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Evaluation of the Performance of a Downward Flow Inclined Gravity Settler for Algae Dewatering

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EVALUATION OF THE PERFORMANCE OF A DOWNWARD FLOW INCLINED GRAVITY SETTLER FOR ALGAE DEWATERING

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ABSTRACT

With recent concerns over the environmental implications of burning fossil fuels coupled with the depletion of fossil fuel reserves an alternative source of energy is needed. Algae derived biofuels may be an effective replacement for transportation fuels as they are carbon neutral and have a high area productivity. Algae is superior to terrestrial plants as a biofuel source due to its high oil productivity and efficiency along with the fact that it will not displace food production. Currently the largest obstacle to the implementation of commercial algae to biofuel processes is algae dewatering. The separation of algae from water is difficult due to the dilute concentration of the algae suspension and the extremely low settling velocity of the algae biomass. This work investigates recent improvements to the downward flow inclined gravity settler which has the potential to unlock this much needed process. Additionally, an investigation into algae settling velocity, a field which has received little attention, is also discussed.

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CHAPTER I

INTRODUCTION

The development of carbon neutral renewable energy sources is a critical issue meriting study. While solutions like nuclear, solar, and wind energy production have received much attention in recent years these technologies face many obstacles to replace fossil fuels to power transportation systems. While there have been many recent advancements in battery and electric motor technology geared to creating practical electric vehicles, it is unlikely that electric propulsion can completely replace the internal combustion engine in the near future. Biologically derived hydrocarbon fuels, called biofuels, have the potential to replace fossil fuels for use in automotive and aviation applications.

Biofuels main advantages over electric powered cars are the use of existing infrastructure and technology. Transportation power sources have requirements that are difficult to meet, most notable of which are energy density and refueling time, and by using existing infrastructure and technology, few new inconveniences should be added by changing from fossil fuels to biofuels.

Though there are many plant species that can be used to create biofuels, such as corn, soybeans, and palm trees. Microalgae is one of the most promising candidates currently being actively researched. This is because algae is highly efficient at

converting sunlight to compounds that are easily converted to biofuels, thanks to its lack of structural components that are high in lignin and cellulose. This efficiency translates into reducing the land area needed to cultivate algae for fuel production. The major drawback to algae is the difficult separation required to remove the algae biomass from the water in which it is grown. The combination of its low concentration, small size, and density similar to water make this an especially difficult separation.

The downward flow inclined gravity settler is a simple apparatus that is capable of separating the algae from the water it was grown in. It is essentially a long thin box that is held on an angle while an algae suspension is pumped through it. The algae in the liquid will settle onto the lower surface of the settler where it will then slide to a second outlet. Previous work involving the downward flow inclined gravity settler separating algae from water found a five fold increase in the algae concentration at moderate flow rates and angles (Hou, 2011).

1.1 Objectives

The separation of algae from its growth medium is known to be a difficult process. This is because of many factors, chief among them is the low concentration of algae biomass, at best a few milligrams per liter, which can be produced from any commercially viable system (Chisti, 2007). Additionally, the small cell size of algae, in the range of 10 to 30 μm, makes separation with filter systems extremely difficult (Sawayama 1995). Finally, the similarity of the density of the algae cells to that of water, a trait necessary to keep algae in suspension during cultivation, makes sedimentation based separations extremely difficult (Chisti, 2007). The downward flow inclined gravity

settler system allows a simple low energy method to process large amounts of algae suspension at low cost. The original downward flow inclined gravity settler was intended for perfusion cultures of mammalian cells and needed several modifications to be effective for use in an industrial algae to biofuel process. This study focused on the simplification of the settler system to simplify manufacturing and assembly while testing the new design to ensure that those changes had no effect on the effectiveness of the settler. The settler outlet was changed to be in line with the flow chamber in addition to the settler being modified to simplify the cleaning and repair of the settler. As such a new prototype inclined settler was designed and tested.

This study also examined the performance of the settler over a range of flow rates and angles as opposed to previous studies which used relatively few angles. This more comprehensive analysis will enable the development of operating guidelines for the commercial use of this settler system. In addition a study of the settling velocity of *Scenedesmus Dimorphous* was undertaken, an action that was omitted from the previous study using the downward flow inclined gravity settler system in conjunction with algae.

A settling velocity study also examined the settling velocity of the algae *S. Dimorphous* under a variety of circumstances. The influence of the growth phase on algae settling velocity was subject to preliminary investigations while another part of the settling velocity study examined the effect of saltwater acclimation on settling velocity.

1.2 Significance

With the recent development of the downward flow inclined gravity settler, additional information is required to develop this technology for the use as part of a

commercial algae to biofuel process. This study has illuminated some of the roadblocks in the implementation of a downward flow inclined gravity settler. While more research into algae adhesion to solid surfaces and methods to prevent that adhesion is needed this study has found promising operating conditions that attempt to maximize throughput and separation effectiveness while minimizing the adhesion problem. This study has also produced a settler design that while similar to the original model in internal geometry is far easier to assemble, clean, and maintain. The study found that the changes to the design have caused no observable deterioration in the effectiveness of its separation. Additionally, the settling velocity study achieved two major objectives.

The settling velocity component of this study achieved important milestones. With the discovery that the settling velocity increases after an algae culture ceases growth and improved harvesting schedule can be developed. Previous work in this area has always assumed continuous growth and harvesting will be the most efficient method of algae biomass production (Chisti, 2007). This result has found that from a separation perspective that would be a terrible idea and that batch or semi-batch farming, with the reduced difficulties from contamination, may prove to be superior.

This study has already enabled other studies attempting to create a computer model of the inclined gravity settler. Preliminary data concerning the settling velocity of growing algae cultures and the size distribution that was provided to Scott Hug allowed him to produce accurate simulations of exponents performed by Wang and Hou (Hug, 2011). Finally, the confirmation that the adaptation of *S. Dimorphous* to brackish water has no observable influence on the settling velocity of the culture is of great importance. This final piece of information enables the confirmation of the viability of the use of

adapted algae in biofuel production. Additionally, the fact that the adapted algae exhibits the same trend relating growth phase to settling velocity further proves that no major change in settling velocity properties occurs.

CHAPTER II

BACKGROUND

The use of microalgae for the production of biofuels is a new and exciting target of research. No company has been able to successfully commercialize the algae to biofuel process. Currently there are many companies that use microalgae to produce valuable products such as beta carotene and other diet supplements or food additives (Chisti, 2007). Pharmaceutical processes like beta carotene production are both small scale and sell relatively high priced products. A biofuel facility must be able to sell biofuel at a cost at or below the cost of traditionally produced fuels.

Microalgae has been cultivated for thousands of years with the first documented instance being in present day China (Wang, 2009). Currently the largest use of algae culture is for production of beta carotene for use in food supplements. The largest production facilities are located in Israel while the largest concentration of production is in Southeast Asia centered in Thailand (Chisti, 2007).

2.1 **Biofuels**

With recent changes in the economy and governmental regulations, in response to global climate change caused by the greenhouse effect, there has been much interest in

finding a renewable and environmentally friendly energy source. Solar, wind, nuclear, geothermal, and hydroelectric generation of electricity are currently being implemented but are not practical for the complete replacement of transportation fuels. Biologically derived liquid fuels are the leading candidate for replacement of petroleum for transportation purposes (Morweiser, 2010).

Recently, due to legislation, corn-derived ethanol has been added to gasoline to reduce net greenhouse gas emissions. This has led to some serious consequences most noticeable of which was a price increase, first in corn, then other food products. This was because of the dramatic increase in corn demand followed by the repurposing of farmland to grow corn. In addition to causing food prices to rise, the addition of ethanol to gasoline led to many issues of deterioration of the fuel system of many older vehicles (Adriance, 2012). Biodiesel derived from various sources has been found to have no major negative side effects. The only noticeable consequence of using biodiesel was that fuel filters would have drastically shortened lifespans over the first few tanks of biodiesel as the biodiesel would clean the fuel system. Once the initial changeover was accomplished, fuel systems using biodiesel experienced fewer issues than prior to the change to biofuel (Bennink, 2012).

Unfortunately, most sources of biofuels require extremely large amounts of land to produce necessary amounts of biofuel. For many biofuel sources such as corn, soybeans, canola, jatropha, and coconuts to completely replace petroleum, for domestic transportation use, a land area greater than what is currently farmed in the United States would be required. It has been found that there is no terrestrial crop that is efficient

enough to replace petroleum without requiring unfeasible large areas of land; only microalgae is efficient enough to realistically replace petroleum (Chisti, 2007).

Production of biofuel from algae is usually accomplished by a four step process. The first step is the cultivation of the algae, producing the biomass that will be processed to make the oil. The second step is the separation of the algae from the culture medium. This separation is currently the largest roadblock in the implementation of commercial algae to biofuel production. The third step is the extraction of lipids from the recently dewatered algae. The final step is the transterification of the extracted lipids to produce a biocrude close to diesel fuel.

2.1.1 Algae Culture

2.1.1.1Factors Affecting Algae Growth

The growth of most algae species is dependant on three major factors. The first and potentially most important factor is the availability of light. If there is insufficient light intensity algae growth will be slowed. As a general rule, the increasing of light intensity, while below a certain threshold, will cause algae growth to increase. At light intensities above the threshold, algae growth will be inhibited by temporary damage to the chlorophyll. If the algae is exposed to a cycling light source of greater intensity than the threshold the growth inhibition will not be experienced if the rate of cycling is high enough and the periods of darkness are long enough. In bulk algae cultures, the liquid near the surface in direct light will have the highest light intensity and as the light passes through the culture the light intensity will decrease at a rate proportional to the algae concentration. Therefore, in most algae cultures in direct sunlight there exists a region,

near the surface, where the algae is light inhibited, a deeper region with near optimal light concentration, and a region near the bottom which is mostly dark with the algae experiencing extreme light deprivation. Optimal bioreactor performance requires adequate mixing of algae to move the cells from one region to another allowing the light that enters the reactor to be used most effectively (Chisti, 2007).

The second factor affecting algae growth is the presence and concentration of CO_2. If the CO_2 concentration is below a certain concentration the growth of algae is drastically reduced (Fernandez, 2001). All aquatic organisms, including algae, have an optimal pH and any major deviation from that pH is detrimental to the organism. Therefore, the acidity caused by excessive CO_2, causing high carbonate ion concentration, is detrimental to the growth of algae cultures in addition to causing an additional expense.

The third factor affecting algae growth is the concentration of nutrients in the growth medium. Certain basic nutrients like phosphorus, sulfur, nitrogen, potassium, and iron are required for most organisms and as such are required in any growth medium. Magnesium is necessary as it is essential for the production of chlorophyll. Lastly, the presence of certain vitamins in the growth medium, while not essential to algae growth, can simplify the synthesis of organic compounds needed for algae growth and increase the growth rate of the algae culture. Much research has been dedicated to optimizing the composition of the algae growth medium to either increase the growth rate or minimize excess concentrations of more expensive constitutes to reduce the cost of growing algae. One of the most commonly explored routes for the reduction of algae growth media cost is the use of sewage or anaerobic digester effluent as a feedstock (Schwenk, 2012). This

provides valuable nutrients, such as phosphates, sulfates, and nitrates, while also allowing the algae production facility to double as a water treatment facility. Many algae species have been found to uptake many common heavy metals such as chromium. The ability of certain algae species to uptake heavy metals can be useful if a proposed facility is to double as a waste treatments facility to lower the cost of media and offset the oil production costs. Unfortunately, the uptake of heavy metals in algae may be detrimental for the use in animal feed, one of the more commonly proposed byproducts of an algae biofuel facility (Chisti 2007).

2.1.1.2 Open Pond Bioreactors

Algae culture is currently performed in one of two methods: in a bioreactor or in an open pond. Open pond cultures are of three forms: the first is a large lagoon, the second is a circular agitated pool, and the third is a raceway. Open lagoon algae cultivation is the oldest form of algae aquaculture and requires the least resources and energy input for cultivation (Pulz, 2001). Due to the lack of agitation open lagoon algae culture for biofuel production remains economically nonviable due to the low productivity and possible algae concentration, based on current separation technology. If future advances in algae separation allow inexpensive separation of dilute algae solutions this method of aquaculture may become effective in regions with extremely low land value and abundant water resources.

Agitated pool cultivation is most often used in Southeast Asia. Agitated pools are almost always open to the environment and circular in order for simple agitation equipment to be used. Most agitated pools are only between 10 and 20 meters in

diameter (Sim, 1988). Typically, agitated pools are between 3 to 5 feet deep but in some operations can be as shallow as 1 foot deep. Some low budget operations use small manually agitated pools often of natural origin (Sawayama, 1995).

The third open pond culture method and the one receiving the most attention for new algae culture projects is the racetrack pond. Racetrack ponds are long oval or serpentine shaped channels filled with water between a depth of 1 to 3 feet, typically 2 feet deep. Simple installations can be constructed from earthen ditches covered with plastic sheeting while more permanent facilities will have a layer of concrete forming the bottom of the raceway. Racetrack ponds have been found to require little energy to maintain mixing while still having an extremely high productivity for an open pond system. Mixing is maintained by a paddle wheel that moves the algae suspension through the raceway (Chisti, 2007).

2.1.1.3 Closed System Bioreactors

Bioreactor culture for algae has typically been reserved for use in the production of high value products. While some species of algae grown in bioreactors are fed sugar most are grown in clear photobioreactors. Photobioreactors are split between three different designs: stirred tank, tubular, and column. Stirred tank reactors are typically for small scale due the low surface area to volume ratio. Early experimentation for nutrient requirements or other trial research for full scale algae culture was performed using stirred tank reactors. Additionally, sugar fed algae cultures for the production of extremely valuable products are typically grown in stirred tanks due to the ease of operation.

Tubular photobioreactors are the most productive form of photobioreactor and are capable of growing algae at the highest concentrations. Tubular photobioreactors are comprised of a long series of clear pipes or tubes through which the algae solution is pumped. Due to buildup of oxygen causing photoxidation, pressure drop in the pipes, and the depletion of CO2, tubular reactors can only have continuous runs of about 80 m for most tube diameters (Chisti 2007). Scale up of a tubular photobioreactor system is accomplished by having tubes run in parallel commonly with tubes located on racks suspended in a grid while smaller or lower cost systems will have tubes located on the ground. While stirred tank reactors experience a decrease in surface area to volume ratio upon scale-up, tubular photobioreactors can maintain a constant surface to volume area upon scale-up.

Pumping the algae suspension and facilitating the gas transfer present unique challenges for tubular bioreactors. Unlike in open ponds or even stirred tanks, algae present in a tubular photobioreactor system are unable to transfer gas while in the tube runs. This causes the buildup of oxygen, which when combined with the high intensity of light experienced by a large fraction of the algae, will cause the degradation of the algae and drastically slow algae growth. This photo-oxidation based growth inhibition will even affect properly mixed cultures. Sheer is also a major concern which affects both the tube dimensions to reduce sheer damage to algae while in the tubes and the pump design as most pumps will damage algae as it passes through the pump. A new pump design, the airlift pump was developed to avoid damaging the algae (Fernandez, 2001).

Column photobioreactors are noted for their high productivity and simplicity (Molina Grima, 1999). The column bioreactor combines the gas transfer properties of a stirred tank reactor with the light transfer properties of the tubular reactor. The column photobioreactor is a narrow vertical tube, about 8 inches in diameter, which has an aeration system of some kind in the bottom. This system will not have oxygen accumulation and sheer stress limitations like in the tubular reactor. Scale up can be performed by creating taller columns but is more often accomplished by more of these columns in a gird arrangement.

2.1.2 Algae Dewatering

Separation of the algae from the culture medium, also know as algae dewatering, is a difficult process due to its unique challenges. Any proposed system must be of low cost both for initial purchase and for operating costs while being able to separate the dilute algae biomass from large amounts of water. Currently the major separation systems of interest are drum filtration, chemical flocculation, air froth flotation, centrifugation, and sedimentation. All systems have their advantages, disadvantages, and design challenges that must be overcome.

Filtration of algae is a difficult process due to the large amount of water that must be processed and the small pore size required to contain the algae. Algae caking on the filter and infiltration into the pores reduce the volume of algae suspension that can be processed by filtration. The use of sufficiently small pore sizes or even membrane filtration is infeasible due to the large energy requirements that are necessary to process that volume of water. Currently drum filtration is the only filtration method that has been

found to be effective at processing algae. Drum filtration has been found to be effective at processing large celled algae species and those that are composed of clusters of cells. Drum filtration has proven ineffective at processing small celled algae due to the cells penetrating the belt, lowering throughput and entering the filtered water side (Sawayama 1995).

Chemical flocculation is a preprocessing technique that relies on the addition of chemicals to cause the algae to cluster into large groupings, enabling easier separation with another method. While changing the temperature or removal of carbon dioxide has been found to result in some flocculation, the effects are minimal. The changing of the pH by large degrees by addition of sodium hydroxide, addition of certain specialized polymers, or chitosan have been found to be the most effective methods of chemical flocculation. The most cost effective of these chemicals is chitosan which is extracted from the crushed shells of crustaceans (Sim, 1988). This method works best when combined with filtration, air frothing, and sedimentation. The use of chemical flocculation to prepare an algae suspension for filtration provides the most benefit when used on small-celled algae species, decreasing the penetration by algae cells of the filter pores. The benefits of this processing system with large algae cells are outweighed by the additional expenses. The flocculation of algae causes it to have a higher settling velocity enabling the use of smaller settling tanks, greatly reducing the footprint of the equipment. The chemicals required to induce flocculating are quite expensive and are currently not fiscally feasible. Additionally, the inability to remove the chemicals from the processed growth medium prevent the recycling of water, greatly increasing the water requirements of any facility that uses this separation process. The presence of chemicals

used to induce flocculating limit possible uses of the residual biomass after the extraction of the lipids. This is especially disadvantageous as one of the most profitable uses of the residual biomass is for sale as animal feed.

