EFFECTS OF DIFFERENT LIGHT TREATMENTS ON A MICROBIAL MAT PHOTOBIOREACTOR

by

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This thesis is dedicated to the women in my family, my mother, Zelfije and my sisters, Leonora and Merita. They are the most kind-hearted and intelligent women who have given me unconditional love and support. I am dedicating this manuscript to them because I wish they had the same opportunities in life that I did. I feel so lucky to be able to pursue my education in Canada – a country where women have their place in society and people feel safe every day. I feel grateful and I never take it for granted.

My mother never had the opportunity to pursue higher education simply because she was a woman growing up in the 1960s in Kosovo. My sisters gave up on their dream of pursuing higher education due to displacement as refugees during the war in Kosovo in 1998. When they were my age, surviving was the only goal for one's future, higher education was simply inaccessible. Being the first female in my family with a university degree, let alone pursuing a Master's degree, makes me feel extremely fortunate and makes me wish that they had the same opportunities that I do. Earning this degree means as much to them as it does for me.

Do të dëshiroja ta dedikoj këtë temë të diplomës së masterit femrave në familjen time, nënës time Zelfijes dhe motërave të mija Leonores dhe Merites. Ato janë femërat më zemërmira që më kan përkrahur në gjdo aspect të jetës. Kisha pasur dëshirë qe edhe ato ti kishin pasur numdësitë në jetë që i pata unë. Ndihem aq me fat që e kam pasur mundësinë të shkollohem ne Kanada – një shtet ku femërat kan një rrol themelor në shoqëri dhe secili njeri ndihet i sigurt. Ndihem aq mornjohëse dhe nuk e asgjësoj.

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Abstract

One of the most exciting scientific innovations in fish feed and waste remediation systems has been the use of microbial mats for biomass production. This study aimed to improve productivity of biomass in an open microbial mat bioreactor. Biomass production was compared under fluorescent and light emitting diodes (LEDs) using 24 h photoperiod and similar radiance. Although the biomass production was similar under both treatments (1.8–2.0 g m^{-2} d^{-1}) the power consumption of LED light treatment was 58.5% lower compared to fluorescent light treatment. The biomass productivity decreased significantly (0.5 g m^{-2} d^{-1}) when pulsed LED lights were used. In addition, the effect of the different light regimes on microbial matt biodiversity was analyzed using phylogenetic analysis. In this open system, no significant differences were found at the class level. Overall, LED could potentially replace fluorescent lights in the production of microbial mats reducing annual costs significantly.

List of Abbreviations Used

16S Small subunit ribosomal RNA gene in prokaryotes

18S Small subunit ribosomal RNA gene in eukaryotes

24L:0D 24 hours light, 0 hours dark

bp Base pairs

DNA Deoxyribonucleic acid

DW Dry weight

OTU Operational taxonomic unit

PBR Photobioreactor

RNA Ribonucleic acid

LED Light Emitting Diodes

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Chapter 1 Introduction

1.1 Rationale and Significance

Microalgae are microscopic organisms that share the capacity to perform oxygenic photosynthesis. They inhabit fresh water and marine environments with a wide distribution of species and abundance around the world. In recent years, microalgae have received much attention due to their wide variety of uses including medicine, waste remediation, and one of the most popular applications – biodiesel production as a source of renewable energy (Carvalho, Silva, Baptista, & Malcata, 2011).

To make such endeavors possible, different types of industrial scale bioreactor designs have emerged, such as suspended cultures, biofilms and microbial mats. Suspended culture bioreactors generally grow one specific strain of interest. In contrast, biofilms and microbial mats grow attached to a surface and can grow as monoculture or a community of organisms, respectively. Despite the differences, all the bioreactors use light as a source of energy. Light is an essential component of photosynthetic microalgal growth. Optimizing light conditions can improve the rates of growth (Carvalho and colleagues, 2011) leading to an increase in productivity of the bioreactors.

1.2 Microbial Mats

Microbial mats are multi-layered ecosystems that contain diverse populations of prokaryotic organisms as well as eukaryotic organisms such as diatoms and other unicellular algae (Stolz J. F. 2000; Franks, & Stolz, 2009). Microbial mats are found in shallow sediments of fresh or marine waters where they form cohesive microbial communities. They are largely self-sustaining and are often seen as boundaries between

liquid and solid substrates (Gereaand colleagues,2012, Castenholz, 2009). The bacterial communities are spatially arranged in ways that facilitate metabolic cooperation (Costerton, 2009; Van Gemerden, H 1993). They may be only a few millimeters thin, known as ephemeral (seasonal) microbial mats, but can also acquire greater thickness and are called perennial (several years old) microbial mats. The physical appearance of a microbial mat is a leathery sheet and the upper layer is comprised of organisms that provide organic material through photosynthesis (Costerton, 2009). The layers underneath are comprised of various types of bacteria that aid in sulfide oxidation such as the Alphaproteobacteria *Rhodobacter*, sulfate-reducing bacteria such as the Deltaproteobacteria Myxoccocales (Couradeau and colleagues, 2011); and colorless sulfur bacteria and purple sulfur bacteria (van Gemerden, 1993). Phototrophs are the primary producers of the microbial mat system. They use light energy to fix inorganic carbon (CO_2) into organic carbon through photosynthesis. Photosynthesis is the process of using light energy to convert carbon dioxide and water to organic compounds and release oxygen – typically, the organic compound is glucose (Baumgartner, 2006; Castenholz, 2009). A photobioreactor (PBR) is a system that provides an artificial environment to grow phototrophic microorganisms such as algae (Kommaredy and Anderson, 2003). The microbial mat defined above is one that best describes the film growing in the PBR used in this study. The horizontal nature of the bioreactor allows for multiple layers of biomass growth.

Biofilms are generally comprised of single strain microorganisms, including bacteria and algae, attached on a growth surface (Ravelonandro, Ratianarivo, Joannis-Cassan, Isambert & Reherimandimby, 2008, and Xue, Su and Cong, 2011). The term

"biofilm" refers to mats composed of variable amounts of attached algae, bacteria, fungi, organic and inorganic detritus, as well as small protozoans and metazoans (Des Marais, 1990; Trevors, 2011; Wetzel, 1983). However, "microbial mat" is a more accurate term to refer to a community of different taxonomic groups of eukaryotes and prokaryotes that grow adhered to a surface. Microbial mats can be viewed as complex biofilms – no matter how complex they develop, by definition they begin as biofilms. Microbial mats play a fundamental role in aquatic food webs as a main resource of primary consumers (Wetzel, 1983). The composition of microbial mats is influenced by chemical changes (pH, salinity) and physical changes (temperature, light and water content) (Stal, van Gemerden, & Krumbein, 1985).

1.3 Biological Factors Influencing Microbial Mat Growth

Microbial mats are complex and the species that comprise the mat interact with one another. Tolker-Nelson and Molin (2000) suggested that in close-knit microbial mats, cell-to-cell signaling allow for the structural organization of the community in response to environmental factors such as temperature, pH and salinity. These factors are believed to predict the shape and phenotype of the microbial mat (Elias & Banin, 2012).

Cyanobacteria generally form the top layer of the microbial mat (Castenholz, 2009) and provide the structure and energy for other organisms (van Gemerden, 1993). They are photosynthetic prokaryotes known for their diversity and can grow under different climates and environments such as fresh water and sea water. Cyanobacteria can grow as plankton (free floating) or as biofilm. The filamentous nature of certain groups of

cyanobacteria allow for the creation of biofilms as the filaments intertwine with one another to form a mat.

Organisms have been reported to have different metabolic properties in single-species reactors compared to communities. Mechanisms of protection employed by organisms in microbial mats may involve the release of compounds that could paralyze or poison grazing protozoa since microbial mats continue to persist and dominate in even extreme environments (Castebholz, 2009; Costerton, 2009).

1.4 Physical Factors Affecting the Microbial Mat

Microbial mats thrive in a wide range of habitats that vary in temperature, salinity and pH. Their survival is a result of metabolic versatility and physical adaptability to different conditions (Franks & Stolz, 2009). Light, temperature and available nutrients are the most influential factors that control primary production (Canfield and Des Marais, 1993).

1.4.1 Quality of Light

The quality of light plays a big signaling role in plant development. However, less is known about the effects of quality of light on aquatic photosynthetic microorganisms (Hultberg, Jonsson, Bergstrand and Carlsson, 2014). Energy absorbed from light supports microalgal growth which can be used for many applications. Light intensity, frequency and type of light can directly affect the type and amount of lipid, protein and carbohydrate production in microalgal cells (Markou, 2014 and Simionato, Basso, Giacometti, & Morosinotto, 2013).

1.4.1.1 Light Intensity

The intensity of light, also known as radiance, is expressed as the number of photons passing through a unit area per unit time, namely the photon flux (Katsuda, Lababpour, Shimahara, & Katoh, 2004). This is normally measured using μ mol m^{-2} s^{-1} which is an SI unit used in the literature interchangeably with μ E m^{-2} s^{-1} (microEinstein per second square meter) (1 μ E $m^{-2}s^{-1}$ = 1 μ mol m^{-2} s^{-1}, Asrar, Myneni, & Kanemasu, 1989).

Light intensity can be separated into regions according to its relationship with the specific growth rate of the microbial mat. The light-limited region (< 44 μ E m^{-2} s^{-1}) promotes an increase in specific growth rate indicating first-order kinetics. The light intensities used by Ravelonandroand colleagues (2008), and Xueand colleagues,(2011) to grow *Spirulina platensis* were of light-limiting region (< 44 μ E m^{-2} s^{-1}) and achieved desirable growth rates.

The quantity and quality of light is very important for the growth of a microbial mat. Notably, different species have different demands. Even though the intensity of sunlight is 1,000-2,000 μ E m^{-2} s^{-1}, optimal light intensity for cyanobacterium *Oscillatoria* is 15-150 μ E m^{-2} s^{-1}. Purple phototrophic bacteria have even lower light requirements, 5-10 μ E m^{-2} s^{-1}, so they are normally not found on the surface of the microbial mat (Stal, et al, 1985). Algae such as Chlorophyta and Rhodophyta which utilize light for photosynthesis are able to acclimate to a wide range of light intensities (Debloisand colleagues, 2013 and Levy and Gantt, 1988).

1.4.2 Type of Light

1.4.2.1 Fluorescent Light

Fluorescent illumination is achieved when electrical current passes through a glass tube filled with mercury vapor. This causes the electrons in the gas vapor to emit ultra violet light frequencies which are picked up and transformed into fluorescence by a phosphor coating along the inside walls of the tube (Yen, Shionoya and Yamamoto, 2006). Fluorescent lights have been used in research to promote biomass growth in PBR (Genin, Sitchison & Allen, 2013; Johnson and Wen, 2010; Katsudaand colleagues, 2006; Kommareddy and Anderson, 2003; Ozkan, Kinney, Ketz, & Bergeroglu, 2012; Sukačová, Trtílek, & Rataj, 2015). White fluorescent lights emit light in the visible spectrum of 400-700 nm. They are known to produce high energy output which is emitted in all directions and does not necessarily aid in photosynthesis. (Kommareddy and Anderson, 2003). Some of the energy used by the fluorescent lights is turned into heat (Carvahloand colleagues,, 2011), which results in energy waste.

1.4.2.2 Light Emitting Diode (LED) Illumination

LED is a light source that uses electroluminescence in a semiconductor material to create light. The wavelength or color of light emitted by a particular type of LED is determined by the properties of the semiconductor material used (Moreno and Sun, 2008). The use of LEDs in the microalgae industry has increased dramatically in recent years. They are more economical than fluorescent or incandescent light bulbs due to their narrowband wavelength and higher efficiency in the use of energy (Iluz, Alexandrovich, & Dubinsky, 2012; Pilon, Berberoglu & Kandilian, 2011; Sforza, Simionato, Giacometti, Bretucco & Morosinotto, 2012; Yan, Lou and Zheng, 2013).

Biomass growth in g m^{-2} d^{-1} is one of the most common ways to measure productivity of a system. Using different wavelengths of LED lights to promote biomass growth has been explored in many studies. This has been especially true for blue, red and cool white LED lights as the quality of light absorption differs depending on the species (Pilon and colleagues, 2011). Blue LED lights emit light in the wavelength of 460-470 nm (Yan et a. 2013). Some studies that used blue LED lights reported highest biomass growth for some species such as *Haematococcus pluvialis* (Katsuda and colleagues,, 2006), *Chlorella vulgaris* (Hultberg and colleagues, 2014), *Tetraselmis sp*. and *Nannochloropsis sp*. (Teo and colleagues, 2014). Productivity under blue intermittent lighting versus white lighting produced higher overall biomass and cells contained a greater amount of neutral lipids, glycolipids and phospholipids (Hultberg and colleagues, 2014). Using blue LED flashing lights with an intensity of about 8 $\mu Em^{-2}s^{-1}$ produced the highest amount of astaxanthin (keto-carotenoid byproduct) in *Haematococcus pluvialis* (Katsuda and colleagues, 2006) and substantially reduced energy consumption (Jansson, 2012 and Teo and colleagues, 2014).

Red LED lights emit light in the 620-630 nm wavelength (Yan and colleagues, 2013). Studies that used various LED lights to promote microalgae growth such as those by Markou (2014) with *Arthrospira platensis*, Yan and colleagues (2013) and Zhao and colleagues (2013) with *Chlorella vulgaris*, reported highest biomass growth under red LED light treatments. Cool white LED lights have a wider-spectrum wavelength (380-760 nm) which encompasses the blue, red and green light spectrum (Yan and colleagues, 2013).

