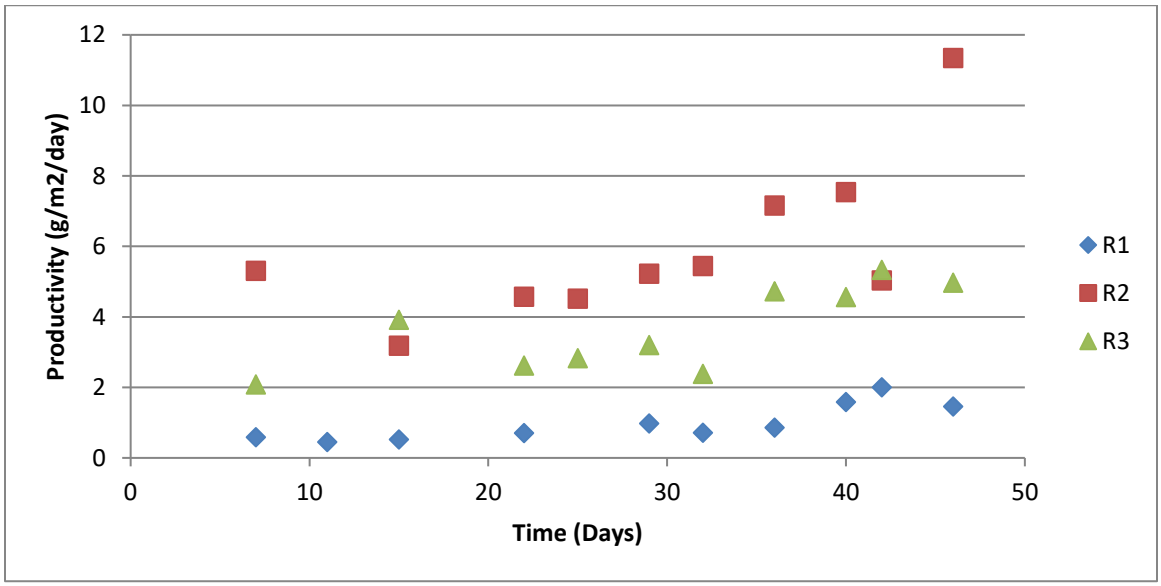

Figure 38: Productivity of all reactors. R2 had the highest productivity, R3 the second highest and R1 the lowest. The addition of sodium bicarbonate and incorporation of a settling phase resulted in a higher productivity in R2. Experiment 4.

Biomass productivity as calculated using Equation 6 is shown in Figure 39, with R2 having the highest productivity. The R1 productivity was 5.3 g/m^2/day on day 7 followed by a decrease to 3.9 g/m^2/day and gradually increased throughout the experiment. The R2 productivity generally increased throughout the experiment, ending at 11 g/m^2/day for the final measurement, and was almost always consistently higher than that of R1 and R3. The R3 productivity also generally increased and was 5.0 g/m^2/day at the end of the experiment. R1 had the lowest productivity values, which was due to lack of sodium bicarbonate in the feed. The higher effluent TSS in R2 at the beginning of the experiment, and subsequent decrease (Figure 37) could explain the decrease in

productivity during the first 15 days. R2 and R3 had the same loading of sodium bicarbonate, but the inclusion of a settling phase in R2 may have resulted in higher biomass and productivity than in R3.

ii. Settled volume, sludge volume index, and settleability

The settled volume, SVI, and settleability (Equation 4) for all reactors are shown in Figure 39, Figure 40, and Figure 41. R2 overall had better performance in SVI and settleability.

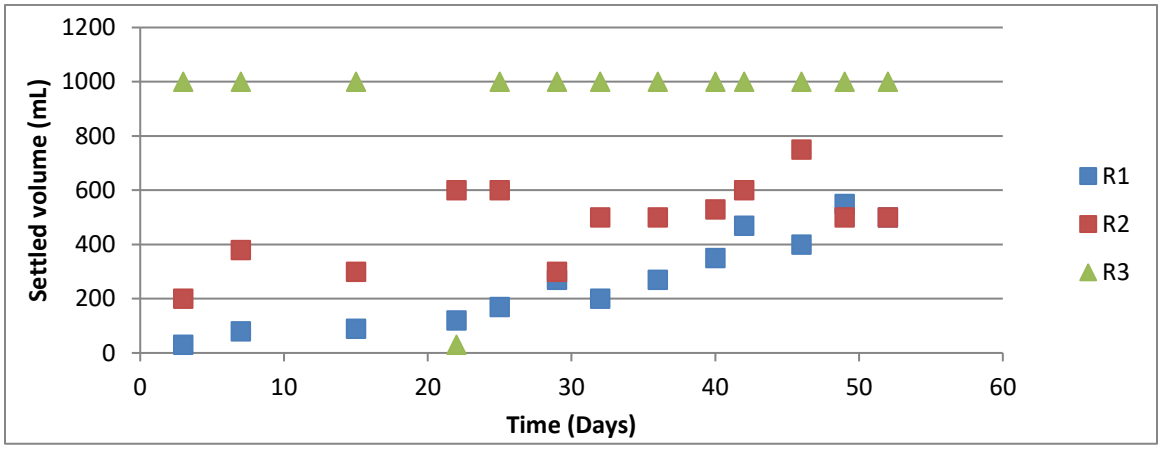

Figure 39: Settled volumes of reactors. R3 on day 22 had a small layer of biomass settle. R1 and R2 had increased settled volumes during the experiment. Experiment 4.