One novel form of flocculation which has only recently come to light is the field of magnetophoretic algal separation technologies. This method uses expensive magnetically active nanoparticles of magnetite or a similar ferromagnetic material which can be recovered and reused reducing the cost of flocculation. This method is not without its drawbacks as recovery has only been proven at the laboratory scale, the particles require frequent recoating to maintain effectiveness and extremely high loadings are required to maintain effectiveness (Lim, 2011).

The use of air froth flotation has proven effective for the separation of algae. An algae suspension within a long vertical tube is fed air from the bottom. The algae is drawn to the interface between the bubbles and air by surface tension and is carried up with the bubbles. At the surface, the foam created by the bubbles contains a large amount of algae biomass with small amounts of water. While froth flotation can be an effective separation tool (Levin, 1962), for a long time it has been ignored in favor of techniques more suited to high value products. Due to the lack of recent studies, it is unknown whether the process would be economically competitive given the costs associated with energy use for air compression.

Traditionally, for the concentration of high-value cells in suspension, centrifuges have been the method of choice. Centrifuges require little space when compared with traditional sedimentation methods even when chemical flocculation is implemented. Centrifuges can effectively take the dilute algae suspension and concentrate it sufficiently

for lipid extraction. Unfortunately, centrifugation of algae requires an extraordinary amount of power, so much so that one study found that it was impossible to extract enough energy from algae harvested to power the centrifuge (Sym, 1988).

The method of separation with the lowest separation cost is sedimentation. Sedimentation requires relatively little power to operate and only requires simple equipment relative to the other harvesting systems. Traditional sedimentation separation technology uses a large tank with a long residence time. Such systems are effective for use when the solids can be periodically removed, but the when the solid product can deteriorate with time and is the actual object of the separation, large tanks are not feasible (Sawayama, 1995). Inclined sedimentation has the potential to require a far smaller footprint while allowing continuous recovery of the biomass (Wang, 2009). Additional discussion about inclined sedimentation is contained in Section 2.2.

2.1.3 Lipid Extraction

The lipids can be removed from the algae biomass once the biomass has been separated from the growth medium. The lipid extraction is accomplished by first breaking the cell walls of the algae cells, followed by a solvent extraction. If the cell walls are not broken then the lipid recovery of the solvent extraction would be at unsatisfactory levels. While most laboratory groups completely dry and then grind algae samples prior to lipid recovery, this would not be effective for industrial scale processes (Gigante, 2014). One effective method of breaking the cell walls industrially is by sonication (Mercer, 2011).

Once the cell walls have been burst a solvent extraction can then take place. While there are many possible solvents the leading candidates are chloroform and hexane. Chloroform has been found to have the highest lipid recovery but presents a large health risk for the workers at a plant that processes the algae biomass. Additionally, any residual chloroform present in the algae biomass after the extraction is complete would prevent many uses of the biomass. Hexane, while having a lower lipid recovery, would be the better choice of solvent for large-scale use (Mercer, 2011).

The most profitable use of the biomass would be to have it sold as animal feed. That necessitates having a minimal concentration of harmful chemicals. The other proposed use of the leftover biomass would be to use it as a feedstock for an anaerobic digester. The anaerobic digester would produce methane which could either be sold or burned to generate electricity, with the exhaust from the generator providing additional carbon dioxide, enabling less expensive culture of the algae. Unfortunately, the bacteria cultures present in an anaerobic digester cannot survive in an environment with excessive chloroform concentrations (Clu-In.org).

2.1.4 Lipid Processing

The majority of oils and other hydrocarbons extracted from most plants are unable to be used in conventional diesel engines due to various difficulties such as vapor pressure and viscosity. These oils, especially fatty acids, must be processed such that they can be used in conventional engines. The most common method to render these fatty acids useable is to use a transesterification reaction. In a transesterification reaction triglycerides are reacted with an alcohol such as methanol to form glycerol and methyl

esters at high temperatures and pressures in the presence of a catalyst. The resulting biocrude is useable as a biodiesel once catalysts are removed, or it can be further processed to yield a range of other products. It is possible to change the properties of the final product by varying the alcohol used with larger alcohols yielding a heavier ester (Merher, 2004).

An alternative to extraction and transesterification of the fatty acids would be to process a concentrated algae slurry using pyrolysis (Agrawal, 2013). This method can be performed in one of two different methods, the first could be as part of an electrical generation process where the algae, possibly combined with other solid fuels such as coal, would be completely combusted in two stages as part of the generation of electricity (Penney, 2012). The second method would be to use fast pyrolysis to generate a low grade fuel of about 40 octane rating based on the results of various terrestrial plants (Bartis, 2008). It is highly likely that algae biomass, which has significantly less lignin and cellulose, would yield a biofuel from fast pyrolysis that is superior to that produced using terrestrial plants as a feedstock.

2.2 Gravity Settler Background

Gravity settler technology has been under development since the 1920s (Ponder, 1925). The original gravity settlers were little more than large tanks where solids would settle to the bottom for periodic removal. Modifications to the original design to reduce the size and residence times of such systems include a small scale vertical column, a conical tank, and the inclined gravity settler. Inclined gravity settlers were developed to reduce the volume and footprint of the settler. Early inclined gravity settlers were

comprised of an angled rectangular box with a number of dividers running along the length of the box. The fluid would be fed near the bottom of one side of the box and the clarified liquid would be removed from the opposite side near the top. The inclusion of the dividers acts to reduce the distance that the solids would need to settle to reach a surface; effectively turning one large settler into many more efficient smaller settlers (Batt, 1990).

Currently upward flow inclined gravity settlers are used in older sewage treatment plants as a secondary solids removal process, though they have been largely replaced with large combined settling/fermentation tanks. Since the 1970s, Parson Co. has sold a Lamella Ecoflow inclined plate settler for water treatment use. This upward flowing settler uses one tenth of the space required for traditional treatment methods (Parson Lamella Ecoflow Brochure). Additionally, upward flow inclined gravity settlers have proved promising for use in experimental continuous ethanol fermentation processes (Maia, 1992). Upward flow settlers are limited to applications where long residence times for the solids to be separated are acceptable. Any applications where the solids can strongly attach to the surfaces of the settler require frequent cleaning, as is true with algae, reducing the attractiveness of this technology. The upward flow design of gravity settler has effectively remained unchanged since its development in the early 20th century. The original downward flow gravity settler was designed to provide a low-stress cell retention device for perfusion bioreactors. The cell residence time of upward flow inclined gravity settlers was found to be too long, potentially leading to decreased productivity and cell death. In order to reduce the residence time the clarified and inlet streams were reversed. The reversal of the flow direction yielded many positive results,

most notable of which were the drastic reduction of residence time of solids, a small increase in efficiency of the separation, and greater system flexibility. It proved effective in the perfusion culture of mammalian cell cultures and in preliminary tests with algae dewatering (Wang, 2014).

2.2.1 Downward Flow Settler Studies

The large spherical mammalian cells used by Wang provide little to no difficulties for the downward flow inclined gravity settler system. By using these easy to separate and process cells in the initial development of the system severe limitations were imposed regarding repurposing the system to other cell lines. The work by Wang for mammalian cells was complete even including iterative prototyping and settling velocity studies, which would enable simulations. The later collaboration with Hou was by comparison was rather limited in scope. This work proved that the settler system was compatible with algae cell lines opening the door for future work. Additionally, it was proven that the system would be able to yield a five fold increase in algae concentration and the settlers could be linked in series to provide an extremely concentrated final product. Unfortunately, the limitations in the scope of this work delayed follow up studies. The lack of a settling velocity study of the algae prevented simulation of the settler with this new cell line preventing exploration of many modifications simplifying the subsequent iterative prototyping.

2.3 Settling Velocity

To develop a complete understanding of the separation of algae using an inclined gravity settler system, knowledge of the settling velocity is critical. While this information is vital for the optimization of algae separation processes, there has been little research on this topic (Hug, 2011).

Equation 1: $U_i=SQRT((2 * G * M_p * (P_p - P)) / (P * P_p * A_p * C_D))$

Well established relationships are known for dilute suspensions of spherical, slowly settling particles. An equation for settling velocity is shown in Equation 1 (Green, 1997). This equation was derived for particles of simple geometries, though it can be applied to more complex geometries, experiencing free fall in Newtonian fluids and ignores skin drag. It is based on the velocity of the particle in question, U_i being based on several other factors of the fluid and particle. These factors include the local gravitational acceleration G, the mass of the particle M_p, the densities of the particle P_p and the fluid P, the cross sectional area op the particle perpendicular to the direction of motion A_p, and the drag coefficient C_d which is based on a number of factors including geometry of the particle and the Reynolds number. It should be noted that the drag coefficient must be found experimentally except for the simplest of geometries. While the modification of Stokes Law for particles of various simple shapes has been performed, the applicability of this is unknown when applied to complex irregular shapes (Green, 1997). Understanding of the settling velocity of complicated shaped particles is limited and mostly empirical in nature (Smith, 1998). From the limited information available, it has been shown that larger algae particles settle faster than smaller algae cells though the settling velocities of various algae species vary immensely. However,

most algae sedimentation studies were performed as part of environmental studies on populations comprised of numerous algae species, in only a semi-quantitative manner (Larocque, 1995).

Two major types of settling velocity measurement apparatus are in use by different fields. The first type of apparatus is comprised of a small tube with a divider to split the tube into an upper and lower portion or a clear side wall to easily view observable particles. This type of settling measurement apparatus does not use periodic sampling and is more common in medical diagnostics. Most commonly this method is used to measure the settling velocity of red blood cells but new methods have been developed to measure the settling velocity of other solids. Recent advancements in cameras and dynamic light scattering allow individual particles to be tracked in real time yielding actual settling velocity distributions (Malarkley, 2013). The other type of settling velocity measurement apparatus common in environmental analysis is a large tube from which samples are periodically drawn from the bottom of the tube, and the mass of dry solids measured from each sample. The settling velocity apparatus developed by Wang et al. (Wang, 2010) is a hybrid of those two types of apparatus, combining the small sample size used in the first type with the ability to quantitatively measure settling velocities of particle distributions of the second. Details of this method will be described Chapter 3 of this thesis.

CHAPTER III

MATERIALS AND METHODS

This thesis was comprised of two separate sections, a review of the performance of an improved gravity settler prototype and a settling velocity study. The gravity settler study investigated the effect that changes in angle and flow rate have on the settler's performance. The settling velocity study initially sought to find a relationship between algae clustering and settling velocity. Additionally, the study determined a link between culture growth states and settling velocity.

3.1 Algae Cell Line

S. Dimorphous samples (#417 and #1237) were acquired from "The Culture Collection of Algae" at University of Texas at Austin for use in this study. *S. Dimorphous*, pictured in Figure 1A, was chosen as the algae species in this study as it is a promising candidate for commercial biofuel production, due to its medium growth rate coupled with a medium lipid content. There are many algae species that have either a higher growth rate or a higher lipid content but few that have both. Additionally, S. Dimorphous is a very robust and hardy algae species capable of surviving stresses, such as sheer, salinity changes, pHchanges, and temperature swings, which would kill most

cells. A second cell line of *S. Dimorphous* that had been adapted to grow in brackish water was also used in this study (Gigante, 2013).

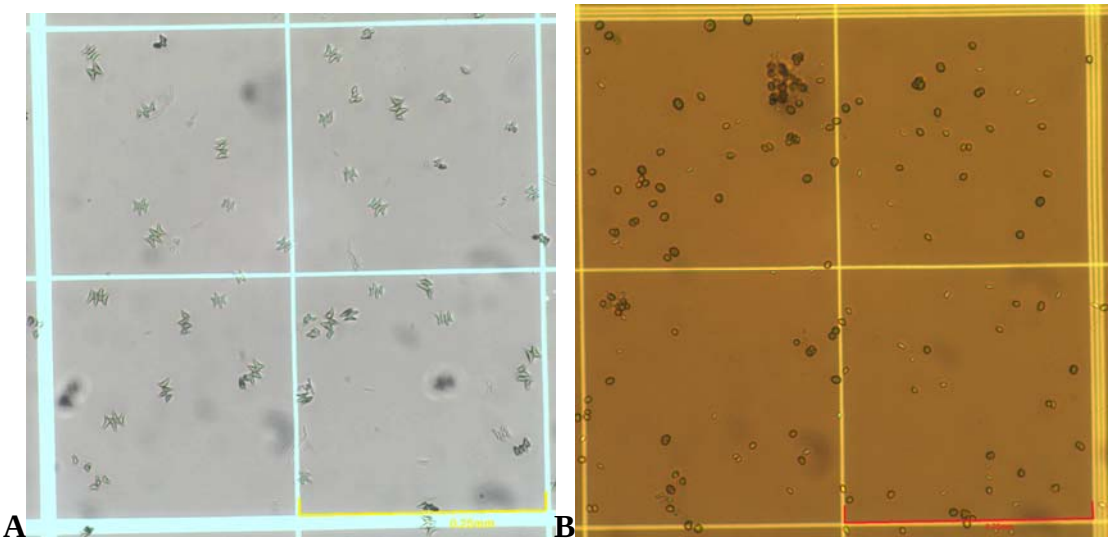


Figure 1A - Photograph of *S. Dimorphous* on hemocytometer slide. Figure 1B - Photograph of 1.015 TSG Brackish water acclimated *S. Dimorphous* on hemocytometer slide. Small squares visible are 0.25 mm by 0.25 mm.

3.2 Gravity Settler Design

The gravity settler used in this study was based on the design developed by Zhaowei Wang (Wang 2009). This design has been modified to be simpler to manufacture, inherently stackable, reusable, designed for disassembly and reassembly, and less prone to blockage. A schematic of Wang's gravity settler design is shown in Figure 2A while the current design is shown in Figure 2B. The first of the major modifications to the system was the changing of the outlet from a downward pointing flow separator to a flow separator that was in line with the rest of the settler. This greatly simplified the design and structural integrity of the settler and allowed the design to be inherently stackable as each plate in a stack is flat and required no offsetting.

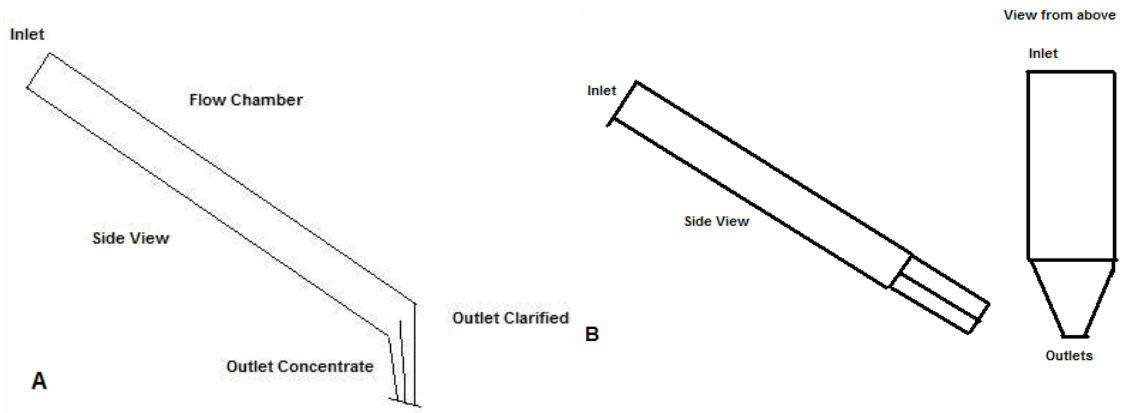


Figure 2A - Schematic of Wang's downward flow inclined gravity settler design. Figure 2B – Schematic of current gravity settler design used in this study.

The second major modification was altering the settler such that it was able to be rebuilt and cleaned. The previously used settlers were constructed of polycarbonate plates assembled using epoxy, and as such were difficult to repair or clean. When a leak developed, the entire settler had to be completely dissembled and then rebuilt. By using a design based on machine screws and gaskets it is now possible to partially dissemble the settler for cleaning or to tighten certain sections to stop leaking. The use of screws allowed the settler to be assembled much faster than with the epoxy constructed version. While the new design is slightly more likely to develop a leak due to either improper construction or mishandling, any leaks that do develop are much easier to stop.

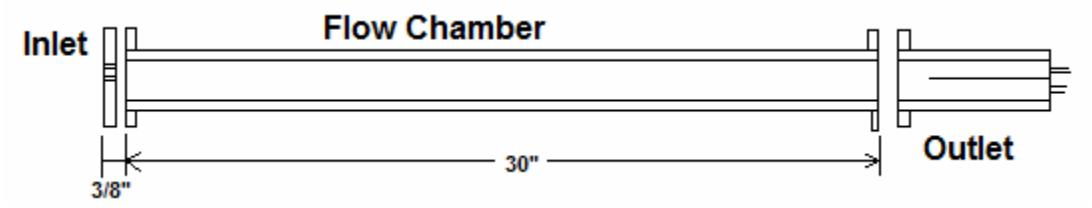


Figure 3: assembly view of entire settler system. Not to scale.