1.4.3 Photoperiod

1.4.3.1 Full Light Photoperiod

In nature, algae are exposed to dual cycles of light and dark, which change in duration according to seasonal shifts. However, some studies report that LED lights used at full light 24-hour on, zero off (24L:0D) produces the highest biomass production when compared to other regimes for certain algae (Kommareddy and Anderson, 2003; Yoshioka, Yago, Yoshie-Stark, Arakawa, & Marinaga, 2012; Sukačová and colleagues, 2015).

In microbial mats, light should be able to penetrate the surface layer to reach the photosynthetic cells underneath the surface of the water. Low light intensity or flux does not provide enough light to penetrate beyond the surface layer (Gordon and Polle, 2007). On the other hand, using high-intensity light and a 24L:0D photoperiod, might cause oxidative stress due to overexposure to light or UV radiation. This can cause damage to the chlorophyll and pigment, and decrease metabolic activity (Castenholz, 2009, Sforza et a. 2012). Overexposure to light may also cause light inhibition for the surface layer of microbial mats (Carvalho and colleagues, 2011).

1.4.3.2 Pulsed Light

A number of researchers have explored different variables of light such as the type of light, photoperiod and frequency of light. It was found that prolonged exposure to light such as 24L:0D could reduce photosynthetic productivity (Sforza and colleagues, 2012). As an alternative, short durations of high intensity light allow for the regeneration of light receptors and recycling or dissipation of harmful by-products such as reactive oxygen species - making pulsed or intermittent lighting more ideal for increased growth

rates without damaging the cell (Xue and colleagues, 2011). Photoacclimation to low or high light intensity environments involves a change in the photosynthetic apparatus such as photosystem I and photosystem II as well as other changes in cell structure (Falkowski and La Roche, 1991). Using pulsed lighting as an alternative to prolonged light or dark periods leads to photoacclimation therefore, increasing photosynthetic efficiency (Deblois, Marchand and Juneau, 2013). When organisms are not exposed to constant light, they are able to utilize their photosynthetic apparatus at a higher rate (Iluz and colleagues, 2012; Yan and colleagues, 2013; Walter, de Carvlho, Soccol, and Ghiggi, 2011).

Yoshiokaand colleagues (2012) observed how different light regimes affect the eukaryotic microalga *Isocrysis galbana*. They tested full light regime (24 hours on), half-light regime (12 hours on, 12 hours off) and intermittent or pulsed light and found that high intensity intermittent or pulsed light produced higher growth rates than continuous light-dark regimes. High intensity pulses promoted higher oxygen production rates, minimized carbon dioxide in the air and improved cell concentration by 20% which increases overall biomass production by 20% (Park & Lee, 2000; Park & Lee, 2001). Limited light seeks to mimic the intensities of light experienced by algae in the environment. Studies also found that “limited light” of $< 44\ \mu E\ m^{-2}\ s^{-1}$ produced high growth rates with the lowest power consumption (Ooms Graham, Nguyen, Sargen, and Sinton 2017; Xue and colleagues, 2011; Ravelonandro and colleagues, 2008). The ability to grow algae at a fast and affordable rate makes it advantageous for fish feed production and waste remediation, a process which already naturally occurs in oceans and lakes (Christenson & Sim, 2011).

1.4.4 Temperature

Seasonal temperature fluctuations play an important role in the overall composition of a bacterial and algal community. Temperature increases in the summer months promote the growth of some organisms such as cyanobacteria (Vincent, 2009). Zhaoand colleagues (2016) also compared similarities among bacterial community compositions between seasons and revealed that temperature had the most important impact on the composition of a bacterioplankton community. Furthermore, they analyzed the co-occurrence of actinobacteria with cyanobacteria during unsuitable environmental conditions and found that the abundance of cyanobacteria decreased drastically, while actinobacteria and betaproteobacteria experienced minor fluctuations. In natural systems, cyanobacteria play a different role in the ecological network depending on the season (Castenholz, 2009). Most cyanobacterial cultures in microbial mats grow extremely slowly at low temperatures (4-10^{0}C), however other species such as *Synechococcus* can tolerate extreme temperatures of up to 74^{0}C (Vincent, 2009).

1.5 Bioreactor uses

Different types of PBRs have emerged for industrial purposes. One type is an enclosed chamber surrounded with a light source such as the one described by Hultberg and colleagues (2014). This type of PBR was maintained at constant temperature (20°C) and was used to cultivate a single species of *Chlorella vulgaris*. Some of the benefits of enclosed systems are that external factors such as temperature do not influence growth rates and it is possible to maintain a pure culture with a very low risk of contamination. Some limitations of these systems are that they generally produce lower growth rates due to shading and are costlier because of controlled parameters and labor-intensive

harvesting techniques. Other types of PBRs use open systems with flat growth surfaces such as ones described by Xue and colleagues (2011) and Abu-Gosh, Fixler, Dubinsky and Iluz, (2015). Open-system PBRs facilitate direct light exposure on the growth surface without creating shading and are generally cheaper to maintain as parameters such as temperature are not controlled. Harvesting is also simplified by scraping the biomass from the growth surface. The drawbacks of open systems are that changes in community dynamics are possible due fluctuations in temperature and salinity due to evaporation. Ozkan and colleagues (2012) created a bioreactor that was made up of a flat surface with a 0.2-degree incline. Water was collected at a common tank and run through the system while light was shone from above, directly onto the mat. The light radiance shining down on the mat was 55 μ E m^{-2} s^{-1}. The system created for our study was similar to this one with similar parameters.

1.5.1 Aim of the Study

The aim of our study was to compare the effects of different light treatments in the productivity of a microbial mat PBR. This was tested using different types of light (fluorescent and LED) and photoperiods (24L:0D and pulsed), and cost analysis were performed to determine which light regime was more economically viable. Additionally, the dynamics of the microbial mat taxonomic composition were explored to see whether light treatment had an effect on the community.

Chapter 2 Materials and Methods

2.1 Culture

The system was seeded with a community culture containing eukaryotic and prokaryotic organisms collected from a pre-existing microbial mat. The microbial mat was formed in a laboratory at a local clean technology company, SabrTech Inc®. This microbial mat was developed and grew in a photobioreactor over a three-year period with continuous harvesting and maintenance. The composition of the microbial mat varies from harvest to harvest, depending on external factors such as temperature and light regime. The design of the bioreactor studied here was similar to one described by Ozkan and colleagues (2012).

2.2 Design of the Photobioreactor

2.2.1 Structural Design

The experimental system consisted of four horizontal, flat runways stacked on top of one another with lights mounted above each level with approximately 75 cm between the lights and the growth substrate below. Each level was divided in three sections, each 46 cm x 81 cm separated by 5 cm high walls on two sides, and waterproofed with pond liner (Figure 2-1). The panels were angled at 1.5 ° downwards, allowing for water to flow from the water-manifold (top) (Figure A-1, label 2) of each panel to the drain (downhill) (Figure A-1, label 4). Each level was supplied with water by a pump fed from a shared water reservoir filled with 500L of water (Figure 2-2). Each section had an independently controlled water manifold with 6 mm holes spaced at 2.5 cm intervals (Figure A-1, label

2 and 3). Water that washed down each panel collected on a 15 x 15 cm gutter (Figure A-1, label 4) and recycled back into the water reservoir Figure 2-2.

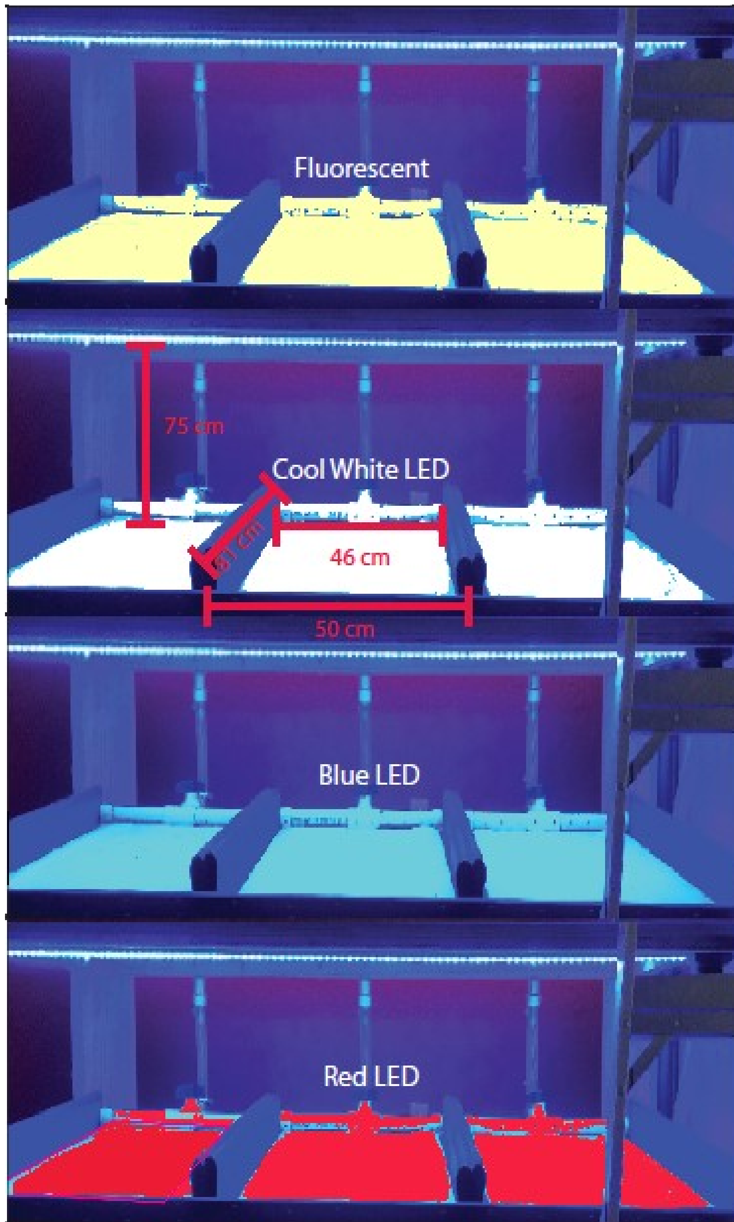

Figure 2-1. A front-view of the photobioreactor's structural design and dimensions. Preliminary data was collected on a photobioreactor made up of four levels. Each level of the photobioreactor uses a different light treatment and had three replicates identical in size. Levels were labeled with the corresponding light treatment. The false coloring was used to emphasize the different lights used.

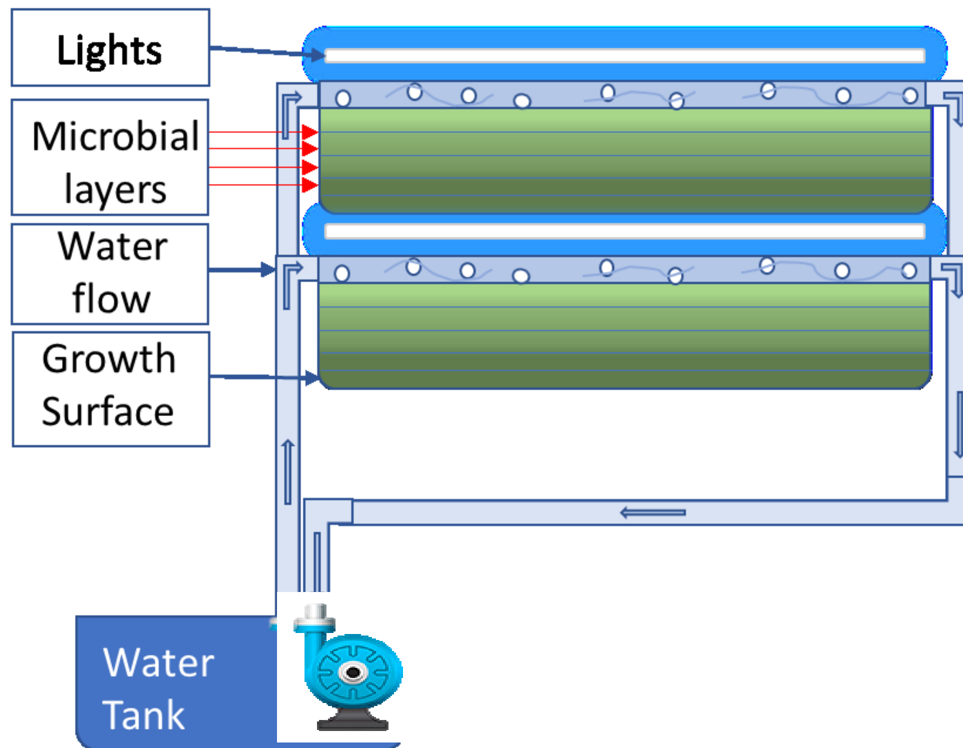

Figure 2-2. A simplified side-view of the photobioreactor's structural design. This graphic shows a side view of the design of the bioreactor. This simplified graphic shows two levels instead of four and two water pumps in the water tank instead of four that were used in the actual system. Pipes were connected to each pump to helped water flow through each level and collect back in the tank. Lights were mounted on top of each level and shine downward onto the growth surface.