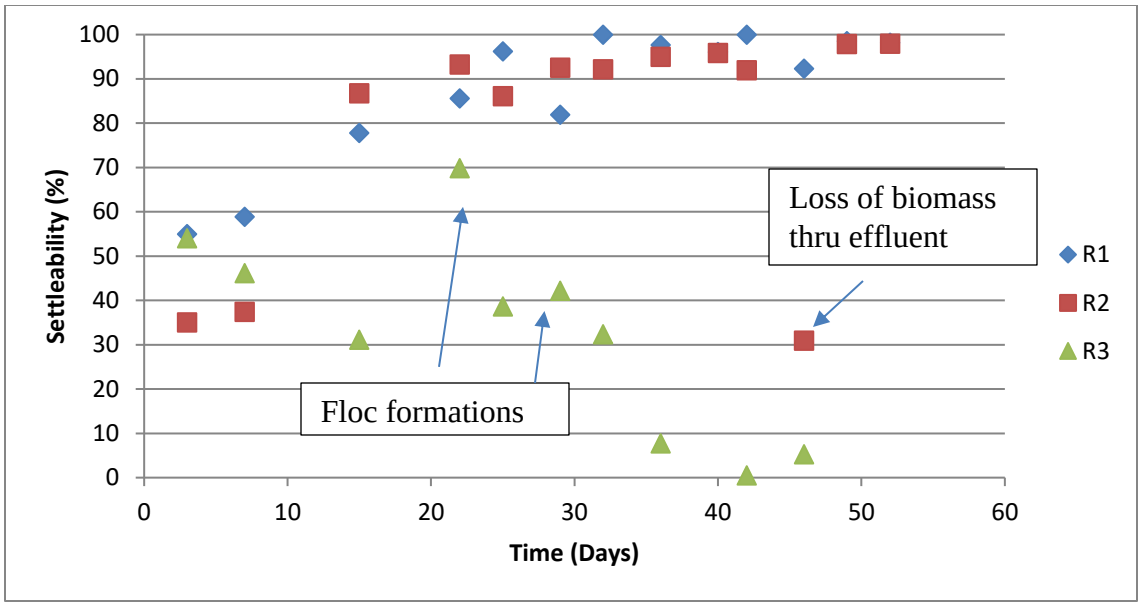

Figure 40: Settleability (%) in R1, R2, and R3. The settleability in R3 was due to a higher SRT and presence of biomass flocs for the first 32 days. Experiment 4.

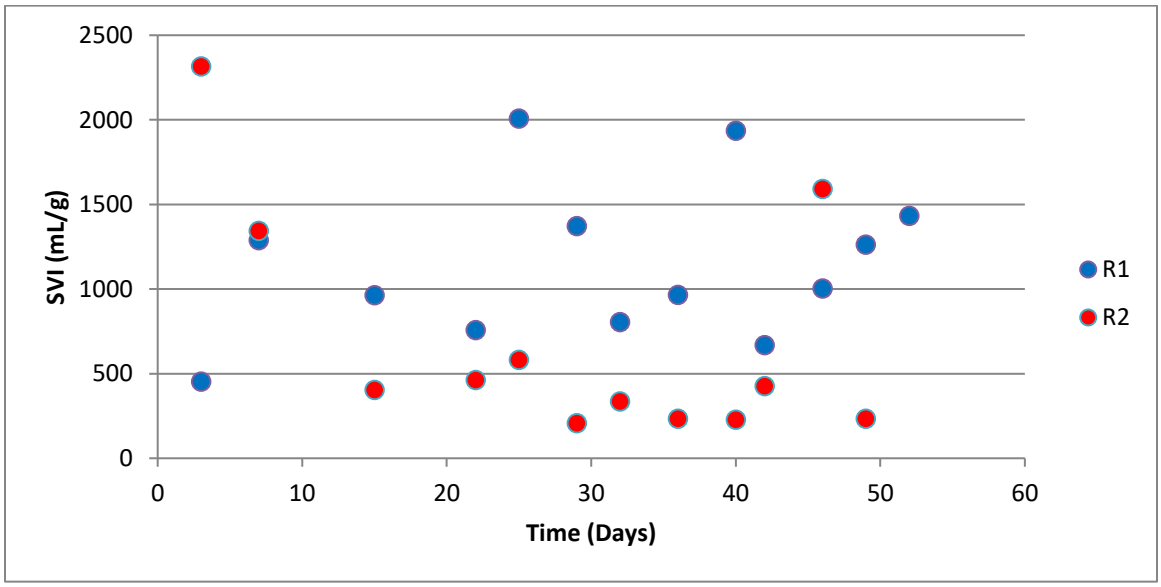

Figure 41: SVI of R1 and R2 adjusted for settleability. R3 values are not shown because it did not settle. Addition of sodium bicarbonate improved the SVI of R2 over that of R1. Experiment 4.

R1 had lower settled volume than R2, but worse settling performance in terms of SVI.

The R1 settled volume increased over the duration of the experiment from 80 to 550 ml

(Figure 40). The settleability and biomass concentrations increased throughout experiment 4 (Figure 41 and 37 respectively), which contributed to the increasing settled volume. Settled volume illustrates the differences in performance in the reactors, but can be misleading when quantifying the differences. SVI is a better indicator of settleability, as it is the settled volume divided by the mass of algae settled. Figure 41 is adjusted for the biomass that settled during the SVI test; SVI values for R1 increased moderately throughout the experiment. Not accounting for the biomass settled in the SVI test will give a false impression of better settling with smaller SVI values.

R2 had higher settled biomass than R1, but better SVI performance. The R2 settled volume was higher than for R1 throughout the experiment (Figure 40), which was consistent with the higher R2 biomass concentration throughout the experiment (Figure 37). On day 46, R2 experienced a loss of biomass because the settled volume was greater than the intake for the effluent. The settleability in R1 and R2 were similar throughout the experiment (Figure 41), with both increasing to greater than 90%. The R2 SVI was consistently lower than the R1 SVI (Figure 42), suggesting that CO_2 addition improved settleability. The R3 settled volume was consistently 1000 mL (Figure 39) (except for day 22, where there was a small layer of settled biomass that was subsequently removed from the reactor) indicating the biomass did not settle. These results demonstrate that the inclusion of a settling phase selected for a relatively well-settling biomass in R1 and R2, while omission of the settling phase in R3 produced biomass with essentially no settleability.

iii. Carbohydrates

The amount of carbohydrates expressed as a percent of total dry biomass is shown in Figure 42; R1 did not have high enough biomass concentrations to accurately measure its carbohydrate content. The carbohydrate content in the R2 and R3 biomasses decreased throughout the experiment.