A third major change to the settler was the development of a modular system for research purposes. The settler is composed of three separate components, all of which can be substituted separately in a different design. The three components are shown in

the assembly drawing in Figure 3. These three elements will be described briefly here, and in detail in the following section. The first component is the flow chamber located in the middle of the settler. It can be replaced with a longer or shorter flow chamber as necessary. The second component is the inlet. The inlet is a polycarbonate plate that has holes and fittings for both the vacuum line that is used to fill the settler and the inlet line. The third component is the outlet which effectively connects to the flow chamber and has connections for both the clarified and concentrated streams. The three components all contain flanges that permit them to be bolted together.

3.2.1 Gravity Settler Inlet.

The gravity settler inlet is made of a single 3/8th inch thick sheet of polycarbonate, with dimensions of 2”x4”, as seen in Figure 4. The inlet has two main small holes drilled into it, one centered and the other near one of the sides slightly offset to the top of the piece. Each of the holes has a fluer silicone tubing connector, with the threads removed from the large end, inserted into it and sealed with RTD Silicone Sealant. The centered tubing connecter is the algae inlet. The off-center connecter is the vent. In addition to the holes with the tubing connecters there are eight holes for No. 8 machine screws, four on the top and four on the bottom.

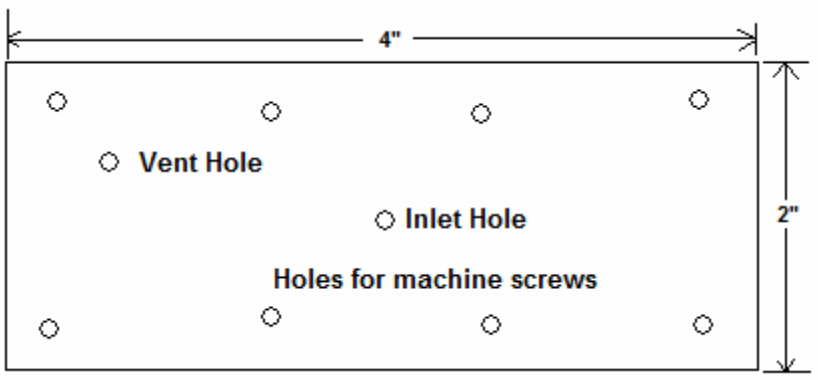


Figure 4: schematic of inlet for gravity settler.

3.2.2 Gravity Settler Flow Chamber

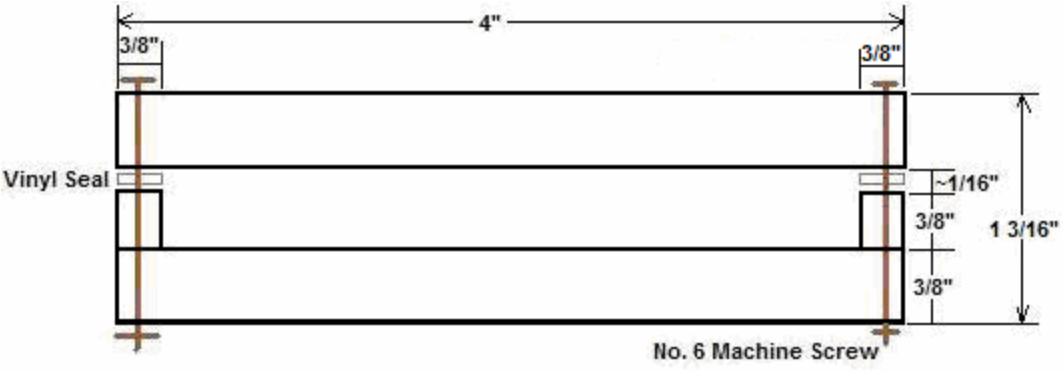


Figure 5 – Schematic of cross section of flow chamber; flanges are not shown. Side view with flanges shown can be seen in Figure 3.

The gravity settler flow chamber is made from two 3/8th inch thick polycarbonate sheets, each 3 and 3/8^{ths} inches wide by 30 inches long. One of the sheets has two 3/8th inch square pieces by 30" long glued to the upper side on the edges using a polycarbonate bounding chemical. A cross sectional schematic of the flow chamber is shown in Figure 5. A strip of vinyl M-D Building Products premium garage door weather-stripping, that was cut to fit, nominally 3/8^{ths} of an inch wide, was placed between the upper and lower polycarbonate sheets. Ten holes, five on each long side, are drilled at equal intervals through both of the sheets and the 3/8th inch insert. The flow chamber was held together

using brass No. 6 machine screws inserted into each of the ten holes, held in place with nuts and washers. Additionally, two pieces of 3/8th inch thick by four inches wide by 7/16th inch long polycarbonate were attached to the ends of each large sheet to act as a flange to hold the flow chamber to both the inlet and the outlet. The flange pieces each have four holes, suitable for No. 8 machine screws, positioned to match the placement on the inlet.

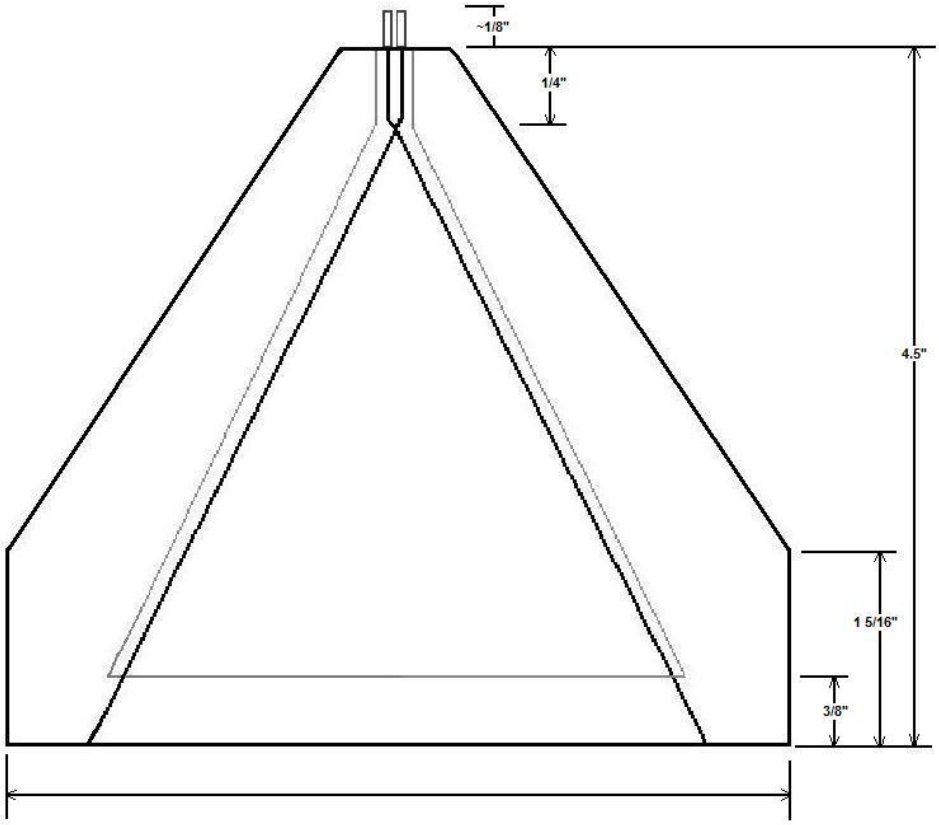
3.2.3 Gravity Settler Outlet

A  **B** 

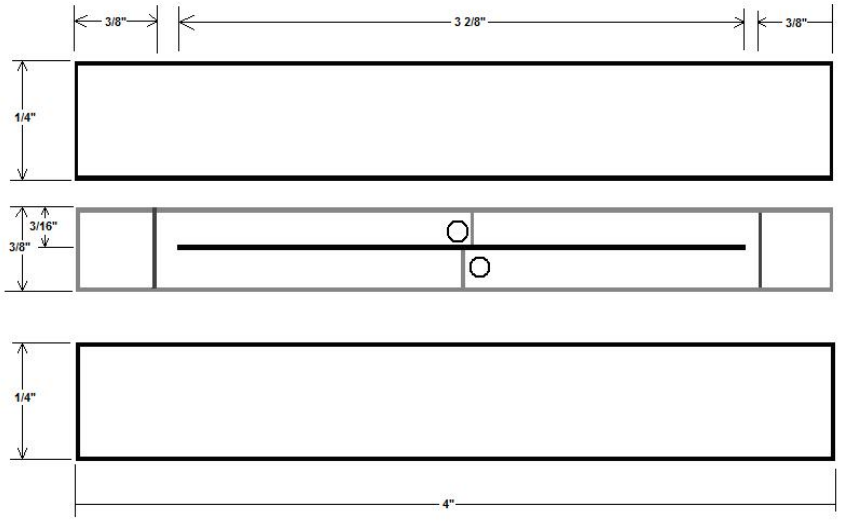
Figure 6A – Photograph of medium size settler with two concentrate outlets and one clarified outlet (Wang, 2014). Figure 6B – Photograph of small sized settler with only one concentrate and one clarified outlet. Small size settler is about ½ the size of medium settler (Wang, 2009).

When designing the new settler outlet it was determined from preliminary testing that the small size settler outlet, shown in Figure 6B, was far less likely to experience blockage than the outlet of the medium size settler, shown in Figure 6A. This was attributed to the presence of only one concentrate stream outlet and one clarified stream

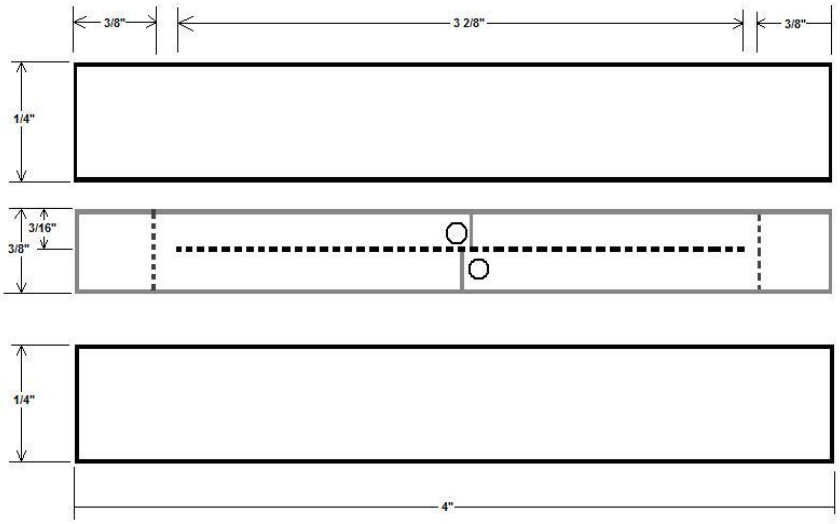
outlet. The outlet design shown in Figure 7 is not as complex as the original design for a medium scale settler but is far less likely to experience a compete blockage of the concentrate outlet stream due to the presence of only one concentrate outlet port. While the interior geometry has not changed drastically the assembly of the new design requires far less dexterity than the construction method used by Wang et al. 2014.



A.



B.



C.

Figure 7 – A: Top view schematic of the outlet. Shown with top plate and flanges removed for clarity especially of the flow separator component. B: Back view schematic of the outlet. Flanges omitted for clarity. C: Front view schematic of outlet. Flanges omitted for clarity. All components are constructed from polycarbonate. For view of how flanges attach to outlet see Figure 3.

The outlet for the new improved settler was constructed from seven individual polycarbonate pieces, of which only four are unique. See Figure 7 for schematic of how the outlet was assembled. There are two identical six-sided top or bottom pieces that have the shape of the outer outline shown in the Top View of Figure 7A. There are two identical side wall pieces of cut polycarbonate that have a small squares cut out for the outlet modified fluer silicone tubing connector inserted into it and held in place by RTD Silicone Sealant. Additionally, there are two identical flange pieces that are quite similar to those found on the flow chamber that are to be attached to the front end of the outlet to hold it to the flow chamber. The last piece is the flow separator which is made of a thin ½ mm thick piece of polycarbonate that has the shape of the gray outline from the top view of Figure 7A. The flow separator fits into a small grove located on the side wall pieces. Four holes on each side of the outlet were drilled into the upper surface, side walls, and lower surface such that No. 8 machine screws could be used to hold the

assembly together. During assembly, a thin layer of RTD Silicone Sealant was applied to all places where the upper surface, lower surface, and the side wall pieces connect. The sealant was allowed to cure while there were small gaps between the pieces such that this sealant would be compressed when the screws were tightened completely.

3.3 Bioreactor

A New Brunswick Bioflow 5000 Micro Fermenter was used as a photobioreactor for the culture of algae. The reactor assembly is comprised of a 7.5L stirred tank reactor coupled with a control tower. This reactor allows a five liter culture volume minimizing the effect of algae accumulation in the gravity settler during testing. The various inlets, outlets, and features of the stirred tank are shown in Figure 8. A photograph of the bioreactor without algae growing in it is shown in Figure 9B and with algae growing in it in Figure 9A. The bioreactor was illuminated using between three and five lights each with two Coralife 24 inch 10K 14 Watt T-5 Fluorescent Lamp, model number 58560. These lights provided about 560 foot candles on the outer surface of the bioreactor. The experiment started with using three lights and additional lights were added to minimize variation of the brightness on the outside of the bioreactor as they became available.

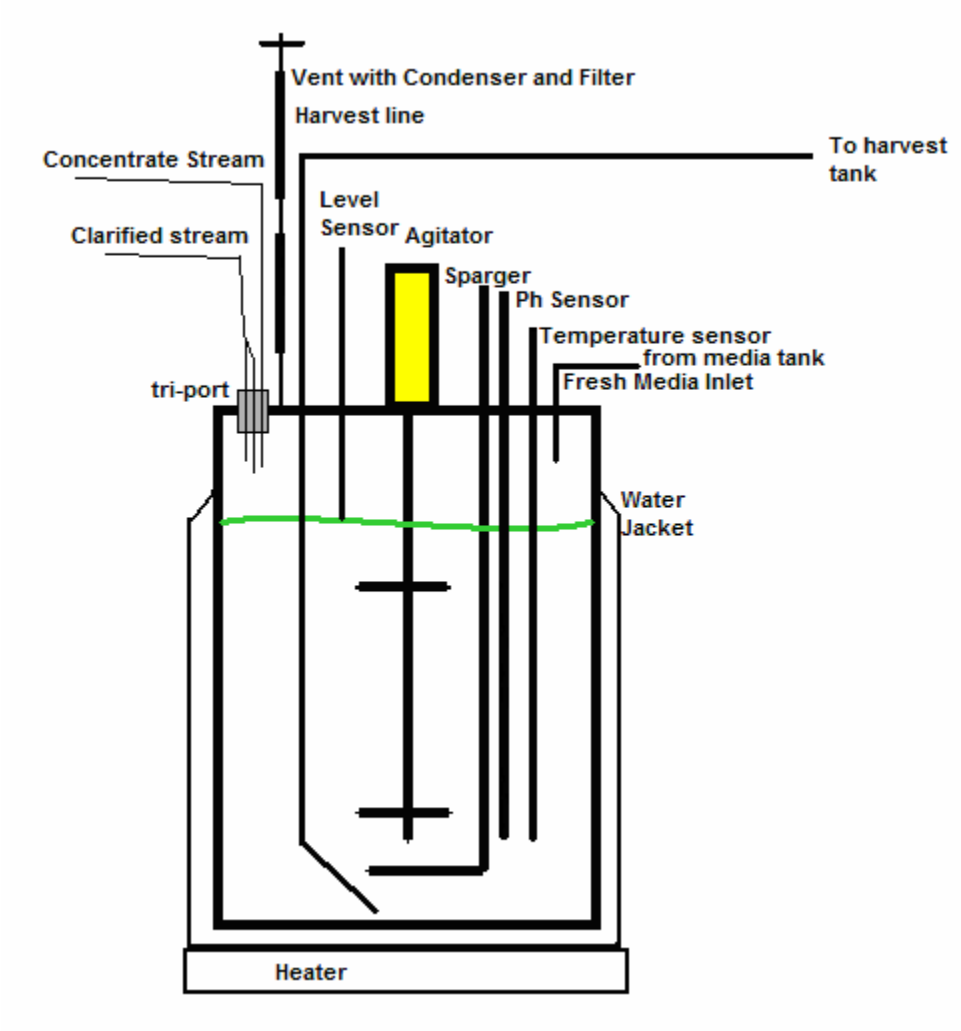


Figure 8 - Schematic of New Brunswick bioreactor as used in this experiment. Not shown are the control tower, fresh media tank, harvest tank, and the lights surrounding the reactor.

The reactor was equipped with a temperature controller that would control both the flow of cooling water to the water jacket and the heater located below the water jacket. Unfortunately, due to issues with the water supply for the cooling jacket the cooling functionality was inoperable. The water supply would routinely be a temperature of 30˚C or greater and occasionally the water would have large amounts of dark brown particulate matter that could reduce light levels in the bioreactor. In keeping with previous experiments using the bioreactor the temperature controller was set to 28˚C. Early in the experiment the reactor was commonly above the specified temperature

especially during the batch algae growth prior the start of the trial. During the remainder of the experiment the temperature varied between 26.5˚C and 31˚C.