2.2.2 Water Supply

Seawater was collected from Sandy Cove, Ketch Harbour, NS. The seawater was sand-filtered in the pump hose to a nominal level of 25 microns where it flowed through a drum filter with 90-micron screens, and was then transported in a 1000L tank into the lab was filled with 500 L of water.

2.2.2.1 Water Top-Up

Salinity was maintained at around 35 units (35g of salt/1kg of water). The water reservoir was topped up with distilled fresh water when the salinity meter read over 40 units.

2.2.3 Nutrients

The nutrients used to supplement the cultures were provided in the form of Guillard's (f/2) Marine Water Enrichment Solution (Guillard, 1975). The system was fed by measuring 100 mL of nutrients in a beaker and depositing it inside the water reservoir. This allowed for the nutrients to be mixed and distributed throughout the system. The nutrient recipe is included in Appendix B.

2.3 Inoculation Process

The initial inoculation of the experimental system was broken up into four steps. First, distilled water was circulated throughout the system for 24 hours to ensure that any impurities (i.e. dust, wood chips) present in the growth substrate were flushed out. Second, 100 mL of a microbial mat sample acquired from our industrial sponsor was transferred onto the virgin material of the new system. Third, the biomass was smeared into the material (Figure 2-3, label A) and water flow commenced 30 minutes after smearing. This seeded the rest of the material that was previously not covered. Fourth, the system was run for 14 days to allow the community to establish on the growth substrate before harvesting (Figure 2-3, label B).

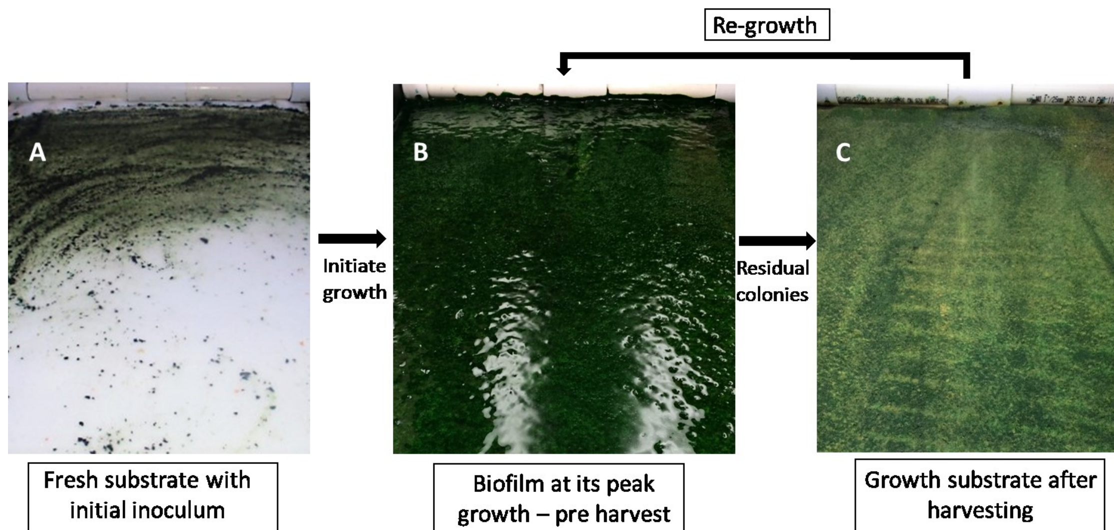

Figure 2-3. Images of the stages of growth of the microbial mat leading up to the first harvest. (A) Initial seeding of the virgin growth substrate with 100 mL of microbial mat by smearing it on to the surface. (B) An image of the microbial mat two weeks after seeing, minutes before harvesting. (C) An image of the harvested growth substrate. Harvesting was done by pressing and dragging a squeegee through the mat and collecting it at the bottom. The green color of image (C) is given by remaining cells that were not picked up by the squeegee and help initiate re-growth.

2.4 LED and Fluorescent Lights

LED illumination was provided by waterproof LED strips, 171.5 cm in length, with LED lights spaced every 6 cm, built to order and purchased from LilyLEDs.com. The strips were glued to thin wooden beams for structural support and mounted to the structure and suspended 75 cm above the growth substrate. The light strips were distributed equidistant from one another, 15.2 cm apart. The LED lights used 2 Amperes (A) of power with a life expectancy of 50,000 hours (lilyled.com). The LED lights were powered with a TC420 1.4" LED Programmable Time Controller (12~24V) supplied by BORUIT® purchased from Amazon.com.

2.4.1 Blue, Red and White Low-Intensity LED Lights

Low intensity blue, red and cool white LEDs were used for preliminary data collection. The light regime was 24L:0D, and the treatments were run for 2 months resulting in a total of 4 harvests. After preliminary analysis, three more strips of cool white LED lights were added to the second level and the lights were lowered to 45 cm, as shown in Figure 2-4. The red and blue LED treatments were turned off completely.

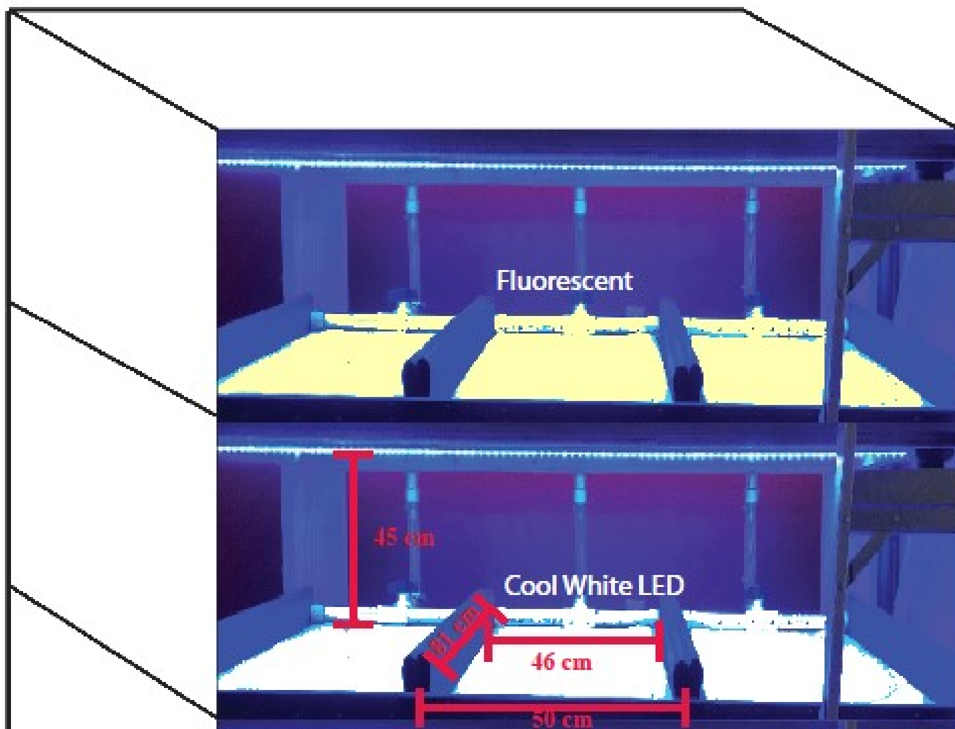

Figure 2-4. A front view of the structural design of the photobioreactor with higher intensity LED lights. The top level shows the fluorescent light treatment, which remained unchanged after preliminary data collection. The lights of the lower level (LED light treatment) were lowered by 30 cm in order to increase the light intensity reaching the surface.

The fluorescent lights used in this system were purchased from Lighting Direct Canada, and consisted of one fluorescent tube centered above each panel and oriented parallel to the water flow (Figure A-2), totaling three light tubes across the entire treatment. The fluorescent lights were mounted on the wooden frame at a 75 cm distance that made the radiance close to that of the LED lights.

2.5 Radiance Measurement

Light radiance measurements were taken using three different instruments. The first was the Traceable™ Dual-Range Light Meter from Fisher Scientific which measures LUX (lumen per square meter) in three ranges from 0 to 1999, 2000 to 19,990 and 20,000 to 50,000 lux with ±5% accuracy plus two digits. It is capable of measuring wavelengths that are detectable by the human eye. The second was the Quantum Solar Laboratory (QSL) Radiometer QSL – 2100 manufactured by Biospherical Instruments Inc. The units of this probe were μ E m^{-2} s^{-1}. Radiance inside the water tank was measured by the third instrument, a submerged light probe Ll-250A and Quantum Q40477 sensor (Li-Cor Biosciences, Lincoln, NE, USA) measuring in lux. Positions at which measurements were taken from each of the sections of the PBR are shown in Figure 2-5, recorded daily for one week, and averaged to get the average light intensity.

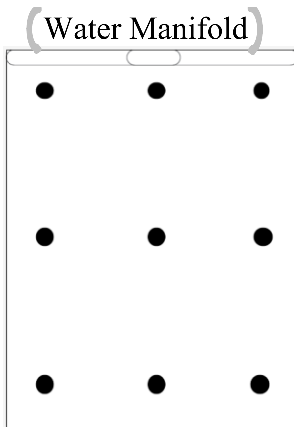

Figure 2-5. Points of measurement of the light intensity for each growth surface. Light intensity was recorded by measuring 9 points on each microbial mat replicate. The average was taken and reported as treatment radiance. This is a top view of one such surface.

2.6 Photoperiod of the Light Treatments

2.6.1 Continuous light treatment

Blue, red and white LEDs and the fluorescent treatments consisted of 24L:0D (continuous light) for six consecutive harvests at 14-day intervals or until the biomass yield was consistent in each light experiment.

2.6.2 Pulsed Lighting Treatment

A duty cycle is a full on-off cycle of a signal and it is sometimes used to describe the frequency of pulsed light. Katsuda and colleagues (2006) used duty cycles from 17-67%. A 35% duty cycle used by Vejrazka Janssen, Streefkand and Wijfels (2012) was found to give the highest growth rates for *Chlamydomonas reinhardtii* cultures. Lunka and Bayless (2013) obtained the highest biomass per unit power at 20% duty cycle. Since most studies that used pulsed light used a duty cycle between 10% and 50%, we decided to use a duty cycle of 30%. The pulsing regime was set to 43 milliseconds of light and

100 milliseconds of dark photoperiod (30% duty cycle) as it was reported to be successful by previous studies. An Arduino Uno Rev3 (Figure A-4) microcontroller board was used to program a rapid photoperiod for the LED treatment. The fluorescent treatment remained unchanged, at 24L:0D. The script for the desired output was written in Arduino IDE (Integrated Development Environment) software and then installed in the device. Once the code was installed, the microcontroller was able to control the light output at the desired photoperiod.

2.7 Data Collection

2.7.1 Pictures

Photographs were taken daily with an Apple iPhone5 8-megapixel camera with flash, held 18 inches above the base of the growth substrate, centered 12 inches from the sides. The camera was held straight with the manifold positioned as the top border of the photo and the start of the growth material positioned as the bottom border.

2.7.2 Temperature

The water temperature of the reservoir was measured daily using an alcohol thermometer placed in the center of the tank and recorded 15 seconds after being placed in the reservoir. The lab temperature was measured daily by reading the top of the alcohol thermometer meniscus mounted on the wall.

2.7.3 Salinity and pH

Salinity was measured with a salinity refractometer provided by Brix® (model: REED R9500) with measurements of 0-100 mg of salt per liter of water (mg L^{-1}). A transfer pipet was used to collect water from the tank and deposit it on the device.

The pH was measured using a Nutrafin® kit. Three drops of pH solution were added to 1mL of water collected from the tank. After shaking it well, the color was compared to the pH scale provided in the kit. Salinity and pH values were recorded on a daily data sheet.

2.8 Biomass Harvesting and Analysis

2.8.1 Harvesting of Biomass

Biomass was collected every 14 days. Prior to harvesting, water circulation was turned off for 30 minutes to allow for any excess water to drain off. The microbial mat was scraped with a squeegee (Figure 2-3 label C and Figure A-3) and a small wet sample of 20 mg was put aside for DNA extractions from each replicate. The biomass was then transferred into a container with punctures to allow drainage for 24 hours, after it was weighed and the weight recorded as wet biomass. After weighing, the biomass was dried in an oven at 80°C for 24 hours. The dry biomass was then weighed and recorded as dry weight (DW). Dry weight biomass is one of the most commonly used ways to determine bioreactor performance.

2.8.2 Sampling for DNA Extractions

Wet biomass samples of 20 mg were taken using a transfer pipet every 14 days and transported to the lab for analysis. If the samples were not analyzed immediately, they were stored in a -20°C freezer for up to a week before proceeding to DNA extraction. The remainder of the sample was saved for future extractions.

2.9 Protocols and Analysis

2.9.1 DNA Extraction, Quantification and Analysis

Total DNA was extracted from 20 mg of biomass for each sample using the ZR Fungal/Bacterial DNA Miniprep® kit (Zymo Research, Catalogue: D6005) following the manufacturer's instructions. DNA quantification was performed using an Epoch™ Microplate Spectrophotometer from BioTek® with the Nucleic acid quantification program. Two µL of the elution buffer from the extraction kit was used as blank for calibration. After calibration, 2 µL of sample was added to the plate and the results were displayed using BioTek's Gene5 software.

