Development and optimization of algal cultivation systems

Martin Gross
Iowa State University

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Development and optimization of algal cultivation systems

by

Martin Anthony Gross

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

Master of Science

Major: Food Science and Technology

Program of Study Committee:
Zhiyou Wen, Major Professor
Lawrence Johnson
Tong Wang
Shihwu Sung

Iowa State University
Ames, Iowa
2013

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ABSTRACT

This thesis describes research done on two novel algae cultivation systems. The first system was an attached algal growth system to facilitate biomass harvest with improved biomass yield. In the attached system, algal cells were grown on the surface of a material rotating between the nutrient-rich liquid phase and the carbon dioxide-rich gaseous phase. The algal cells from the attached growth system were harvested by simply scraping off the algal biofilm. The operation conditions of the attached growth system were optimized to improve biomass productivity. A harvesting frequency of 7 days with a rotational speed of 4 rpm resulted in the highest cell productivity. Changing the CO2 content from atmospheric CO2 level (~300ppm) to 3000 ppm did not significantly change growth performance. The attached growth system resulted in a biomass productivity of 10.5 g*m^-2*day^-1. The biomass harvested from the attached system had higher carbohydrate content, but lower lipid content compared to the suspension culture system.

Other research presented in this thesis was to grow the microalga Chlorella vulgaris under simulated microgravity conditions to evaluate CO2 consumption and O2 generation rates. The effects of hydraulic retention time, gas flow rate, and CO2 concentration on algal growth were investigated. All the three factors significantly influenced CO2 consumption and O2 generation rates. A statistical response surface design was used to optimize these two parameters. The optimal conditions for CO2 consumption and O2 generation were determined to be 6.59 days hydraulic retention time, 0.153 vvm gas flow rate, and 0.80% CO2 concentration. Algae growth and CO2 consumption rates in microgravity were not significantly different than growth at earth (1 g) gravity. A hollow fibre membrane photobioreactor was also developed which enhanced CO2 consumption rates.
CHAPTER 1: INTRODUCTION

1.1 Project Description

This thesis presents a comprehensive review of algae cultivation and two research projects, including (i) development and optimization of an algae culture system that simulates microgravity conditions, and (ii) a biofilm-based cultivation system that maximizes biomass productivity while reducing biomass harvesting costs.

1.1.1 Oxygen Generation by Microalgae Under Simulated Microgravity Conditions

The United States National Aeronautics and Space Administration (NASA) is interested in developing a sustainable O\textsubscript{2} generation system for long duration space missions. Microalgae was chosen as a candidate to address this issue as they consume CO\textsubscript{2} and convert it into O\textsubscript{2} via photosynthesis. They can also be grown in a relatively high density and can produce more O\textsubscript{2} in a given area than any other known organism, which make them an ideal organism for this project.

Research was conducted on how microgravity affects microalgae cells. This was done on a novel microgravity simulating algae photobioreactor. We also conducted research to identify the optimal conditions (hydraulic retention time, inlet gas flow rate, and CO\textsubscript{2} % of inlet gas) for O\textsubscript{2} generation by the microalga Chlorella vulgaris by using a statistically-based central composite design.
1.1.2 Development of a Novel Revolving Algal Biofilm Photobioreactor (RABP) for Easy Biomass Harvest

Current large-scale microalgae production is inhibited by the large capital and operational costs. One major issue is cost effectively harvesting algae cells from a solution that is approximately 99.98% water. The approach we designed to address this issue was to grow algae on a biofilm so that the algae are naturally concentrated and can be easily harvested in situ during the culturing process. We developed a revolving algae biofilm-based photobioreactor (RABP) system for easy harvesting and maximum biomass productivity.

In our RABP system, algal cells were attached to a material that is rotating between nutrient-rich liquid phase and CO₂-rich gaseous phase for alternating absorption of nutrients and CO₂. The algal biomass was harvested by scrapping the biofilm from the surface. Compared to suspended culture systems, the new system had several advantages including, simple harvesting, improved CO₂ mass transfer, and increased light utilization. Our research focused on optimizing various operational parameters to maximize biomass productivity, including attachment material, rotational speed, harvesting frequency and atmospheric CO₂ concentration.

1.2 Thesis Organization

Chapter 2 is the literature review outlining various aspects related with microalgae culture from algal biology to mass cultivation systems. Chapter 3 is the analysis and optimization of microalgae culture in simulated microgravity conditions. Chapter 4 describes developing a novel revolving algal biofilm photobioreactor (RABP) for easy biomass harvest.
CHAPTER 2: LITERATURE REVIEW

2.1 Algae – Classification and Chemical Composition

Traditionally, algae have been classified by two major characteristics; ability to perform photosynthesis, and ability to live completely submerged in an aquatic environment (both fresh and marine). Algal cells can be single or multicellular. They also can be prokaryotes or eukaryotes. Today, algae are most commonly split into three major groups. These groups are macroalgae, cyanobacteria and microalgae.