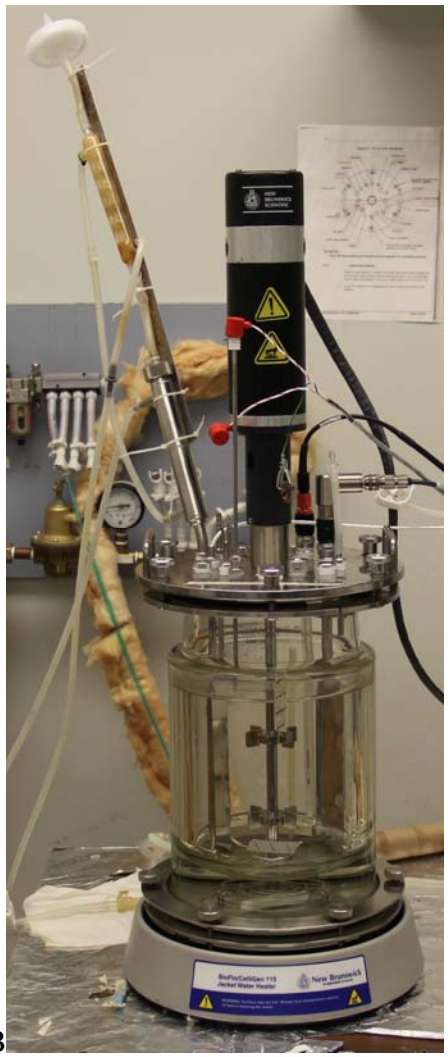
A  B 

Figure 9A – Photograph of bioreactors during algae culture when four lights were in use. Figure 9B - Photograph of bioreactor when empty.

The reactor control tower was equipped with three pumps. The first pump was carefully calibrated and used to slowly and continuously remove algae suspension from the reactor into a collection bottle. A flow rate of one liter per day was used for the downward flow trials and a flow rate of two liters per day was used for the upward flow

trials. These flow rates were chosen to yield dilution rates of 0.2 day^{-1} and 0.4 day^{-1} respectively. The 0.2day^{-1} dilution rate allowed the algae within the bioreactor to slowly increase in concentration, allowing the culture to recover from accumulation in the settler or any leaks that developed in the tubing. The 0.4 day^{-1} dilution rate caused the algae concentration in the bioreactor to decrease slowly, since the algae growth rate was unable to keep up with algae lost to the harvest stream as well as accumulation in the settler. The second pump was controlled by the level controller, which was placed at the 5 L position in the bioreactor (Figure 8). This pump drew algae or media from a 20 L carboy jug that was autoclaved just prior to use in order to maintain the level constant in the bioreactor. The third pump was left unused except when a small two liter bottle of media was used to provide media to the reactor while primary media jugs were being cleaned and sterilized.

An air/CO_2 gas mixture was fed to the reactor to enable photosynthesis, provide pH control, and to improve mixing in the reactor. Prior studies had found that maintaining the air flow rate at 1 LPM while adjusting the CO_2 flow based on the algae culture provided adequate mixing in the reactor while reducing evaporation from the reactor (unpublished data). The CO_2 flow rate was adjusted to maintain a pH of about 6.30. Previous trials showed that a CO_2 flow rate that yielded a pH much greater than or less than 6.3 would slow algae growth and alter algae morphology (Observation from preliminary trials). Often alterations in algae morphology caused the algae to attach to the walls of the bioreactor and settler, limiting light and decreasing settler performance.

The agitation system on the bioreactor was maintained at 300 RPM. Previous unpublished studies had found excellent mixing of the algae culture, without cell damage,

was obtained at 300 RPM. If algae buildup on the glass surface of the bioreactor was noticed the impeller speed was increased to the maximum speed of 1200 RPM for a period of 5 minutes. This high speed agitation did not damage the culture except over a long period of time but managed to dislodge most of the algae buildup on the glass walls of the bioreactor ensuring that the light was not blocked.

The bioreactor was initially filled with 3N-BB media which has been found to be effective at growing algae. 3N-BB media was prepared using deionized water obtained from a reverse osmosis filter system with 0.75 g $NaNO_3$, 0.075 g K_2HPO_4, 0.175 g KH_2PO_4, 0.075 g $MgSO_4*7H_2O$, 0.025 g $CaCl_2*2H_2O$, 0.025 g NaCl and 6 mL of a trace metals stock solution is added for each liter of media. The metals solution contains 0.75 g Na_2EDTA, 0.097 g $FeCl_3*6H_2O$, 0.041 g $MnCl_2*4H_2O$, 0.005 g $ZnCl_2$, 0.002 g $CoCl_2$, and 0.004 g $NaMoO_4$ for each liter of solution (Schwenk, 2012).

3.4 Combined Settler and Bioreactor System

The combined settler-bioreactor system is shown in Figure 10. The settler was attached to a custom made stand that allowed the setter to operate over a wide range of angles. The inlet to the settler was installed to the bioreactor with a "T" connector on the harvest line allowing the settler inlet to draw directly from the bottom of the bioreactor. Drawing from the bottom of the bioreactor minimized the amount of bubbles that entered the settler, which would accumulate and effectively reduce the length of the settler. A metal plug was removed from the top bioreactor and was replaced with a "tri-port" fitting that had three small metal tubes that passed into the bioreactor. Two of the tubes were used for the clarified stream while only one was used for the concentrate stream. This

arrangement prevented backflow from one stream to another during sample collection. Two Masterflex L/S Easy Load Peristaltic Pumps were used to pump the clarified and concentrate streams. The tubing to and from the settler was minimized to reduce algae accumulation in the tubes but excess tubing was required to allow the settler to operate at various angles. While attempts were made to reduce the amount of algae that settled in the tubing that connected the bioreactor and settler there was a noticeable amount of algae buildup in the lines.

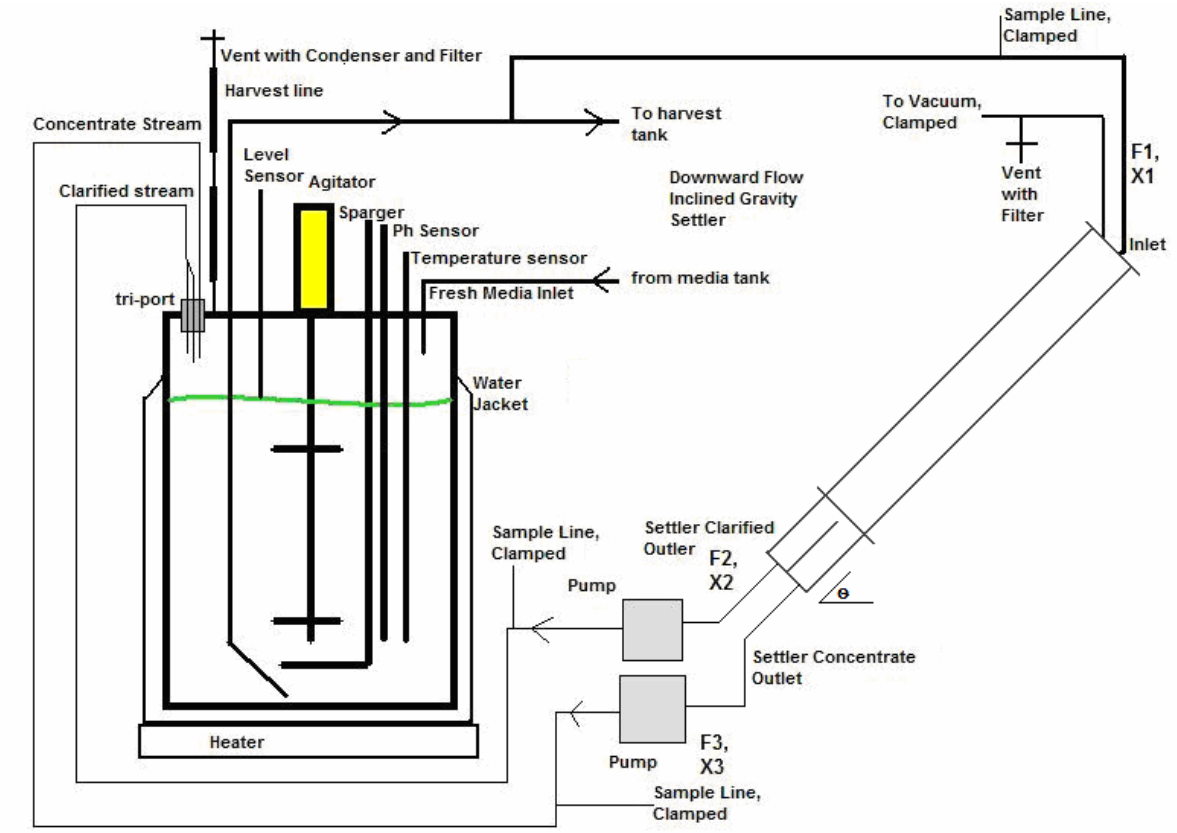


Figure 10 - Schematic of combined settler and bioreactor system. Not shown are the lights surrounding the reactor, control tower, harvest tank, media tank, and vacuum flask. F1, F2, and F3 represent the inlet, clarified, and concentrate streams flow rates respectively. X1, X2, and X3 represent the concentration of algae in the inlet, clarified, and concentrate streams.

3.5 Definition of Important Parameters in this Study

Important parameters that are controlled in this study are the angle of inclination of the settler, the split ratio, and the total flow rate. The angle of inclination is adjusted by adjusting the stand on which the settler is mounted. The split ratio and total flow rate are controlled by adjusting the speed of the peristaltic pumps; changing the values of F2 and F3. The total flow rate F1 is the sum of F2 and F3. The split ratio is the ratio of F3:F2.

Equation 2: $R_{cl} = F2 * X2/(F1 * X1)$

Equation 3: $Rco = F3 * X3/(F1 * X1)$

Equation 4: $TR = (F3 * X3 + F2 * X2) / (F1 * X1) = R_{cl} + R_{co}$

Equation 5: $R_C = X3 / X2$

Equation 6: $C_{oF} = X3 / X1$

Equation 7: $C_{AF} = X2 / X1$

Important measures of the settler performance are calculated from the flow rates and algae concentrations. The three types of recovery are: the total recovery (R_t), the clarified recovery (R_{cl}), and the concentrate recovery (R_{co}). The clarified recovery is a measure of the fraction of algae that exits through the clarification stream. It is calculated by the formula Equation 2. The concentrate recovery is a measure of the fraction of algae that exits through the concentrate stream. This value is highly important because if this process is implemented on a larger scale the concentrate stream would be harvested as opposed to being recycled. It is calculated by Equation 3. The total recovery is a measure of how much algae accumulates within the settler and has the potential to be used to determine the extent of settler fouling. This is calculated by Equation 4. In

addition to the recoveries, the relative concentration, concentration factor, and the clarification factor were also calculated from the values of F1, F2, F3, X1, X2, and X3. Relative concentration (R_C) is the ratio between the concentration of the concentrate stream and the clarified stream calculated by Equation 5. Concentration factor (C_{oF}) measures how effectively the settler concentrated the inlet stream algae into the concentrate stream and is calculated by Equation 6. The clarification factor (C_{AF}) is the measure of the concentration of algae remaining in the clarification stream compared to that in the inlet. The clarification factor is calculated by Equation 7.

3.6 Algae Sample Collection

To gather proper data from the settler, sample collection had to be performed without inducing large disturbances to the system. As such, care had to be taken to avoid disturbing the settler itself during sampling. If the settler was bumped or otherwise moved prior to the collection of the final sample, at each time point, the sample collection would be aborted and the samples would be collected at least an hour, preferably two hours, afterward. When the settler was bumped a large amount of algae that was near the verge of sliding would then break free prematurely, sliding rapidly down the settler causing the concentrate stream to have far more algae in it than would be present at steady state conditions.

The sample collection for each data point was started by spraying ethanol on the ends of the sample lines to kill any bacteria that had accumulated in the tubing outside of the clamp used to seal the tube. Prior to the collection of the sample, the first 30 mL syringe was used to draw all of the liquid and algae from the sample line. This was done to prevent the clarified liquid, which was present in the tube remaining from the previous

sampling, from lowering the algae concentration of the collected sample. Additionally, while that liquid was collected the sample line was pinched along its length to resuspend any of the algae that had settled and attached to the wall of the tube as the algae was likely to break free during the collection of the sample.

The concentration of algae samples would be measured in 4 mL polystyrene cuvettes in a Genesys 10S UV-Vis Bio spectrophotometer at 600 nm. A calibration of algae dry biomass to spectrophotometer absorbance had previously been created indicating a slope of 0.56 gdw L^{-1} $A600^{-1}$ (unpublished data). This calibration is only valid for absorbences of less than 1.0. If the algae was at a concentration that would correspond to an A600 greater than 1.0, the sample was diluted prior to measurement.

First a sample was taken from the inlet line by drawing about 10 mL into the 30 mL syringe from the recently cleaned sample line. The only requirement for the drawing of the inlet sample is that the sample is drawn smoothly as any large changes of flow rate will dislodge algae from the tube walls. This sample was immediately measured. Next, the concentrate stream was cleaned. There existed two different procedures for the collection of samples from the concentrate stream. The first procedure was performed during the first several samplings and was identical to the procedure for the inlet collection except that the algae had to be collected over a period of between 2 and 5 minutes. Due to the very slow flow rate of the concentrate stream it became difficult to draw algae at a similar rate with a syringe. Any mistake made in drawing the sample would cause algae from between the sample line and the bioreactor to be dislodged from the walls of the tube and drawn into the syringe. Therefore, a second procedure was developed. In the second procedure the tube between the sample line and the bioreactor

was clamped and the algae being pumped through that line was diverted through the sample line into a 50 mL centrifuge tube. Lastly, the algae was drawn using a syringe from the clarified stream slowly and carefully at a rate matching the flow rate of that stream.

3.6.1 Filtering of Collected Data

The data collected during the course of the experiment was subject to a number of factors that reduced its usefulness. A number of outlier data points, deviating significantly from trials where there were no difficulties in sampling, indicated that most of the error was induced by sampling technique. The development of a procedure to remove the erroneous data points, that increased the precision of the study results, was necessary. This analysis was primarily performed on the relative concentration metric as this was subject to the most errors in sampling, while least likely to be affected by changes in the concentration of the algae in the inlet stream. A secondary analysis was performed on the concentration factor to double check the results of the primary analysis. The first step in this process was to determine the quality of the raw dataset. This was done by first plotting all the values of the concentration factor and relative concentration while also calculating an error value. The error value was calculated by dividing the standard deviation of the group by the average of the group. The removal of data was performed by the combination of both a visual inspection of the data to determine which data points were candidates for removal and an analysis of the error value. The error value was observed when the data point was removed and if the value of the error value reduced sufficiently with the removal of that data point then the data point was judged to be erroneous and not required in the analysis. The value of the error was used to

determine what reduction of error was needed. When the error values were high indicating that clustering was not as tight, more stringent requirements were needed to remove suspected outliers. Low error values coupled with tight clustering of the remaining data points relaxed the requirements for the removal of data points. This filtering was performed based on the principal that as few data points as possible should be removed while minimizing the error value of the remaining data sets.

3.7 Management of Settler Fouling

One of the biggest issues with the collection of data over long periods of time with the settler was managing settler fouling. Once the settler becomes fouled, algae will cease to slide and begin to build up, resulting in a greatly reduced separation. With the small bioreactor used in the study algae recycling was necessary. As such bioreactor washout occurred any time settler fouling reached critical levels. When algae came in contact with the lower surface for an excessive period of time it would attach to the surface. Additionally, if the algae culture became excessively stressed the algae would attach rather quickly and fouling would become difficult to manage. The most effective way to clean the settler, without opening it, was to detach it from the bioreactor and bleach the settler with a 10% bleach solution followed by aggressive agitation to remove any solids still adhering to the surface. If such a cleaning procedure was unable to remove algae deposits the settler was dissembled and manually cleaned.

Due to the long periods of time required to remove settler fouling, once it had reached excessive levels and impacted settler performance, procedures to delay and reduce the rate of settler fouling were developed. It has been found that if the algae

located on the lower surface, was removed daily, settler fouling was greatly reduced. Therefore, each morning the settler was be drained by blocking the inlet and opening the vent to the atmosphere through the filter located along the vent line (Figure 10). Once the settler was half drained it was removed from the stand and gently shaken by allowing the liquid inside to flow back and forth across the algae removing it from the lower surface. Once the algae solution inside the settler, which was at an extremely high concentration, was completely drained, the settler was refilled. The inlet stream was reopened and algae suspension was poured in from the bioreactor. The valve connecting the house vacuum to the settler was opened and the settler would be rapidly refilled. The vacuum valve along with the vent were closed after the settler was completely filled.