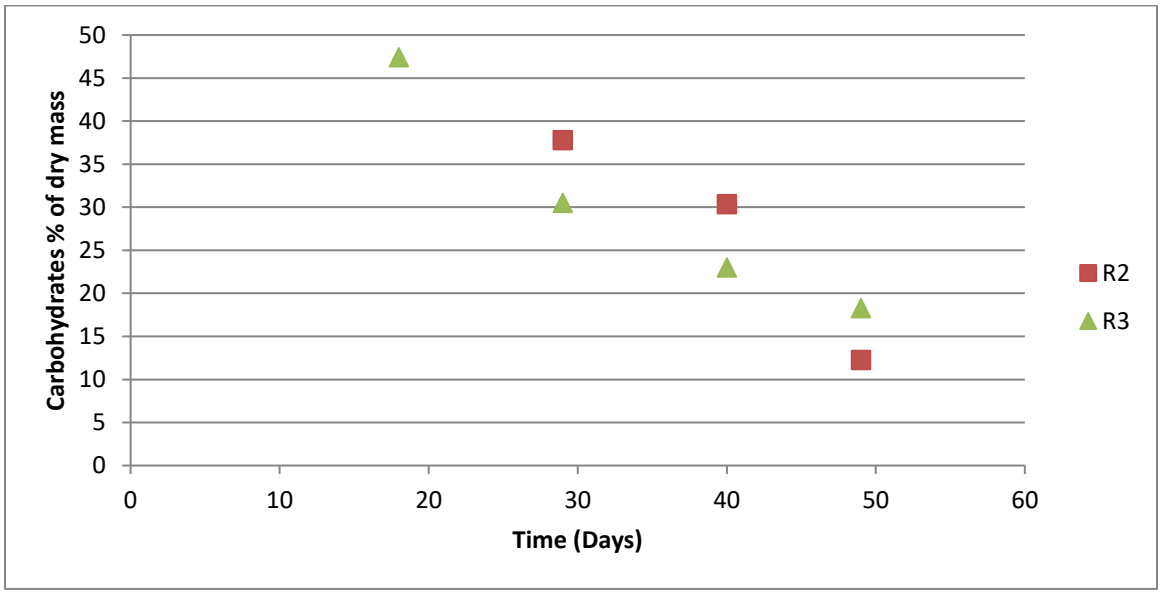

Figure 42: Percentage of carbohydrates in total biomass in R2 and R3. Both reactors had a decrease in carbohydrates during the experiment. Experiment 4.

The carbohydrate contents in R2 and R3 decreased during the experiment. The similar downward trends in both reactors suggest that loss of carbohydrate content is not operation dependent (since both were operated differently) but nutrient dependent, however analysis on macro-nutrient uptake (e.g. nitrogen and phosphorus) would be needed for confirmation. For comparison, Möllers, Cannella, Jørgensen, and Frigaard (2014) found the carbohydrate content as percent of total dry mass ranged from 60 to 20% in mixed algal cultures (cyanobacterium *Synechococcus sp*).

iv. Illumina sequencing and phase contrast microscopy

The Illumina sequencing results are shown in Figure 43, and phase contrast microscopy images are shown in Figure 44.

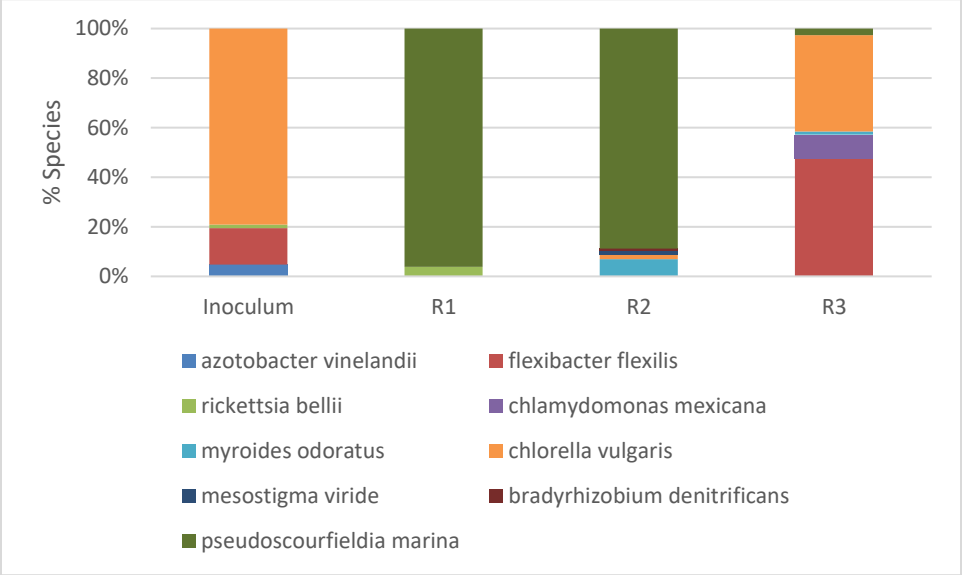

Figure 43: Illumina sequencing showing species. Samples of R1, R2, and R3 taken on day 52. X-axis is source. Inoculum dominated by the green algae *Chlorella vulgaris.* R1 and R2 were dominated by *Pseudoscourfieldia marina.* R3 was dominated by *Chlorella vulgaris* and *Flexibacter flexilis*. Experiment 4.

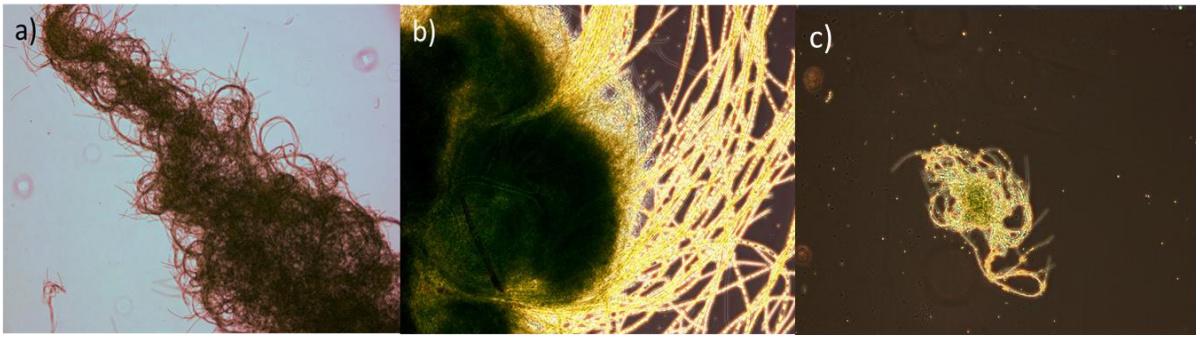

Figure 44: Phase contrast microscopy images of R1 (a), R2 (b), and R3 (c). Magnification of 100x. Taken on day 30. R1 and R2 had the presence of filamentous biomass while R3 had both dispersed and filamentous growth. Experiment 4.

Figure 43 indicates that before startup, the dominant algae organism in the inoculum was *chlorella.* After one month of operation, the dominant species in R1 and R2 was *Pseudoscrourfieldia marina,* and *Flexibacter flexilis* and *Chlorella vulgaris* in R3 (Figure 43). *Pseudoscrourfieldia marina* and *Chlorella vulgaris* are genera of green algae and have spherical morphology. *Flexibacter, Rickettsia*, and *Myroides* are genera of bacteria. *Flexibacter* is a chemo-organotroph that is typically filamentous. *Rickettsia* can be rod or wire-shaped and *Myroides* are rod-shaped. The shape of the biomasses in R1 and R2 were large filamentous flocs and in R3 there was dispersed and spherical biomass (Figure 44). These results indicate that algae were found in all reactors, but the presence of *Pseudoscrourfieldia marina* was found only in the settling reactors (R1 and R2). In addition, the Illumina results indicate that sodium bicarbonate addition did not have any influence on species selection, suggesting it was more dependent on the operation conditions.

