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Utilizing optical light filters and biofilm based cultivating to enhance microalgal growth

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Utilizing optical light filters and biofilm based cultivating to enhance microalgal growth

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ABSTRACT

The main objective in this research is to explore two separate approaches for improving the growth of microalgae that can be potentially used for producing fuels and chemicals. One approach was to use optical light filters, which selectivity filter natural light allowing certain wavelengths into the culture systems. The other study is to use an algal growth system with different bio-renewable materials being used as attachment materials. Both studies demonstrated increased biomass productivity and the ability to scale-up.

In the first part of the study, the research involving the use of optical light filters for microalgal growth in different cultivation systems included flask culture systems, flat panel photobioreactors, and rotating algal biofilm (RAB) systems. The optical light filters allow a specific range of wavelengths to pass through, while reflecting harmful wavelengths such as UV and IR. Two thin film light filters were fabricated for this research. One light filter allows both red and blue wavelengths to penetrate through (termed as blue filter) while the other allows the penetration of wavelengths in the red spectrum (termed as red-filter). Both filters resulted in increased biomass productivities when microalgae were grown in the flat panel photobioreactors and the RAB systems. When the blue filter was used for the culture, the biomass productivity increased 31.9% in flat panel photobioreactors and 99.8% in RAB systems; while the red filter resulted in an increase of 34.1% in the continuous operation of the flat panel photobioreactor. These results provide evidence that the use of these filters have the ability to enhance algal yield.

The second part of this study used different bio-renewable materials as an attachment substrate for algal growth. An initial test was performed to screen the material that can result in the highest amount of attached biomass. It was found that the soy protein based sheet showed the greatest amount of algal attachment. Compared to the suspended cell growth of the control,

the biomass productivity from the soy protein based sheets increased by 294%. However, significant deterioration was observed from the soy protein based sheet after 20 days. To alleviate the dissolving of materials and enhance the integrity, poly-lactic acid was integrated into the soy protein based sheet. After this integration the attachment material displayed a significant amount of rigidity allowing for a longer duration of experimentation. However, the productivity from the PLA integrated soy protein based sheet displayed a certain degree of decreasing biomass yield compared to the 100% soy protein based sheet. Collectively, the PLA integrated sheet still demonstrated adequate performance in terms of cell growth improvement and material longevity.

In summary, this study shows that the use of the optical light filters and bio-renewable based sheets for attached algal growth are two viable approaches for enhancing algal growth performance. However, these two approaches need to be further optimized in order to be implemented at a large scale.

CHAPTER 1. OVERVIEW AND MOTIVATION

Rational & Significance

With increases in the price of fuel and industrial commodities, there is an urgent need to explore a sustainable alternative as a substitute for petroleum based products. Microalgae have proven the potential to fulfill this requirement. However, several key issues need to be addressed before a commercial algal production process can be developed. This would involve the ability to produce a high amount of biomass, more efficient conversion of raw algal biomass to end products, and ultimately a lower the cost of operations. The specific issue that this research focused on was to increase biomass yield and productivity through manipulation of light illumination and the development of an attached algal growth system.

Light quantity and quality are main factors affecting the production of microalgae at the commercial scale. This work focuses on using an optical light filter to manipulate the light spectrum for algal culture. The light filter can play a critical role in algal production as specific wavelengths such as UV and IR are detrimental to algal growth (Holzinger & Lutz, 2005). In addition, it has been reported that specific wavelengths in the blue and red spectrum can increase productivity and specialty products (Blair et al., 2014) (Schulze et al., 2014). Therefore, selectively providing preferable wavelengths of light to algal cells can result in higher specific growth rates as well as larger biomass productivities.

The other aspect this research focuses on is the utilization of bio-renewable resources as attachment substrates for algal growth. After an assessment of several bio-renewable materials, a soy protein based sheet demonstrated the highest amount of algal attachment. The attached algal growth was performed in a rocking photobioreactor; the amount of attached algal biomass

was optimized in this type of photobioreactor in terms of harvesting time. However, the pure soy protein based sheet did not display sufficient longevity for supporting algal growth. The integration of poly-lactic acid into the soy protein based sheet increased the rigidity of the bio-renewable sheet allowing for a longer longevity and increased biomass productivity. This technology has the unique ability to increase the productivity of microalgal growth as well as being bio-degradable once growth is completed.

Hypothesis

The hypothesis of the light filter study (1st part) is that algal biomass productivity can be increased with the use of optical light filters that serve the function of reflecting harmful wavelengths and allow beneficial wavelength bands to pass through. The hypothesis of the attached algal growth research (2nd part) is that an attached growth system can greatly enhance the biomass productivity, while a bio-renewable material as attachment materials can reduce the production cost and is environmental friendly.

Objectives

1.) To demonstrate that optimal light filters can increase the biomass productivity and specialty products in the microalgae *Chlorella vulgaris*.

2.) To utilize the bio-renewable resource of soy protein isolate to enhance and simulate the growth of the microalgae *Chlorella vulgaris*.

CHAPTER 2. LITERATURE REVIEW

Overview of Algae

Algae are considered some of the oldest living species to live upon the Earth. Algae are classified into two major groups; Prokaryotes and Eukaryotes. Prokaryotic algae, commonly called cyanobacteria, are single cell organisms with a simple cellular structure without nuclei. Eukaryotes are more advanced in cellular structure and therefore typically require more nutrients in order to survive. Presently, algae are classified into three general classes; cyanobacteria, microalgae, and macroalgae.

Cyanobacteria

Cyanobacteria are one of the oldest forms of life on Earth presently known. They are credited with converting Earth's atmosphere into more suitable conditions by producing oxygen, and allowing for the atmosphere that there is today. Due to this environmental change it is thought that cyanobacteria gave rise to chloroplasts allowing higher plants to be created. Unlike the other microalgae and macroalgae, cyanobacteria have the ability to live in very adverse environments, with very limited nutrient requirements. This has allowed cyanobacteria to endure through natural catastrophes and still be a viable organism. Today cyanobacteria are being grown for various researches which include biomass production for biofuels, and fertilizers for agriculture (Rodgers et al., 1979).

Macroalgae

Unlike cyanobacteria and microalgae, macroalgae are large multiple cell species. Commonly known as seaweed, macroalgae are simply harvested by filtration out of water and utilized for several various end products. Additionally, several different colors of macroalgae exist which also result in different products. For instance, golden and red macroalgae can be used for medicinal and pharmaceutical purposes (Smit, 2004). While other commonly known products derived from macroalgae include food for consumption and energy production (Rodriguez-Bernaldo de Quiros et al., 2010).

Microalgae

Microalgae are single celled organisms and can be found in association with one another to form masses of cells. The major reason microalgae have been studied intently is due to the large variation among algal species and the different productivities and compositions among these various species. Due to this wide plethora of microalgae, it is reported that over 15,000 compounds can originate from algal biomass (Cardozo et al., 2007). Another very important aspect of microalgae is that they can be both autotrophic and heterotrophic. This leads to the potential for heterotrophic microalgae to be fermented and produced in large cell densities (Xu et al., 2006). This characteristic allows industry to utilize microalgae in order to produce large quantities of specialty products such as DHA.

However, autotrophic algae are receiving considerable attention in recent years. Phototrophic algae have the ability to be scaled up to a much larger scale than heterotrophic algae due to not having to be confined to a large fermenter. This led researchers to believe that autotrophic microalgae can adopt similar practices as modern agriculture. However, unlike

agriculture today, microalgae have a much faster reproduction rate while having the ability to grow in non-suitable environments for terrestrial crops. This makes microalgae a very attractive option for biomass production.

The ability to tailor microalgae for mass production, with enhanced yield and productivity is critical for the fuel industry. With microalgae having the ability to produce high amounts of lipids and carbohydrates it is believed that algae can replace petroleum based fuels. Additionally, culturing microalgae for biofuels can decrease CO_2 emissions and boost the economy due to lowered fuel prices and jobs being created. As a result, the research of this thesis mainly focused on increasing microalgal growth.

Microalgal chemical composition

The major constituents of microalgae include lipids, proteins, and carbohydrates. Other essential compounds that may be present in algae would include carotenoids, omega-3 fatty acids, and amino acid residues. The high reproduction rate of microalgae allows for researchers to manipulate the cultivation conditions such as nitrogen starvation, light wavelength specificity, and altered pH (Chisti, 2007) (Ravelonandro et al., 2008) in order to produce a more favorable end product.

Lipids

Large species variation leads to certain microalgae having the ability to produce a high lipid content of up to 75% dry weight (Chisti, 2007). Lipids are a valuable commodity due to their ability to be converted into biodiesel. However, biodiesel must be produced from specific characteristics of the fatty acids that make up a lipid. Fatty acids are normally broken into two groups, saturated and unsaturated. The difference lies within their chemical makeup with saturated fatty acids containing no double bonds, and unsaturated fatty acids contain one or more double bonds. In regards to biodiesel, a good mixture of saturated and monounsaturated fatty acids are usually desired (Spolaore, 2006). This will give the fuel desirable qualities such as a good fire point and favorable viscosity to be ran as a fuel.

The main fatty acid makeup of microalgae in *Chlorella*, a green alga widely used in research and development of algal biofuel, is C16:0, C18:0, C18:1, C18:2, and C18:3. However, other species of microalgae are able to produce a wide assortment of fatty acids. Fatty acids that have very long chains (C >18) and multiple double bonds starting at the omega-3 end are considered very beneficial in human nutrition. These omega-3 fatty acids have shown to increase brain development in infants and adults, as well as being a promising food source for aquacultural applications. Presently several companies are working to produce specific omega-3's for human consumption such as DSM and Monsanto.

Proteins

The proteins and amino acid profiles that make up microalgae are currently a popular research topic across the scientific community. With many of the protein sources used today becoming over farmed or over fished there is a current need for a high quality protein substitute.

Similar as lipids, the proteins and amino acids found with microalgae are very diverse. Microalgae that produce a large percentage of protein are being most readily studied for their ability as a food supplement for agricultural and aquacultural feed. Having a large percentage of protein being produced is not the only advantage, as most microalgae have a very good amino acid profile. When compared to other feeds sources such as corn and soybeans, microalgae have an impressively higher percentage of essential amino acids such as lysine, phenylalanine, and methionine (Becker, 2007). Therefore, using microalgae as a food supplement could increase the health profile of livestock animals, leading to an overall increase in quality.

Carbohydrates

Carbohydrates found within microalgae are as diverse as the lipids and proteins. This diversity depends on the percentages of starch and cellulose found within the biomass. With starch being the most attractive, research has focused on the ability to produce more starch than cellulose. This is due to the simple breaking down of starch and easily converting it into ethanol through fermentation of the sugars. However, most microalgae contain large percentages of cellulose, up to 25%, which cannot be broken down as easily as starch (Bra'nyikova et al., 2011). Therefore, cellulose must be processed further in order to ferment the sugars making up the cellulose structure. This could become very crucial as the ability to utilize microalgae becomes clearer for years to come.

Environmental Factors and Influential Nutrients

Phototrophic microalgae culture requires specific environmental conditions for the species of algae being researched. The main factors include temperature, light, gas exchange,

nutrients, and fluid mixing. When these factors are altered, certain characteristics of the microalgae can change within the culture system.

Temperature

The temperature used in a culture can vary greatly depending on the algal strain. In general, most microalgae require that the temperature to be in between 21-26 ^{o}C for optimum growth conditions. An environmental temperature is usually influenced by the amount of solar radiance and ambient temperature (Borowitzka, 2005). It is desirable to perform the algal cultivation, particularly at large scale, in an area where microalgae can produce biomass year round. Temperature is also a very effective way for manipulating algae to produce specialty products from algal biomass. When put under stressed temperature conditions certain microalgae have been reported to behave differently, producing more or less lipids, proteins, or carbohydrates.

Light

Sunlight or artificial light is the main energy source in phototrophic algae culture and therefore the most important influencing factor. In algal culture, low light intensities result in a low amount of cell growth and minimal biomass productivity. As light intensity is increased, algal biomass productivity is increased up to a certain threshold. Once this threshold is reached and surpassed the light intensity becomes too high resulting in photo-inhibition (Lee, 1999). Light penetration is also a problem in open pond cultivation systems. Open pond systems are often too deep not allowing for light to penetrate to the algal cells at the bottom of the pond. This results in cells on the top becoming stressed, causing photo-inhibition, while cells on the

bottom receive little or no light. Extensive research has been performed in pond depth and turbulence in order to achieve even cell distribution (Grobbelaar, 1994).

It is accepted that natural sunlight is not optimized for algal growth due to the large amount of UV and IR rays (Holzinger & Lutz, 2005). This is due to UV rays being directly detrimental to algal cells due to damaging their membranes causing cell death, while IR rays are harmful indirectly through overheating of the culture conditions. The research performed in this study focused on how light affects algal growth and the potential to increase algal biomass productivity through manipulation of light. In general, specific wavelengths of light such as blue (420-470) and red (650-680) are considered beneficial to algal cells (Schulze et al., 2014). Several studies have confirmed this by the use of blue and red light emitting diodes (LEDs) resulting in increased growth or specialty products compared to a control (Blair et al., 2014) (Wang et al, 2007). However, to our knowledge no previous study has been performed to use optical filters with natural sunlight on microalgal cultivation systems.