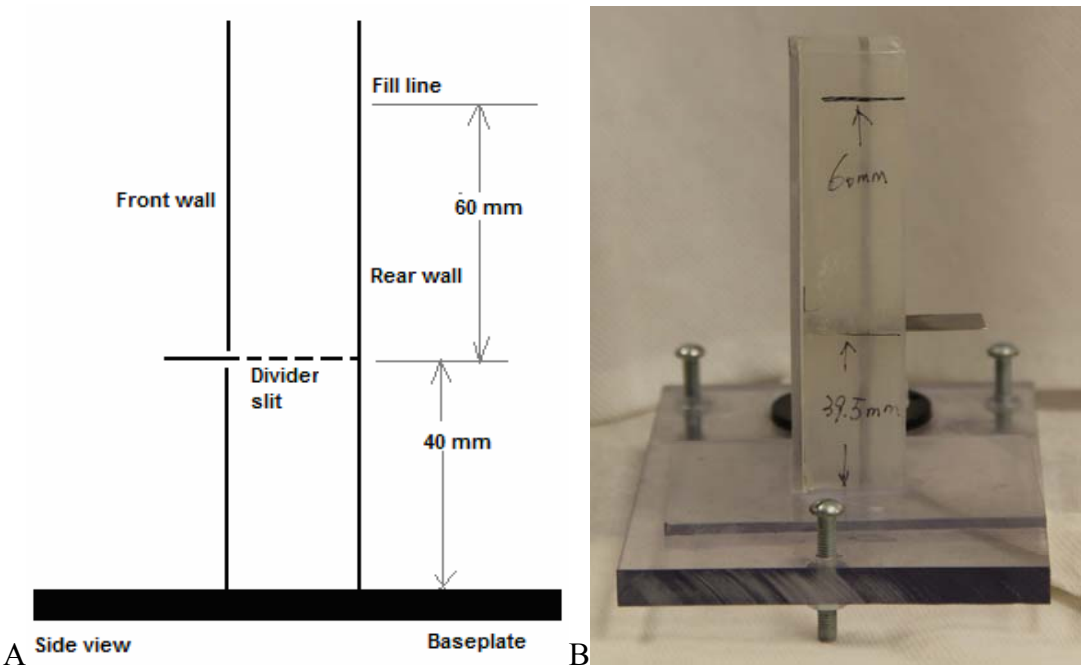
The system was operated in the downward flow setup using combinations of four angles of inclination and four flow rates for a total of 14 states. A ration of 5 mL/min clarified stream to 1 mL/min concentrate stream was chosen to maximize the number of flow rates within the range of interest in this study. The four flow rates were at 24, 30, 36, and 42 mL/min. The four angles of inclination were at 44, 52, 57.5, and 66°. Table 1 lists the order in which the trials were performed. For the 44° trial only the 36 and 42 mL/min flow rates were able to be tested as during the 30 mL/min trial the rate of algae accumulation was too great. In addition, one upward flow configuration trial was attempted using a flow rate of 24 mL/min at 66°. In each trial it was attempted to gather between three and five data points that would pass the filtering methods mentioned in Section 3.6.1.

Table 1: Order of Collection of Data Points.

	44°	52°	57.5°	66°
24 mL/min	N/A	#7	#2	#13
30 mL/min	#10 Blockage	#6	#1	#12
36 mL/min	#9	#5	#3	#11
42 mL/min	#15	#8	#4	#14

3.8 Settling Velocity Study

Measurements of settling velocity were performed using a simple settling velocity system which was developed by Zhaowei Wang based on similar devices for different purposes and shown in Figure 11A. The settling velocity column is a 1.4 cm wide by 2 cm deep rectangular chamber which has a height of 11.5 cm. Exactly 39.5 mm from the bottom of the apparatus cut into the front is a small slit. A thin piece of metal can be inserted into the chamber, through the slit, separating it into a lower and upper portion. At a level 10 cm above the bottom of the chamber there is a line on the front of the device indicating the level to which the chamber would be filled.



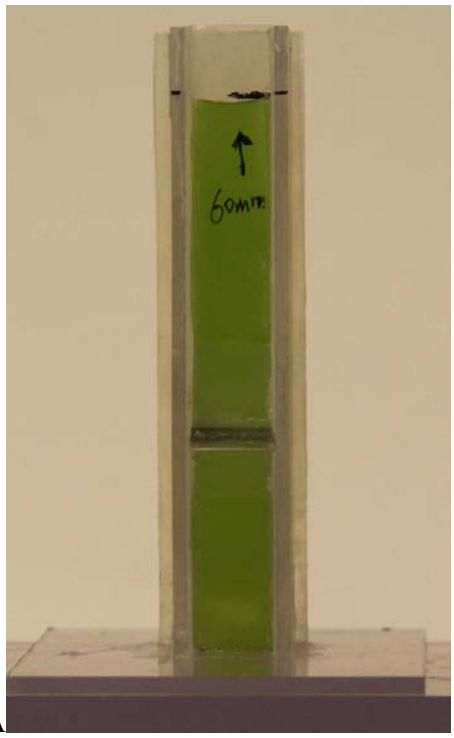
A B

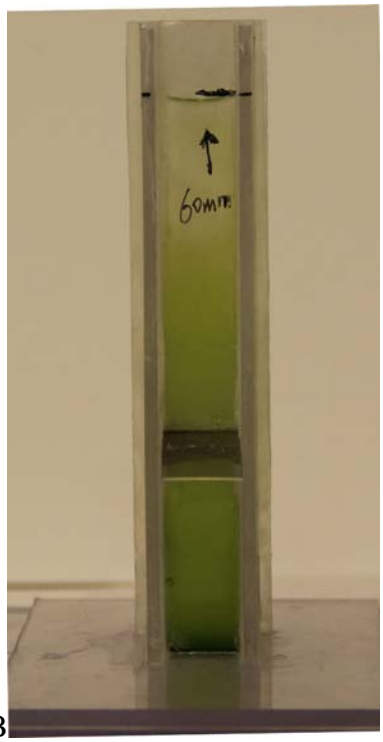
Figure 11A - Schematic of settling velocity column used to measure settling velocity of algae in this study.
Figure 11B - Settling velocity column on leveling stand prior to start of experiment with metal slot fully inserted.

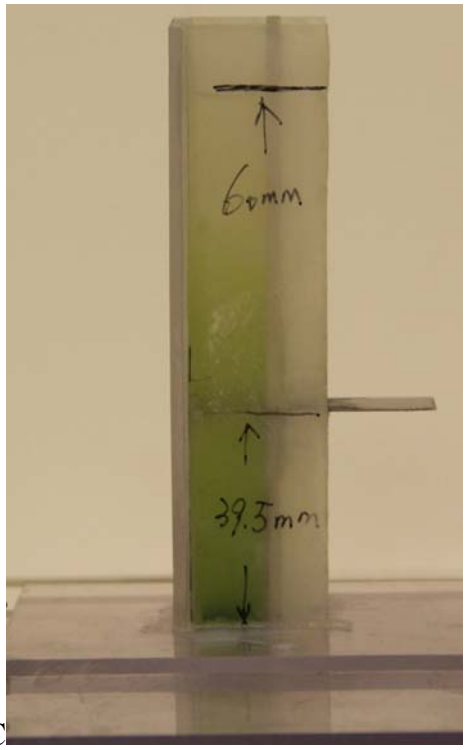
3.8.1 Settling Velocity Procedure

The column was first placed on a level surface. If a level surface was unavailable a small stand with three screws as legs and an attached level was be used to level the column. The stand is visible under the column shown in Figure 11B. An algae suspension of approximately 30 mL was drawn from an algae culture and mixed well. If the algae sample was of excessive concentration it could be diluted with pure growth medium to simplify the algae cell counting process. This procedure is not advised or often practiced due the possible effect cell concentration can have on settling velocity. The algae sample was then added to the column using a 10 mL pipette. Once the column was filled to the 10 cm line the pipette was used to mix the algae a final time prior to starting the timer. A recently filled settling velocity column is depicted in Figure 12A. A small portion of the algae sample is retained to measure the cell concentration. After 3

hours passed the metal tab was inserted fully into the slit completely separating the upper and lower regions of the column. Figure 12B, depicts the settling velocity column just prior to the insertion of the metal tab. Figure 12C, depicts the column just after the metal tab was inserted. After the tab was moved the 10 mL pipette was used to remove the algae suspension from the upper section of the column; Figure 12D depicts the column after the upper section was drained. Afterward, the metal tab was returned to its original position and the alga suspension contained in the lower section was collected.

A 

B 

C 

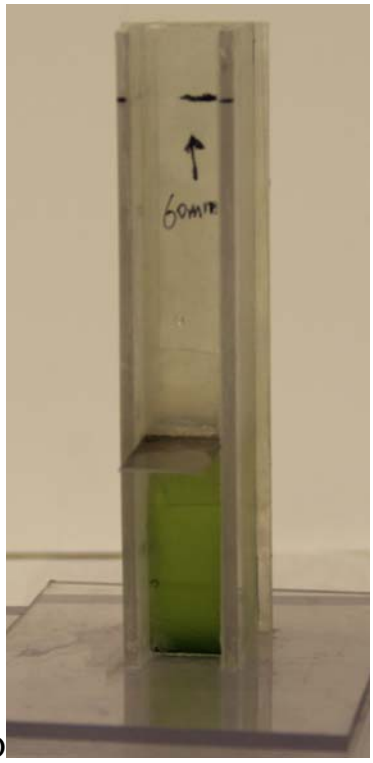
D 

Figure 12A – Settling velocity column just after being filled. Figure 12B - Settling velocity column after three hours; the change in the concentration of algae at top of column is noticeable. Figure 12C – Settling velocity column after the metal tab has been fully inserted prior to the removal of the upper fraction of algae suspension; Figure 12D – Settling velocity column after the upper fraction has been removed prior to the removal of the lower fraction.

The concentration of algae was determined by two different methods. The first and simplest method was to use a spectrophotometer. Three absorbance measurements were performed on each of cell suspensions (the initial algae sample, the upper algae fraction, and the lower algae fraction). The second method is to count individual algae cells in a Hausser Scientific hemocytometer, using a Nikon Eclipse E400 microscope. The cells in the four corners and the middle large squares were counted over three different slides. Instead of counting the total number of cells, each population of cell clustering (e.g. single cells, doublets, triples, and quads) was counted individually. Additionally, in response to the large size distribution of the four-cell clusters, the population was split into the large sized and the small sized quads. In total, five

populations were counted: single cells, double cells, triple cells, small quads, and large quads. This allowed a detailed analysis of the settling velocity of the various fractions of the algae culture but required a large amount of time and effort to complete.

3.8.1.1 Calculation of Settling Velocity

The settling velocity of the algae is calculated using Equation 8. This equation calculates the settling velocity V from the height of the metal tab h, the initial algae concentration X1, the final algae concentration in the lower section of the column X2, and then amount of time t the algae was permitted to settle prior to the closing of the metal tab. The equation is derived from a mass balance of the algae settling through the interface between the top and bottom sections of the column. This equation assumes that the total amount of algae in the column stays constant (Wang, 2010). It can be used to measure the average settling velocity of a sample or any sub populations that can be measured separately. This equation ignores any effects cell concentration and time have on settling velocity.

Equation 8: $V = h * (X2 – X1) / (t * X1)$

3.8.2 Batch Algae Growth for Settling Velocity Study

Algae grown for the settling velocity study was grown by batch culture in 2 liter bottles, containing a stopper with three holes (Figure 13A). The sparger is a long metal tube made of stainless steel that nearly reaches the bottom of the bottle. Some stopper assemblies use a stiff piece of silicone tubing instead of the stainless steel tubing. The end of the metal tube was capped with a cut down 2mL pipette tip to promote the formation of smaller diameter bubbles to enhance mixing. The sparger was connected to

a manifold system allowing up to 20 different bottles of algae to be sparged with a 5% CO_2 and 95% air mixture.

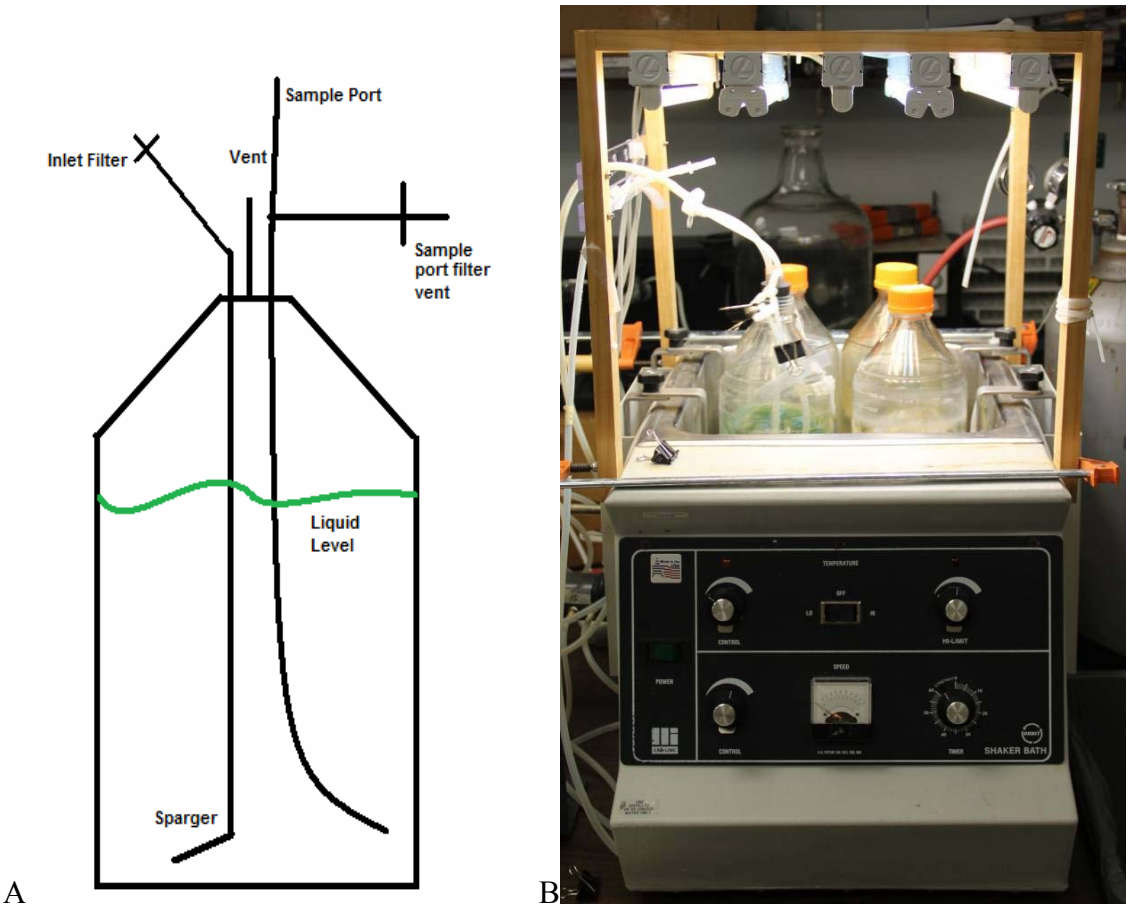


Figure 13 A – Schematic of 2 L bottle used for batch algae growth. During normal operation both the sample port and the sample port filter were closed using medium size binder clips. Figure 13 B – 2 L bottles of algae in Orbit brand shaker bath. The three bottles with caps are counterweights used to balance the shaker tray.

The vent was comprised of a short length of silicone tubing, about 3 to 4 inches, connected to a short metal tube that goes through the stopper and stops just on the inside of the bottle. Near the upper end of the silicon tubing a small wad of cotton is used to filter the outgoing gas while simultaneously preventing bacteria from entering the bottle thought the vent. Condensation in the tubing causes a buildup of water in the vent tube therefore, reducing the amount of evaporation the bottle experiences. Unfortunately, some of the condensation was blown up into to the cotton ball thereby, increasing the

pressure drop across the system potentially affecting the sparged gas rate. Additionally, if nothing prevents the movement of the cotton ball when it is wet it can be blown out of the tube allowing bacteria to contaminate the culture. A "Y" or "T" connector was inserted into the upper end of the silicone tube to prevent the cotton wad from becoming dislodged.

The sample port consists of a long piece of silicone tubing traveling from the bottom of the bottle to the top of the bottle. The top of the silicone tubing was connected to a small metal tube that extended through the stopper and was identical to that used for the vent. The other end of the metal tube was connected to a short piece of silicone tubing that was about 1 to 2 inches long. That piece of silicone tubing connects to either a "Y" or "T" connector of which the other two ends of the connector are connected to similar sized lengths of silicone tubing. One of the pieces of silicone tubing was clamped using a medium sized binder clamp while the other has a sterile 20 micron PTFE filter on the end with another binder clamp blocking that tube.

A 2 L bottle was filled with 1.8 L of 3N-BB media and autoclaved. After being removed from the autoclave and cooled in a sterile environment, algae would be introduced to the bottle. The introduction of algae to the bottle was accomplished via one of two methods; the first was the addition of a liquid sample of algae from another bottle and the second was the use of a ring sparger to remove some algae from a culture on an agar slant. The first method was preferable as there was great certainty that the algae would survive the transfer and the lag phase of algae growth would be minimized. When inoculating a bottle in this manner a small sample would be drawn using a sterile syringe and be inspected using a hemocytometer and a microscope. If the sample was found to

contain healthy and uncontaminated *S. Dimorphous* a second sample would be drawn using another sterile syringe. The sample port of the second bottle was sterilized with ethanol and this second sample was fed into that port. Afterward a volume of sterile deionized water was fed into the port to clean out any remaining media from the tubing.

When a source of clean healthy algae growing in liquid was unavailable, the second method was used. First a ring sparger is sterilized with the flames from a Bunsen burner in a biological cabinet. After cooling to near room temperature the ring sparger was used to scrape some of the algae growing on an agar slant. The tip of the ring sparger was inserted into the liquid in the bottle of media and the bottle was sealed. This method was rarely used due to the long duration of time after inoculation required to determine whether the inoculation actually worked. Additionally, the risk of contaminating the agar slide limits the use of this inoculation method.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gravity Settler Study

The gravity settler was redesigned significantly from the version developed by Wang et al. (Wang, 2009). This analysis of the gravity settler was concerned with determining the effect that changes in angle of inclination and flow rate have on settler performance. Additionally, a comparison between previous studies with a more complicated design will determine whether the simplification of the outlet affected the performance of the settler.

4.1.1 Notes about Settler Performance

Settler performance is a function of three operating parameters: the total flow rate, the angle of inclination, and the split fraction. The total flow rate influences the cell residence time thereby affecting how many cells have sufficient time to reach the lower surface of the settler before they are removed via the clarified stream. Additionally, the total flow rate influences the force that cells lining the bottom of the settler experience causing them to slide at different speeds.