Chapter 5 Conclusions

This study has provided knowledge on reactor operation variables on mixed algal/bacterial biomass productivity and solids separation. In this study an examination of operation parameters (solids separation times, light/dark cycle, solids retention time, and hydraulic retention time), density, and settleability were evaluated.

The following conclusions can be drawn from our study:

1. Density was not a good measure for comparison of reactor performance. The density of the algal floc in the system does not play a major role in the ability of the system to enrich for a settling biomass (experiments 1 and 2).
2. Reactor operation with wasting from the top half of the reactor can enrich for settling algae with high biomass concentrations (experiments 1,2, and 4). Reactors with selection for settling biomass enriches for large filamentous floc formation (experiments 1,2, and 3).
3. Settling performance of the reactors was not influenced by the dark or light phases (experiment 2). The density of the algal biomass is not significantly different in the light or the dark phase (experiment 1 and 2). An increase in density does not have any effect on the sludge volume index of the reactor, suggesting that floc morphology plays a larger role in settling performance.
4. Reactors operated with a settling phase and sodium bicarbonate addition resulted in better overall performance (experiment 4). Under identical nutrient loading, a reactor with a settling phase performs better than a continuously mixed system in productivity and settleability. The settleability was found to be independent of nutrient loading, and in particular to carbon loading.

5. The inclusion of a settling phase in reactors selected for filamentous biomass dominated by *Pseudoscrourfieldia marina.*

Further research needs to include the following:

- Evaluation of lipid productivity in the systems in this study
- Evaluation of nitrogen and phosphorous removal
- Nitrogen stress conditions with *Pseudoscrourfieldia marina*
- Investigation of reactor operation to enrich for high lipid producing algae

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Appendix A: Total Suspended Solids for All Experiments

Table 14: Total suspended solids (TSS) and effluent TSS for experiment 1

Reactor 1				
Day	Reactor TSS (mg/L)	Standard deviation (mg/L)	% Error	Effluent TSS (mg/L)
6/9/2015	86.67	10	59	20.00
6/16/2015	842.22	25	59.45	73.33
6/18/2015	113.33	15		93.33
6/22/2015	626.67	10	18	93.33
6/29/2015	1053.33	13	11	100.00
7/2/2015	1380.00	3		13.33
7/6/2015	1780.00	6		0.00
7/9/2015	1813.33	9	33	0.00
7/16/2015	2966.67	10	32	0.00
7/20/2015	3713.33	97	7	0.00
7/27/2015	4080.00	10	12	28.89
7/30/2015	3290.00	5	10	11.11
8/3/2015	4130.00	8	10	66.67
8/17/2015	4000.00	4		0.00
8/20/2015	4210.00	7		35.56
8/25/2015	3970.00	75	3	15.56
8/27/2015	4530.00	90	4	66.67
9/1/2015	3950.00	67	6	44.44
9/3/2015	3760.00	56	8	108.89
9/7/2015	3770.00	43	7	88.89
9/10/2015	2655.00	24	7	97.78
9/15/2015	2910.00	15	3	140.00

9/17/2015	3320.00	24	4	153.33
9/22/2015	2870.00	23	7	80.00
9/24/2015	3420.00	56	8	122.22
9/29/2015	3070.00	154		84.44
10/1/2015	2790.00	113		44.44
Reactor 2				
Day	TSS (mg/L)	SD	% Error	TSS Effluent (mg/L)
6/9/2015	166.67	19	11.4	13.33
6/16/2015	80.00	5	6.25	80.00
6/18/2015	140.00	27	19.28571	220.00
6/22/2015	20.00	3	15	253.33
6/29/2015	1320.00	114	8.636364	353.33
7/2/2015	60.00	10	16.66667	0.00
7/6/2015	380.00	65	17.10526	0.00
7/9/2015	46.67	13	27.85714	20.00
7/16/2015	60.00	8	13.33333	8.89
7/20/2015	60.00	13	21.66667	6.67
7/27/2015	253.33	56	22.10526	86.67
7/30/2015	153.33	34	22.17391	266.67
8/3/2015	180.00	43	23.88889	126.67
8/17/2015	440.00	113	25.68182	80.00
8/20/2015	140.00	27	19.28571	86.67
8/25/2015	520.00	96	18.46154	37.78
8/27/2015	773.33	154	19.91379	71.11
9/1/2015	1540.00	213	13.83117	226.67
9/3/2015	180.00	78	43.33333	88.89

9/7/2015	46.67	3	6.428571	62.22
9/10/2015	73.33	9	12.27273	66.67
9/10/2015	80.00	7	8.75	
9/15/2015	93.33	13	13.92857	91.11
9/17/2015	140.00	34	24.28571	33.33
9/22/2015	140.00	56	40	60.00
9/24/2015	293.33	67	22.84091	111.11
9/29/2015	520.00	134		144.44
10/1/2015	1150.00	168		
Reactor 3				
Day	Reactor TSS (mg/L)	SD	% Error	TSS Effluent (mg/L)
6/9/2015	46.67	10		60.00
6/16/2015	200.00	25	21.4	180.00
6/18/2015	153.33	15	12.5	146.67
6/22/2015	66.67	10	9.8	106.67
6/29/2015	113.33	13	15.0	66.67
7/2/2015	33.33	3	11.5	6.67
7/6/2015	33.33	6	9.0	20.00
7/9/2015	66.67	9	18.0	66.67
7/16/2015	53.33	10	13.5	40.00
7/20/2015	786.67	97	18.8	60.00
7/27/2015	80.00	10	12.3	53.33
7/30/2015	46.67	5	12.5	33.33
8/3/2015	86.67	8	10.7	66.67
8/17/2015	46.67	4	9.2	40.00
8/20/2015	80.00	7	8.6	48.89

8/25/2015	346.67	75	8.8	304.44
8/27/2015	186.67	90	21.6	53.33
9/1/2015	433.33	67	48.2	306.67
9/3/2015	173.33	56	15.5	115.56
9/7/2015	140.00	43	32.3	180.00
9/10/2015	120.00	24	30.7	91.11
9/10/2015	80.00	15	20.0	
9/15/2015	280.00	24	18.8	126.67
9/17/2015	166.67	23	8.6	168.89
9/22/2015	246.67	56	13.8	268.89
9/24/2015	1086.67	154	22.7	113.33
9/29/2015	266.67	113	14.2	776.00
10/1/2015	1530.00	208	42.4	