For 16S and 18S ribosomal DNA (rDNA) PCR amplicon sequencing, 5 µL of each rDNA sample (concentration >0.2 ng/µL) was sent to the Integrated Microbiome Resource (http://cgeb-imr.ca) to be amplified using PCR primers for the sequence encoding a variable region in the gene for the rRNA gene for bacteria and eukaryotes (Table D-5). The PCR product was sequenced using an Illumina MiSeq instrument with the v3 chemistry (2x300 bp PE) as described by Comeau and colleagues (2017). Illumina makes possible the assembly of paired amplicon reads into one high-quality sequence. The sequences were then run through the QIIME (Quantitative Insights Into Microbial Ecology) pipeline. QIIME is an open-source pipeline designed to perform microbiome analysis from raw DNA sequences that are generated by Illumina. QIIME provides a platform where start-to-finish workflow can be performed, starting with quality filtering, taxonomic classification and diversity analysis (Caporaso et a. 2010).

2.9.2 Ananke – UI: Time Series Clustering Algorithm

Ananke – UI is a time-clustering algorithm that was used to display microbiome diversity in a time-series fashion. This program was developed by Mike Hall, a graduate student from the Dalhousie University Faculty of Computer Science and his supervisor, Dr. Robert Beiko in an attempt to resolve the difficulty with which time-series microbiome data are presented and how to make sense of it. Ananke – UI is an interactive interface that uses an open source programming language and software RStudio. RStudio can be launched by downloading RStudio and the Shiny library, which is a package that has pre-built widgets to display plots and tables (RStudio, 2013). After the data is uploaded, Ananke-UI forms clusters with sequences that are the most related to one another using operational taxonomic units (OTUs) that are 97% or more alike and groups them into groups or clusters. This web-based platform allows the user to choose the desired parameters such as time point and clusters and updates them instantly. Cluster parameters depend on sequence depth and the number of sequences. Sequences were clustered according to the time series cluster in which they belong (Hall, 2016). This algorithm was used to explore interactions between the species in the community.

2.9.3 pplacer

pplacer is an algorithm that uses existing eukaryotic rDNA gene sequence reference trees to identify 18S sequences that are too short, 400 base pairs (bp) or less, to be identified by QIIME. pplacer attempts to map short sequences into an existing tree at the highest likelihood. The sequences deemed as "unknown" by QIIME pipelines were run through pplacer for more accurate taxonomic assignment.

2.10 Statistical Analysis

The normality of the harvest data was tested through a Shapiro Wilk test performed in RStudio (RStudio, 2013). A two-way ANOVA was used to check for significant differences between two treatments. An unpaired, two tailed t-test was used to test for a significant difference between the temperature of the lab and the water tank. A one-way ANOVA was used to measure differences between harvests as well as within treatments. GraphPad Prism®, version 5.01 was used as the statistical analysis tool. The α level was set at 95% and the p – value of 0.05 was used to indicate if the difference was significant or not.

To test whether overall taxonomic composition from harvest-to-harvest was normally distributed, a Kolmogorov-Smirov (KS) test was performed using the free statistical computing software RStudio (RStudio, 2013). KS is used to test if a sample comes from a continuous distribution and uses a chi-square goodness of fit test.

Multidimensional Fuzzy Set Ordination (MFSO) is a mathematical function of ordination which was used to explore whether changes in temperature, salinity or pH affected the taxonomic composition of the microbial mat. This statistical analysis was performed using RStudio. Each variable (temperature, salinity and pH) was assessed to determine its influence in the taxonomic composition. Results were given as p values.

Chapter 3 Results

3.1 Blue, Red and White Low-Intensity LEDs

Preliminary productivity data were collected using low light intensity cool white, blue and red LED lights. Biomass productivity was significantly lower under blue, red and white LED light treatments compared to fluorescent light. (Table D-1). Figure 3-1 shows images of the growth surface after fluorescent light treatment and the lack of growth for the three LED light treatments. Due to these preliminary results, data collection was terminated for the blue and red LED light treatments. Cool white LED light intensity was increased to become similar to that of the fluorescent treatment and data collection continued with cool white LEDs.

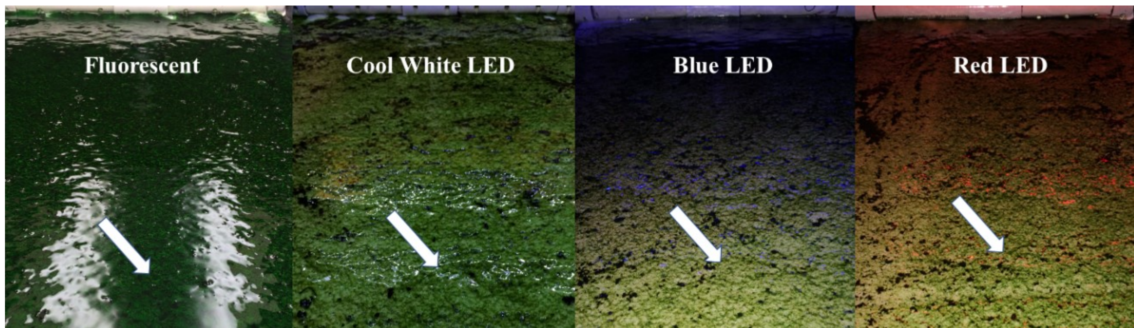

Figure 3-1. Images of the microbial mat under fluorescent light treatment, cool white LED, blue LED and red LED light treatments. The side-by-side images show the surface of the microbial mat at 14 days of growth under fluorescent light treatment and the three LED light treatments. The white arrows focus on the growth substrate which is completely covered with a green, leathery sheet of microbial mat in the fluorescent treatment, but is almost mostly exposed in the three LED light treatments.

3.2 Biomass Growth Rate During 24L:0D Photoperiod

To assess the productivity of the cool white LED lights with increased intensity and compare it to the productivity of fluorescent lights with similar intensity, biomass

was harvested and the dry weight was recorded. The 24L:0D photoperiod was applied to each treatment for 12 weeks, with biomass harvesting every 14 days (6 harvests total). Biomass growth rates for each treatment are shown in Table 3-1. Average DW productivity for the fluorescent light treatment was 1.98 g m^{-2} d^{-1} at 11.82 μ E m^{-2} s^{-1} radiance measurement. The average DW productivity for the LED light treatment was 1.76 g m^{-2} d^{-1} with radiance 8.39 μ E m^{-2} s^{-1}. Dry weight biomass productivity and radiance levels are shown in Figure 3-2.

Harvest data were normally distributed ($p>0.05$) (Table D-2). A one-way ANOVA was performed to check for variance of growth rates measured in g m^{-2} d^{-1} within replicates. The LED and fluorescent treatment had p values of $p=0.80$ and $p=0.38$, respectively. Since there is no significant difference, the three replicates were true replicates in this study. Variances between treatments did not significantly differ with one exception (Harvest 2) ($p<0.05$).

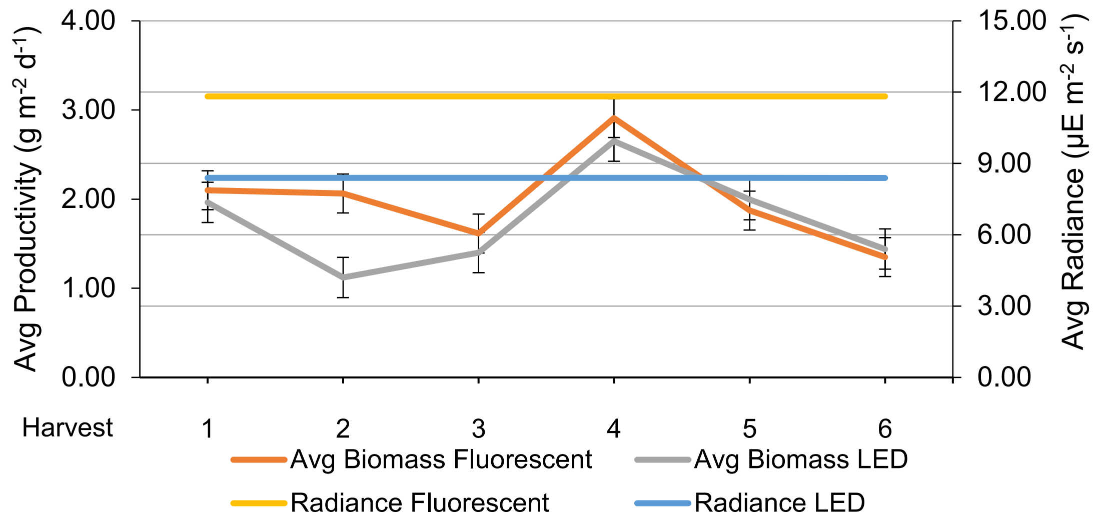

Figure 3-2. Productivity and radiance of the LED and fluorescent light treatments during the 24L:0D photoperiod. The average dry weight biomass production was divided by the total growth surface (0.36 m^2) and the number of days of growth (14 days) for each harvest point (1-6). The radiance of fluorescent and LED lights was measured at 9 points for 7 days in a row and averaged. The average radiance was plotted on the secondary axis.

Table 3-1. Productivity of fluorescent and LED lights with a photoperiod of 24L:0D.

Harvest	Productivity of replicates of light treatments and averages (g m^{-2} s^{-1})						
	Fluorescent 1	Fluorescent 2	Fluorescent 3	Fluorescent avg	LED 1	LED 2	LED 3	LED avg
1	1.92	2.43	1.95	2.10	2.14	2.29	1.45	1.96
2	2.03	2.12	2.04	2.06	0.94	1.10	1.32	1.12
3	1.45	1.79	1.60	1.61	1.39	1.69	1.12	1.40
4	2.47	3.34	2.92	2.91	2.49	2.95	2.51	2.65
5	1.65	2.07	1.89	1.87	2.21	2.00	1.77	1.99
6	1.21	1.53	1.31	1.35	1.92	1.42	0.99	1.44

3.3 Biomass Growth Rates During 24L:0D Fluorescent and Pulsed LED Photoperiod

Fluorescent and LED light treatments with the 24L:0D photoperiod were compared to a pulsed LED light regime. Biomass was harvested every two weeks and dry weight determined (Table 3-3). The DW biomass growth rate of harvests 7 to 12 under the fluorescent treatment averaged 2.02 g m^{-2} d^{-1} using 11.82 μ E m^{-2} s^{-1} radiance. The DW biomass production under the pulsed LED treatment was significantly lower, averaging 0.52 g m^{-2} d^{-1} compared to 24L:0D photoperiod (1.76 g m^{-2} d^{-1}). The exact radiance of the pulsed light was not possible to measure due to the frequency of pulses. The difference in biomass production between the continuous fluorescent light treatment and pulsed LED light treatment was significant (two-way ANOVA; $p<0.001$), indicating that light photoperiod had an effect on biomass growth. The effect of the difference in radiance levels on average productivity for each treatment is shown in Figure 3-3.

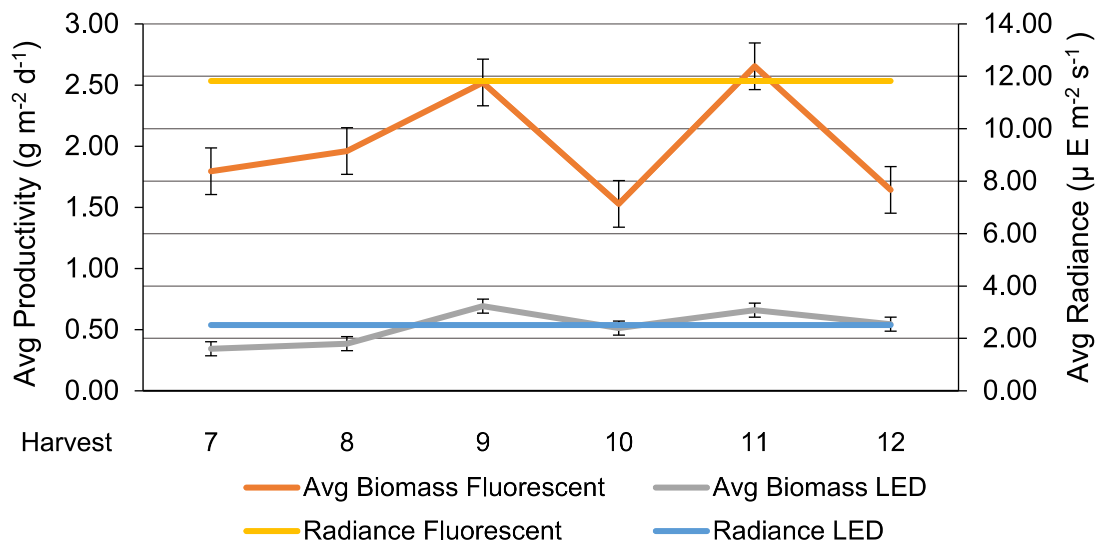

Figure 3-3. Productivity and Radiance in for Pulsed LED Treatment and 24L:0D Fluorescent Treatment. Average dry weight biomass production was divided by the total growth surface (0.36 m^2) and the number of days of growth (14 days) for each harvest point (7-12). Average radiance was plotted on the secondary axis

Table 3-2. Biomass growth under fluorescent and pulsed LED light treatments.