The split fraction affects how much total liquid is included in the concentrate stream thereby affecting the maximum separation possible. Most of the algae biomass that enters the concentrate stream is a sliding mass. The addition of any liquid beyond what is needed to prevent blockage of the outlet will dilute the algae contained in the stream, thereby reducing the effectiveness of the separation. A high split fraction can yield an extremely concentrated product but may experience a cell buildup and possibly a blockage in the outlet region of the settler. A lower split fraction may not yield as concentrated product but will be less likely to have cell accumulation in the outlet region. Due to the limitations of the pumps a lower split fraction of 5:1 (ratio of clarified to concentrate stream flow rates) ratio was used as opposed to the 9:1 ratio of previous studies. The 5:1 ratio was selected to allow more data points to be taken over the range of total flow rates that were of interest. Creating a method to directly compare the performance of trials operating with these different split ratios is impossible due to the lack of knowledge about the gradient of algae concentration at the outlet region of the settler.

4.1.1.1 Gravity Settler Governing Equation

The performance of an ideal gravity settler is governed by Equation 9. This equation is a modified version of that used to predict the performance of an upward flow settler used to remove solids from a liquid product stream. The performance of the settler P is calculated from the settling velocity V, the width of the settler W, the length of the settler L, depth of the settler D, the total flow rate F, and the angle of inclination of the settler θ. Considering that the settler is far longer than it is deep the term associated with

the death of the settler is normally ignored. This causes the predicted performance of the settler to be superior at a lower angle of inclination. For a fixed settler design the only variables will be the settling velocity, angle of inclination, and the flow rate. In normal operation of the settler, the settling velocity can not be controlled and often is unknown so the performance of the setter will be controlled by the total flow rate and the angle of inclination. This equation ignores the effect of algae accumulation on the settler performance (Wang, 2009).

Equation 9: $P = V * W * (L * \cos(\theta) + D * \sin(\theta)) / F$

4.1.2 General Observations of Gravity Settler Operation

From periodic observations of the gravity settler during sampling it was observed that there were two different methods by which the algae would slide along the lower surface. The first was the continuous bulk motion of algae along the entire surface of the settler. This mechanism was the primary sliding mechanism for the majority of the study and was observed to yield more consistent measurements for the concentrate stream.

The second mechanism involved the stationary buildup of algae on the lower surface of the settler. Once certain areas achieved sufficient thickness, an entire area normally less than 1cm wide and 0.25 cm in length, would begin to slide. These sliding flocs of algae would then disturb the algae buildup that was encountered during the downward slide, adding that algae to the floc, in a manner quite similar to that of an avalanche. This mechanism yielded discontinuous results and any outside disturbance such as the settler being bumped during sampling would cause a large number of flocs to break free prematurely. This resulted in both unusually high and low concentrations being present in the concentrate stream.

Attempts to measure the speed at which the algae slid along the lower surface were met with failure. The first method of sliding had no reference point that moved with the sliding algae, preventing the measurement of the algae sliding speed. While the second method of algae sliding was easy to track, this behavior was unpredictable and did not occur each time a high quality camera was available to record the motion.

The data collected from the gravity settler was somewhat chaotic in nature and as a result much of the data had to be discarded, in the manor described in the materials and methods section. The data points that were discarded were occasions when sampling errors went unnoticed causing erroneously high values of the concentrate stream to be recorded. Most of the data points that were discarded were from the beginning of the study, in the first 23 days, and can be seen in the comparison between Figure 14 and Figure 15. This proves that efforts to collect data of higher quality were effective at reducing the number of disturbances introduced to the system.

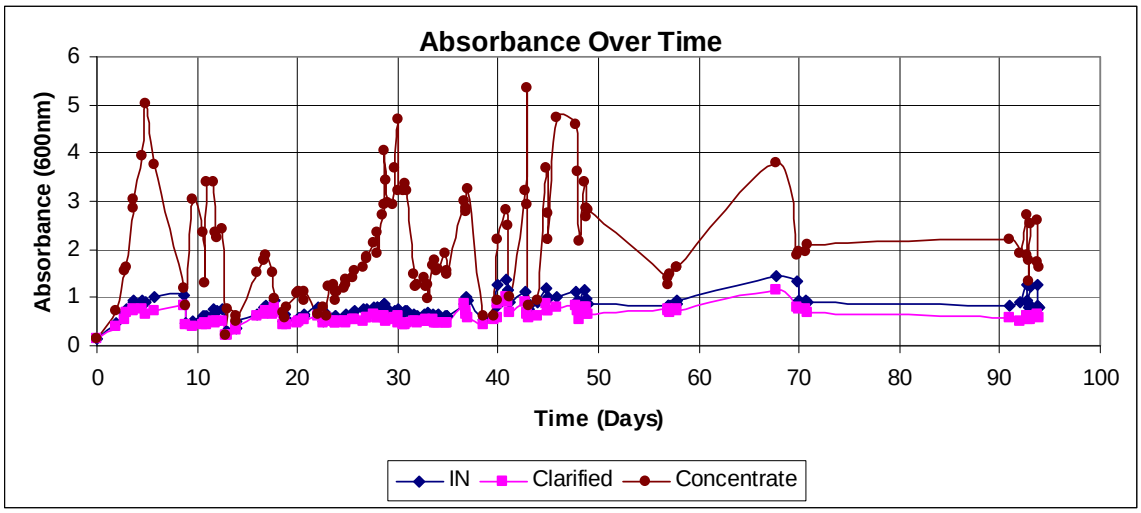


Figure 14 - Algae concentration data for all streams leading into and from the settler over the course of this study. It can be seen that the concentrate stream is subject to many large variations that are not present in either the clarified or inlet streams. Data represents various flow rates and angles at a constant split fraction.

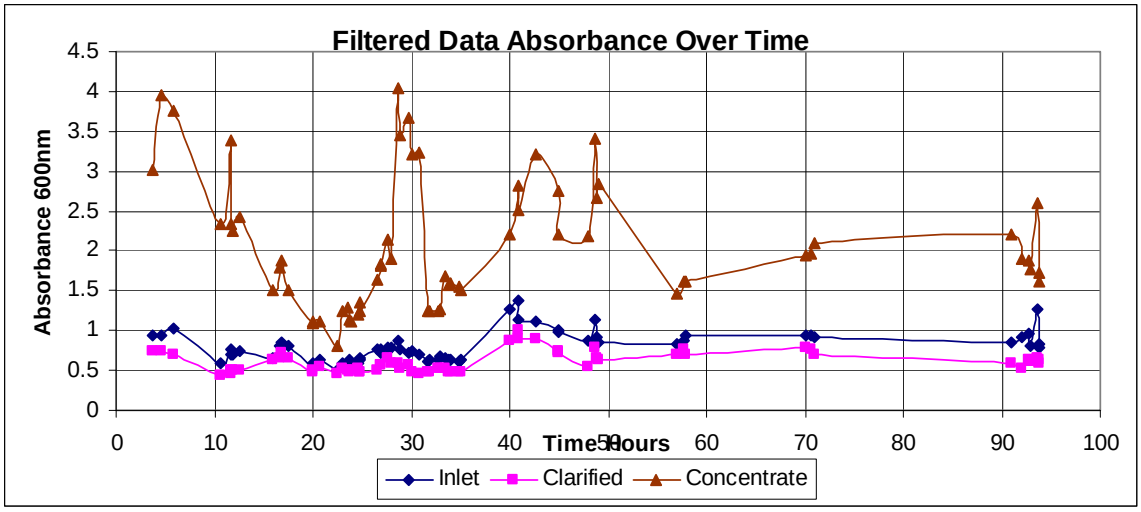


Figure 15 – Data from Figure 14 which was filtered according to criteria described in the Materials and Methods section.

When comparing Figure 14 to Figure 16 it can be noticed that, in general, variations in inlet stream concentrations are reproduced in both the clarified and concentrated streams. The mirroring of changes between the inlet and concentrate stream is even more apparent when the filtered data are compared in Figure 15 and Figure 17. Discarding of data had little effect on the relationship between the clarified and inlet streams, indicating that the majority of sampling errors were associated with the concentrate stream.

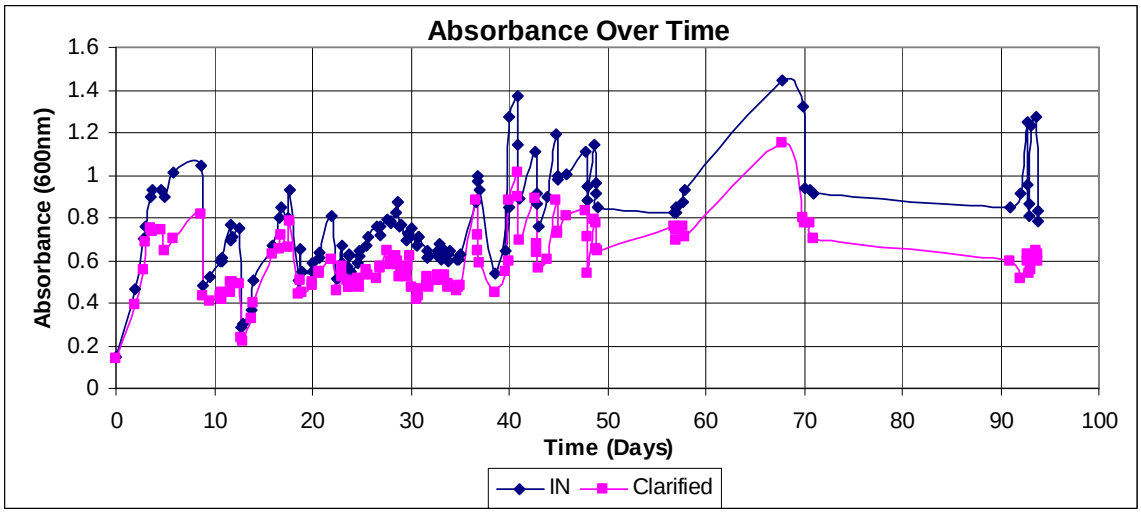


Figure 16 - Algae concentration data for the inlet and clarified streams for over the course of this study. It can be seen that the same trends are present in both streams.

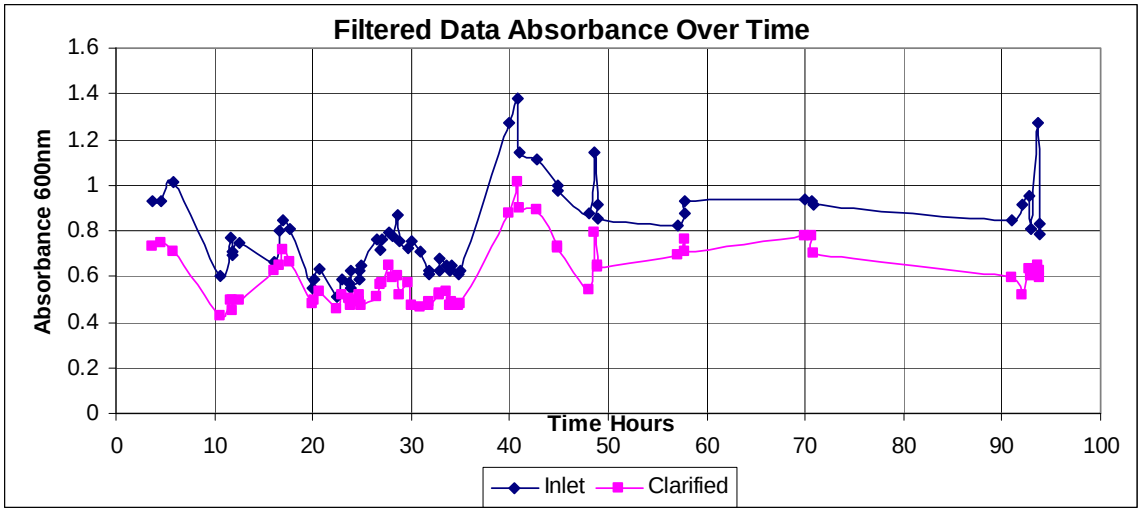


Figure 17 – Data from Figure 16 that after it had been passed through the filtering methods discussed in Chapter 3.

4.1.3 Algae Recovery

Recovery of algae throughout the experiment is shown in Figure 18. The recovery of algae from the gravity settler was unusual as 71% of the data showed that more algae exited the settler than entered the settler. For the majority of the points close to a value of 100% total this can be explained by growth of the algae that had settled on the lower surface of the settler. .

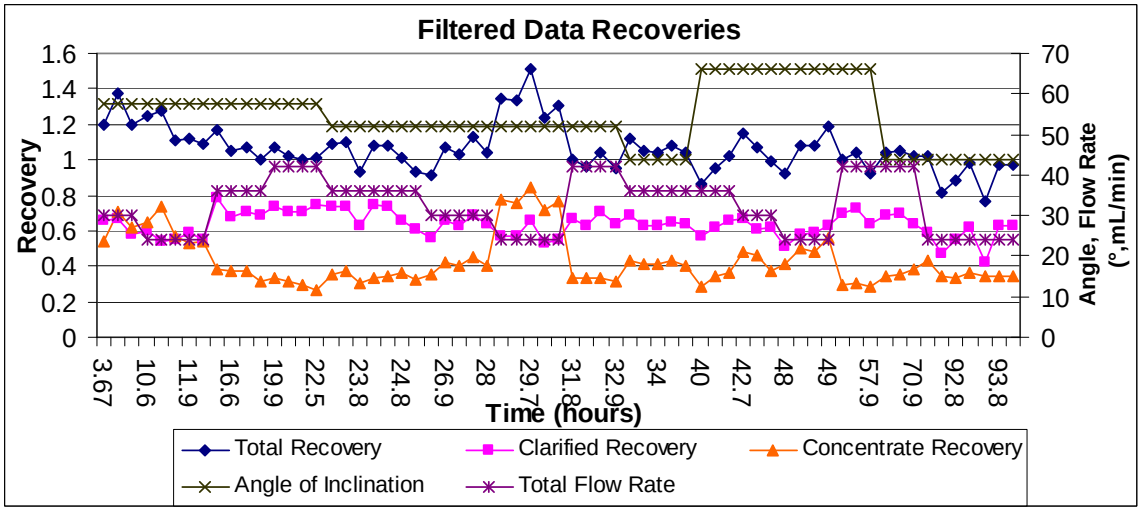


Figure 18 – Concentrate recovery is the percentage of the algae that enters the settler that exits the settler through the concentrate stream. Clarification fraction is the percentage of the algae that enters the settler that exits through the clarified stream. The total recovery percentage is the fraction of the algae that manages to exit the settler after entering it.

Additionally, it can be seen that for all angles and flow rates, the fraction of algae that exits through the clarified stream is almost constant. This constant high value of the clarification fraction is most likely an artifact of the experimental design, causing the majority of algae that enters the settler to pass through to the clarified stream outlet. This is due to the rapid cycling of algae through the settler drastically altering the nature of the algae found in the bioreactor. The algae cells that do not settle in the settler will spend far less time in the settler causing the bioreactor algae population to shift to cells that will pass straight through the bioreactor. The lowest flow rate for the settler yielded a bioreactor residence time of only 3.5 hours which indicates that the settler has a profound effect on the composition of algae in the bioreactor (5000 mL / 24 mL/min = 3.47 hours). This low residence time is vastly different from the 5 day residence time used in normal algae perfusion culturing.

4.1.4 Settler Performance

In this study three outcome measures were used to evaluate the performance of the gravity settler: relative concentration, concentration factor, and clarification factor. Relative concentration is the ratio of algae concentration of the concentrate stream to that of the clarified stream. This parameter measures the ability of the settler to separate the algae from the water. This outcome is the primary measure of settler performance.

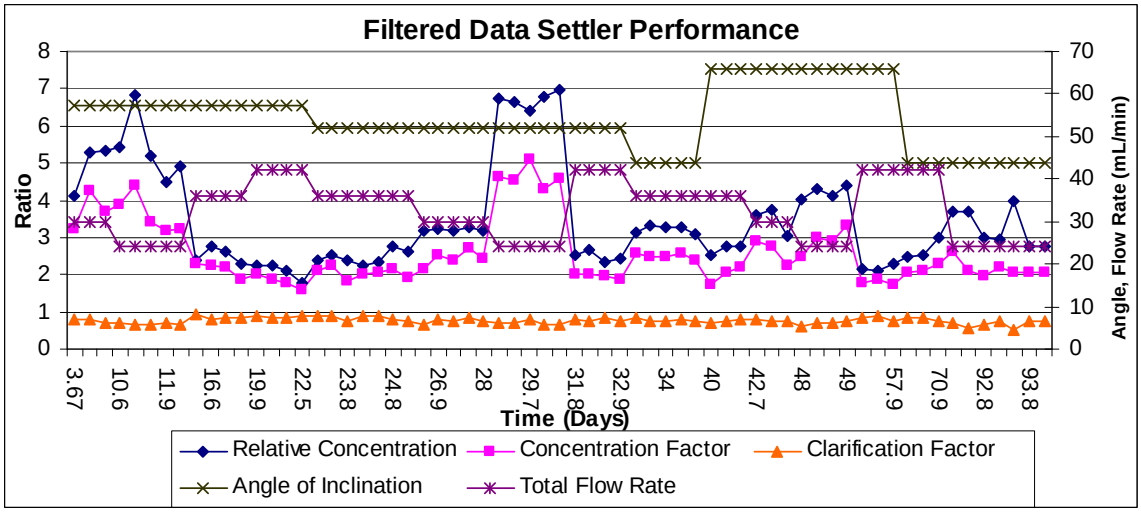


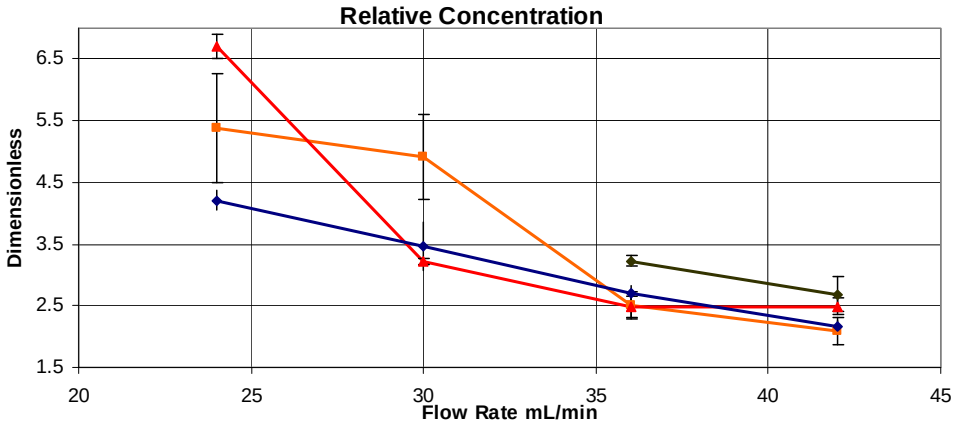
Figure 19- Collection of all filtered data points organized by angle and flow rate.