Table 15: Average reactor TSS for experiment 2

Reactor 1				
Date	Time	Average reactor TSS (mg/L)	SD	% Error
1/12/2016	8:00 AM	1453.33	56.57	3.89
1/12/2016	8:00 PM	1233.33	66.00	5.35
1/13/2016	8:00 AM	1193.33	28.28	2.37
1/13/2016	8:00 PM	1370.00	70.71	5.16
1/14/2016	8:00 AM	1286.67	52.92	4.11

1/14/2016	8:00 PM	1070.67	14.05	1.31
1/15/2016	8:00 AM	1050.00	73.99	7.05
1/20/2016	8:00 AM	873.75	132.58	15.17
1/21/2016	8:00 PM	866.67	30.14	3.48
1/21/2016	8:00 AM	1035.00	30.41	2.94
1/28/2016	8:00 PM	973.33	115.58	11.87
1/28/2016	8:00 AM	1061.67	93.85	8.84
2/3/2016	8:00 AM	735.00	39.05	5.31
2/3/2016	8:00 PM	598.33	63.51	10.61
2/4/2016	8:00 AM	1013.33	183.46	18.10
2/11/2016	6:30 AM	895.00	238.17	26.61
2/11/2016	6:30 PM	843.33	115.14	13.65
2/16/2016	8:00 AM	895.00	203.90	22.78
2/17/2016	7:30 AM	710.00	115.33	16.24
2/18/2016	7:00 PM	806.67	238.24	29.53
2/25/2016	7:30 AM	1537.50	272.24	17.71
3/3/2016	7:30 AM	1175.00	754.20	64.19

3/11/2016	7:30 AM	1551.67	887.92	57.22
3/12/2016	6:00 AM	814.44	219.25	26.92
3/14/2016	8:00 AM	1215.56	329.35	27.09
3/17/2016	7:30 AM	313.33	230.96	73.71
3/22/2016	7:30 AM	535.56	65.18	12.17
3/24/2016	8:00 AM	398.89	81.81	20.51
3/29/2016	6:00 AM	427.78	38.63	9.03
3/31/2016	6:00 AM	317.78	38.49	12.11
4/3/2016	6:00 AM	300.00	96.15	32.05
4/5/2016	6:00 AM	303.33	32.15	10.60
4/7/2016	6:00 AM	282.22	17.11	6.06
4/29/2016	6:00 AM	807.78	90.21	11.17
Reactor 2				
Date	Time	Average TSS (mg/L)	SD	% Error
1/12/2016	8:00 AM	1480.00	9.43	0.64
1/12/2016	8:00 PM	2046.67		
1/13/2016	8:00 AM	1390.00	61.28	4.41

1/13/2016	8:00 PM	1590.00	61.28	3.85
1/14/2016	8:00 AM	1460.00	94.04	6.44
1/14/2016	8:00 PM	1226.67	147.64	12.04
1/15/2016	8:00 AM	1001.67	27.54	2.75
1/20/2016	8:00 AM	972.50	144.96	14.91
1/21/2016	8:00 AM	1090.00	140.00	12.84
1/21/2016	8:00 PM	1201.67	20.21	1.68
1/28/2016	8:00 AM	1360.00	84.85	6.24
1/28/2016	8:00 PM	1236.67	70.06	5.67
2/3/2016	8:00 AM	1090.00	106.07	9.73
2/3/2016	8:00 PM	761.67	156.07	20.49
2/4/2016	8:00 AM	1295.00	238.80	18.44
2/11/2016	6:30 AM	1051.67	128.48	12.22
2/11/2016	6:30 PM	1015.00	57.66	5.68
2/16/2016	8:00 AM	1050.00	82.61	7.87
2/17/2016	7:30 AM	861.67	68.07	7.90
2/18/2016	7:00 PM	966.67	80.98	8.38

2/25/2016	7:30 AM	635.00	296.98	46.77
3/3/2016	7:30 AM	823.33	112.40	13.65
3/11/2016	7:30 AM	975.00	187.55	19.24
3/12/2016	6:00 AM	878.89	671.25	76.38
3/14/2016	8:00 AM	1058.89	268.75	25.38
3/17/2016	7:30 AM	398.89	21.69	5.44
3/22/2016	7:30 AM	353.33	17.64	4.99
3/24/2016	8:00 AM	288.89	88.46	30.62
3/29/2016	6:00 AM	390.00	202.68	51.97
3/31/2016	6:00 AM	764.44	263.36	34.45
4/3/2016		157.78	29.88	18.94
4/5/2016		232.22	40.18	17.30
4/7/2016		80.00	3.33	4.17
4/29/2016		1262.22	497.06	39.38
R3 (Control)				
Date	Time	Average TSS (mg/L)	SD	% Error
1/12/2016	8:00 AM	816.67	80.14	9.81
1/12/2016	8:00 PM	506.67	28.28	5.58
1/13/2016	8:00 AM	570.00	33.00	5.79

1/13/2016	8:00 PM	346.67	18.86	5.44
1/14/2016	8:00 AM	411.11	10.18	2.48
1/14/2016	8:00 PM	214.67	47.72	22.23
1/15/2016	8:00 AM	366.67	2.89	0.79
1/20/2016	8:00 AM	220.00	7.07	3.21
1/21/2016	8:00 AM	393.33	7.64	1.94
1/21/2016	8:00 PM	221.67	2.89	1.30
1/28/2016	8:00 AM	497.50	31.82	6.40
1/28/2016	8:00 PM	310.00	10.00	3.23
2/3/2016	8:00 AM	452.50	3.54	0.78
2/3/2016	8:00 PM	283.33	7.64	2.70
2/4/2016	8:00 AM	418.33	10.41	2.49
2/11/2016	6:30 AM	510.00	17.32	3.40
2/11/2016	6:30 PM	331.67	7.64	2.30
2/16/2016	8:00 AM	338.33	15.28	4.51
2/17/2016	7:30 AM	476.67	5.77	1.21
2/18/2016	7:00 PM	383.33	25.17	6.57

2/25/2016	7:30 AM	680.00	0.00	0.00
3/3/2016	7:30 AM	558.33	36.17	6.48
3/11/2016	7:30 AM	473.33	109.81	23.20
3/12/2016	6:00 AM	604.44	12.62	2.09
3/14/2016	8:00 AM	492.00	18.33	3.73
3/17/2016	7:30 AM	400.00	14.42	3.61
3/22/2016	7:30 AM	596.00	6.93	1.16
3/24/2016	8:00 AM	450.67	12.22	2.71
3/29/2016	6:00 AM	382.00		
3/31/2016	6:00 AM	262.50		
4/3/2016		655.00	134.35	20.51
4/5/2016		595.00	35.36	5.94
4/7/2016		500.00	0.00	0.00
4/29/2016		1290.00	14.14	1.10