DW biomass growth rates ($g\ m^{-2}\ d^{-1}$) 24L:0D fluorescent and pulsed LED treatment								
	24L:0D				Pulsed			
Harvest	Fluorescent 1	Fluorescent 2	Fluorescent 3	Fluorescent avg	LED 1	LED 2	LED 3	LED avg
7	1.74	1.76	1.88	1.80	0.24	0.41	0.39	0.34
8	1.73	2.19	1.96	1.96	0.34	0.42	0.40	0.39
9	2.32	2.69	2.56	2.52	0.74	0.72	0.62	0.69
10	1.41	1.65	1.53	1.53	0.49	0.54	0.51	0.51
11	2.27	2.67	3.02	2.65	0.66	0.68	0.64	0.66
12	2.12	1.61	1.20	1.64	0.52	0.54	0.57	0.55

3.4 Grazer Episodes

The system in this study suffered grazer invasions that lasted a few days at a time. Grazing led to loss of biomass in a circular fashion as seen in Figure 3-4. At the beginning these circular shapes were quite small and spread out, but as time went on and more predation instances occurred, they grew in number and size.

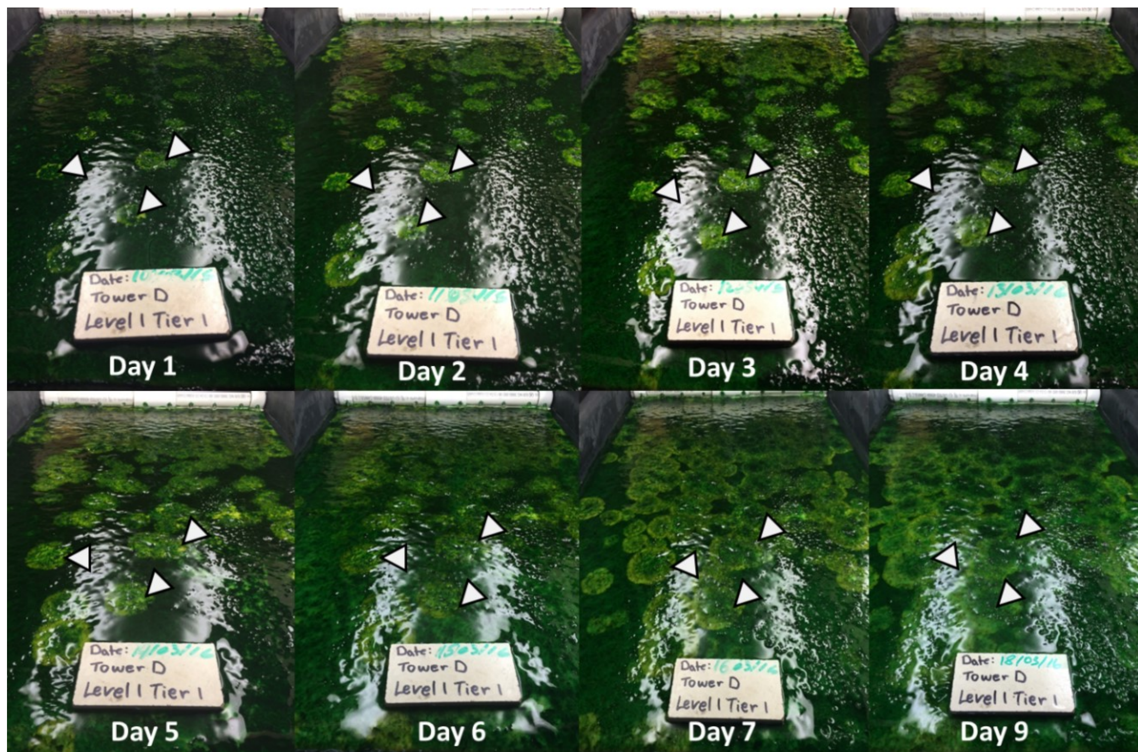

Figure 3-4. Patterns of a grazer episode and its progression throughout a 9-day period. Images of the same microbial mat replicate during a grazing episode are shown to highlight the progression of the circular grazing patterns. Arrowheads follow the progression of three circles from day 1 (top left) to day 9 (bottom right). The circles become larger in size from day 1 and begin to overlap around days 7 to 9. The exposure of the growth substrate reflects the reduction in biomass that is being consumed by the grazers.

3.5 Microbial Community Analysis

3.5.1 Community Composition Analysis

Multispecies communities have not been thoroughly appreciated in the field of industrial algal culturing until recently, however, more research is now focusing on these (Elias & Banin, 2012). It is difficult to identify organisms that comprise the microbial mat using conventional light microscopy. Molecular biology procedures such as polymerase chain reaction (PCR) and sequencing of the small subunit of the ribosomal RNA gene (16S, bacteria and 18S, eukaryotic) have become very useful in identifying the organisms present in a complex community.

The taxonomic composition of the microbial mats collected during this study was assessed across all samples using a targeted metagenomic approach through sequencing a variable region of the small-subunit ribosomal RNA gene. The sequences were analyzed (see section 2.9.1) and showed that taxonomic composition of the microbial mat did not differ between treatments ($p>0.05$) but did differ from one harvest to the next within the same treatment. Taxa composition at the class level of the fluorescent and LED treatments was normally distributed, as shown by Kolmogorov–Smirnov test (Table D-3). It is worth noting that eukaryotic abundance in the total sample was much smaller than the prokaryotic groups.

To test whether temperature, salinity, pH or average biomass yield ($g\ m^{-2}\ d^{-1}$) had any impact in taxa composition, a Multidimensional Fuzzy Set Ordination (MFSO) test was performed. The factors mentioned above were not found to influence one another or taxa composition ($p>0.05$) (Table D-4). Therefore, light treatment did not have an effect on taxonomic composition.

3.5.2 Prokaryotic Taxonomic Composition

PCR primers targeting the V4-V8 region of the small-subunit ribosomal RNA gene in prokaryotes amplified sequences from a wide range of prokaryotic taxa from the microbial mat sequences (Table D-5). The relative abundance of the most common prokaryotic groups are presented in Figure 3-5. The most abundant prokaryotic organisms present in the sample were Proteobacteria (Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria), Cyanobacteria, Sphingobateria, Anaerolineae, Flavobacteriia, Cytophaga and a varying proportion of unassigned sequences (sequences with no close matches in databases). Deltaproteobacteria in harvest 11 and 12 of the fluorescent treatment appear to be significantly more prevalent than in the LED treatment.

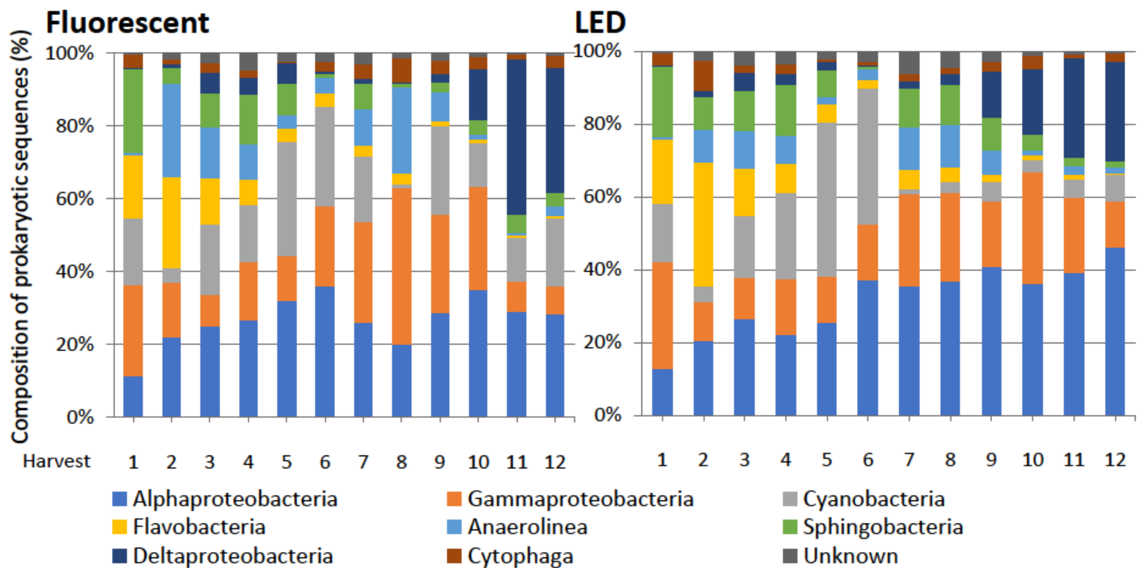

Figure 3-5. Taxonomic distribution of bacterial small subunit rRNA gene sequences in the community of the microbial mat. This graph shows the phylogenetic distribution of higher-level taxa of bacteria for each harvest of the fluorescent treatment and LED treatment. Each bacterial group presents the percentage it makes up out of the total bacterial community in one sample.

3.5.3 Eukaryotic Taxonomic Composition

Eukaryotic diversity was assessed by sequencing PCR amplicons obtained using primers targeted to the V4 region of the eukaryotic gene for small-subunit ribosomal RNA (Table D-5). The relative abundance of the most abundant eukaryotic groups in the samples are depicted in Figure 3-6. The most abundant eukaryotic organisms included algae (Rhodophyta, Chlorophyta and Ochrophyta) and several heterotrophic groups such as Ciliophora, Nematoda, Fungi, Labyrinthulomycetes and a varying proportion of unassigned sequences (sequences with no close matches in the QIIME database). Harvest 1 is substantially different in taxonomic composition compared to the rest of the harvests with greatest abundance of Ochrophyta and Labyrinthulomycetes. Rhodophyta and Chlorophyta make up the majority abundance of harvests 2 to 10, and in harvests 11 and 12, Nematoda (*Heterodera*) was extremely prevalent even though they did not appear in previous harvests.

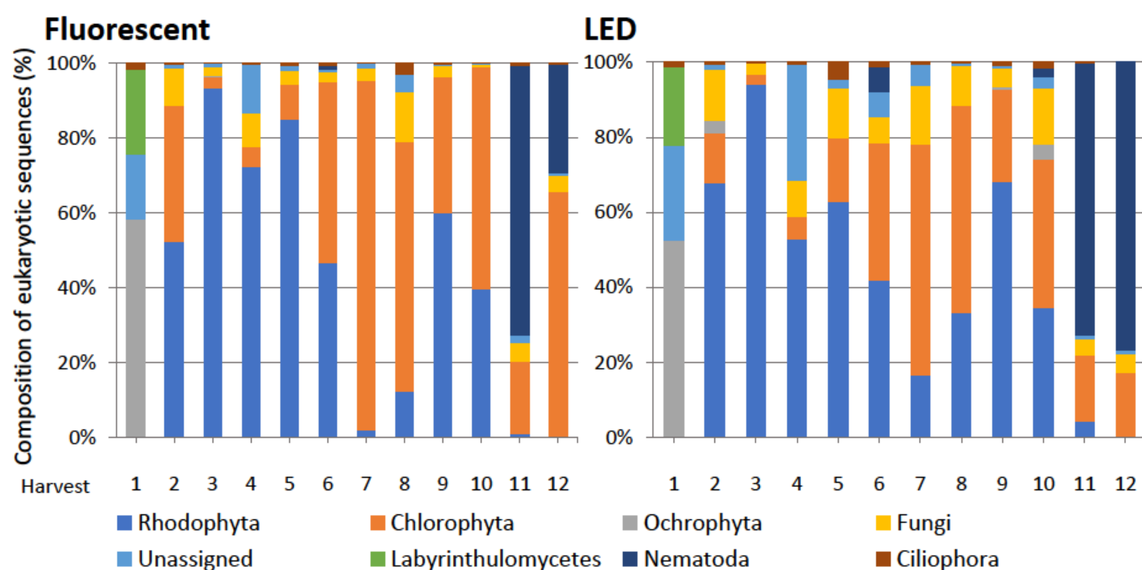

Figure 3-6. Taxonomic distribution of the eukaryotic small subunit rRNA gene sequences in the community of the microbial mat. This graph shows the phylogenetic distribution of higher-level taxa of eukaryotes for each harvest of the fluorescent treatment and LED treatment. Each eukaryotic group presents the percentage it makes up out of the total eukaryotic community in one sample.

3.5.4 Sequences of Undetermined Affinity

Another way of gaining a better understanding of the microbial mat community that was used in our study was to explore the unassigned taxa. The presence of unassigned groups for both eukaryotic and prokaryotic taxa was determined (Figure 3-7). The sequence reads that were gathered under the "unassigned" group could be unclassified species or short reads (less than 400 bp) that due to their length could not be assigned to one particular taxa with any degree of certainty. To overcome this difficulty, the program pplacer was employed in an attempt to identify at least some of the unassigned taxa.