Concentration factor is the ratio of algae concentration from the concentrate stream to the inlet stream. While this parameter was a primary method of analysis in previous works, its usefulness was limited in this study due to algae accumulation that did not result in the algae sliding.

The clarification factor is the ratio of algae concentration of the clarified stream to that in the inlet stream. It measures the ability of the settler to remove algae. Unlike the other outcome parameters in this study, a lower value of the clarification factor is indicative of superior performance. It was found that this parameter remained consistent even when data points were discarded due to obvious disturbances. This was because most disturbances affected only the concentrate stream.

The angle of inclination has been found to affect the settler performance between 52° to 66° The 44° trials are incomplete due to excessive buildup of algae in the settler at lower flow rates. At lower angles the settler achieves greater performance, as seen in the maximum values of the various curves (Figure 20A and Figure 20B). Additionally, the angle was found to influence the flow rate at which the performance begins to decrease. At shallower angles the drop off occurs more sharply and reaches a consistent low value

at a higher flow rate. Higher angles have a more gradual drop off with the 66° curves from Figure 20A and Figure 20B appearing linear. It can be seen from the 57.5° trials that between the 36 mL/min and 30 mL/min total flow trials that there is a large increase in performance of the settler. Likewise in the 52° trials a similar trend can be seen between the 36 mL/min and 24 mL/min total flow rate with the majority of the increase occurring at the between 30 mL/min an 24 mL/min.



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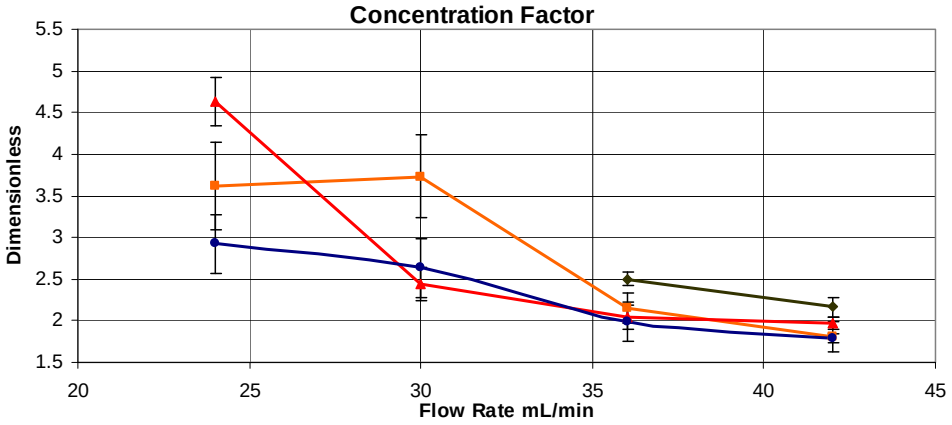

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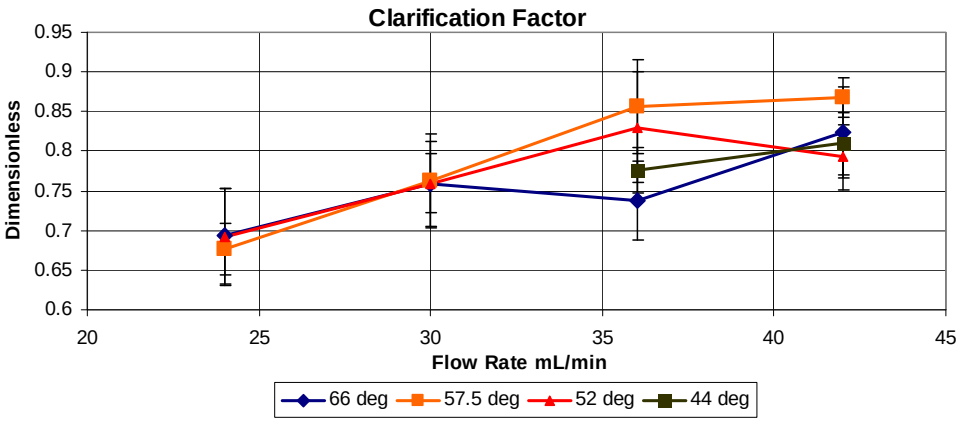

C

Figure 20A: Comparison between relative concentration and flow rate for various angles of inclination. The values of the relative concentration found in this graph can be found in Table 6 in the appendix. Figure 20B: Concentration factor at four total flow rates and four angles of inclination. The data can be found in Table 7 in the appendix. Figure 20C - Relationship between clarification factor and flow rate for various angles of inclination. The values of the clarification factor found in this graph can be found Table 8 in the appendix.

All angles appear to have near identical clarification factor at all flow rates except 36 mL/min where the 66° trial deviates strongly from the 57.5° trial and the 52° trial maintaining the same value as at 30 mL/min (Figure 20C).

Previous studies and sedimentation theory have found that the flow rate and the angle of inclination of the settler will have a profound effect on the performance of the settler and the results of this study agree. At any constant angle as the flow rate is increased the quality of separation the settler yields is decreased (Wang 2009).

4.1.5 Upward Flow Configuration

A trial was performed where the settler was operated with the direction of flow in the flow chamber reversed to flow upward as opposed to the downward flow used in the rest of this study. The settler was held at a 44° angle of inclination with a total flow rate of 24 mL/min using the same split fraction, 1 mL/min concentrate to 5 mL/min clarified, as in the downward flow configuration study. It should be noted that the angle and flow rate used for this trial were selected to give the best performance possible from the settler while minimizing the risk of settler fouling and blockage. When the settler was operated in an upward flow configuration the performance of the settler alternated between two different states which are shown in Table 2. The first state, which was more common and the data was more closely clustered, had extremely poor performance when compared to most of the downward flow trials, especially those at a similar flow rate. The value of the relative concentration was only 68% of the corresponding downward flow trial with the concentration factor and the clarification factors being only 71% and 105% respectively. The second state while not having as tight fitting data, had performance only slightly

worse than that of the downward flow settler trials. Additionally, managing settler fouling with the settler in this configuration was extremely difficult, when compared with the downward flow configuration trials, with the settler rapidly accumulating a biofilm that proved difficult to remove after the experiment.

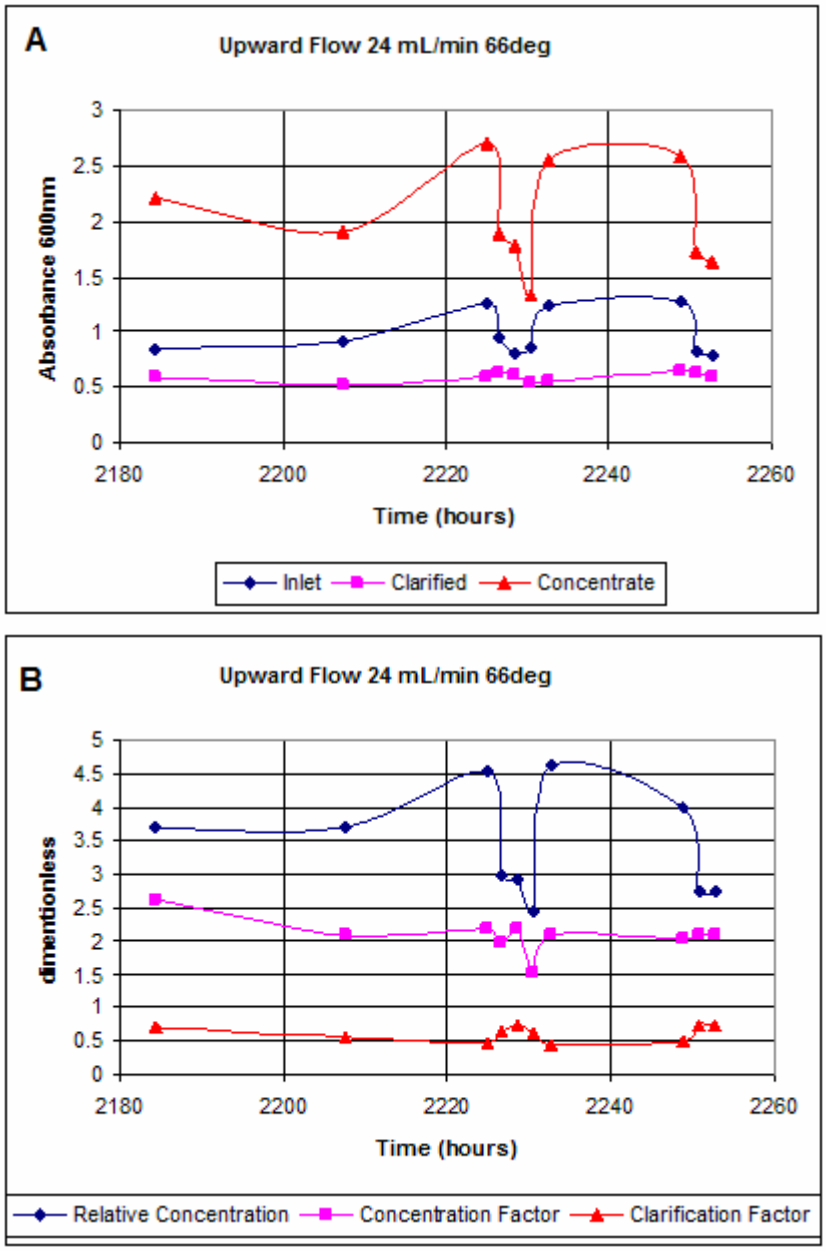

Figure 21: data collected during the upward flow trials. A: Concentration over time data as collected by a spectrophotometer. B: Data from A was used to calculate the relative concentration, concentration factor, and the clarification factor.

Table 2 - Upward Flow Configuration, 44°, 24 mL/min.

	State 1	State 2
Relative Concentration	2.85±0.043	3.80±0.045
Concentration Factor	2.08±0.041	2.24±0.14
Clarification Factor	0.73±0.06	0.59±0.16
# of Data Points	4	3

Table 3 - Dimensions of Downward Flow Inclined Gravity Settlers

Settler	Width	Length	Depth	Surface Area	Notes
Small Settler (Hou, 2011)	4.5 cm	59 cm	1 cm	265.5cm^2	Single clarified and concentrate outlet. Volume = 266mL
Medium Settler (Hou, 2011)	9.5 cm	59 cm	1 cm	560.5cm^2	Two concentrate and one clarified outlets. Volume = 561mL
Current Study	7.5 cm	76 cm	1 cm	570cm^2	Single clarified and concentrate outlet. Volume = 570mL

4.1.6 Previous Data for Comparison

Previous work with downward flow inclined gravity settlers was performed on two gravity settlers; the dimensions of which are shown in Table 3 (Hou, 2011). A comparison of the performance of the two studies can be seen in Table 4. The comparison between the results of the two studies is obscured by the major differences in the two studies. The primary difference is that the previous study used a more effective split ratio as mentioned in section 4.1.1. The second difference was that the angle of inclination used in the previous study is unknown. A third difference of unknown effect is that the previous study fed a more concentrated algae suspension to the settler. When comparing the settlers most similar in size, the current and medium settler from the previous study, it can be seen that the medium settler, at a 252 min residence time compared to a 23.8 min residence time, was capable of a much more effective separation as expected. When the comparing the trials of the medium and current settler at the

closest residence times available the current design was able to concentrate the algae biomass twice as effectively with a residence time almost 43% shorter even with the disadvantage in the split ratio. When the 16.2 min residence time trial from the small settler is compared to the 15.8 min residence time trial from the current study the small settler achieved a relative concentration 2.9x greater than the of the current study. It can be seen that the current settler can achieve a performance similar to that in the previous studies but no definitive conclusions can be made due to the differences in the operating conditions of the studies.

Table 4 - Comparison between Previous and Current Study (Hou, 2011)

Settler	Flow Rate mL/min	Residence Time (min)	Inlet abs nm(600)	Relative Concentration	Concentration Factor	Clarification Factor
Small	4.67	56.9	2.40	42.0	7.80	0.18
Small	16.4	16.2	2.97	7.25	4.51	0.62
Small	27.9	9.52	2.31	4.25	3.22	0.76
Medium	2.22	252	4.11	28.0	6.85	0.25
Medium	7.78	72.0	4.55	5.09	3.58	0.71
Medium	7.82	71.7	2.25	3.69	2.81	0.75
Medium	13.3	42.1	4.25	2.56	2.14	0.83
Current	24	23.8	0.53	6.70	4.63	0.69
Current	30	19.0	0.76	3.21	2.44	0.76
Current	36	15.8	0.60	2.48	2.05	0.83
Current	42	13.6	0.63	2.49	1.97	0.79

4.2 Settling Velocity Study

Research into the factors affecting the settling velocity of algae is necessary for improving settler design. There has been little investigation into algae settling velocity outside of ecological studies and as a result any new information on algae settling velocity may prove important.

4.2.1 Settling Velocity Overview

Ten trials to measure the settling velocity of *S. Dimorphous* were performed and the results of those studies are shown in Figure 22. Four of the trials were performed using a brackish water acclimated species of algae while the remaining six were used a regular culture of *S. Dimorphous*. Large variations in the settling velocity of the algae prompted further investigation.

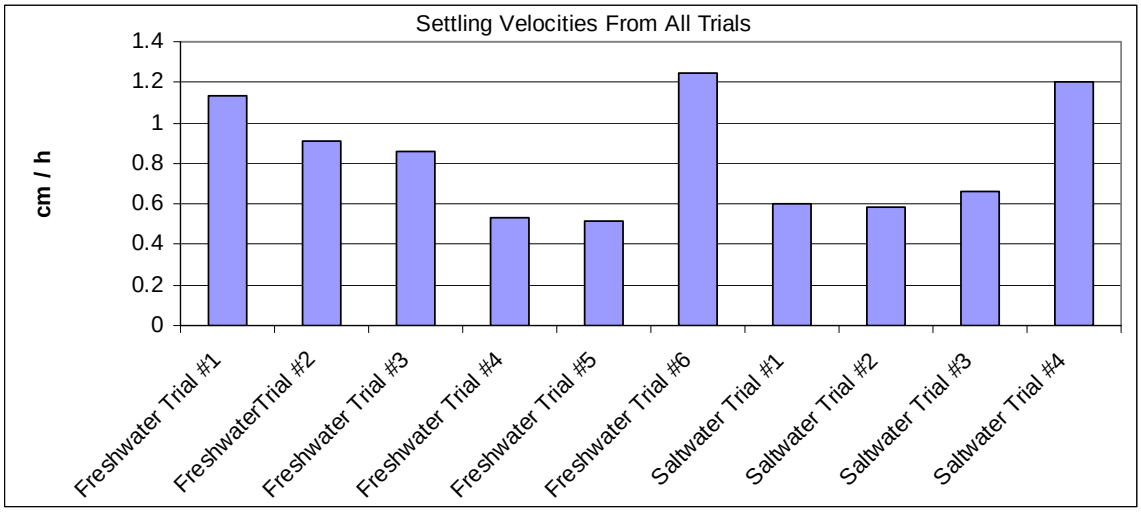

Figure 22 - Bar graph of settling velocities recorded in various studies; based on measurements using a spectrophotometer.

Table 5 – Settling Velocity of Algae at Various Stages of Growth and Salinity

Trial #	Settling Velocity		Growth State
Freshwater			
1	1.13	cm/h	early stationary phase
2	0.913	cm/h	late exponential
3	0.855	cm/h	growing
4	0.532	cm/h	growing
5	0.514	cm/h	growing
6	1.25	cm/h	late exponential
Saltwater			
1	0.6	cm/h	growing
2	0.587	cm/h	growing
3	0.658	cm/h	growing
4	1.2	cm/h	late stationary phase

4.2.2 Effect of Culture Growth State on Algae Settling Velocity

It was noticed that for trials performed on algae cultures that had been allowed to finish growing and approach the stationary phase, that the settling velocity was far greater than for cultures that were still growing. It was found that there was a 54% difference in the settling velocity between the two types of cultures with a p-value of 0.018. This can be seen in both Table 5 and Figure 23. This result is important for organizing an algae production facility, as gravity settlers will have greater volumetric efficiency separating algae cultures grown in batches that have reached stationary phase as opposed to continuously grown cultures.