2.1.1 Major Groups of Algae

2.1.1.1 Macroalgae

Macroalgae are commonly known as seaweed. They are macroscopic, multicellular, benthic algae. These algal species are used in food, medicine, fertilizer, and energy production. Most macroalgae are commonly grown in the wild but they can also be cultivated. They can appear red, brown or green due to different photosynthetic pigments each group possesses.

2.1.1.2 Cyanobacteria

Cyanobacteria are an ancient phylum of bacteria that obtain their energy via photosynthesis. These cells are prokaryotic and unicellular. This group of algae is unique in that they have the ability to live in terrestrial environments such as in soil and on rocks. Many cyanobacteria species form visible colonies. Cyanobacteria are considered one of the most important groups of organisms because they are credited for converting the earth’s early reducing atmosphere into an oxidizing form through photosynthesis. This triggered a drastic change in the biodiversity of life on earth. Also, according to endosymbiotic theory all
chloroplasts in plants and eukaryotic algae evolved from the endosymbiosis of cyanobacteria cells.

2.1.1.3 Microalgae

Microalgae are unicellular and can be found in solitary cells or in groups of single cells connected together. Microalgae produce approximately one-half of the oxygen generated on earth while simultaneously consuming carbon dioxide during photosynthesis. Over 200,000 species exist while only about 50,000 have been described. Over 15,000 novel compounds originate from algae biomass (27).

One interesting aspect of microalgae is that some species can be both phototrophic and/or heterotrophic depending on environmental conditions. Due to this phenomenon microalgae can also be grown in fermenters supplemented with organic carbon sources such as sugars, organic acids and alcohols. Heterotrophic microalgae culture commonly results in much higher cell densities than phototrophic culture because there is no light limitation in heterotrophic culture. (28). In some cases, mixotrophic cultures (a combination of heterotrophic and autotrophic culture) have been used. These cultures contribute to much higher cell densities than exclusively phototrophic systems (29).

The interest in microalgae stems from its unique biological properties. Microalgae can grow very rapidly, much faster than terrestrial crops. In addition to fast growth rate, algae can be cultivated anywhere that receives sunlight including areas with poor soil quality. Lastly, microalgae can have a high lipid content, which can be converted to biofuels through various techniques. Due to these properties (high growth rate, flexibility in location, and high lipid content), algae has been considered a leading contender in minimizing our need for fossil fuels.
2.1.2 Major Chemical Composition of Microalgae Biomass

The majority of microalgae biomass is made up of proteins, carbohydrates, and lipids. In general, algae biomass contains 20-30% carbohydrate, 10-20% lipid, and 40-60% protein (10). In addition to the three main components, cells can contain smaller amounts of nucleic acids and pigments such as carotenoids. In many instances, researchers have been able to manipulate these levels to favor a particular end product. For example, if nitrogen is starved, microalgae tend to produce more lipid than carbohydrate (11).

2.1.2.1 Lipids

Lipids are a very attractive feedstock for biofuel production due to their high energy density and easy upgrading to biodiesel. Lipids are generally classified into two main groups: polar and neutral lipids. Polar lipids consist mainly of free fatty acids, glycolipids and phospholipids. Neutral lipids consist mainly of triacylglycerol (TAG) but also include monoacylglycerol, diacylglycerol and sterols. In general, under optimal growth conditions algae produce predominantly polar lipids. When under stressed conditions, algae produce significantly higher concentrations of neutral lipids. Certain species have been found to contain up to 77% of total dry weight (DW) of biomass as lipids and 80% of which are neutral lipids (11).

A significant amount of research has been done to characterize the fatty acid (FA) profiles of different species of algae, because different chain lengths will have different biofuel or nutritional properties. In general, FAs can be characterized as saturated or unsaturated. Saturated FAs contain no double bonds in the carbon chain. Unsaturated FAs contain either one double bond (monounsaturated) or multiple double bonds (polyunsaturated). The major fatty acids in most microalgae cells are C16:0, C18:1, and C18:2 or C18:3. However, some species are
capable of producing FAs with longer chain lengths. Species that can produce longer chain FA (such as omega-3 polyunsaturated fatty acids) have received additional attention because of their nutritional benefits.

2.1.2.2 Proteins

As described above proteins commonly constitute approximately one-half of the dry weight in microalgae cells. However, protein levels vary greatly from species to species, from 15-71% of dry biomass. Currently, the major use of algae protein is animal feed. In 2007 30% of the world’s algae was estimated to be grown for a protein source for domesticated animals (30).

Algal protein has a very good amino acid (AA) profile. When comparing algae to the “well-balanced protein” as defined by World Health Organization (WHO) & Food and Agriculture Organization (FAO) algae is a very promising protein source (31). Table A shows that the AA profiles of many algae species compares favorably to other common animal feeds such as soybeans (30).