Table 16: Reactor TSS and effluent TSS for experiment 3

Reactor 1				
Date	Reactor TSS (mg/L)	SD	% Error	Effluent TSS (mg/L)
5/14/2016	734.44	65.01	8.85	
5/16/2016	433.33	109.29	25.22	0

5/18/2016	241.90	174.88	72.29	0
5/20/2016	300.74	12.24	4.07	40
5/23/2016	278.33	7.64	2.74	10
5/25/2016	442.50	22.22	5.02	10
5/27/2016	353.33	10.41	2.95	0
5/30/2016	371.67	36.43	9.80	0
6/1/2016	277.04	17.82	6.43	3.33
6/3/2016	265.93	40.39	15.19	3.33
6/6/2016	309.63	31.92	10.31	0.00
6/15/2016	128.15	16.68	13.02	13.33
6/20/2016	135.56	18.19	13.42	53.33
6/24/2016	74.07	11.40	15.39	26.67
6/29/2016	16.67	3.06	18.33	
7/6/2016	17.78	8.89	50.00	6.67
7/13/2016	159.33	97.17	60.98	33.33
7/15/2016	137.33	153.73	111.94	
7/18/2016	136.00	5.29	3.89	
7/20/2016	82.00	3.46	4.22	3.33
7/25/2016	150.67	22.30	14.80	
Reactor 2				
Date	Reactor TSS (mg/L)	SD	% Error	Effluent TSS (mg/L)
5/14/2016	734.44	65.01	8.85	
5/16/2016	207.78	22.69	10.92	
5/18/2016	202.86	5.71	2.82	0
5/20/2016	155.56	8.01	5.15	50
5/23/2016	304.17	55.30	18.18	23.33

5/25/2016	290.00	36.31	12.52	20
5/27/2016	179.17	16.65	9.29	13.33
5/30/2016	197.50	21.36	10.82	13.33
6/1/2016	154.81	14.46	9.34	40
6/3/2016	100.74	12.83	12.74	50
6/6/2016	187.41	18.90	10.08	143.33
6/15/2016	157.04	7.80	4.97	146.67
6/20/2016	149.63	3.39	2.27	160
6/24/2016	122.96	2.57	2.09	126.67
6/29/2016	90.67	4.16	4.59	
7/6/2016	67.41	26.32	39.05	20
7/13/2016	142.67	72.23	50.63	3.33
7/15/2016	173.33	102.32	59.03	
7/18/2016	205.33	30.55	14.88	
7/20/2016	192.67	34.08	17.69	3.33
7/25/2016	206.00	16.37	7.95	
Reactor 3				
Date	Reactor TSS (mg/L)	SD	% Error	Effluent TSS (mg/L)
5/14/2016	734.44	65.01	8.85	
5/16/2016	625.56	103.35	16.52	0.00
5/18/2016	553.33	15.74	2.84	0.00
5/20/2016	503.70	7.80	1.55	223.33
5/23/2016	513.33	18.76	3.66	133.33
5/25/2016	375.00	7.50	2.00	76.67
5/27/2016	338.33	17.02	5.03	70.00
5/30/2016	324.17	13.77	4.25	76.67

6/1/2016	267.78	12.62	4.71	53.33
6/3/2016	196.67	3.33	1.69	63.33
6/6/2016	208.89	23.20	11.11	36.67
6/15/2016	131.11	11.55	8.81	26.67
6/20/2016	151.11	19.75	13.07	63.33
6/24/2016	248.15	16.68	6.72	73.33
6/29/2016	332.67	9.87	2.97	
7/6/2016	365.19	19.42	5.32	70.00
7/13/2016	318.67	27.15	8.52	66.67
7/15/2016	201.33	42.72	21.22	
7/18/2016	145.33	1.15	0.79	
7/20/2016	242.00	15.62	6.45	23.33
7/25/2016	82.67	4.16	5.04	

Table 17: Reactor TSS, Effluent TSS, and volatile suspended solids (VSS) for experiment 4

Reactor 1						
Date	Reactor TSS (mg/L)	SD	% error	Effluent TSS (mg/L)	Reactor VSS (mg/L)	VSS/ Reactor TSS (%)
8/9/2016	104.00					
8/12/2016	120.00					
8/14/2016	38.00					
8/16/2016	105.33			43.33		
8/17/2016	80.00					
8/20/2016	56.00				92.00	164.29
8/24/2016	120.00			26.67	120.00	100.00
8/31/2016	185.00			26.67	162.00	87.57

9/3/2016	88.00			3.33	113.00	128.41
9/7/2016	240.00	40.84	17.02	43.33	201.33	83.89
9/10/2016	248.00	17.44	7.03	0.00	205.33	82.80
9/14/2016	286.00	21.63	7.56	6.67	236.00	82.52
9/18/2016	507.33	2.31	0.46	20.00	394.00	77.66
9/20/2016	702.00	21.17	3.02	0.00	512.67	73.03
9/24/2016	432.00			33.33	343.00	79.40
9/27/2016	442.22	55.51	12.55	6.67	400.00	90.45
9/30/2016	491.33	53.27	10.84	6.67	355.33	72.32
Reactor 2						
Date	Reactor TSS (mg/L)	SD	% error	Effluent TSS (mg/L)	Reactor VSS (mg/L)	VSS/ Reactor TSS (%)
8/9/2016	294.00					
8/12/2016	246.67					
8/14/2016	146.00					
8/16/2016	756.00			473.33		
8/17/2016	853.33					
8/20/2016	440.00				456.67	103.79
8/24/2016	853.33			113.33	730.00	85.55
8/31/2016	1386.67			93.33	1353.33	97.60
9/3/2016	1196.67			166.67	1236.67	103.34
9/7/2016	1560.00	196.41	12.59	116.67	1528.89	98.01
9/10/2016	1613.33	85.11	5.28	126.67	1582.22	98.07
9/14/2016	2246.67	445.23	19.82	113.33	2200.00	97.92
9/18/2016	2413.33	211.97	8.78	100.00	2342.22	97.05
9/20/2016	1486.67			120.00	1386.67	93.27