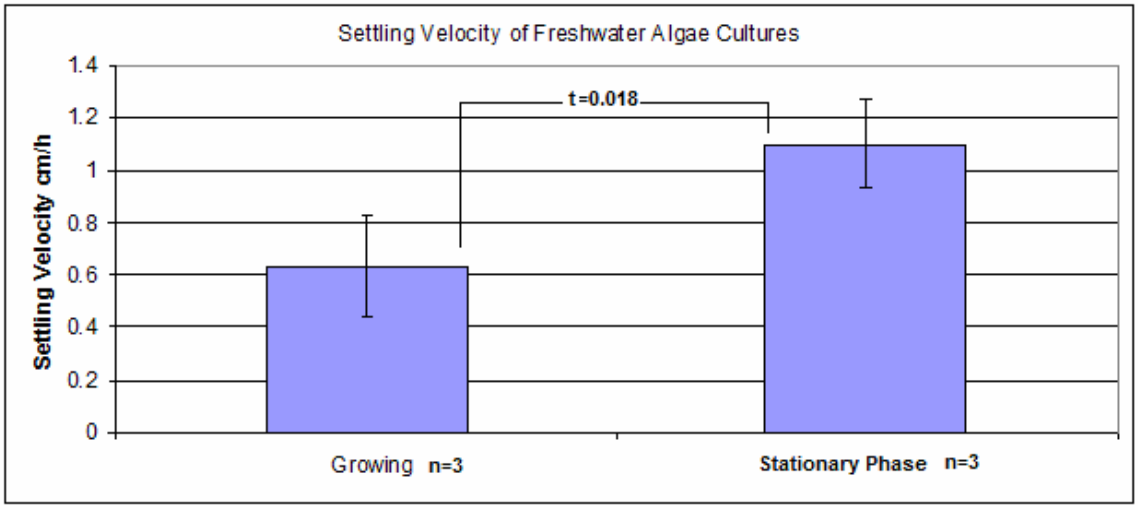

Figure 23 - Average settling velocity of actively growing algae cultures and cultures that had entered or approached stationary phase. Error bars denote standard deviation of measurement.

4.2.3 Effect of Saltwater Acclimation on Settling Velocity

An algae strain of *S. Dimorphous* that had previously been adapted to grow in 1.015 TSG salinity brackish water was tested to determine if the adaptation had influenced potential dewatering processes. It was found that there was no noticeable change, t=0.44, in settling velocity when comparing algae cultures at similar growth states. This is demonstrated by Figure 24. It was found that the adapted algae has the

same increase in settling velocity as the culture approaches stationary phase which can be seen in Figure 25.

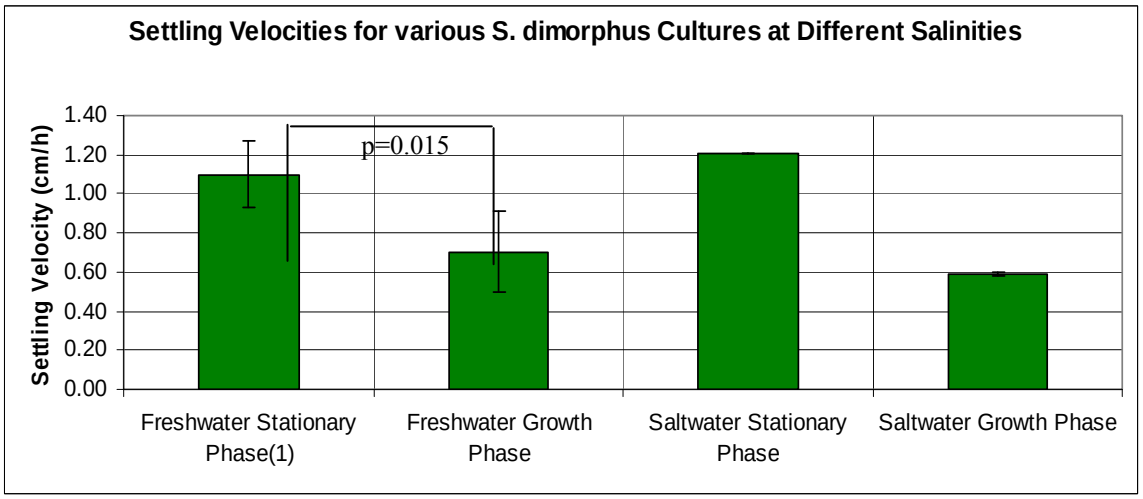

Figure 24 - Comparison between the settling velocities of stationary saltwater algae cultures and that of freshwater cultures. Comparison between the settling velocities of growing saltwater algae and freshwater cultures.

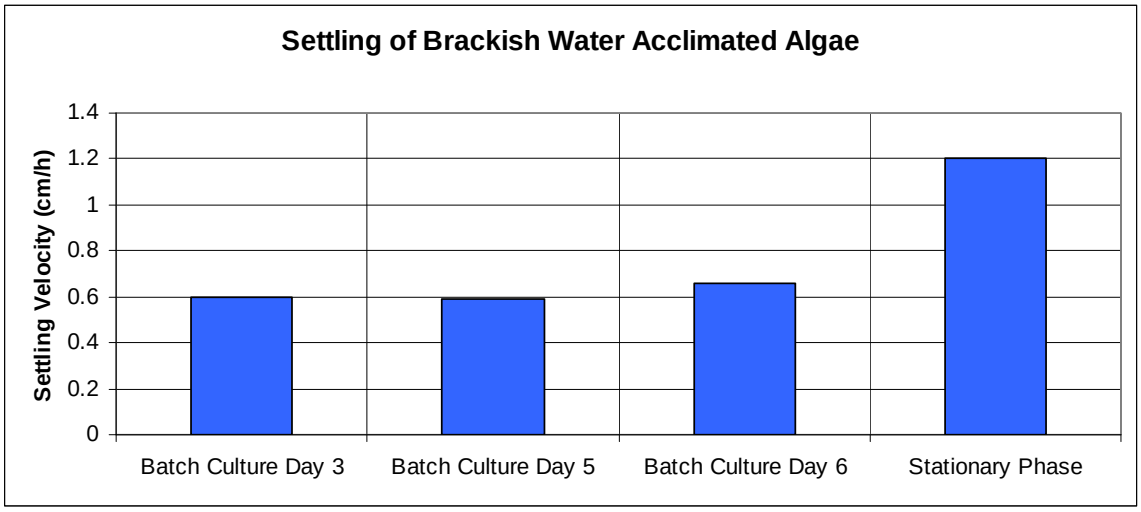

Figure 25 - Variation of settling velocity for a 1.015 TSG acclimated culture over time. Stationary phase measurement was performed on inoculums used to start this culture to reduce time required for this study.

4.2.4 Algae Clustering

Due to the fact that the algae species *S. Dimorphous* forms irregular shaped cell clusters instead of individual cells, any settling velocity model will require knowledge of both the distribution of various types of clusters and the settling velocity of each type of

cluster. Unlike bulk settling velocity measurements, which can be rapidly performed using a spectrophotometer, these studies were performed using manual cell counting in a hemocytometer.

4.2.4.1 Distribution of Algae Cell Clusters

Understanding the size and shape distribution of algae cells can be instrumental in the development of an effective theoretical model of the gravity setter. This is because both particle size and shape have a major influence on the settling velocity. From cell counting settling velocity experiments a relationship between the number of various cell clusters has been found. Figure 26 shows the fraction of the total cell cluster population each grouping occupies. Quads are by far the most common cell cluster, representing 89% of cell clusters. The remaining types of cell clusters were of approximately equal numbers. Quads also appeared to have the largest variation in size ranging from extremely small but tightly connected groupings to loosely connected giant groupings.

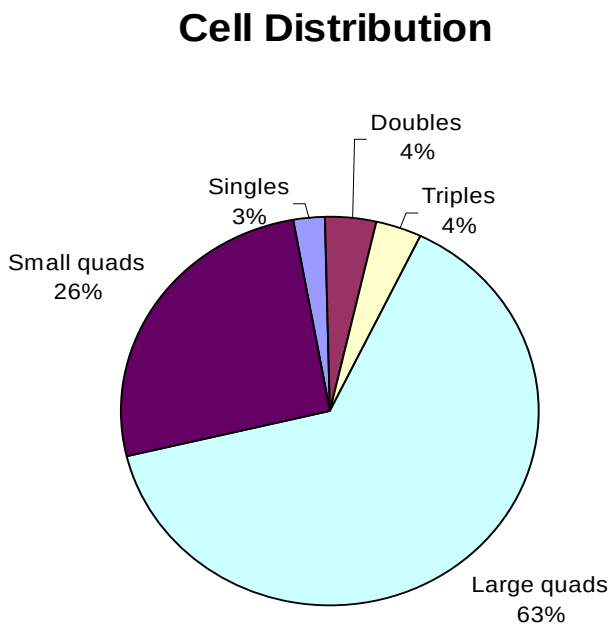

Figure 26 – Distribution of number of cells in cell clusters; taken from the average of three cell counting experiments: Trial # 1, Trial # 4, and Trial # 5 from Figure 22.

4.2.4.2 Settling Velocity by Cell Cluster Type

Figure 27 shows the settling velocity of various cell clusters and the large variations found in the three experiments. Large variations in the settling velocity of individual algae cluster types were noted between Trial # 1 and that of Trial # 4 and Trial # 5 as shown in Figure 22 and Table 5. This may indicate that there is a major difference between the settling velocities of various types of algae clusters based on growth state. This result is only conclusive for the quads as the number of cells, other than quads, counted in Trial # 4 and Trial # 5 was small enough that there may be a large amount of error in those calculations as demonstrated by the negative settling velocity of the doubles from Trial # 5 (Figure 27).

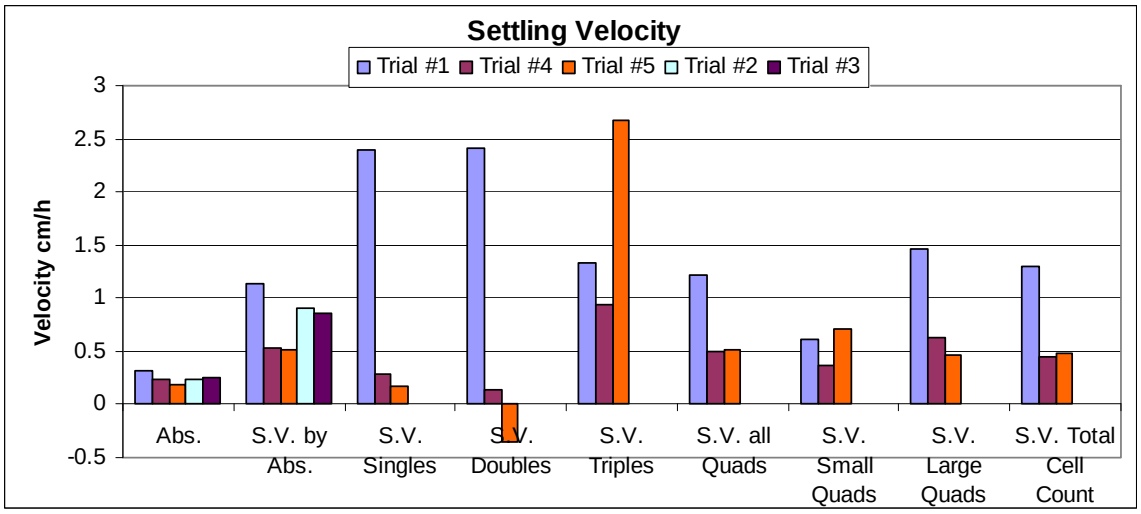

Figure 27 – Variation of settling velocity of various types of cell clusters over three trials. Abs. is the absorbance measured for each trial.

4.3 Discussion

While the data collected in this study does not match that collected by Hou, any differences can be explained by the different ratio between the clarified and concentrate stream flow rates that were selected for this study. The fact that similar concentration factors were achieved while having almost twice the amount of liquid present to dilute

the solids that enter the concentrate stream indicates that the modifications made in the new prototype did not hinder the separation (Hou, 2011). Another difference between the studies was that the residence time within the settler during this study was far longer than that used in the previous work. The fact that the current gravity settler design was able to exceed the performance of the most comparable trial of its predecessor with those two handicaps should indicate that the changes had no effect or a positive effect on the separation.

This study was limited by the volume of the bioreactor. The small size of the reactor allowed it to enter a washout condition which drastically changed many properties of the algae population. Additionally, this prevented the algae from reaching the concentrations present in other studies.

The settling velocity data though interesting cannot be directly compared to any literature for algae due to the lack of velocity measurements. When compared to the settling velocity of Hybridoma it has been found that algae generally settles at a lower velocity. Hybridoma of various cell lines were found to range from 3.5 cm/h to 0.9 cm/h with the average from the study being 2.3 cm/h. A T test found that the settling velocities found in each study were vastly different with a value of $t=0.003$. Additionally, a comparison to theoretical values is complicated by the large variability in both size and shape in addition to lacking information on the density of the cells in a algae culture, a factor that most likely changes with culture state.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The development of a new and improved downward flow inclined gravity settler has made it easier to scale up while also improving maintenance. These changes greatly simplify the outlet design and enable the settler to be dissembled and reassembled without the use of power tools. The changes to the gravity settler have been found to have no negative effects on the performance of the downward flow inclined gravity settler. The settler was found to produce enhanced performance at lower flow rates and lower angles of inclination just as would be predicted by theoretical relationships that were established for upward flow settlers. The best separation should occur at a flow rate between 24 mL/min and 30 mL/min and between angles of 57.5 and 66°. The results of this study are complicated by accumulation of algae which made comparison between theoretical models and the collected data difficult. Additionally, the downward flow configuration was found to have enhanced performance and experienced a slower rate of fouling than an upward flow configuration at the same operating conditions.

It has been found that actively growing algae cultures have a much lower settling velocity, 54% lower, when compared to cultures that have approached or entered the

stationary phase. This result was found to be statically significant with a p-value of 0.018. Additionally, it has been found that algae cultures of *S. Dimorphous* that have been adapted to brackish water have the same settling velocity properties as those that were not adapted. Lastly, it has been found that the vast majority of algae clusters of *S. Dimorphous* have four cells per cluster but the large size variation amongst quads leads to a large variation in settling velocity for those cell clusters.

5.2 Recommendations

It is recommended that any future researchers continuing with this project should use a bioreactor that is larger than that used in this study. The bioreactor should have a residence time sufficient to allow cells that have exited the settler to recover before being drawn back into the settler. Future studies should concentrate on mitigating or minimizing the attachment of algae to the settler surfaces, as this is one of the most critical flaws with this system. The most effective way to do this would be to test various surface treatments to find a treatment that will not harm the algae culture while preventing attachment to the surfaces of the settler.

Due to the operational difficulties of operating a settler at a shallow angle of inclination it is recommended to sacrifice the volumetric efficiency of the settler by operating it at a high angle where there is a far lesser chance of the algae sticking to the surface. It is also recommended that any gravity settler in industrial use should be regularly drained and an antifouling procedure should be developed. Research into various antifouling techniques will be helpful and have much potential to improve the performance of the gravity settler system especially if they require fewer shutdowns than

currently used methods. Due to the discovery of the increase of settling velocity as a culture approaches stationary phase, investigation into batch culture and harvesting of algae should be revisited.

One proposed modification for commercial use settlers would be to have the outlet constructed as part of the flow chamber using the upper and lower plates of the flow chamber for structural support. This would require six fewer separate pieces to be fabricated, greatly simplifying the construction and sealing.

Due to algae clusters being predominantly composed of four cells each, investigation of the size distribution of algae cells should be investigated. This may yield a full settling velocity distribution that will be of great use for finite element analysis of the gravity settler geometry.

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APPENDIX

APPENDIX A

Table 6 – Performance of Settler: Relative Concentration

Relative Concentration								
Angle	44 deg	Std. Error	52 deg	Std. Error	57.5 deg	Std. Error	66 deg	Std. Error
24 mL/min	N/A	N/A	6.70	0.202	5.38	0.891	4.21	0.168
30 mL/min	N/A	N/A	3.21	0.0443	4.91	0.696	3.47	0.387
36 mL/min	3.22	0.0898	2.51	0.180	2.51	0.209	2.69	0.147
42 mL/min	2.68	0.281	2.49	0.132	2.09	0.222	2.17	0.0837

Table 7 – Performance of Settler: Concentration Factor

Concentration Factor								
angle	44 deg	Std. Error	52 deg	Std. Error	57.5 deg	Std. Error	66 deg	Std. Error
24 mL/min	N/A	N/A	4.63	0.524	3.62	0.524	2.93	0.351
30 mL/min	N/A	N/A	2.44	0.201	3.74	0.506	2.63	0.346
36 mL/min	2.50	0.0802	2.05	0.141	2.15	0.182	1.99	0.230
42 mL/min	2.16	0.120	1.97	0.0700	1.81	0.174	1.79	0.0610

Table 8 – Performance of Settler: Clarification Factor

Clarification Factor								
angle	44 deg	Std. Error	52 deg	Std. Error	57.5 deg	Std. Error	66 deg	Std. Error
24 mL/min	N/A	N/A	0.692	0.0617	0.676	0.0322	0.694	0.0602
30 mL/min	N/A	N/A	0.763	0.0542	0.763	0.0591	0.759	0.0375
36 mL/min	0.776	0.0289	0.830	0.0702	0.856	0.0596	0.737	0.0495
42 mL/min	0.809	0.0385	0.792	0.0408	0.792	0.0249	0.824	0.0571