CO_2 concentration

The main chemical component that makes up algae is carbon. Accounting for up to 50% of the entire organism, carbon must be replenished continuously in order for successful growth (Becker, 1994). Phototrophic algae are able to get carbon through CO_2 aeration from the ambient environment or CO_2-rich gases. Some phototrophic culture systems such as closed photobioreactors are not able to access the atmospheric CO_2 and must have it supplemented. In this situation, CO_2 is supplied to the system through aeration. This leads to CO_2 being absorbed and gas exchange occurring, with an O_2 molecule is released. If this gas exchange rate remains

high, a high amount of oxygen may become present and photo-oxidation may begin to occur. This can be very detrimental to the culture causing decreased growth and death.

Fluid mixing/turbulence

The turbidity of a system is an important factor. In large volume culture systems, settling and shading are common problems occurred when turbulence is too low, resulting in a congregation of cells at the bottom of the reactor. This is detrimental to the culture as lack of nutrients and shading will lead to decreased cell growth. Shading often happens when light is unable to penetrate to the bottom of a system due to either the depth or the density of the culture. On the other hand, when turbulence is too high, the algae can become damaged from the hydrodynamic shear stress. In order to avoid those problems, turbulence must be controlled at an appropriate range.

Nutrients

When considering nutrients and phototrophic microalgae, nitrogen is almost always considered to be a limiting factor (Brzenzinski, 1985). In addition to nitrogen, other nutrients important for successful growth include phosphorous, magnesium, and calcium. These nutrients are delivered to the system in the form of salts, and are normally mixed with water to allow for a dilute mixture. This leads to a uniform medium where all cells can come in contact with the same amount of nutrients as the other cells. Other nutrients supplemented for a healthy metabolism would include trace metals, vitamins, and a form of chelated metal. Most culturing practices usually over-supply nutrients to ensure no nutrient limitation. But the levels must be carefully controlled to avoid substrate/nutrient inhibition.

Algal Cultivation Systems

New technologies are constantly being developed in terms of algal cultivation systems. The selection of cultivation system depends on the type of research and target products. If a large quantity of biomass is desired with contamination not being an issue, a system such as open pond raceway or a pilot scale rotating algal biofilm (RAB) system would suffice. However, if the culture contamination is a concern, a closed photobioreactor system is preferred, although this type of photobioreactor cannot completely avoid contamination either.

Flask culture

The most common use with flask culture is to make several replicate flasks and run a series of experiments on them. Due to a small amount of culture and easy to manipulate, this is a very attractive system used for fundamental studies in algal culture. Also, flask culture is the first step in the production chain of large scale algal culture, and is often used for preparing seed.

Plastic bag photobioreactor

Closed photobioreactors are a very usefully culture system used at both laboratory and commercial scales for algal production. These systems allow for the algal culture to be easily and precisely controlled for various parameters such as pH, evaporation, and nutrient levels. Some photobioreactor designs include tubular and helical shaped reactors (Molina et al., 2001) (Carvalho, 2006). Although there are several styles of closed photobioreactors, the two discussed are the most popular at commercial scale. Over the past few years algal companies such as Algenol have began using the plastic bag photobioreactor for their algal production. The plastic

bag photobioreactor system is considered attractive due to low material cost as well as a small aerial footprint. The system also allows for easy manipulation of the algae and increased light penetration due to each individual bag system being considerably small in volume. This allows companies to produce numerous reactors in a small area of land with a high production rate. However, the downsides to this reactor involve the stability of the plastic, poor insulation from temperature extremes, and very labor intensive.

Flat panel photobioreactor

The flat panel photobioreactor system is used for various researches such as comparative analysis to raceway ponds or simply for scaling up to a larger culture system (Richmond, 1999). Flat panel photobioreactors are mainly composed of glass or plexiglass and usually found in the shape of a rectangular box. The size of the reactor depends on the research experiments being performed. Large flat panel reactors are normally used for preparing the seed culture to transition into a larger scaled system such as open raceway ponds (Borowitzka, 1999). Smaller photobioreactors may be utilized for scaling up to a larger flat panel, or utilized to study algal growth and behavior in a closed reactor setting. Studies are conducted on flat panel reactors due to having increased biomass productivity and lowered contamination issues. They function by being a completely enclosed system having CO_2 pumped from the bottom for proper mixing. This allows for nutrients to be distributed evenly as well as the light source to penetrate more readily reaching all the algal cells. Similar to the plastic bag photobioreactors, the flat panel reactors allow for researchers to easily manipulate the system in order to run a variety of experiments. However, the flat panel reactors have more capital cost in terms of materials than the plastic bag reactors, although being more resilient and having better insulation to adverse

temperature conditions. As mentioned previously, the flat panel system is a good model for manipulating algae at a somewhat larger sized scale. The research in this study utilized the flat panel system in order to manipulate the quality of light allowed to pass through to the algae in the photobioreactor.

Open raceway pond culture

The raceway pond culture system is the most highly used commercial scale algal cultivation system used today. This system allows for a large amount of biomass to be produced without the high material cost of other photobioreactors. These ponds are found to be a few feet deep and hold the shape of a raceway while having a paddle wheel circulate the liquid. The scalability of the raceway pond up to thousands of square feet is reasonable due to being formed digging the shape out of the ground and covering with a tarp (Singh et al., 2011). However, with any system there are flaws such as wind, rain, and natural contamination of native species. Natural contamination is the biggest issue with the open raceway system. This is mainly due to the algae being exposed to other native species of algae, which contaminate the system and take over the algae being cultured. Therefore, only a number of microalgae can be grown in the outside environment. These algae are usually salt or heat tolerant, enabling them to outperform other native species (Laws et al., 1988). The open raceway systems also have a poor CO_2 exchange rate, leading to the algae in raceway ponds being less dense then photobioreactors. The ability for light to penetrate to the bottom of the raceway is another limiting factor resulting in a lower cellular density of algae in a raceway system.

Rotating algal biofilm (RAB) system

This newly developed technology shows great promise for large scale algal production. The system utilizes the raceway pond by retrofitting vertical attachment materials rotating through the liquid reservoir. The biomass productivity from the RAB system displays a large increase in terms of daily growth rate and overall biomass compared to the raceway system. There are several factors that are believed to have an impact on the increased biomass productivity. Light penetration is greatly increased with the biofilm system as the algae come out of the liquid and have direct access to the sunlight. Additionally, the gas exchange is also increased due to the direct contact between the gaseous CO2 molecules and the algae cells in the biofilm.

In addition to the increase in productivity, the RAB system lowers the cost of harvesting cells as the cells can be directly scraped from the biofilm (Johnson & Wen, 2010). On the contrary, centrifugation is a costly process that involves a large portion of the energy it takes to harvest and dewatered algae in a raceway pond or photobioreactor. However, there are several factors that still need to be taken account for in this system such as rotation speed and attachment materials. For the research in this study cotton duct and bio-renewable materials were used as an attachment substrate as it showed success in previous research (Gross et al., 2013).

Harvesting and Dewatering of Algal Biomass

Harvesting and dewatering of microalgae from the suspended culture system usually requires high energy input. Today, new designs are attempting to lower these costs and develop an effective new way to harvest and dewater algae. With that being said, many designs do not address this problem, as the majority of the algal industry produces microalgae in suspension.

The microalgae that are found in suspension are very dilute, only contributing about 0.02% of the total liquid content (Borowitzka, 1988). The algae must then require further steps such as centrifugation in order to remove the water and harvest the algae. However, other technologies have developed effective models that reduce the dewatering and harvesting cost at pilot scale. Gross & Wen, (2014) have reported technologies that not only increase the biomass productivity compared to other systems, but also reduce harvesting and dewatering costs significantly. The expense for harvesting and dewatering contributes up to 30% of production cost (Molina et al., 2001). Therefore, industry must address the significant energy input from these processes and develop new technologies in order for algae to be viable.

Filtration

Filtration is normally used at lab scale for harvesting algae cells. For large sized microalgae cells, filtration is very effective and therefore an attractive choice due to being inexpensive. However, the issues with filtration involve the ability to scale up to a larger scale. In order to accommodate large scale operation, a large filtration unit is needed, as well as a method to prevent clogging of the filter. This would prove to be very difficult as the screen pore size would be miniscule, resulting in a poor flow rate.

Flocculation

Like filtration, flocculation is used to harvest suspended algal cells. Flocculation is usually performed by a neutralization process on the algal cells which hold a negative charge. This process is essentially forming a conglomerate of cells which can then be easily removed due settling in the system (Golueke & Oswald, 1965). There are several mechanisms for the

flocculation in a suspended cultivation system. One way is the use of inorganic molecules such as aluminum sulfate or lime to neutralize algal cells for removal from the system (Barclay et al., 1994). However, this can lead to a buildup of inorganic chemicals which cause problems with downstream processing. Additionally, this adds further steps to remove the chemicals, unless the algae are to be used as fertilizers. A safer method is the use of highly charged organic molecules called polyelectrolytes.

Unlike the inorganic molecules mentioned previously, polyelectrolytes are organic compounds, making them less toxic and more attractive. They function by not only neutralizing the algal cells but also by forming linkages among themselves which causes a stronger flocculation (Barclay et al., 1985). Unfortunately, the costs of polyelectrolytes are high, resulting in the use to be limited among all cultivation scales. Other forms of flocculation that are not as reliable include electroflocculation, bioflocculation, and dissolved air flotation. Electroflocculation is simply the process of introducing electric currents into the liquid culture. This results in the cells becoming less negative and forming flocculates for the potential to be harvested. Bio-flocculation has been found to be the best form in regards to cost and quality end products. However, the process involves altering the pH or limiting nitrogen which can result in the flocculation of cells to be time consuming, therefore unattractive (Benemann & Oswald, 1996). The dissolved air flotation process is widely accepted and used in wastewater treatment facilities. It is commonly performed by having flocculates form and then pumping air through the system allowing the conglomerated cells to float to the surface, allowing easier harvest.

Centrifugation

In light of a large portion of industry using suspended cultivation systems to produce algal biomass, centrifugation is very popular, particularly when the centrifuge is operated in a continuous mode, allowing for the removal of liquid at a high rate without pause. However, the largest problems with centrifuges are the high energy input. This energy input can account for up to 30% of the operational cost for algal processing (Molina et al., 2003). In addition to high operation cost, centrifuging only decreases the water content to be 80-90% of the sample (Johnson & Wen, 2010). Therefore, new alternatives for centrifugation must be developed at large scale algae harvesting and dewatering in order to make the harvest of biomass economically feasible.

Scrape Harvest

Scrape harvest is a newly found technique that is only available for use with algae that are attached to a substrate. Of all the harvesting techniques mentioned previously, the scrape harvest is the simplest. A specially designed scraper is fabricated to fit the biofilm system that is being used. This scraper is then brought across the surface of the attached substrate while collecting the algal biomass. The biomass collected is in the form of a paste, with similar water content as centrifugation (80-95%), so other dewatering methods such as freeze drying are still necessary. However, this harvest method is highly effective with the use of biofilm technologies to effectively collect algal biomass. Novel research has been performed demonstrating how effective this harvesting method is with biofilm systems (Gross et al., 2013).

Microalgal Applications

Microalgae have received a great deal of attention in regard to industrial applications and the wide array of possible products that can be produced. Of these end products the most important involves biofuels as a potential replacement of petroleum based fuels. Other applications include the use as feed, nutraceuticals, and bio-plastics.

Biodiesel

Of all applications biodiesel is considered the most targeted fuel product because of the high percentage of lipids contained in algal biomass. Biodiesel is produced from the microalgae by transesterfication of the lipids (Mata et al., 2010). Nonetheless, only the neutral lipids in the algal biomass can be converted into biodiesel. This leads to most biodiesel production to require some form of pretreatment to convert the negative charged lipids into neutral lipids. The biodiesel produced from this algal biomass has a high quality with several favorable characteristics such as high energy content, and a low viscosity to avoid gumming of the engine. Due to the biodiesel being of such high quality it allows the fuel to be directly combusted into the engine, not requiring to be mixed with other diesels. In general, biodiesel production generates crude glycerol as a byproduct, which can be used for several applications such as a feedstock for bacterial fermentation (Gong & Jiang, 2010).

Ethanol

In addition to lipids, many microalgal species can have a large content of carbohydrates. These carbohydrates are often in the forms of starches, cellulose, or hemicelluloses. Therefore, technologies based on using terrestrial lignocellulosic biomass for fuel production can be adapted

for the use of microalgae. However, algal cells are very miniscule making the breaking of the cell to be done by mechanical grinding and then dissolving the carbohydrates with solvent (Olguin, 2003). Once the carbohydrates are accessed, either saccharification with enzymes or bacterial fermentation can be used for producing the ethanol, which is then distilled to be concentrated into the final product. The production of ethanol via starch is a rather straightforward process while the production from cellulose and hemicelluloses requires further steps. Cellulose is a very rigid and difficult molecule to break apart. Nonetheless, new technology has made this viable with the ability to produce ethanol from lignocellulosic biomass (Harun et al., 2009).

Certain companies in the algal industry have recognized this potential and applied it to genetically modifying algal species to directly produce ethanol. For example, Algenol has developed a strain to successfully produce ethanol with no fermentation. This technique has the ability to be a frontrunner for replacing petroleum based fuels and becoming and economically based option. The company claims that their genetically modified species is able to produce 9,000 gallons of ethanol/acre of land. They continue further by claiming that the production cost would only be 0.85 cents per gallon.