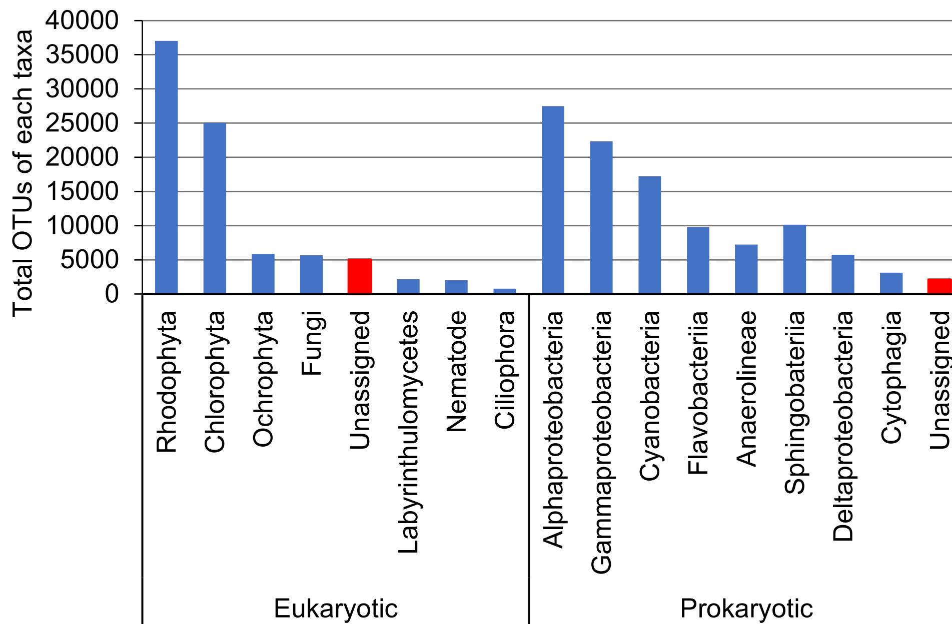

Figure 3-7. Total OTUs of each taxa of the prokaryotic and eukaryotic sequences as well as the unassigned OTUs. Combined OTUs for each group were added together to get a total number of OTUs for each taxa. The unassigned OTUs were also added together to display the large number of unassigned sequences in each domain.

3.5.4.1 pplacer

In order to explore what some of the unknown taxa were, the pplacer algorithm which can be used as a tool to map short sequences to a pre-existing tree, was used (Figure 3-8). This tree was derived for analysis of unpublished sequences that the creator of the tree, Yana Eglit (Dalhousie University), intends to use in future publications. Only sequences which matched 100% on the tree, or had a likelihood of “one” were included in the tree. This analysis suggests that the microbial mat harbours a large diversity of eukaryotic organisms of unclear or unknown affinity, opening the opportunity for further investigation into the functional and ecological roles of those taxa. The prokaryotic unassigned taxa could also be mapped with the help of pplacer to get a better idea of the total taxonomic make up of the mats.

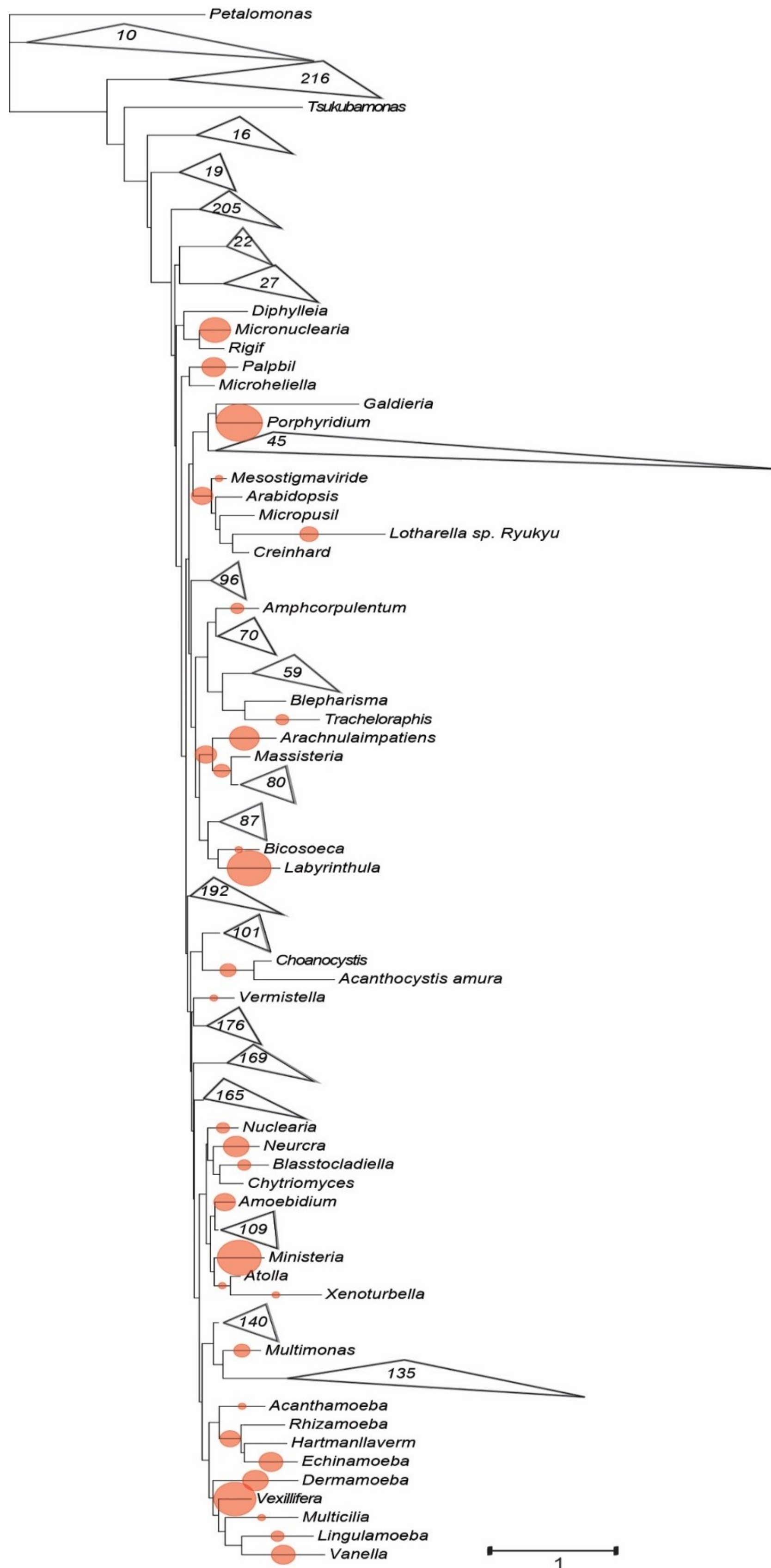
Petalomonas
10
216
Tsukubamonas
16
19
205
22
27
Diphylleia
Micronuclearia
Rigif
Palpbil
Microheliella
Galdieria
Porphyridium
45
Mesostigmaviride
Arabidopsis
Micropusil
Lotharella sp. Ryukyu
Creinhard
96
Amphcorpulentum
70
59
Blepharisma
Tracheloraphis
Arachnulaimpatiens
Massisteria
80
87
Bicosoeca
Labyrinthula
192
101
Choanocystis
Acanthocystis amura
Vermistella
176
169
165
Nuclearia
Neurcra
Blasstocladiella
Chytriomyces
Amoebidium
109
Ministeria
Atolla
Xenoturbella
140
Multimonas
135
Acanthamoeba
Rhizamoeba
Hartmanllaverm
Echinamoeba
Dermamoeba
Vexillifera
Multicilia
Lingulamoeba
Vanella
1

Figure 3-8. Short, unassigned sequences placed at the highest likelihood on the branches of a eukaryotic tree using pplacer. The red bubbles represent the highest likelihood of where the sequences belong. The size of the red bubbles shows how many sequences belong in that specific branch. The numbers on the collapsed branches represent the number of species in the branch. The scale bar presents the inferred number of nucleotide substitutions per site.

3.5.4.2 Ananke – UI

In order to better understand interactions between taxa in a microbial community, researchers are developing software and algorithms that could aid in painting a clearer picture of such interactions. One such researcher is Michael Hall from the Beiko Lab at Dalhousie University who developed Ananke - UI. Ananke - UI is an algorithm that at first combines sequences that are 97% or more alike. Then, it counts the sequences across the time points, generating a time-series for each sequence. Using the short time-series distance measure, it is able to compute the similarity in patterns of occurrence between each pair of sequences. One can import taxonomic and sequence-identity clustering information as metadata for the Ananke UI and explore the patterns or contrast the patterns within sequence-identity clusters.

An example of how this algorithm can be used in the future for microbial community explorations is presented (Figure 3-9). Different clusters are selected by cluster number. The two treatments are lined up side-by-side with their relative abundance of each taxa cluster. Cluster graphs are then lined up to show the same time frame from one harvest to the next in order to observe any changes between treatments as well as changes among taxa. Using this analysis, Flavobacteria was found to be present in the system at the same time in the fluorescent light as well as LED light treatments (Figure 3-9). *Colinelliacea,* which is a Gammaproteobacteria, on the other hand, had two

instances of occurrence during the fluorescent light treatment. The first one was around Harvest 8, and the second around Harvest 11. Under the LED light treatment, however, it only had one occurrence around Harvest 8. *Anaerolineae*, a Chloroflexi, had two occurrences under the fluorescent light treatment and none under the LED light treatment. This is a very interesting phenomenon to explore further in order to determine if the behaviour of this group was due to the light treatment or to other factors. Stolz (2000) reported that species within a microbial mat community act very differently from ones in suspended cultures in terms of their metabolic pathways in response to oxygen and light. This sort of phenomenon could potentially be further explored using technologies such as Ananke – UI.

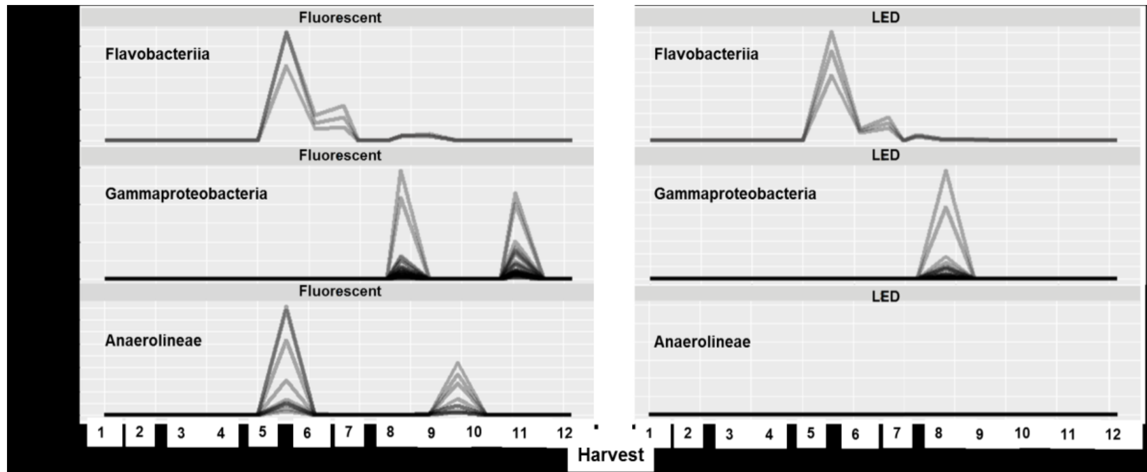

Figure 3-9. An Ananke-UI analysis of prokaryotic groups present at different harvest points. This image shows an example of the how Ananke-UI algorithm displays the results. The peak trends of increase and decrease of certain taxa show abundance of that taxa at each time point. The color intensity of the lines expresses the number of identical OTUs that make up each peak.

3.6 Power Consumption

Power consumption was calculated to assess the economic output of each treatment. An online power consumption calculator was provided by saskpower.com. Fluorescent light power consumption was calculated at 24L:0D for one year at a rate of 15.03 ¢/kWh; drawing 102W of power. At this rate and usage, the total cost of this light regime for one year was $132.50 CAD. The LED light power consumption was also calculated at 24L:0D for one year at a rate of 15.03 ¢/kWh, drawing 42.5W of power. At this rate and usage, the total cost of this light regime for one year was $55 CAD. These values predict that LED lights would use 58.5% less electricity than fluorescent lights using the same photoperiod.

Chapter 4 Discussion

The aim of this research project was to improve parameters for microbial mat biomass growth using different light properties and photoperiods for more efficient large-scale production systems that provide reliable and flexible parameters to grow a variety of cultures. Such parameters include the most suitable light intensity and radiance, temperature, pH, carbon dioxide and nutrient requirements (Schoepp and colleagues, 2014).

4.1 LED Light Compared to Fluorescent

Phototrophs, which are photosynthetic organisms, are the primary producers of the microbial mat and require light as a source of energy (Wagner, Besemer, Burns, Battin, & Bengtsson, 2015). This makes light quality and intensity extremely important for the productivity of the microbial mat. Light exposure directly affects the photosynthetic microorganisms present in the microbial mat, but also the overall bacterial composition (Wagner and colleagues, 2015).

Blue, red and white LED lights have been used in studies to increase biomass production, lipid and protein concentration. Zhao, Wang, Zhang, Yan and Zhang (2013) used red, blue and white LED lights with light intensities ranging from 800 – 1600 μ E m^{-2} s^{-1} to improve growth rates of the microalgae *Chlorella* sp. and found that red LED lights produced the highest biomass production.

4.1.1 Productivity with 24L:0D Light Exposure

4.1.1.1 Fluorescent Light Treatment

The biomass productivity of the fluorescent treatment used in this study was 1.98 g m^{-2} d^{-1} for microbial mats exposed to 12 μ E m^{-2} s^{-1} light radiance in an open system PBR (Table 3-1). Okzanand colleagues (2012) reported 0.71 g m^{-2} d^{-1} growth of algal biofilm PBR at radiance of 55 μ E m^{-2} s^{-1}. This bioreactor was almost identical in design to the one used in our study in which fluorescent light and produced less biomass even though it was exposed to almost 5 times greater radiance. Other studies conducted in open raceway systems with similar design such as that by Johnson and Wen (2010) reported growth rates of 2.5 g m^{-2} d^{-1} of dry biomass weight, which is only slightly higher in productivity. Similarly, Genin and colleagues (2013) reported biomass growth similar to that obtained in our study using an open pond bioreactor. Therefore, this study accomplished similar productivity to others but with lower radiance levels.