9/24/2016	1524.44	71.91	4.72	1053.33	1484.44	97.38
9/27/2016	2171.11	454.38	20.93	46.67	2088.89	96.21
9/30/2016	2308.89	160.05	6.93	46.67	2235.56	96.82
Reactor 3						
Date	Reactor TSS (mg/L)	SD	% error	Effluent TSS (mg/L)	Reactor VSS (mg/L)	VSS/ Reactor TSS (%)
8/9/2016	244.00					
8/12/2016	420.00					
8/14/2016	136.00					
8/16/2016	712.00	155.90	21.90	383.33		
8/17/2016	786.67					
8/20/2016	656.67				573.33	87.31
8/24/2016	1220.00			840.00	983.33	80.60
8/31/2016	1060.00			320.00	853.33	80.50
9/3/2016	923.33			566.67	890.00	96.39
9/7/2016	1066.67	116.24	10.90	616.67	986.67	92.50
9/10/2016	748.89	42.86	5.72	506.67	655.56	87.54
9/14/2016	1293.33	70.24	5.43	1193.33	1116.67	86.34
9/18/2016	1162.22	44.39	3.82	1240.00	1055.56	90.82
9/20/2016	1406.67			1400.00	1190.00	84.60
9/24/2016	1344.44	3.85	0.29	1273.33	1195.56	88.93
9/27/2016	1666.67	109.75	6.58	1666.67	1477.78	88.67
9/30/2016	1744.44	191.64	10.99	1744.44	1693.33	97.07

Appendix B: Density Measurements

Table 18: Density measurements for experiment 1

Date	Time	R1	R3	R2
7/20/2015		1.039	1.037	1.039
7/29/2015		1.055	1.078	1.055
8/3/2015	6:30 AM	1.052	1.055	1.052
8/3/2015	8:30 AM	1.049	1.068	1.078
8/3/2015	10:30 AM	1.049	1.050	1.052
8/3/2015	12:30 PM	1.050	1.049	1.065
8/3/2015	2:30 PM	1.050	1.055	1.059
8/3/2015	4:30 PM	1.050	1.050	1.059
8/3/2015	6:30 PM	1.050	1.055	1.062
8/3/2015	8:30 PM	1.049	1.059	1.068
8/3/2015	10:15 PM	1.049	1.062	
8/3/2015	11:15 PM	1.049	1.078	1.078
8/17/2015	8:20 AM	1.065	1.055	1.068
8/20/2015	6:00 AM	1.068	1.055	1.062
8/20/2015	1:00 PM	1.067	1.065	1.067
8/20/2015	8:30 AM	1.063	1.062	1.063
8/27/2015	5:30 AM	1.076	1.075	1.078
8/27/2015	6:50 AM	1.065	1.073	1.075
8/27/2015	8:20 AM	1.065	1.068	1.068
8/27/2015	12:30 PM	1.065	1.049	1.075
8/27/2015	4:30 PM	1.059	1.047	1.052
8/29/2015	9:45 PM	1.068	1.067	1.072
8/29/2015	10:30 PM	1.068	1.067	1.068
9/7/2015	4:40 AM	1.065	1.085	1.076

9/7/2015	6:30 AM	1.070	1.078	1.076
9/7/2015	8:30 AM	1.065	1.088	1.097
9/7/2015	10:45 AM	1.070	1.080	1.070
9/10/2015	1:30 PM	1.062	1.088	1.075
9/17/2015	7:30 AM	1.065	1.088	1.089
9/17/2015	9:00 AM	1.065	1.091	1.089
9/17/2015	12:50 PM	1.057	1.088	1.094
9/17/2015	3:25 PM	1.055	1.065	1.065
9/17/2015	5:15 PM	1.059	1.075	1.072
9/17/2015	7:25 PM	1.059	1.078	1.068
9/17/2015	9:00 PM	1.065	1.098	1.078
9/24/2015	7:15 AM	1.065	1.089	1.065
9/24/2015	9:15 AM	1.052	1.104	1.059
9/24/2015	1:05 PM	1.070	1.104	1.078
9/24/2015	3:30 PM	1.065	1.102	1.088
9/24/2015	6:30 PM	1.065	1.104	1.098
9/24/2015	9:00 PM	1.075	1.111	1.094
10/1/2015	7:45:00 AM	1.065	1.065	1.089
10/1/2015	1:00:00 PM	1.055	1.076	1.089
10/1/2015	3:15:00 PM	1.052	1.076	1.083
10/1/2015	5:00:00 PM	1.059	1.080	1.086

Table 19: Density Measurements for experiment 2

AM Density				
Date	Time	R1	R3	R2
1/12/2016	8:00 AM			
1/13/2016	8:00 AM	1.0455	1.0715	1.05525
1/14/2016	8:00 AM	1.04875	1.06825	1.052
1/15/2016	8:00 AM	1.04875	1.076375	1.05525
1/21/2016	8:00 AM	1.052	1.079625	1.056875
1/28/2016	8:00 AM	1.050375	1.095875	1.0715
2/3/2016	8:00 AM	1.052	1.099125	1.073125
2/11/2016	6:00 AM	1.052	1.099125	1.069875
2/15/2016	9:00 AM	1.050	1.099125	1.067
2/25/2016	6:30 AM	1.054	1.101	1.067
3/3/2016	6:45 AM	1.054	1.098	1.067
PM Density				
Date	Time	R1	Control	R2
1/12/2016	8:00 PM	1.052		1.052
1/13/2016	8:00 PM	1.039	1.0585	1.0455

1/14/2016	8:00 PM	1.050375	1.06825	1.053625
1/15/2016	8:00 PM			
1/21/2016	8:00 PM	1.052	1.06825	1.06175
1/28/2016	8:00 PM	1.052	1.091	1.069875
2/3/2016	8:00 PM	1.05525	1.073125	1.073125
2/11/2016	6:00 PM	1.050375	1.091	1.0715
2/18/2016	6:00 PM	1.0455	1.089375	1.073125
2/25/2016	7:30 PM	1.050	1.095875	1.072
3/2/2016	8:00 PM	1.049	1.088	1.075

Appendix C: Settled Volume, Sludge Volume Index, and Settleability

Table 20: Settled Volume, SVI and Settleability for experiment 3

Reactor 1			
Date	Settled volume (mL)	SVI (mL/g)	% Settled
5/16/2016	60	138.46	
5/18/2016	100	413.22	
5/20/2016	170	565.27	86.70
5/23/2016	230	826.35	96.41
5/25/2016	230	519.77	97.74