Bio-oil, biochar, and syngas

In addition to ethanol production from algal cellulose or starch, algal biomass can also be used as a feedstock for producing bio-oil, biochar, and syngas through thermochemical processes such as pyrolysis, gasification and hydrothermal liquefaction. Pyrolysis is essentially the process of burning dried biomass at a temperature of 400-500 C with no oxygen present. The products from this process are normally bio-oil and biochar. Bio-oil has great potential to become a drop-

in fuel as it can be upgraded through processing to become various transportation fuels. Biochar is often times recycled back to agricultural fields as it is considered great at carbon sequestration. The process of gasification is similar to that of pyrolysis but is run at higher temperatures and allows a limited amount of oxygen. This limited amount of oxygen results in the production of large amounts of CO, which can then be combined with H_2 and produce several products. These products are produced through various reactions of combustion, water shift, and catalysis to produce thermal power, hydrogen fuel, and various fuels and chemicals (Brown, 2011). The last and newest technology used to produce energy from biomass is hydrothermal liquefaction. The largest benefit of using hydrothermal liquefaction is that the biomass does not have to be dried, reducing the cost going into the system. It is the process of using moist biomass, such as algal paste, to produce bio-crude. This is essentially produced with high pressure and use of various solvents at a lower temperature compared to gasification and pyrolysis.

Animal feed

Biofuels are not the only product that microalgae can be processed into. Many industrial companies are beginning to utilize algae as a potential animal feed substitute. In fact, the year of 2007 reported that almost 30% of the cultivated algae in the United States were used for the feed industry (Becker, 2007). In particular, microalgae are attractive in the potential to replace percentages of fishmeal and fish oil used in aquaculture The price of these two commodities has increased dramatically over recent years due to over fishing. Algae have the potential to decrease the dependence of fishmeal and fish oil from fisheries by the ability to produce omega-3 fatty acids, as well as high protein and lipid contents. In addition to decreasing the amount of over fishing, algae can serve as an optimum feed for various aquacultures such as mollusks,

crustaceans, and of course fish (Wang, 2010). Another benefit of microalgae for the use of animal feed is that it can be coupled with the biodiesel or ethanol industry. In these industries they are only removing one aspect of the microalgae, either lipids or carbohydrates. The residual biomass left is still high in protein and other attractive components of the cell. Due to this, the same algal cell can be able to facilitate various applications such as providing lipids for biodiesel and in turn providing proteins to potentially replace fishmeal (Barclay et al., 1985).

Nutraceuticals

Like any natural product, algae have been researched for various nutraceuticals that can be produced. Today the supplement from algae that receives the most attention is omega-3 fatty acids. This is due to omega-3's being beneficial to a large percentage of the population that are overweight and have poor health. Omega-3 fatty acids are proven to increase both heart and brain function which can lead to a better quality of life. Among various omega-3 fatty acids, docosahexaenoic acid (DHA) being produced commercially by DSM is being recognized as essential for fetal and infant brain development and therefore is a great nutraceutical success. Other compounds that are being researched for nutraceuticals would include certain carotenoids that have antioxidant properties. Many of these carotenoids are found exclusively in microalgae, making the research and production of these species necessary for these specialty products.

Wastewater treatment & fertilizer

Some of the newest technologies to be developed in the algal industry are to integrate the algae as a wastewater treatment. Algae have the ability to take nitrogen and various heavy metals out of its surrounding environment to facilitate growth (Luz & Yoav, 2010). This ability

has lead research to focus on technologies that enable microalgae to survive in a wastewater setting while sequestering nitrogen and heavy metals. This is of great importance as the recycling of wastewater has become an increasing issue that needs to be resolved. Technologies such as the RAB system mentioned previously have demonstrated promise in being able to remove the problematic compounds found in wastewater. Unlike other nitrogen or heavy metal removal systems, the algae can be recycled back to the environment as fertilizer (Rodgers et al., 1979). This algal fertilizer would then decrease the amount of fertilizer produced annually for agriculture, being an economically viable option. In addition to the fertilizers being produced as a by-product from wastewater, algal biomass can also be used for producing biodiesel, ethanol, and other applications mentioned previously.

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CHAPTER 3. GRADUATE RESEARCH: PART 1

Abstract

Microalgae growth highly depends on light intensity as well as wavelength spectrum. Traditional studies on the algal growth and light spectrum mainly used artificial lights emitting specific wavelength ranges. In this work a thin film nano-material is used to filter light to selectively transmit certain wavelengths from natural sunlight to algal culture. A red light filter (620-710 nm) and blue filter (450-495 nm and 620-710 nm) were evaluated. Algae were grown in shake flasks, flat panel reactors, and Rotating Algal Biofilm (RAB) system. The finding shows that the light filters significantly ($P<0.05$) improved biomass yield (13-34%) in flat panel reactors and biomass productivity (70-100%) in RAB system, depending on the growth mode and lighter filters, while they did not improve algal growth in flask cultures. The research shows a great potential of using light filters to improve microalgal growth.

Introduction

Microalgae represent a group of photosynthetic organisms that are capable of growing rapidly while only requiring light and basic nutrients. As a promising biomass feedstock, microalgae can be used for producing a variety of bio-based fuels, feeds, and chemicals. The most common fuel produced from microalgae is biodiesel produced from transesterfication of algal lipid. Other types of algal derived biofuels include alcohol (Wang et al., 2011), biogas (Gunaseelan, 1997), and bio-oil (Yang et al., 2004). In addition to various types of fuels, algal biomass can also be used for producing fertilizers (Mulbry et al., 2008), aquacultural feeds (Duerr et al, 1998, Hemaiswarya et al, 2010) and nutraceuticals (Hudek, 2014).

In the mass production of microalgae, in either open ponds or closed photobioreactors, light limitation is a major bottleneck restricting cell growth. Due to the mutual shading caused by the algal cells, light penetration is reduced exponentially with depth (Lee, 1999). The poor light penetration often results in an ultra-low cell density. For example, algal cell density in open ponds can be as little as 0.5 g/L (0.05% dry weight or 99.95% moisture content), and 2-6 g/L (0.2-0.6% dry weight or 99.4-99.8% moisture content) in photobioreactors (National Algal Biofuels Technology Roadmap, 2010).

In addition to light intensity, the wavelength of the light source also plays an important role in the algal culture. Algal growth must rely on solar radiation to perform photosynthesis. However, natural sunlight is not optimized for algal cell growth due to the wide light spectra including ultra-violet (UV) and infrared red (IR) rays which can damage the cellular structure (Holzinger & Lutz, 2005). This damage is particularly severe when light intensity is strong; although, in some specific cases a strong light intensity and daylight length can increase production of specific compounds such as protein and β-carotene (Seyfabadi et al, 2010).

In general, specific wavelengths of visible light such as blue (420-470 nm) and red (650-680 nm) are considered beneficial to algal cells (Schulze et al., 2014). Blair et al., (2014) used bulbs emitting blue wavelength (475 nm) for the culture of *Chlorella vulgaris* and reported an improved biomass growth as compared to the red (650nm) and white artificial light. Shu et al., (2011) reported greater lipid content in *Chlorella sp*. illuminated with blue light emitting diodes (LED) compared to red LED. Wang et al., (2007) studied the growth of *Spirulina platensis* as a response to various LEDs, and found higher biomass productivity when grown under red light, (620-645 nm) compared to blue (460-475 nm) and white artificial light (380-760 nm). In the culture of the *Nannochloropsis*, blue LED (470 nm) resulted in better growth than the red (680 nm) and white artificial wavelengths (Das et al, 2011).

All these reports demonstrate that the use of appropriate wavelengths of light is an effective way to enhance algal growth and alter chemical compositions. The effects of wavelength also depend on the species tested. However, one important issue yet to be solved is how deliver the light with the desirable wavelength to the culture system in a cost-effective way. The common approaches as reported in previous research are to use the artificial light sources such as specific light bulbs or LEDs (Fu et al., 2012, Wang et al., 2007). However, natural sunlight is preferred in scalable systems because of the potential of reducing cost.

Our research team recently developed a unique filter using nano-scale coatings that can selectively transmit specific wavelengths from natural sunlight. The optical filter may be applied to a variety of substrates. For the purpose of this study, the filter was applied to plastic sheeting due to its versatile form factor. Despite these unique advantages of the filter, the material has not been used in algal cultivation system. The objective of this research is to evaluate the feasibility of using this specially designed sheet as light filter to deliver specific wavelengths of light to

microalgae culture systems. The effectiveness of enhancing cell growth and producing a specific chemical composition will then be evaluated.

Materials and Methods

Algal strain, medium, and subculture

The microalga *Chlorella vulgaris* (UTEX #236) was used. To prepare seed culture, the cells maintained in anoxic agar slants were transferred to 250-mL Erlenmeyer flasks containing 50 mL Bold's Basal Medium. The medium was autoclaved at 121 ^{o}C for 15 minutes prior to use. The flasks were placed on an orbital shaker set at 200 rpm and 25°C with continuous illumination (110–120 µmol s^{-1} m^{-2}). The cultures were grown for 7-10 days and then inoculated into various culture systems as defined in the later sections.

Optical light membrane filters

Two variations of light membrane filters (cryptolux and phytolux) were used in this work. The phytolux (red) filter is designed to allow the penetration of the wavelengths in the red spectrum (620-710 nm), while the cryptolux (blue) filter is designed to allow the penetration of the wavelengths in both the blue (450-495 nm) and red (620-710 nm) of the visible spectrum. The filters can reflect UV and infrared rays which causes cell damage due to oxidative stress (Malanga et al., 1997). A twin wall polycarbonate sheet allowing the transmission of all solar radiation wavelengths was used as the control.

Algal cultivation greenhouse facility

All the algal cultivation experiments were performed in a greenhouse facility located in Iowa State University Biocentury Research Farm in Boone, IA, USA. This greenhouse is made of corrugated transparent polycarbonate. Natural light was used exclusively throughout the project period. Various algal culture systems described below were used for testing the effect of light filters on the algal growth.

Algal Cultivation Systems

Flask cultures

The flask culture system was performed in 250-mL Erlenmeyer flasks with 100-mL working volume. The flasks were place on an orbital shaker rotating at 200 rpm. As show in Figure 1A, a box assembled with transparent corrugated polycarbonate panels (30 × 30 ×30 cm) was placed over each shaker to create a growth chamber. To create designated wavelength, one chamber was covered with phytolux (red) filter, the second chamber was covered with cryptolux (blue) filter, while the third chamber without any coverage of the light filter was used as a control. The chambers were ventilated (on March 28) by introducing the air from outer environment so the temperature inside the chamber can be maintained at the same level as the greenhouse. The algal culture was performed in three replicate flasks inside each chamber.

To monitor the temperature of the flask culture, an additional flask holding 100-mL of water was placed into the shaker, an Onset HOBO® temperature probe (Onset HOBO data logger, Bourne, MA) was inserted into the water of this flask to record temperature every hour (Figure 3-1A). The temperature of the chamber was reported as average daily temperature of the 24 records. To monitor the PAR (photosynthetic active radiation) of the growth chamber, an

Onset® S-LIA-M003 PAR probe (Onset HOBO data logger, Bourne, MA) was inserted inside the air of the chamber to record the PAR every hour (Figure 3-1A). The PAR of the chamber was reported as the average of the highest seven reading through the 24-hour cycle.

Flat panel photobioreactors

Algal cultures in photobioreactors were performed in three 90 × 90 × 8 cm flat panel photobioreactors made of plexiglass. The wall of two reactors was covered with a blue light filter and red light filter, respectively, while the third reactor without any filter-coverage was used as control. Each reactor had a working volume of 20-L with an inoculum of several 100-mL seeds prepared in the flask culture. The temperature of each reactor was monitored by inserting an Onset HOBO® temperature probe into the broth of the reactor. Similar to flask culture, the temperature was reported as average daily temperature. The algal culture in the reactors was repeated twice in a batch mode, and then switched to a continuous culture mode by removing 2-L of culture broth and replenishing 2-L of the fresh medium daily. The hydraulic retention time of the reactor culture was thus, set as 10 day with the pH maintained at approximately 6-8.

Rotating Algal Biofilm (RAB) culture system

An attached growth system called Rotating Algal Biofilm (RAB) system developed in our group (Gross et al., 2013; Gross and Wen, 2014) was also used in this work to test the effects of light filters on the algal attached growth. The detailed design of the lab-scale RAB has been described previously (Gross et al., 2013). The schematic diagram is shown in Figure 3-1B. As shown in the figure, a flexible cell attachment material (cotton duct) with a surface area of 450 cm^2 was stretched around the two shafts to form the vertical conveyor belt configuration. The

lower end of the belt was submerged in a nutrient-rich medium reservoir (1-L working volume) for nutrient supply, while the rest of the belt was exposed to the atmospheric conditions. The shafts were connected to a motor which rotated the belt between the liquid phase and gas phase. The liquid reservoir was inoculated with suspended seed; the belt material was then rotated for the suspended cells to attach. The suspended algal cells took 10 days to establish the biofilm in the attaching material. After harvesting this initial growth biofilm (by scraping), the residual cells remaining on the attaching material were allowed to re-grow for 5 days to re-establish the thick biofilm and then be re-harvested. This re-growth/harvest cycle was repeated for 4 times. The liquid reservoir was operated in a continuous mode by daily exchange of 250-mL medium with 4 day hydraulic retention time. Throughout the study the reservoir kept a maintained pH of 6-8. Similar to the flask culture set up (Section 2.4.1), two polycarbonate boxes respectively covered with a blue filter and red filters, and a third control box were placed over each RAB system.