4.1.1.2 LED Light Treatment

Preliminary experiments with very low LED light intensity (1-2 μ E m^{-2} s^{-1}) did not promote sufficient biomass growth. Lower intensity LED lights did not promote biofilm growth as Gordon and Polle (2007) predicted. After further investigation, it was discovered that the red LED lights used in this experiment had a wide illumination angle of 160° but a very short illumination distance, thus the light would not have been able to reach the surface of the microbial mat and promote photosynthesis (LEDTronics, Inc, 2017). The blue LED lights had a narrow illumination angle of 8-20° and a long illumination distance, thus reaching the growth surface, but only at narrow points, allowing for dark patches on the rest of the surface (LEDTonics, Inc, 2017).

Due to the nature of the design of the system, it was not possible to lower the red LED lights enough for the light to reach the growth surface which is why no further red LED treatments were undertaken. Blue LED lights were also deemed unfit for our experiment as the amount of LED strips needed to provide enough light would quadruple, which would substantially increase the costs of the bioreactor.

Cool white LED lights have a combination of red, blue and yellow wavelengths and an illumination angel of 120° which makes them more adequate to manipulate for the desired radiance. Four more strips of cool white LED lights were added to the system and lowered 30 cm closer to the growth surface. This adjustment increased the light intensity of cool white LEDs to 8.4 μ E m^{-2} s^{-1} following a study by Katsuda and colleagues (2006) in which similar light radiance was used to grow *Haematococcus pluvialis* and reduced power consumption substantially.

The average biomass produced was 1.76 g m^{-2} d^{-1}. The average productivity for the LED lights was slightly less than that of the fluorescent light regime, however it was not significantly different. This means that even though LED treatment used slightly lower light radiance than the fluorescent treatment, it produced the same average biomass, making it suitable for large scale biomass production.

4.2 Productivity with Pulsed LED Light and 24L:0D Fluorescent Light

Many studies show that 24L:0D radiance versus pulsed is excessive and results in overexposure of cells to light (Iluz and colleagues, 2012; Sforza and colleagues, 2012). Extended periods of time using continuous light have proven to be damaging to the chlorophyll and pigment components of the cell. To prevent this from occurring,

intermittent or pulsed lighting has become a common alternative for research (Carvalho and colleagues, 2011). Anbalagan, Schewede and Nehrenheim (2015) suggested the use of pulsing LED lights in order to combat high energy demands of fluorescent light PBRs.

Even though many studies had great success with pulsed LED light, this study found that overall biomass production was significantly lower in comparison to full light. This could be due to light intensity remaining the same as it was during full light, even though other studies suggested increasing it for the pulsing regime. The rationale was to see if the microbial mat could acclimate to the new environment and produce enough biomass growth despite the pulsing at low frequency. An incentive to not add more lights to increase light intensity was keeping the costs low as this was an important parameter for the industry partner.

When considering the decrease in power consumption, this level of productivity might be viewed as positive; however, for a bioreactor whose primary goal is to produce large quantities of biomass, these results are not as favorable and call for a more comprehensive and systematic study. Such assessments should include using a variety of intensities and perhaps a 50% duty cycle instead of 30%. Longer periods might facilitate greater photosynthetic activity and therefore greater overall biomass growth. As well as, more lights to increase overall radiance levels so each flash of light can actually penetrate the cells and cause an electron transfer.

4.3 Vulnerability to Grazers

Grazing can be a significant problem for mass-culturing of algae, especially in open systems such as the reactor utilized in this study. Some strains experience great

sensitivity to grazers. Schoepp at al. (2014) found that some species such as *Chlorella vulgaris* and *Dunaliella tertiolecta* were more susceptible to rotifers and ciliates. There is a need for outdoor screening of species to be used in an outdoor or open system to discover vulnerabilities. Even strains that are known to be more robust, such as *Scenedesmus dimorphus*, have shown great susceptibility to predation. Laboratory models differ significantly in performance when compared to open outdoor systems. One of the reasons for this difference is that laboratory settings allow for control of all physical factors (Schoepp and colleagues, 2014).

The reasons for the grazer invasion in our study are yet to be investigated as the temperatures in the lab, as well as the instability of the system at that early stage could be contributing factors of these occurrences. Ciliophora, a diverse group of protists that includes many common grazers, were more frequently noticed in taxonomic analysis during the time of the grazer episodes, however, the reason for this increase has yet to be determined. One reason could be physical changes to the microbial mat as time goes on and the community shifts to accommodate temperature changes and salinity changes with the evaporation of water and gives rise to particular groups. Studying how a biofilm reacts to environmental changes can shine light on understanding the effects of climate and environment streams and ponds.

4.4 Microbial Community Analysis

Prokaryotic and eukaryotic taxonomic composition was studied to gain insight into the complexity of the microbial community and to better understand the influence of the different lighting regimes on the taxonomic composition. This analysis was conducted through a targeted metagenomic approach consisting of PCR-amplification of a region of

the small subunit ribosomal RNA gene followed by massively-parallel DNA sequencing. The analysis targeting the prokaryotic fraction analysis showed similar taxa to that reported in other studies conducted by Allen, Goh, Burns and Neilan (2009), Battin, Besemer, Bengtsson, Romani and Packmann (2016), and Wagner and colleagues (2015). This suggests that many microbial mats are similar in composition at a higher-level taxonomic classification.

4.4.1 Prokaryotic Taxonomic Composition

When analyzing the prokaryotic taxa found in the microbial mats (Figure 3-5) some groups appeared to be more consistently abundant than others. For example, *Rhodobacter,* a sulfur-oxidizing photosynthetic Alphaproteobacteria made up 10-45% of total community in harvests 1 to 12 of each light treatment. Similarly, Gammaproteobacteria, Chloroflexi (Anaerolineae – a green non-sulfur bacteria) and Cyanobacteria made up 30-70% of the total community in harvests 1 to 12. Similar abundance rates of these groups were reported by Couradeauand colleagues (2011), from community samples collected in alkaline lakes.

Even though Deltaproteobacteria *Myxococcales* was present, in small traces, in all harvests, its abundance in harvests 11 and 12 of the both light treatments was significantlyincreased (Figure 3-5). Myxococcales also known as Myxobacteria, are gram-negative soil bacteria that are found in slightly alkaline environments and are known to have unique cooperative behavior (cell -to-cell interaction). They synthesize a large number of active secondary metabolites. Perhaps the sudden increase of Myxobacteria in harvest 11 and 12 could be attributed to an increased acidity of the water

in the PBR which made it more favorable for this Deltaproteobacteria in particular to increase in numbers.

4.4.2 Eukaryotic Taxonomic Composition

Studies exploring eukaryotic taxonomic composition have not been as common in the literature, mainly because studies reported that 18S analysis were not as successful. Feazeland colleagues (2007) reported the most common groups of the eukaryotic composition were dinoflagellates such as *Gymnodinium* (which belongs to the Dinophyceae) Fungi, Trebouxiophyceae which is a green algae, and diatoms. As there was variability among microbial mats and our understanding of the interactions of species in the mat is limited, the findings here could not be compared with that of other studies to any degree of significant detail.

The taxonomic composition of eukaryotes in harvest 1 of both light treatments was drastically different from all the other harvests that followed. Harvest 1 was mainly comprised of Ochrophyta, Labyrinthulomycetes and some Ciliophora (Figure 3-6). Differences in abundance of organisms might be attributed to several factors. One explanation could be that the starting inoculum originated from a system that was treated with fluorescent lights only. It is possible that when the newly inoculated community was exposed to LED lights, taxa that utilized LED lights better, established at a faster rate. This advantage at the start might have been the determining factor that established the dominance of some members of the community. Since the PBR was an open system, and had a common water source, it allowed for those abundant groups to prevail in both light treatments. As well, an already-established microbial mat was exposed to a virgin growth

substrate, the drastic change in the chemistry of the new environment might have promoted the growth of some and the decrease in abundance of others.

While the first harvest was mostly composed of Ochrophyta and Labyrinthulomycetes (Figure 3-6), the most abundant groups of harvests 2-12 were Chlorophyta, Rhodophyta and Fungi. Chlorophytes and Rhodophytes made up 20-80% of the total eukaryotes in certain harvests 2 to 12. This was expected since a study by Debloisand colleagues (2013) demonstrated that chlorophytes acclimate to grow under a wide range of light conditions (14-1079 μ E m^{-2} s^{-1}) and achieve high growth rates. Similarly, the red algae *Porphyridium* a species of Rhodophyta, requires a relatively low range of light intensity for growth and is sometimes considered a "shade plant" in the literature (Levy and Gantt, 1988).

Another interesting observation in the eukaryotic taxonomic composition was that in harvests 11 and 12 there was an unexpected presence of sequences with similarity to *Heterodera*, a species of Nematoda (Figure 3-6). This species of nematodes is mostly found in food crops such as potatoes and sugar-beets and causes serious damage to the plant upon infection (Franklin, 1951). It is unclear why these species of nematodes were present in the sample. The likelihood of this species being present in a microbial mat environment is highly unlikely. This was a surprising result, and although we don't have a conclusive explanation of why it appears suddenly in such a large abundance, it is highly probable that it was due to contamination. Possible contamination points could be the water supply, harvesting tools, sample collection or sample processing. Since this is a multicellular organism, and PCR is very sensitive, even a small amount can give a lot of signal. To determine the source of contamination, it would be important to take another

sample of the water and collect more samples of the microbial mat to confirm if these results reproduce. Another possibility is that the sequences do not come from the *Heterodera* genus but from a different but closely related nematode that inhabits marine environments.

4.5 Power Consumption

Power consumption was monitored throughout the duration of the experiment to evaluate the economic benefit in using LED lights instead of fluorescent lights and pulsed light instead of constant light. Since power consumption decreased by 58.5% when using LED lights instead of fluorescents, it would be economically reasonable to use LED lights for commercial PBRs. LED lights are also known for their long life expectancy of up to 50000 hours and have 60% less environmental impact than conventional light (Casamayor, Su and Ren, 2017). Pulsed light is predicted to decrease power consumption even more. It can be hypothesized that power consumption probably decreased with 30% duty cycle pulses, however, the amount it decreased was difficult to calculate. Since we used a 30% duty cycle, we can argue that perhaps the power consumption decreased by 70%. When taking into account this dramatic decrease in overhead cost, perhaps the low biomass growth rates are still economically viable for commercial PBRs.

4.6 Limitations of this Study

Some of the limitations of this study are in the structural design of the bioreactor. This PBR was an open system which did not allow for external factors such as daily fluctuations of temperature to be controlled. This makes it difficult to say with certainty whether temperature changes had an effect on taxonomic composition and biomass growth rates.

Another limitation could be the shared water reservoir of both LED and fluorescent light treatments. As the same water circulated throughout the system, any organisms that were more prevalent on the LED treatment could technically wash through and seed the fluorescent treatment or vice versa. This makes it difficult to determine whether light treatment actually has an effect on taxonomic composition.

4.7 Future Research

There are many questions left unanswered regarding the improvement of microbial mats PBRs for biomass production. Factors such as light intensity and temperature could have a larger impact than presented in this study and these factors are worth exploring in further detail in order to better understand their role in biomass production. Studies such as the ones conducted by Castenholz (2009), Canfield and Des Marais, (1993) and Vincent (2009) have found that temperature did have an effect on growth rates in their respective cultures. Such reports are not conclusive for most open-system microbial mats and biofilm studies as temperature is not controlled in open raceway systems, nor the system used in our study.

In the future I would like to look at how increased intensity of pulsed LED light would affect the biomass productivity of the microbial matt as well as the taxonomic composition. As well, I would like to run parallel fluorescent and LED light bioreactors with controlled temperature and separate water tanks.

Other factors that would be interesting to assess are carbohydrate, lipid and protein profiles produced by the organisms present in the microbial mat. George and colleagues (2013) and Markou (2004) reported that light intensity and the types of light

used in their PBRs affected total lipid, protein and carbohydrate content. Markou (2004) reported that under white LED lights, protein content was highest (49.8%). It would be interesting to see what these profiles would be in a microbial mat system.

Chapter 5 Conclusion

As a result of an increase in interest in recent years, the field of microalgal exploration has expanded into creating artificial biofilms and microbial mats to obtain higher biomass production. The majority of research addressing the productivity of microbial mats is confined to laboratory settings. Companies that move from the laboratory scale into large-scale projects experience a drastic increase in overhead costs. Some of those costs are associated with the increase in power requirements for light radiance, higher nutrient demands and larger biomass amounts that require drying in ovens. Other challenges facing large-scale microbial mats are the increased space and storage requirements and laborious harvesting that is required to collect the biomass. There are also considerable challenges that need to be overcome in order to make larger systems as productive as laboratory-scale projects.