5/27/2016	160	452.83	100.00
5/30/2016	330	887.89	100.00
6/1/2016	280	1010.70	98.80
6/3/2016	220	827.30	98.75
6/6/2016	100	322.97	100.00
6/15/2016	30	234.10	89.60
6/20/2016	30	221.31	60.65
6/24/2016	20	270.00	64.00
6/29/2016	20	1125.05	
7/6/2016	90	5062.72	62.50
7/13/2016	100	627.62	79.08
7/20/2016	140	1707.32	95.93
Reactor 2			
Date	Settled volume (mL)	SVI (mL/g)	% Settled
5/16/2016	60	288.78	
5/18/2016	70	345.08	
5/20/2016	80	514.30	67.86
5/23/2016	50	164.38	92.33
5/25/2016	40	137.93	93.10
5/27/2016	30	165.14	92.66
5/30/2016	50	253.16	93.25
6/1/2016	60	387.56	74.16
6/3/2016	70	694.86	50.37
6/6/2016	1000	5335.89	23.52
6/15/2016	1000	6367.93	6.60
6/20/2016	1000	6683.20	-6.93
6/24/2016	1000	8132.53	-3.01

6/29/2016	1000	11029.41	
7/6/2016	50	741.73	70.33
7/13/2016	70	490.65	97.66
7/20/2016	150	778.55	98.27
Reactor 3			
Date	Settled volume (mL)	SVI (mL/g)	% Settled
5/16/2016	80	127.89	
5/18/2016	80	144.58	
5/20/2016	90	178.68	55.66
5/23/2016	210	409.09	74.03
5/25/2016	240	640.00	79.56
5/27/2016	500	1477.83	79.31
5/30/2016	350	1079.69	76.35
6/1/2016	410	1531.12	80.08
6/3/2016	220	1118.63	67.80
6/6/2016	210	1005.32	82.45
6/15/2016	90	686.44	79.66
6/20/2016	80	529.41	58.09
6/24/2016	110	443.28	70.45
6/29/2016	240	721.44	
7/6/2016	340	931.03	80.83
7/13/2016	180	564.85	79.08
7/20/2016	130	743.80	90.36

Table 21: Settled Volume, SVI and Settleability for experiment 4

Reactor 1				
Date	Settled Volume (mL)	SVI (mL/g)	SVI based on settled biomass (mL/g)	% Settled
8/16/2016	80.00	759.50	1290.33	58.86
8/24/2016	90.00	750.00	964.29	77.78
8/31/2016	120.00	648.65	757.89	85.59
9/3/2016	170.00	1931.82	2007.87	96.21
9/7/2016	270.00	1125.00	1372.88	81.94
9/10/2016	200.00	806.45	806.45	100.00
9/14/2016	270.00	944.06	966.59	97.67
9/18/2016	350.00	1860.82	1937.19	96.06
9/20/2016	470.00	669.52	669.52	100.00
9/24/2016	400.00	925.93	1003.34	92.28
9/27/2016	550.00	1243.72	1262.76	98.49
9/30/2016	500.00	1407.13	1434.04	98.12
Reactor 2				
Date	Settled Volume (mL)	SVI (mL/g)	SVI based on settled biomass (mL/g)	% Settled
8/16/2016	380.00	502.65	1344.34	37.39

8/24/2016	300.00	351.56	405.41	86.72
8/31/2016	600.00	432.69	463.92	93.27
9/3/2016	600.00	501.39	582.52	86.07
9/7/2016	300.00	192.31	207.85	92.52
9/10/2016	500.00	309.92	336.32	92.15
9/14/2016	500.00	222.55	234.37	94.96
9/18/2016	530.00	219.61	229.11	95.86
9/20/2016	600.00	393.53	428.08	91.93
9/24/2016	750.00	491.98	1591.98	30.90
9/27/2016	500.00	230.30	235.36	97.85
9/30/2016	500.00	216.55		97.98
Reactor 3				
Date	Settled Volume (mL)	SVI (mL/g)	SVI based on settled biomass (mL/g)	% Settled
8/16/2016	1000.00	1404.49	3042.60	46.16
8/24/2016	1000.00	819.67	2631.58	31.15
8/31/2016	30.00	28.30	40.54	69.81
9/3/2016	1000.00	1083.03	2803.74	38.63
9/7/2016	1000.00	937.50	2222.22	42.19

9/10/2016	1000.00	1335.31	4128.44	32.34
9/14/2016	1000.00	773.20	10000.00	7.73
9/18/2016	1000.00	860.42	-12857.11	-6.69
9/20/2016	1000.00	710.90	149992.50	0.47
9/24/2016	1000.00	743.80	14062.59	5.29
9/27/2016	1000.00	600.00	3000000001.10	0.00
9/30/2016	1000.00	573.25		

Appendix D: Calibration Curve for Carbohydrate Analysis

Refers to carbohydrate analysis in experiment 4, see Chapter 4, section d.iii.

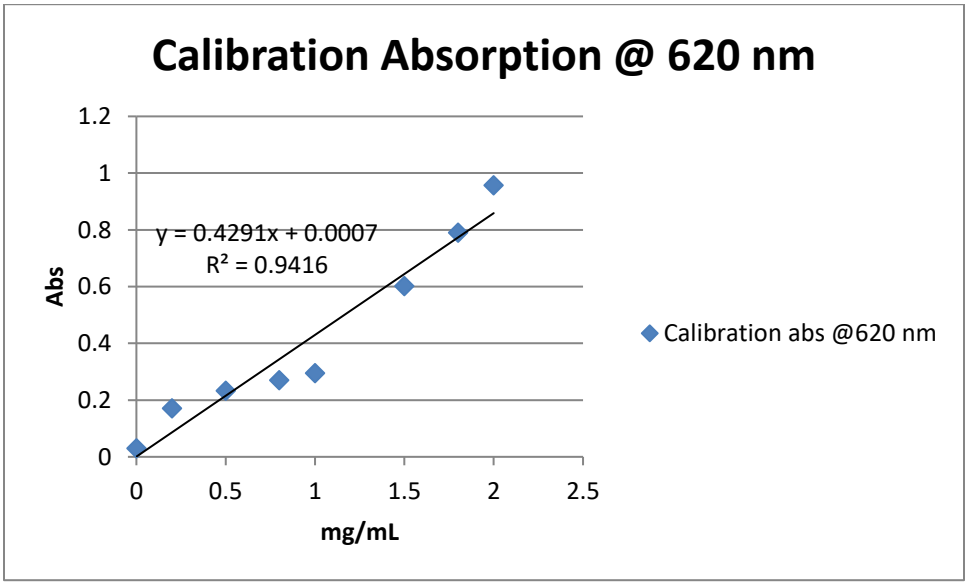

Figure 45: Calibration curve for carbohydrate analysis.