Algal growth, chemical composition analyses, and statistical analysis

The cell growth in the flask cultures and flat panel photobioreactors was determined by measuring the optical density at 680 nm (OD_{680}) using a spectrometer and converting the OD_{680} values into biomass yield (cell dry weight concentration, g/L) through a correlation curve. The cell attached growth in the RAB system was evaluated by measuring the cell dry weight on a certain surface area of the attachment materials and converting the weight into biomass productivity ($g/m^2/day$).

The cells harvested from photobioreactors and RAB system was freeze-dried and then analyzed for ash and chemical composition content. Ash content was measured by heating the

biomass in a 550^{o}C furnace for 8 hours, with the remaining matter weighed. The chemical content was then reported based on ash-free biomass. Crude protein content was analyzed by measuring the total Kjedahl nitrogen (TKN) content of the sample and multiplying by a conversion factor of 5.95 (Lopez, 2010). Lipid content was determined according to the Bligh & Dyer method (Bligh & Dyer, 1959). Carbohydrate content was quantified by subtracting the protein and lipid from the ash-free biomass. Fatty acids were measured with GC-FID following protocols reported previously (Pyle et al, 2008; Liang et al, 2011). The statistical analysis in this study was conducted using JMP^{TM} 9 (SAS Institute Inc., Cary, NC).

Results and Discussion

Effects of light filters on the temperature and PAR variation of the growth chambers

To investigate the effect of light filters on the algal growth in flask culture systems, we assembled transparent chambers covered with different light filters, and placed the chamber over the shakers so each flask inside the chamber could be illuminated with the desired wavelength of light. Such a chamber, however, may create environmental conditions that are different from the open greenhouse environment. To provide an insight of the chamber environmental conditions as a function of the light filters, we monitored the temperature and the PAR level inside each chamber starting from early February until late August, 2014 (Figure 3-2).

As shown in Figure 3-2A, from February 3 till March 27, the temperature inside the three chambers had a significant difference ($P < 0.05$), with blue filter chamber having a higher temperature than red-filter chamber and control chamber. This difference was probably due to the heat generated by the difference spectrums of wavelengths. It has been reported that a higher

frequency of wavelengths such as blue light regime correlates with an increased temperature (Pierrehumbert, R., 2011).

On March 28 we introduced the ventilation to the growth chamber from the outer environment to eliminate the cell growth variance caused by the temperature difference. As shown in Figure 3-2A, the temperature inside each chamber from this day was maintained at the same level throughout all the remaining period of time. We also observed that the temperature of three growth chambers were higher than the greenhouse temperature before ventilation (March 27) but maintained at the same level as the greenhouse temperature when the ventilation happened (data not shown). Figure 3-2B shows the PAR of the three chambers from earlier February to later August. Throughout the entire time period tested, the control chamber tended to have a higher PAR than the blue- and red-filter chambers as the lighter filters block certain wavelengths of lights to pass through.

Effects of light filters on algal growth in flask cultures

Two repeat flask cultures were tested at two different periods. The first is from March 14 to 27 when the growth chambers were not ventilated (Figures 3-3A). The algal growth performance at this period was the response to both temperature and PAR effects resulting from the light filters used (Figures 3-3A and 3-3B). The second period of time for the flask culture was March 29 to April 12 when the ventilation was introduced to the growth chambers (Figure 3-3A). As the temperature variance among chambers was eliminated, the algal growth was therefore only reflecting the PAR effect caused by the different light filters (Figure 3-3B).

As shown in Figure 3-3C, no obvious benefit of the light filter for microalgae growth in flask cultures was observed. The algal growth among three chambers had no statistically

significant difference (P>0.05). Such a growth pattern results are probably due to the reflection of light by the glass wall of the flasks; indeed, once the desirable wavelength of light penetrated through the light filter, they will encounter the glass wall of the flask, which would then cause reflection. As a result, the "net" light entering the liquid culture inside the flask was weak, not enough to lead to a significant difference in cell growth.

We concluded that to increase the light filter effects it was necessary to test the algal growth in flat panel photobioreactors so the light penetrated through the light filter can directly enter the culture solution, without the "second layer" of reflection by the glass wall as found in the flask cultures.

Batch culture in flat panel reactors – effects of light filters on short term algal culture

The flat panel reactors covered with the blue and red-filters, as well as the control membrane were respectively tested for their performances of supporting algal growth. The batch culture was performed twice in the period of early-February to mid- March, 2014, with cells being maintained in flask culture, at regular illumination conditions, being used as seed. As shown in Figure 3-4A, the two batches of algal culture show a similar pattern, i.e., the blue filter resulted in a better performance than the red and the control (P<0.05); while the red and the control had a similar growth performance. The better growth performance of the blue-filter culture may be due to the two wavelength spectrum (450-495 nm and 620-710 nm) being used in the blue-filter while the red filter only had the one spectrum range (620-710); while the control reactor was illuminated with full range the visible spectrum containing ultra-violet (UV) and infrared red (IR) rays which were detrimental to the cell growth (Holzinger & Lutz, 2005). During operational period of time, the temperature of the greenhouse fluctuated; we therefore

monitored the temperature fluctuation of the liquid inside the reactors. As shown in Figure 3-4B, the temperature of the two batch cultures varied widely. However, the temperature of the three reactors was very similar at any given time, indicating the cell growth difference was the consequence of only wavelengths generated by the different light filters.

The chemical compositions of algal biomass obtained from different filtered cultures were also evaluated. As shown in Table 3-1, all three types of biomass displayed high but similar ash content. The high ash content may be due to the large amount of mineral salts used in the synthetic BBM medium. In terms of ash-free biomass based chemical compositions, the control and red-filter based culture had higher protein content than that of blue-filter culture, while the red-filer culture had the highest protein content. The lipid content showed no significant difference (P>0.05) amongst the light filters and control. In the culture of *Spirulina platensis* with a light bulb as a lighting source, Ravelonandro et al. (2008) also reported a higher protein content with the cells exposed to red light wavelength (650-680 nm) compared to those exposed to the blue light. The results obtained in this work using the natural sunlight agreed with previous research.

A more in depth composition characteristic was taken for fatty acid composition of the algal biomass. As seen on Table 3-1, the major fatty acids of the algal biomass are C16:0, C18:1 cis, C18:2, and C18:3. However, there is no statistically significant difference (P>0.05) of the fatty acid composition as well as total fatty acids among the different filter-based cultures.

It should be noted that the algal cultures in Figure 3-4 were performed in a short period (~10 days) of batch mode. Therefore, the effects of light filters on the algal culture as indicated in Figure 3-4 were based on a short term arrangements. The effect of light filters on the algal growth performance also needs to be evaluated in a long term basis, as algal cultures are often

operated in a continuous mode for a sustainable period of time. The long-term effects of the light filters on the algal growth were therefore further evaluated through a continuous algal culture.

Continuous culture in flat panel reactor – effects of light filters on long term algal culture

Continuous cultures of the three reactors were started in mid-March. As the algal cells had been grown in the correspondingly reactors for a period of time in order to build a certain biomass concentration, the algal cells at the time of starting the continuous culture had adapted to the corresponding light filter conditions. As shown in Figure 3-5A, during first 35 days of continuous culture (March 19 – April 23), the blue-filter culture showed a better growth performance than the red-filter culture in the initial 10 days; while this advantage disappeared after 10 days as the red-filter culture "caught up" with the blue-filter culture, resulting in a similar growth performance ($P>0.05$). However, during this first 35-day run, both the blue-filter and red-filter cultures out-grew the control culture ($P<0.05$).

The above results indicated that the long terms effect of light filters on the algal growth were different from the short-term effects. I.e., in the short term batch culture, the blue filter resulted in the best algal growth but the red-filter did not show the growth improvement; while in the long term continues culture, the red-filter gradually showed the beneficial effects on algal growth. Furthermore, we have observed that the seed cells in the short term (batch) culture were maintained in regular lighting conditions, while the cells in the long term (continuous) cultures started from the cells that had been adapted into the filter culture for a certain period of time. All these results indicate that the "history" of the cells may affect their responses to the light filters. To further examine this phenomenon, on April 23, we swapped the blue-filter and red-filter with each other's reactor to determine if a "shocking" effect existed by suddenly allowing or not

allowing certain passage of wavelengths. As shown in Figure 3-5A, both the red- and blue-filter culture still maintained a higher cell density than the control culture; while the algae grown under the red-filter culture appeared to have a greater cell density ($P<0.05$) compared to the blue-filter. For this period of time, the biomass yield of the three cultures steadily increased compared to the initial 35 days culture, probably due to the temperature increase during this period (Figure 3-5B).

The third experiment performed during the continuous culture is that on May 31, both red- and blue-filters were removed from the reactors; the purpose was to study response of certain-wavelength-adapted-cells to regular sunlight upon filters being removed. Figure 3-5A shows that the red-filter adapted cells continued a significantly higher cell density than that of the blue-filter-adapted cells and control cells ($P<0.05$), indicating once the cells adapted to the certain wavelength conditions, they can maintain the existing performance for quite a long time, even under the new solar environment.

During the entire continuous culture period, the temperatures of the three reactors were monitored. Although the temperatures fluctuated widely during the entire period of continuous operations, the three reactors were kept at a similar temperature (Figure 3-5B). This ensured that the cell growth performance, as shown in Figure 3-5A, was the true effect from the lighting condition, without interference from temperature difference.

Chemical compositions of the algal biomass were further analyzed for the weeks immediately before and after swapping blue- and red-filters, and the weeks immediately before and after removing filters. As shown in Table 3-2, at week before the filter-swapping, the control and two-filter based cultures had similar ash content. The protein, carbohydrate, and lipid contents of the ash-free biomass were also similar for the three cultures, without significant

difference (P>0.05). An overall similar trend was also observed for the week after filter swapping although the lipid content of the red- and blue-filter culture tended to be lower. Table 3-2 also shows that there is no significant difference (P>0.05) of the fatty acid composition for the algal biomass among different filter cultures before or after filters swapping.

Table 3-3 shows the chemical composition of the three cultures in the week immediately before and after the filters were removed from the reactors. Prior to the filter removal, the lipid content of the red-filter culture was lower (P<0.05) than that of the blue-filter and control cultures, while the other compositions of the three algal cultures were similar (P>0.05). When filters were removed from the reactors, the chemical compositions of the algal biomass did not exhibit a significant change (P>0.05).

Algal culture in the RAB system – effect of light filters on algal attached growth

The last algal culture used for evaluating the effects of light filters was the rotating algal biofilm (RAB) system, an attached growth system with a great potential of improving biomass productivity and easy biomass harvest (Gross et al., 2013; Gross & Wen, 2014). Similar to the flask culture system, the bench-scale RAB systems were also placed inside growth chambers respectively covered with a blue filter, red filter, and control membrane (Figure 3-1A). The trial was run during the months of June to August, the corresponding temperature and PAR levels for the RAB system can be found in Figure 3-2. As shown in Figure 3-6, the algal cells spent 10 days to establish the biofilm (initial growth) on the attachment materials. The blue-filter base RAB system generated more biomass than the red-filter culture and the control culture. After the initial harvesting, the residual cells were re-grown on the attached materials and re-harvested for every 5 days. For all the four re-growth cycles, the two filter-based cultures consistently

outperformed the control, with the blue filter performing best. The only exception is the 3rd re-growth where the red-filter outgrew the blue –filter culture, which was considered due to an experimental error.

Overall, the results in the Figure 3-6 show a clear benefit effect of the light filters on the algal attached growth. The reason may be that in the blue and red-filter culture systems, the undesirable UV and IR wavelength spectrum in the natural sunlight was blocked by the filters and cannot reach the algal cells. This allows the cell to remain functional under similar conditions of the control, but with less damage caused by UV and IR spectrum.

The chemical compositions of the algal biomass harvested from the RAB system were also analyzed. As shown in Table 3-4, the ash content and ash-free based protein, carbohydrate and lipid contents of the control, red- and blue-filter cultures were similar, without significant difference ($P<0.05$). Compared to the chemical composition of suspended algae in the flat panel reactors (Table 3-2 and 3-3), the composition of attach algal biomass in RAB systems seems to have a lower ash content; and the ash-free biomass contained less protein but more lipid (Table 3-4). Such a difference was probably caused by the different physiological status of the suspended algae vs. attached algae although the precise mechanisms need to be further explored.

Comparisons of algal growth of control, red-filter and blue-filter based cultivation systems

An overall comparison of algal growth performance of control, red-filter, and blue-filter cultures using different cultivation systems is given in Table 3-5. In the flask system, there is no statistically significant different ($P>0.05$) of biomass yield for the three cultures. While the beneficial effect of light filters in the flat panel reactors depended on the culture mode used. When the algae were grown in batch mode, the blue-filter resulted in a 31.9% increase of

biomass yield but the red-filter did not show such a benefit. When continuous culture mode was applied, both the red- and blue-filter resulted in a certain increment of biomass yield, ranging from 13.4% to 34.1%. When the cells were grown in the RAB system, the benefit of the light filters on the cell growth became more significant, as an overall more increment of biomass productivity (ranging from 70.1% to 150%) was observed for both red- and blue filters compared to corresponding light filter cultures in the flat panel reactors.