Existing structures of waste remediation systems paired with low light requirement bioreactors such as the one analyzed in this study may be able to address some of the challenges facing larger scale reactors. For example, using LED lights instead of other, more expensive light sources showed comparable results in biomass production. Hence LED lights seem to be viable and cost efficient light source for such industries. Moreover, using waste water as a source of nutrients and/or using a stackable rack system with automated harvesting mechanisms are some of the ways for addressing such concerns (as in this study's bioreactor setup). In conclusion, this study gave insights into the microbial community dynamics present in a self-sustaining bioreactor and the environmental factors that can influence its production. In the near future, these results can help setting up more productive and cost-efficient versions of this bioreactor.

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Appendix A. Images of the Bioreactor.

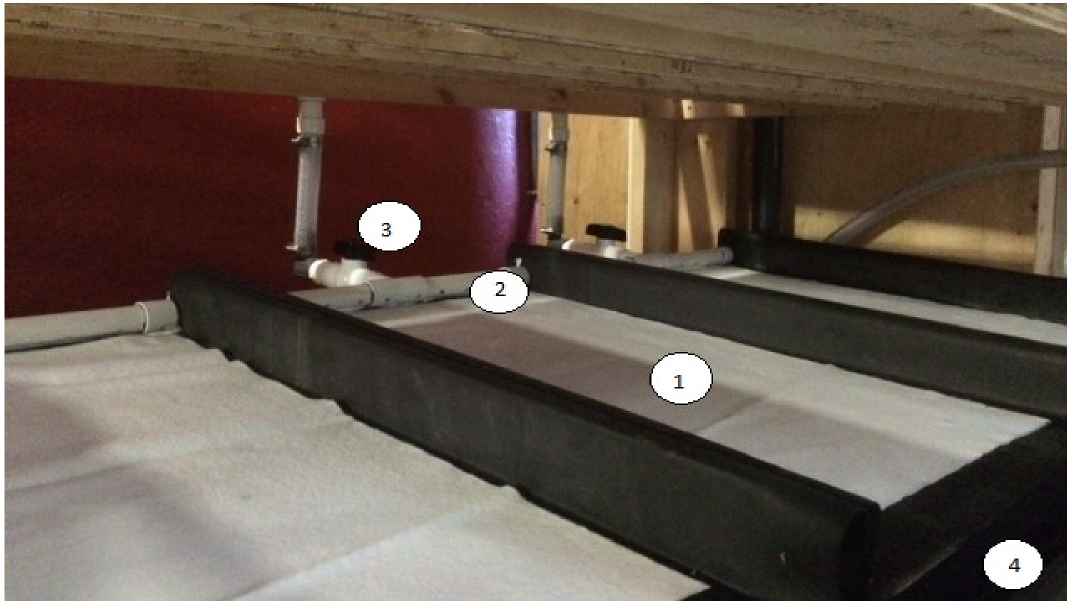

Figure A-1. An image of the replicate design of the bioreactor including manifolds and growth substrate. In this image label (1) shows the growth substrate where the microbial mat grows, label (2) depicts the punctured manifold that supplies the water to the surface, label (3) depicts the water valve used to control the flow intensity and label (4) depicts the placement of gutter where the water collects before it flows back to the reservoir.

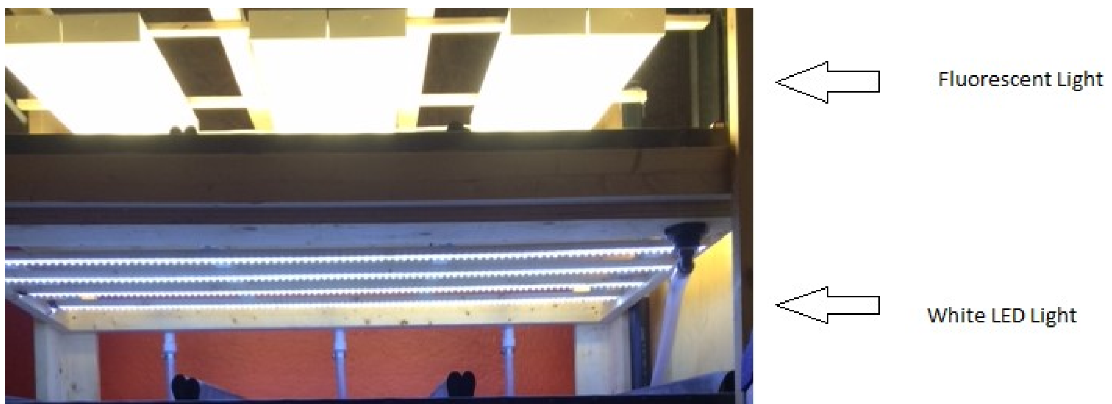

Figure A-2. An image of the LED and fluorescent lights mounted above the growth surface. The fluorescent lights are depicted on the top row and the LEDs underneath.

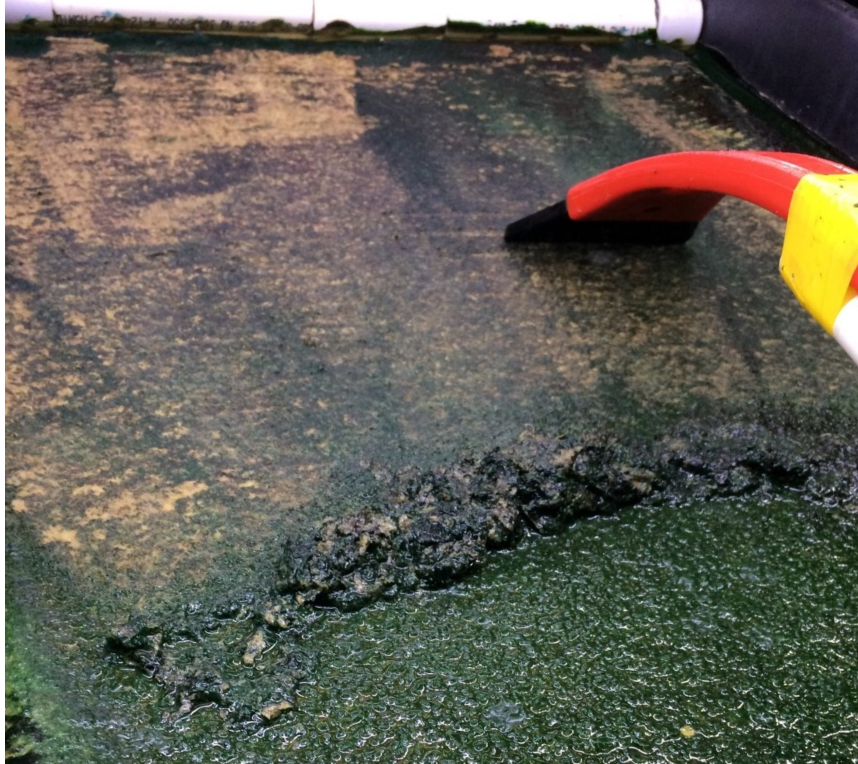

Figure A-3. An image of the harvesting technique of the microbial mat. The microbial mat was scraped from the surface using the squeegee device visible in this image. Some cells remained on the growth substrate and helped to re-seed the system after each harvest. The wet biomass was collected and dried.

Figure A-4. An image of the Arduino Uno Rev3. This device was used to control the pulsing of LED lights. A script was written in Arduino IDE software to get the desired pulse.

Appendix B. Calculation and Preparation of Reagents

B.1 f/2 Medium

To make 1 L of f/2 medium, add the following to 950 mL of 0.22 μM-filtered seawater.

$NaNO_3$ Stock solution ………………………………………………………..........1.0 mL

NaH_2PO_4 Stock Solution ………………………………………………………..1.0 mL

Trace Metals Stock Solution ……………………………………………………1.0 mL

Vitamin Stock Solution …………………………………………………………0.5 mL

To make the stock solutions add the following to 1 L of distilled deionized water:

$NaNO_3$ stock solution

$NaNO_3$ ………………………………………………… ………………………75.0 g

NaH2PO4 stock solution

NaH_2PO_4 ………………………………………………… …………………….5.0 g

Trace Metals stock solution

Na_2EDTA ………………………………………………… …………………...4.36 g

$FeCl_3 \cdot 6H_2O$ (Ferric Chloride) …………………..……………… ………………..3.15 g

Primary Metals Stocks (below) 1ml of each of the five

$CuSO_4 \cdot 5H_2O$ ……………………………..…………………… ………..........1.0 g

$ZnSO_4 \cdot 7H_2O$ ……………………………………..………… ………………..2.2 g

$CoCl_2 \cdot 6H_2O$ ………………..………………………………………………1.0 g

$MnCl_2 \cdot 4H_2O$ ……………………………………………………………….....1.8 g

$NaMoO_4 \cdot 2H_2O$ ……………………………..…………………………..0.63 g

Vitamin Stock solution

Biotin 10.0 mL of 0.1 mg•mL-1 solution ……………………………..........(1mg in 10ml)

Vitamin B12 1.0 mL of 1.0 mg•mL-1 solution ……………………………...(1mg in 1ml)

Thiamine HCl ……………………………………………………………..(2mg in 1ml)

Appendix C. Program Codes

C.1 Arduino Uno Code for LED Pulsing

```
void setup() {
// initialize the digital pin as an output.
// Pin 13 has an LED connected on most Arduino boards:
pinMode(13, OUTPUT);
}
void loop() {
digitalWrite(13, HIGH);            // set the LED on
delay(43);                         // wait for a second ( 1000 milliseconds)
digitalWrite(13, LOW);             // set the LED off
delay(100);                        // wait for a second
}
```

C.2 Shapiro-Wilk Normality Test in R

```
>light <- read.csv(file = “LED and Fluorescent for R.csv”, header = TRUE)
>light
>shapiro.teset(light[,1])
Shapiro-Wilk normality test
data:  light[, 1]
```

Appendix D. Tables and Figures

Table D-1. Biomass production of low-intensity white, blue and red LEDs.

LED type	Avg DW biomass (g m^{-2})
White LED	0.25
Blue LED	1.35
Red LED	1.31
Fluorescent	8.81

These values are averages of four harvests collected during the preliminary data collection. The average dry weight biomass in g m^{-2} was reported. Biomass growth was not divided by the harvest days as it gave extremely low values.

Table D-2. Shapiro-Wilk test for normality values.

Harvest	Fluorescent Treatment		LED Treatment	
	p values	significance α	*p* values	significance α
1	0.10	ns	0.32	ns
2	0.19	ns	0.83	ns
3	0.87	ns	0.94	ns
4	0.96	ns	0.07	ns
5	0.84	ns	0.95	ns
6	0.59	ns	0.92	ns
7	0.25	ns	0.21	ns
8	1	ns	0.46	ns
9	0.67	ns	0.29	ns
10	1	ns	0.78	ns
11	0.93	ns	1	ns
12	0.88	ns	0.78	ns

A Shapiro-Wilk test was performed in RStudio to assess the normality of the data. The symbol (*) signifies significance ($p<0.05$) and the symbol (ns) signifies that ($p>0.05$) there was no significance. It was found that the data was normally distributed.

Table D-3. Assessing significant difference between treatments in taxonomic composition of the class level.

Kolmogorov-Smirnov test					
16S taxa	p value	sig.	18S Taxa	p value	sig.
Alphaproteobacteria	0.13	ns	Rhodophyta	0.95	ns
Gammaproteobacteria	0.95	ns	Chlorophyta	0.36	ns
Synechococcophycideae	0.29	ns	Ochrophyta	0.73	ns
Flavobacteriia	0.56	ns	Fungi	0.05	*
[Rhodothermi]	0.057	ns	Unassigned 18S	0.27	ns
Deltaproteobacteria	0.12	ns	Labyrinthulomycetes	0.09	ns
Anaerolineae	0.52	ns	Metazoa	0.25	ns
[Saprospirae]	0.27	ns	Ciliophora	0.29	ns
Cytophagia	0.42	ns			
Unassigned 16S	0.18	ns			

A Kolmogorov-Smirnov test was performed to assess whether there is any significant difference between the taxonomic composition of the two treatments in the eukaryotic and prokaryotic communities. The symbol (*) represents significance in difference ($p<0.05$) and the symbol (ns) represents non significance ($p>0.05$). The only significantly different taxonomic composition was that of Fungi in eukaryotes.

Table D-4. Multidimensional Fuzzy Set Ordination (MFSO) table.

Variable	*P*-Value
Avg biomass	0.2560
Lab temp	0.3970
Water temp	0.6930
Salinity	0.3250
pH	0.2870

The p values represent the significance of each variable in influencing the taxonomic composition. Since $p>0.05$, the variables did not influence the taxonomic composition of the microbial mat.

Table D-5. Eukaryotic and Prokaryotic Primers Used During PCR Amplification.

Specific Regions		Forward Primer sequences [a]	Reverse Primer sequences [a]
Bacteria	V6-V8	ACGCGHNRAACCTTACC	ACGGGCRGTGWGTRCAA
	V4-V5	GTGYCAGCMGCCGCGGTAA	CCGYCAATTYMTTTRAGTTT
Eukaryotic	V4	CYGCGGTAATTCCAGCTC	AYGGTATCTRATCRTCTTYG
Fungi	ITS2	GTGAATCATCGAATCTTTGAA	TCCTCCGCTTATTGATATGC

[a] sequences are given 5'- 3'. H:[A/C/T]; N:[A/C/G/T]; R:[A/G]; M:[A/C]; W:[A/T]; Y:[C/T]. These sequences were described in more detail by Comeau, Douglas and Langille (2017) (supplementary material Table S1).