Table 3-5 clearly shows that the beneficial effects of the light filters on the algal growth depend on the specific cultivation systems. The reason may be due to the different light pathways caused by the cultivation systems, which resulted in the light intensity difference when reaching the cells. For example, in the flask culture systems, the natural sunlight has to penetrate through the filter, the chamber wall, and the flask glass wall before reaching the suspended cells, at which the light intensity difference among different filers mostly like has been toned down to an insignificant levels.

While in the flat panel reactors, the natural sunlight just needs to penetrate the filter and reactor wall to reach to the suspended cells. In the RAB system, because the cells are attached on the surface of the materials which directly exposed to the growth chamber environment, the light can therefore, directly deliver to the cells after penetrating the filter of the chamber walls, and thus cause much more difference of light intensity difference than the flat panel reactors and flask cultures.

It should be noted that the results shown in Table 3-5 are confined under the specific natural environmental conditions in Boone, Iowa, from February-August 2014. A more thorough evaluation of the light filter performance needs to be tested in more diverse geographical locations and the climate situations in order to get a conclusive conclusion of

whether and how much the light filters can improve the algal growth for each individual cultivation systems. Also, the mechanisms for the effect of light filters on the algal growth need to be further explored to confirm the above hypothesis.

Conclusion

This work reported the use of light-filters to selectively deliver desirable wavelengths of light from natural sunlight to the algal cultures. Among the three algal cultivation systems studied, the light filters significantly ($P<0.05$) improved biomass yield (13-34%) in flat panel reactors and biomass productivity (70-100%) in RAB system. The light filters did not improve algal growth in flask cultures probably due to the toning down of light intensity. Although the results are confined at our specific geographical locations and climate conditions, they do show a potential cost-effective way for improving the algal growth in a variety of algal cultivation systems.

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Table 3-1. Chemical composition of the algal biomass grown in batch culture of flat panel photobioreactors covered with different light filters.

	Control	Red-filter	Blue-filter
Ash (%DW)	25.6 ± 0.4	26.2 ± 0.1	25.39 ± 0.9
Chemical composition (% ash-free DW)			
Protein	51.8 ± 1.9	49.9 ± 1.5	43.6 ± 3.8
Carbohydrate	34.6 ± 6.7	44.9 ± 5.8	38.5 ± 7.2
Lipid	13.6 ± 2.3	11.1 ± 1.8	10.8 ± 1.2
Fatty acid (% TFA)			
16:0	22.4 ± 2.0	24.1 ± 2.0	23.1 ± 1.8
16:1	6.5 ± 3.2	7.4 ± 3.8	6.5 ± 3.1
18:1 cis	19.2 ± 7.5	20.0 ± 5.3	23.6 ± 4.1
18:1 trans	2.8 ± 1.1	3.1 ± 0.9	2.8 ± 1.5
18:2	16.5 ± 3.9	16.6 ± 3.6	16.4 ± 3.4
18:3	32.4 ± 10.8	28.9 ± 8.6	27.6 ± 6.8
TFA (% DW)	3.7 ± 0.5	3.6 ± 0.4	4.0 ± 0.9

Data are means of two batches of culture ± deviations

Table 3-2. Chemical composition of algal biomass in the continuous culture in flat panel photobioreactor in week immediately before and after swapping the red- and blue filters

	Week before filter swapping			Week after filter swapping		
	Control	**Red**	**Blue**	**Control**	**Red**	**Blue**
Ash (%DW)	19.4 ± 1.4	19.2 ± 1.1	20.0 ± 2.8	18.8 ± 0.8	17.31 ± 3.1	16.2 ± 0.6
Chemical composition (% ash-free DW)						
Protein	44.8 ± 1.7	46.9 ± 1.4	48.4 ± 3.5	44.7 ± 1	44.6 ± 0.5	44.9 ± 0.7
Carbohydrate	47.1 ± 6.2	45.6 ± 5.3	44 ± 6.7	46.5 ± 3.3	49.3 ± 5.4	48.9 ± 1.9
Lipid	8.1 ± 2.1	7.5 ± 1.6	7.6 ± 1.1	8.8 ± 0.9	6.1 ± 0.5	6.2 ± 1.1
Fatty Acid (% TFA)						
16:0	30.2 ± 0.2	28.7 ± 0.3	28.9 ± 0.6	27.9 ± 0.4	27.4 ± 1.0	31.2 ± 4.4
16:1	20.7 ± 0.6	20.1 ± 0.3	19.8 ± 0.5	21.5 ± 0.8	19.8 ± 0.4	22.4 ± 4.4
18:1 cis	5.5 ± 0.4	6.1 ± 0.2	6.1 ± 0.6	6.1 ± 0.8	7.3 ± 0.4	4.8 ± 2.9
18:1 trans	1.7 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	2.0 ± 0.2	2.0 ± 0.1	1.7 ±0.3
18:2	25.9 ± 1.1	25.7 ± 0.8	25.7 ±1.1	27.4 ± 0.9	25.3 ± 1.1	19.7 ± 9.8
18:3	15.9 ± 1.3	17.5 ± 1.2	17.6 ± 0.9	15.0 ± 1.1	18.3 ± 0.7	20.2 ± 4.2
TFA (%DW)	2.6 ± 0.4	2.6 ± 0.08	2.6 ± 0.09	2.6 ± 0.1	2.2 ± 0.4	2.2 ± 0.3

Data are means of three consecutive sample analyses during that week ± standard deviations

Table 3-3. Chemical composition of algal biomass in the continuous culture in flat panel photobioreactor in week immediately before and after removal of the red- and blue filters.

	Week before filter removal			Week after filter removal		
	Control	**Red**	**Blue**	**Control**	**Red**	**Blue**
Ash (%DW)	21.4 ± 1.6	19.1 ± 0.8	19 ± 0.8	23.6 ± 0.4	19.1 ± 1.2	21.6 ± 0.8
Chemical composition (% ash-free DW)						
Protein	50.8 ± 0.9	45.1 ± 2.1	48.6 ± 1.2	48.8 ± 1.5	44.9± 1.7	49 ± 1.0
Carbohydrate	44.7 ± 5.9	48.6 ± 4.2	45.5 ± 5.3	44 ± 2.4	46.7 ± 5.8	43.7 ± 4.1
Lipid	8.3 ± 2.2	6.2 ± 1.6	9.5 ± 1.1	7.1 ± 0.9	8.4 ± 0.5	7.2 ± 1.1
Fatty Acid (% TFA)						
16:0	31.3 ± 2.2	28.4 ± 0.7	29.2 ± 1.5	30.4 ± 1.9	27.6 ± 0.8	29.5 ± 1.8
16:1	19.8 ± 1.0	20.0 ± 0.8	20.2 ± 0.7	21.2 ± 0.3	20.4 ± 0.3	21.0 ± 1.1
18:1 cis	5.0 ± 0.5	6.0 ± 1.0	5.3 ± 0.6	6.8 ± 1.8	9.1 ± 0.9	6.6 ± 0.1
18:1 trans	2.1 ± 1.1	2.8 ± 0.7	2.4 ± 0.5	4.0 ± 0.1	4.3 ± 0.2	3.8 ± 0.4
18:2	27.5 ± 3.4	29.5 ± 2.8	29.1 ± 1.2	29.4 ± 1.2	30.6 ± 0.9	31.5 ± 1.4
18:3	13.9 ± 2.6	12.6 ± 3.6	13.4 ± 3.9	8.1 ± 1.4	8.0 ± 1.0	7.6 ± 0.9
TFA (%DW)	2.4 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.4 ± 1.2	2.6 ± .07	2.4 ± .04

Data are means of three consecutive sample analyses during that week ± standard deviations

Table 3-4. Chemical composition of the algal biomass from four consecutive re-growth cycles in the RAB system covered with control chamber, red-filter chamber, and blue-filter chamber.

	Control	Red	Blue
Ash (%DW)	18.1 ± 0.05	15.6 ± 0.05	17.3 ± 0.03
Chemical composition (% ash-free DW)			
Protein	34.9 ± 0.4	38.5 ± 0.5	34.6 ± 0.4
Carbohydrate	49.7 ± 1.8	44.4 ± 1.5	49.5 ± 1.8
Lipid	15.4 ± 1.4	17.1 ± 1.0	15.9 ± 1.4
Fatty acid (% TFA)			
16:0	28.0 ± 3.0	25.8 ± 2.8	29.0 ± 4.6
16:1	5.5 ± 3.2	8.6 ± 0.1	7.4 ± 5.4
18:1 cis	20.5 ± 1.1	20.8 ± 5.6	27.4 ± 8.8
18:1 trans	5.8 ± 1.7	10.5 ± 4.5	5.8 ± 4.0
18:2	13.6 ± 1.8	11.9 ± 0.2	10.8 ± 1.4
18:3	26.7 ± 7.0	22.4 ± 4.4	19.5 ± 2.5
TFA (% DW)	1.5 ± 0.5	1.9 ± 1.2	1.8 ± 0.3

Data are means of four consecutive re-growth sample analyses ± standard deviations

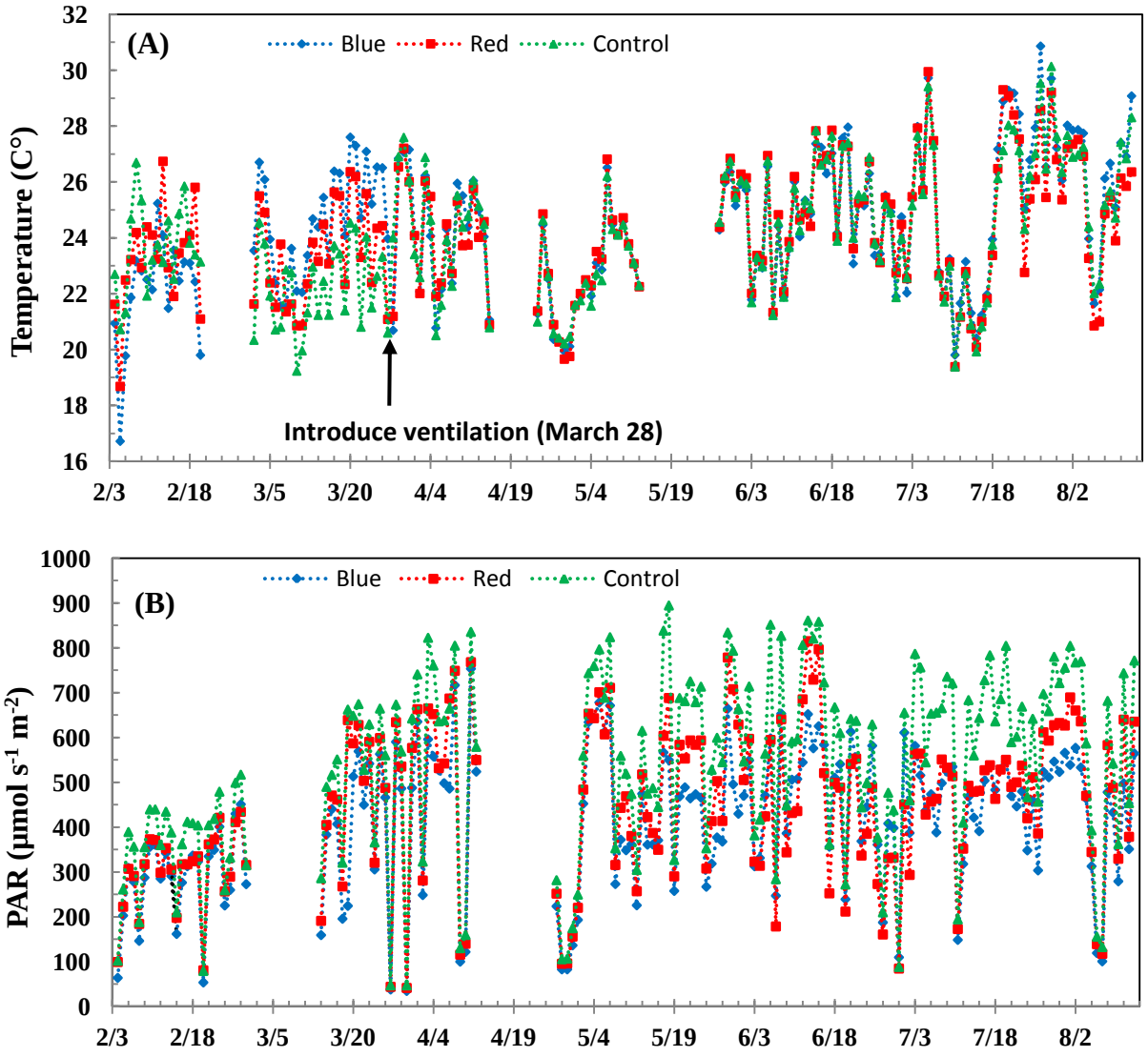

Figure 3-1. Long-term monitoring of **(A)** average daily temperature, and **(B)** PAR of the growth chambers covered with blue- and red-filter and the control chambers. The data missing in the Figure was due to the malfunctions of the probes.

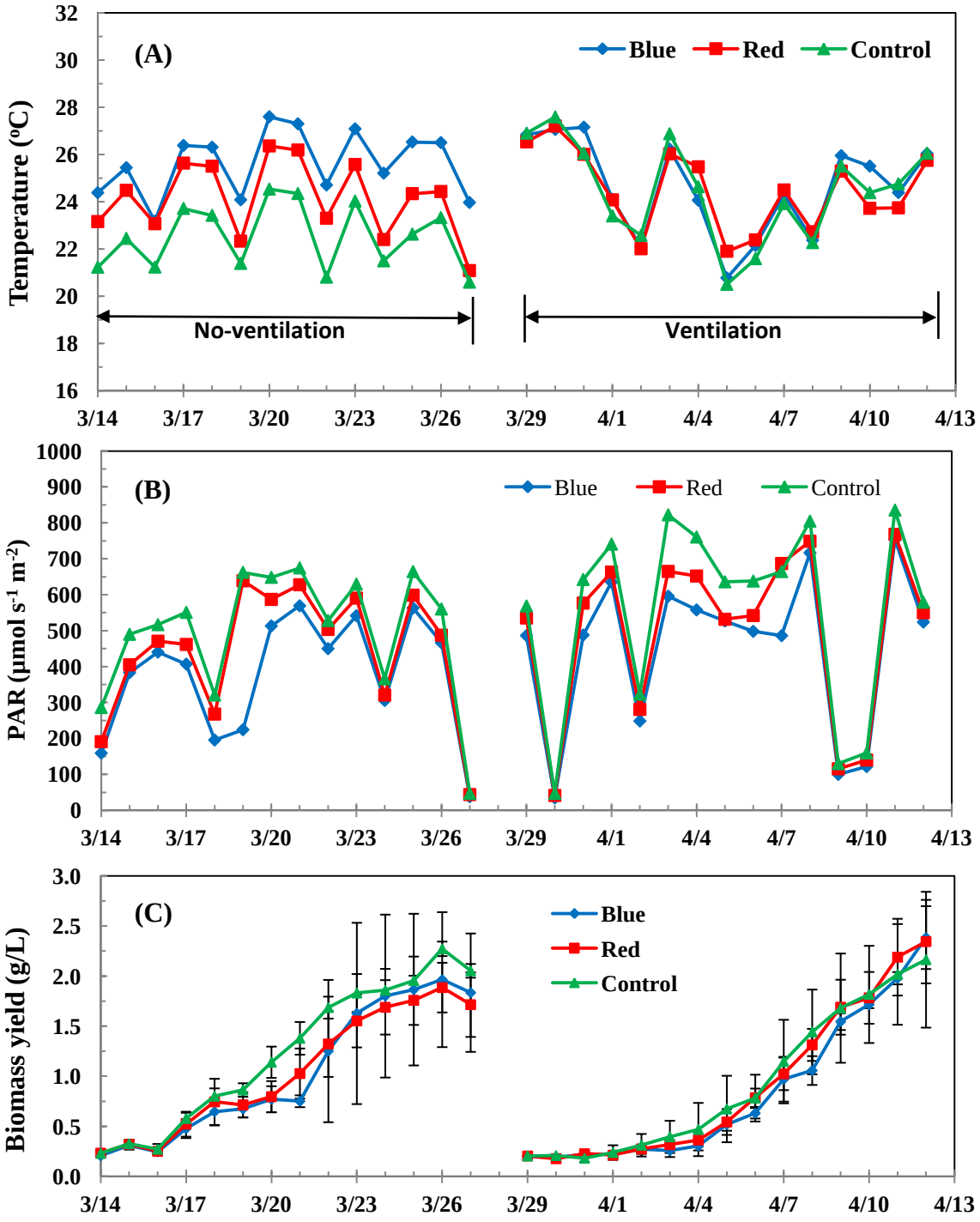

Figure 3-2. Algal growth in flasks under the growth chambers covered with blue- and red-filter and the control chamber. **(A)** daily average temperature; **(B)** PAR of the chambers; and **(C)** Biomass yield (data are means of three replicates and error bars show standard deviations).

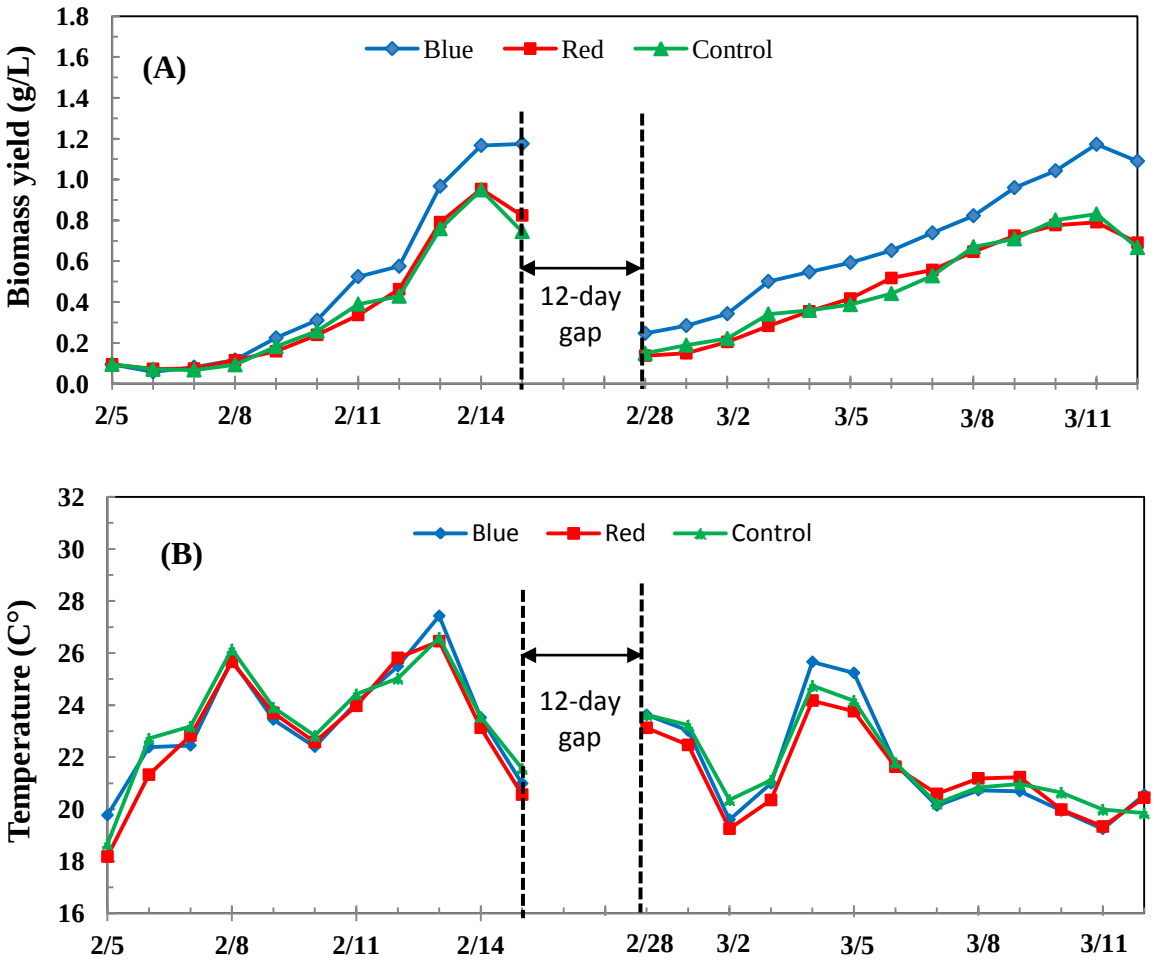

Figure 3-3. Batch algal culture in flat panel photobioreactors covered with blue-filter, red-filter, and control reactor. **(A)** Biomass yield; **(B)** average daily temperature.

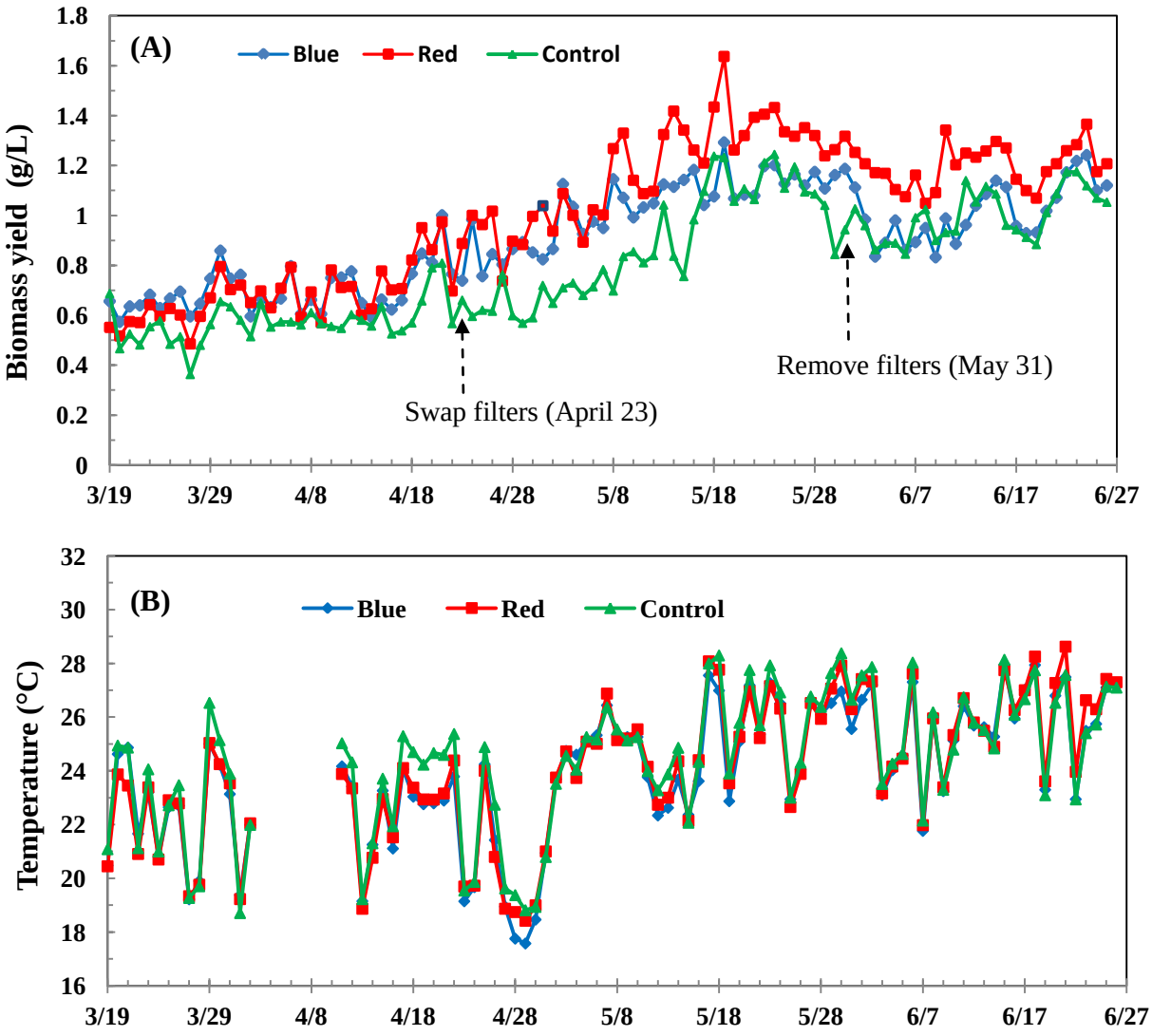

Figure 3-4. Continuous algal culture in flat panel photobioreactors covered with blue-filter, red-filter, and control reactor. **(A)** Biomass yield; **(B)** average daily temperature. **Notes for Figure A**: The red- and blue symbols for the period of April 23-May 31 represent the cultures respectively covered with the red- and blue filters during that period of time (they were covered with each the other filter previously). The red- and blue symbols for the period of May 31-June 27 represent the cultures previously covered with the red- and blue-filters. **Note for Figure B**: The data missing was due to the malfunctions of the probes.

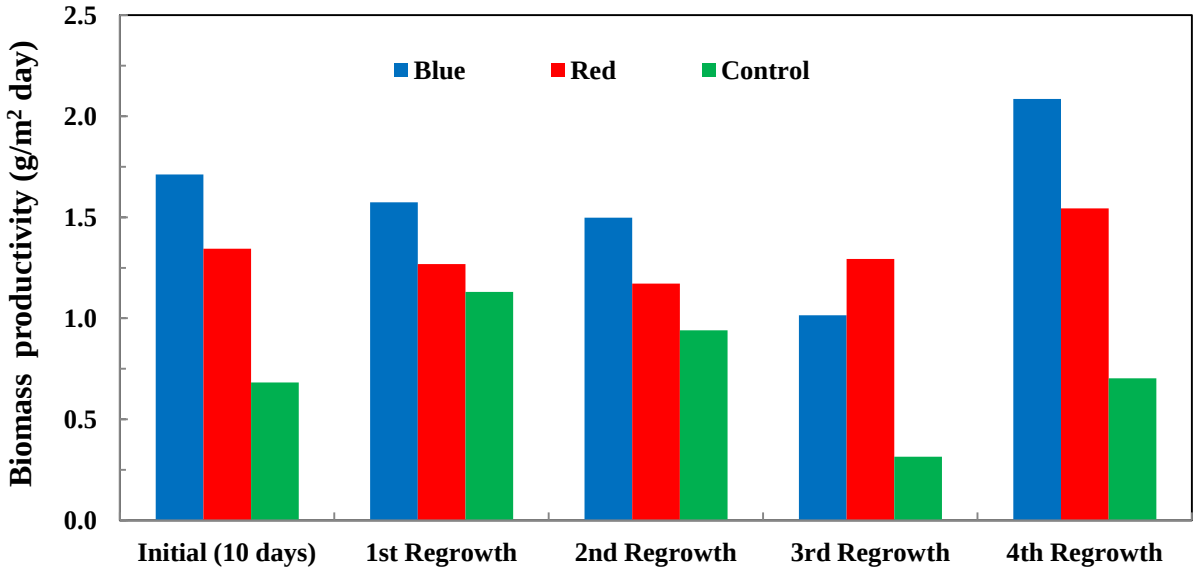

Figure 3-5. Biomass productivity of the RAB systems covered with blue-filter chambers, red-filter chambers, and control chamber.

CHAPTER 4. GRADUATE RESEARCH: PART 2

Abstract

The focus of this study was to utilize a bio-renewable material that can facilitate algal growth and attachment. Recent algal biofilm studies have reported that the degradable cotton sheet and petroleum based plastics can be used as an attachment. The goal of this research was to develop a bio-degradable substrate to be used for microalgal attachment that provided better growth performance and reasonable longevity. Different bio-renewable materials were examined for microalgal attached growth, including soy protein isolate, corn zein protein, sugarcane bagasse, and rice hulls. Poly-lactic acid was added to these feedstocks to increase the integrity of the attachment materials. The highest productivity was obtained in soy protein isolate and the 75/25% soy/poly-lactic acid mixture. These productivities were 273% and 194% higher than the control suspension based growth system, respectively. These results show that the attached algal growth with different materials has the potential to improve the production of algae and microalgal derived products. Further research is required for scaling up this process to a large scale operation.

Introduction

Microalgae have the potential to become a substitute for the production of commodities such as biofuels and animal feed. This is due to microalgae having a fast growth rate while only requiring basic nutrients and light. Therefore allowing algae to produce several biofuel derivatives such as biodiesel (Mata et al., 2010), bio-oil (Yang et al., 2004), syngas (Brown, 2011), and ethanol (Olguin, 2003). Other potential non feed products include animal feed (Duerr

et al, 1998, Hemaiswarya et al, 2010), nutraceuticals (Hudek, 2014), and bioplastics (Hempel et al., 2011). Nevertheless, the mass production of algae to produce these end products is limited due to several key factors such as high harvest costs, low productivities and foreign microbial contamination.

Attachment based cultivation systems have been developed to address high harvest costs and low productivities. The properties of the attachment materials have a large influence on the attached growth performance (Ozkan & Berberoglu, 2013). In general, the mechanisms for cells to attach on the surface of supporting materials (but not limited to) are hydrophobic interactions between the materials and the cells (Palmer et al., 2007), the liquid contact with the substrate surface (Irving & Allen, 2011), and the surface charge found on the biofilm (Christianson & Sims, 2012).

A recent study of an algal biofilm system showed that biofilm systems can have a 300% higher productivity than a standard raceway pond (Gross and Wen, 2014). However, the attachment materials used for biofilm research are either easily degradable, such as cotton (Gross et al., 2013) or are made of non-renewable petroleum based plastics (Przytocka-Jusiak et al., 1984). In order for biofilm technology to succeed at an economic scale and to be environmental friendly, it is necessary to optimize the attachment materials that can provide better algal attachment, and more importantly, degrade naturally while having sufficient longevity. The aim of this research is to explore several renewable materials for cell attachment and promoted microalgal growth. In particular, the low cost corn and soy meal based sheet was tested so that these less expensive agricultural byproducts can be converted into microalgal attached growth substrates, and possibly provide a certain nutrient for the algae. Eventually, this approach can

lead to an environmental friendly, renewable substitute for the production of microalgae and products such as biofuels and other industrially important chemicals.

Materials and Methods

Algal strain, medium, and subculture

The microalgae *Chlorella vulgaris* (UTEX #236) and *Scenedesmus dimorphus* (UTEX 1237) were used. To prepare seed culture, the cells were maintained in anoxic agar slants and then transferred to 250-mL Erlenmeyer flasks containing 50 mL Bold's Basal Medium. The medium was autoclaved at 121^{o}C for 15 minutes prior to use. The flasks were placed on a rotatory shaker set at 150 rpm and 25°C with continuous illumination (110–120 µmol s^{-1} m^{-2}). The cultures were grown for 7-10 days and then inoculated into the attached growth systems.

Bio-renewable materials

The attachment materials were made up of soy protein isolate, corn zein protein, sugarcane bagasse, and rice hulls. The soy protein and corn zein protein based sheets were mixtures composed of specific ingredients in order to be made into attachment materials. Soy protein based materials contained soy protein, soy flour, and phthalic anhydride. The corn zein protein based sheets were made up of zein protein, PEG, and ethanol. The materials were homogenized in the liquid phase and then pressed into the attachment sheets to be cooled and hardened. The materials had a surface area of 25.8 cm^2 and were then placed and sealed in bottom of the designated compartments. After completion of the first experiment, the second trial used soy protein isolate homogenized with poly-lactic acid (PLA) to make a 75/25% soy protein isolate material. This resulted in a more stable material, allowing no deterioration to be

seen during mechanical harvest. In addition to greater stability during harvest, PLA has also been shown to increase rigidity when submerged in liquid (Jamshidian et al., 2010).

Attached growth system

The attached algal culture system was a rectangle chamber made of clear plexiglass. The chamber was divided into 14 equally spaced compartments (7 cm width × 8 cm length × 7cm depth) (Figure 4-1). The compartments were individually sealed to prevent leaking. Twelve of those 14 compartments accommodated the attachment materials, with the remaining two compartments serving as a control for suspended algal growth. The attachment materials were secured to the bottom of each compartment to ensure consistency.

To perform the cell growth, the suspended cultures in the flasks were used to inoculate to each compartment at an inoculation ratio around 20%. The liquid volume of the each compartment was 250mL. Then initial suspended cell concentration in each compartment was 0.31 g/L. The growth chamber was then placed in a rocker shaker at 25°C with continuous illumination (110–120 μmol s^{-1} m^{-2}). The rocker shaker was set at 60 cycles per minutes. The motions of the liquid inside the compartment are illustrated in Figure 4-2. As shown in the figure, the inoculated suspended cells in the compartment were moved back and forth over the surface of material. This motion allows for the attached algal cells to alternatively receive the light illumination and nutrients. Gas exchange can also be increased due to the motion. Therefore, the algal attached growth was improved in this system.

Algal growth metrics and statistical analysis

The attached cell growth in the compartments was evaluated based on the cell dry weight per unit surface area of the attachment materials per unit of time, i.e., biomass productivity (unit: $g/m^2/day$). When harvested, the optical density (OD) of the suspended culture in each chamber (including the control chamber) was determined. To measure the dry weight of biofilm attached on the attachment material, the liquid from the compartment was removed; then, the attachment material was removed from the compartment for scrapping off the attached algal biomass. Upon completion of harvest, the attachment material was placed back to the compartment, and the each compartment was replenished with 250 mL of fresh medium for the next cycle of re-growth. During experimentation the growth chamber was covered with a lid to avoid water evaporation. Gas in the headspace of the chamber was exchanged through an inlet and outlet line to allow sufficient gas transfer.

The suspended cells in the compartments were also monitored by measuring the optical density at 680 nm (OD_{680}) using a spectrophotometer. The cell dry weight was then determined by converting the OD_{680} values into biomass yield (cell dry weight concentration, g/L) through a correlation curve. During the experiment, each experiment setting was replicated in three replicates. The statistical analysis in this study was conducted using JMP™ 9 (SAS Institute Inc., Cary, NC).

Results and Discussion

Evaluation of attachment on different bio-renewable materials

To investigate the attachment of the different types of bio-renewable materials a simple rocker test was performed with the systems shown in Figures 4-1 and 4-2. The microalgae

Chlorella vulgaris and *Scenedesmus dimorphus* were used in the test. After 7 days of incubation, the cell growth in suspension, on the attachment materials, and the combined growth in each compartment was evaluated. As shown in Table 4-1, *Scenedesmus dimorphus* showed a good attached growth with the use of the soy protein sheet, but poor attached growth when the corn zein protein sheet was used (Table 4-1). *Chlorella vulgaris* showed growth in both soy protein and corn zein sheets. Collectively, the soy sheet displayed an increase in biomass yield for both *Chlorella* (4.9 g/m^2/day) and *Scenedesmus* (3.4 g/m^2/day) It should be noted that the sugarcane bagasse and rice hull attachment materials resulted in no significant growth on the biofilm surface (data not shown). In the following study, soy protein sheets were used as the attachment material for the detail kinetic studies of the cell attachment.

Algal growth kinetics on 100% soy protein sheet

Given that the soy protein sheet showed the best growth performance, this material was further used to study the cell growth kinetics and durability for the attached algal growth. Figure 4-3 shows that the effects of harvest frequency (i.e., incubation time) on the biomass yield and productivity. As shown in Figure 4-3A, the yield of biomass attached on the surface increased steadily from day 3 to day 11, while algal cells showed the highest productivity at day 3 (Figure 4-3B). For the control, it was found that the biomass productivity remained constant during all harvest frequencies (Figure 4-3B).

During the experiment, it was found that the 100% soy sheet can only be sustained for a certain period of time. The sheet started to deteriorate after being incubated in the medium for 20 days. This deterioration was caused by both the physical damage due to scrapping of the biomass and the loss of sheet compounds due to dissolving into the liquid. Indeed, the

compounds found within the soy sheet are thought to have dissolved from being constantly soaked in liquid. Both of these two factors caused severe damage to the material (Figure 4-4A). These dissolved materials resulted in insufficient data for the experiment (Figure 4-3A). The harvest frequencies allowing triplicate results before becoming dissolved were the 3 and 5 day growth compartments. While the 7, 9, and 11 day growth compartments resulted in fewer harvests.

Chlorella vulgaris attachment on PLA fortified soy protein- sheet

To increase the durability of the soy protein materials, poly-lactic acid (PLA) was further integrated into the sheet at a 1:4 ratio (PLA vs soy protein). The addition of the hybrid poly-lactic acid (PLA) polymer has shown to increase the stiffness of unstable bio-materials in a mixture (Rasal et al., 2009). In this work, a mixture of soy protein isolate and PLA was blended and prepared into sheets containing a 75/25 ratio (soy protein/PLA). The PLA fortified sheet was tested for algal growth using the same approach as the 100% soy protein sheet having a starting inoculum of 0.297 grams/L. As shown in Figure 4-4B, the stability of the PLA integrated sheet was enhanced, resulting in increased longevity.

This comparison demonstrates that the 100% soy protein isolate sheet outperformed the PLA integrated sheet significantly ($p<0.05$) throughout all harvest frequencies. However, the PLA integrated materials lasted for the entire period of the experiment allowing for a greater collection of algal biomass, and a small amount of dissolved materials. The PLA integrated sheet was further tested for 45 consecutive days with the degree of deterioration examined. It was observed that the PLA integrated sheet did not display a breakdown of materials, displaying sufficient rigidity (Figure 4-4B).

Figure 4-5 shows the comparison of the biomass productivity obtained from the 100% soy protein isolate and PLA/soy protein sheet. The 100% soy sheet resulted in a better growth performance than the 75/25 sheet, probably due to the 100% soy materials providing the algae with more nutrients than the 75/25% soy materials.

In the future, further research is needed for testing the various percentages of poly-lactic acid being integrated into the sheets. This would allow the microalgae to receive the greatest amount of nutrients while the attachment material displays adequate longevity. Other research may include the compositional analysis of the attached microalgae on the assorted soy protein/PLA sheets. As a result, insight to how the sheets may alter the chemical composition of algae may be seen. Further research will need to focus on the process scale up to implement the laboratory results obtained in this work.

Conclusion

This research effort utilized a bio-renewable soy protein isolate to enhance and facilitate microalgal growth via biofilm based cultivation. The two sheets of 100% soy protein and the integrated PLA sheet both outperformed the control compartment by percentages of 273% and 194%, respectively. However, when comparing both sheets, the 75/25 soy protein isolate/PLA sheet proved to have increased biomass yield due to a greater longevity. Although the results are confined to the laboratory scale this research shows the potential to increase the biomass yield of the microalgal species *Chlorella vulgaris*.

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Table 4-1. Growth productivities of raw materials used for initial test of attachment (initial growth).

Species	Growth Style	Material	Dry Weight (g)	grams/square meter	grams/square meter/day
Scenedesmus	Attachment	Soy	0.491	30.42346085	3.042346085
Scenedesmus	Suspended	Soy	0.158	9.796019592	0.979601959
Scenedesmus	Total	Soy	0.648	40.21948044	4.021948044
Scenedesmus	Attachment	Zein	0	0	0
Scenedesmus	Suspended	Zein	0.412	25.57691115	2.557691115
Scenedesmus	Total	Zein	0.412	25.57691115	2.557691115
Chlorella	Attachment	Soy	0.619	38.40907682	3.840907682
Chlorella	Suspended	Soy	0.112	6.937813876	0.693701387
Chlorella	Total	Soy	0.731	45.34689069	4.534689069
Chlorella	Attachment	Zein	0.186	11.53202306	1.153202306
Chlorella	Suspended	Zein	0.450	27.93725587	2.793725587
Chlorella	Total	Zein	0.636	39.46927894	3.946927894

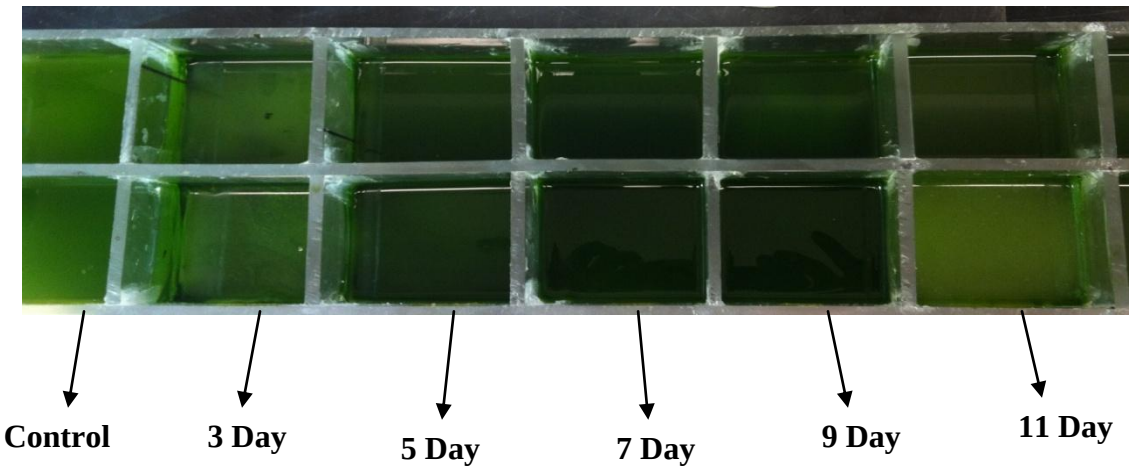

Figure 4-1: Growth chamber setup and harvest frequencies.

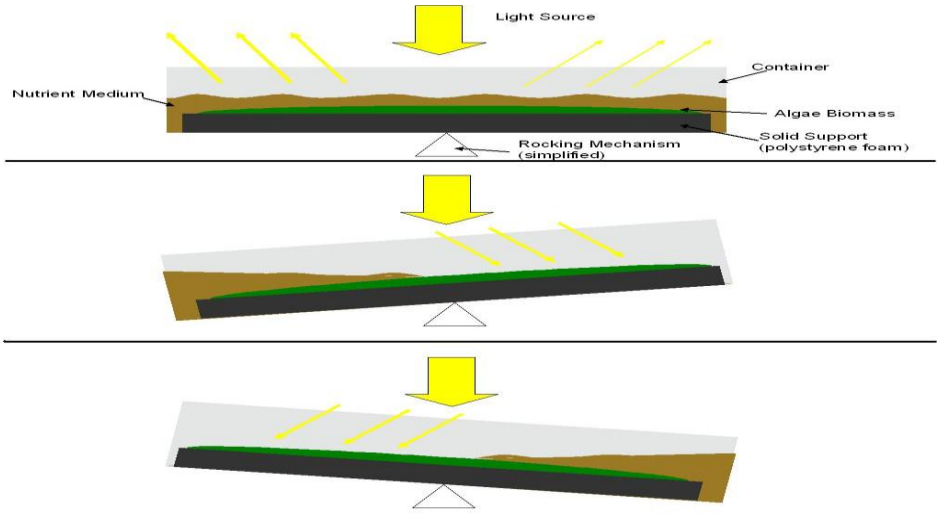

Figure 4-2. Experimental design for growth chamber.

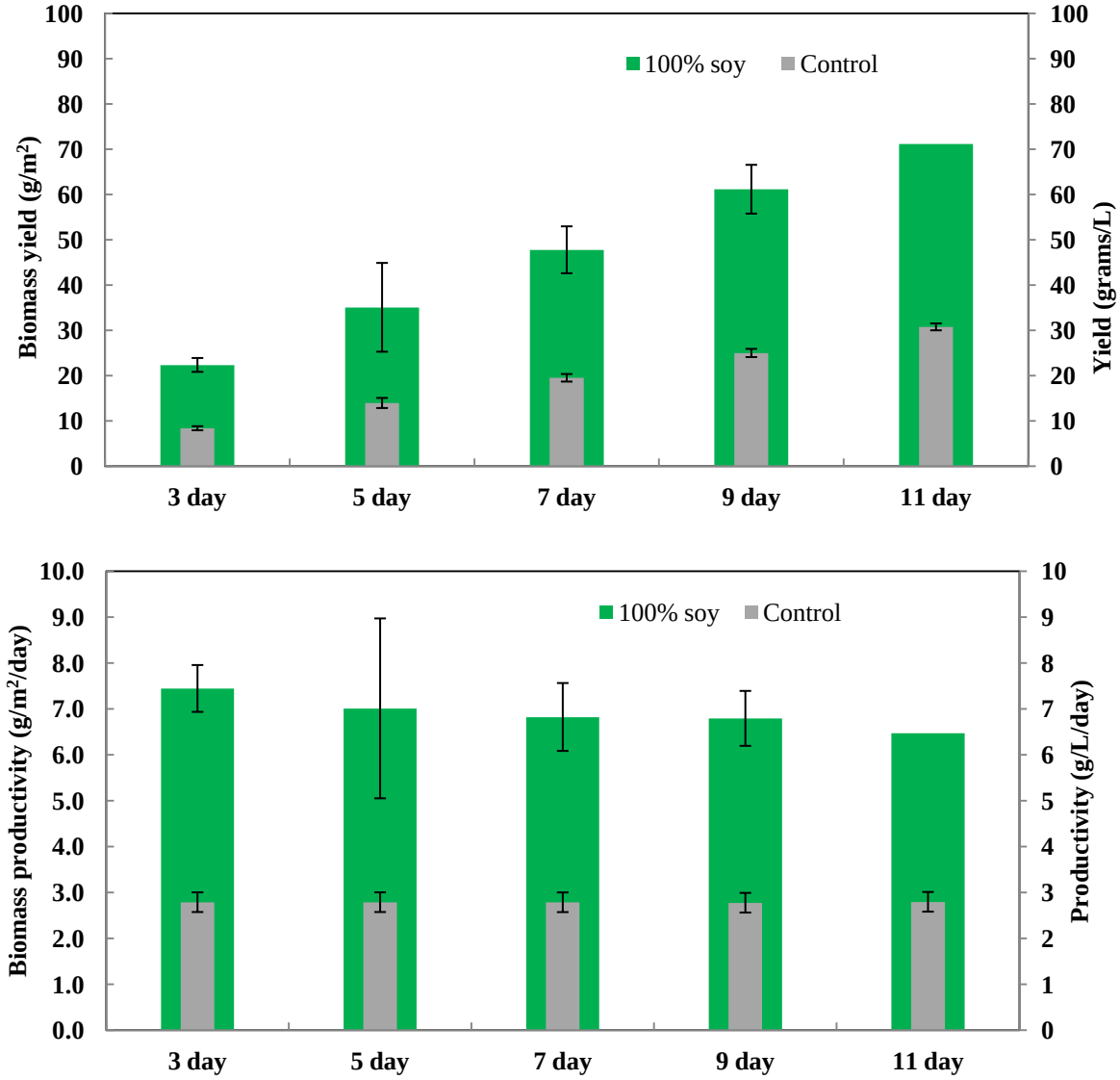

Figure 4-3. (A) Biomass yield of C. vulgaris attachment (g/m2/day) and control (g/L/day) with harvest frequencies**. (B)** Daily productivity of C. vulgaris attachment (g/m2/day) and control (g/L/day). The data include both initial growth and re-growth data.

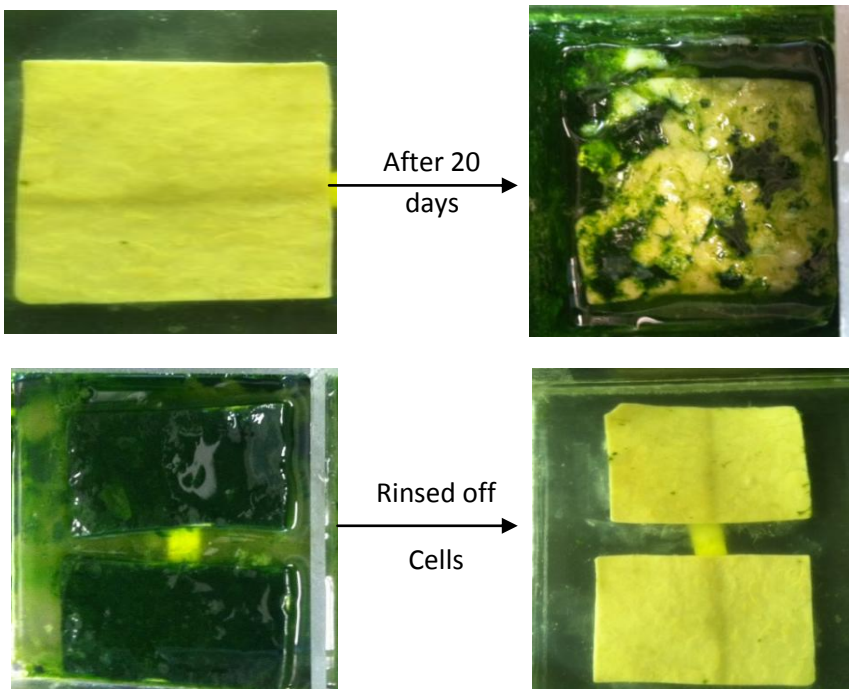

Figure 4-4: (A) Dissolving of 100% soy sheet after 20 days. **(B)** Enhanced structure of PLA integrated sheet after 20 days.

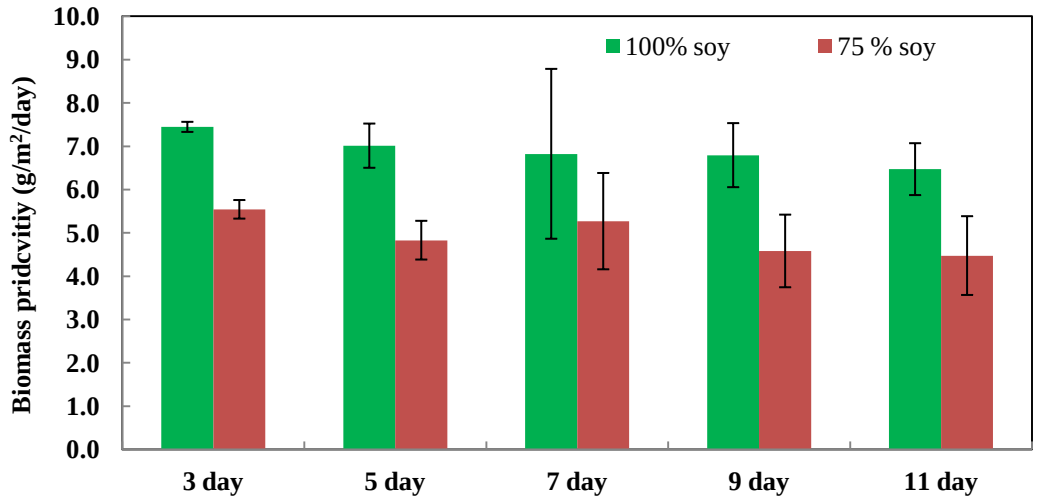

Figure 4-5: Comprehensive comparison of daily productivities between the two fabricated sheets ($g/m^2/day$).

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

The research presented in this thesis presents two approaches for enhancing the growth of the microalgae *Chlorella vulgaris*. This was accomplished with the use of optical light filters for selectively allowing natural sunlight to penetrate with certain wavelengths; and use bio-renewable materials for attached algal growth. With the increased productivity this research demonstrates the potential for microalgal production to be enlarged to the commercial scale is viable.

The research in chapter 3 showed that the biomass productivity of *Chlorella vulgaris* can be optimized with the use of optical light filters. This increase was seen in the two culture systems, i.e., the flat panel photobioreactor and rotating algal biofilm (RAB) system. The enhancement was mainly attributed to the selectivity of wavelengths allowed to penetrate and the reflectance of the harmful wavelengths of UV and IR. The filter in this study allowing both blue and red wavelengths to pass displayed an increase in biomass productivity with the batch flat panel photobioreactor and the RAB system. Additionally the filter allowing only red wavelengths showed an increased biomass performance with the use of the continuous flat panel photobioreactor. Together these two filters significantly outperformed the control (no filter) with the use of these two algal cultivation systems. There was no significant compositional difference seen amongst all of the filter conditions in addition to the various culture systems.

The research in chapter 4 demonstrated the use of bio-renewable materials to facilitate microalgal attached growth is viable. The growth on these bio-renewable biofilms was shown to have an increased attachment productivity of 234% (100% soy) and 193% (75:25% soy). This attachment productivity was compared to an identically sized compartment that only contained suspended algal cells having a yield of 2.78 g/L/day. Poly-lactic acid was used in the study to

provide rigidity to the soy protein mixture and allow prolonged growth. Although productivity was not as prominent as the 100% soy sheet, as shown previous, the PLA sheet still showed a significant biomass increase compared to the control. Further work for this research could be various percentages of PLA integrated into the soy protein mixture.

The research performed in this thesis displays an overall increase in biomass yield and biomass productivity with the use of optical light filters and bio-renewable substrates as attachment materials. The continued study on these objectives has the potential to unveil a greater understanding of microalgal production. In conclusion, these studies have shown the ability to be a feasible technology at a larger scale.