Table A. Amino acid profile of different algae as compared with conventional protein sources and the WHO/FAO reference pattern (g per 100 protein source)

<table>
<thead>
<tr>
<th>Source</th>
<th>Ile</th>
<th>Leu</th>
<th>Val</th>
<th>Lys</th>
<th>Phe</th>
<th>Tyr</th>
<th>Met</th>
<th>Cys</th>
<th>Try</th>
<th>Thr</th>
<th>Ala</th>
<th>Arg</th>
<th>Asp</th>
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<th>Gly</th>
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<tr>
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<td>4</td>
<td>7</td>
<td>5</td>
<td>5.5</td>
<td>6</td>
<td>6</td>
<td>4</td>
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<td>Egg</td>
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<td>5.3</td>
<td>5.8</td>
<td>4.2</td>
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<td>1.7</td>
<td>5</td>
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<td>6.2</td>
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<td>13</td>
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<td>2.4</td>
<td>4.2</td>
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<td>6.4</td>
<td>5</td>
<td>3.7</td>
<td>1.3</td>
<td>1.9</td>
<td>1.4</td>
<td>4</td>
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<td>7.9</td>
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2.1.2.3 Carbohydrates

The predominant form of carbohydrates in microalgae can be very diverse. For example, many green algae have plant-like carbohydrate profiles, composed of mostly cellulose and starch, while diatomic algae accumulate laminaran, fucoidin and maannitol. Carbohydrate content in algae may be as high as 75% dw of cells with starch accounting for 60% dw (32). Starch content in algae can be readily manipulated by macronutrient (sulfur, phosphorus and nitrogen) level limitation. Cyclohexamide has also been used to block protein generation and increase carbohydrate levels (32). Algal carbohydrates could serve two main purposes. The first is to use as a feed source for livestock or fish. One concern with this approach is that the cellulose cannot be utilized, as non-ruminant animals do not have cellulose degrading enzymes. The second use of algae carbohydrates is to use them as a sugar source in an alcohol fermentation process.

2.1.2.4 Nutraceuticals

Over the past decade, microalgae has been investigated for various nutraceutical products. The major nutraceuticals produced by algae include: omega-3 fatty acids, improves both brain and heart health; carotenoids, which have have antioxidant properties and can contribute to eye health; sulfated polysaccharides which have have anticoagulant, anti-tumour and antiviral activities; fucoxanthin which have antioxidant and anti-obesity effects; and sterols which show anti-diabetic properties (34). Of these compounds omega-3 fatty acids and carotenoids have been the most frequently investigated.
2.2 Algae Mass Cultivation Systems

2.2.1 Factors Affecting Algal Culture

Microalgae cultivation requires specific environmental conditions including temperature ranges, light intensities, mixing conditions, nutrient composition, and gas exchange. Different species have different requirements. The following gives a brief overview of these algae cultivation requirements.

2.2.1.1 Temperature

Like many microorganisms, growth rate of microalgae generally increases exponentially with increasing temperatures until it reaches an optimum. Once this level is reached the growth rate declines with further temperature increases. This is very important when considering outdoor culture where the ability to control temperature is often limited and is often dictated by ambient temperatures and solar irradiance. Algae culture temperatures fluctuate up to 20 °C between day and night (1). This fluctuation makes it very difficult to efficiently maintain the optimum growth temperature.

In general, temperatures below the optimal range will not kill the algae until the water freezes. However, temperatures above the optimum can kill the algae. Algae have been shown to be particularly fragile to high temperatures in times of darkness (2). Due to the impact that temperature variation has on algae culture, it is important to select an appropriate species for the environmental conditions it will be growing in.
2.2.1.2 Light

Sunlight or artificial light is the main energy source for phototrophic algal cells. The availability of light to the algae is crucial for algae cultures. At very low light intensities the net growth of algae is also very low. The photosynthetic activity of algal cells increases with light intensity until reaching a threshold point, where further increases in light intensity no longer increases photosynthesis (light saturation point). Intensities higher than this point can damage light receptors in the chloroplasts of the cells and decrease the photosynthetic rate, which is known as photoinhibition (3).

Mutual shading is a common problem for high cell density culture because the cells closest to the surface of the liquid receive the majority of the available light and the cells below are left with very little available light. Microalgae are adapted to be very efficient at harvesting light even at low light intensities, which gives them the ability to reach very high cell densities. Extensive research has been conducted to create culture conditions where light can be evenly distributed. To achieve even light distribution, parameters, such as liquid depth and mixing, play key roles (4,5).

2.2.1.3 Mixing

Mutual shading is a common problem limiting high cell density culture and mixing plays an important role to ensure all cells in the culture receive an equal amount of sunlight. Mixing also plays a role in allowing cells to better uptake nutrients by minimizing the film boundary layer surrounding the cell.
2.2.1.4 Nutrients

Algae require various inorganic nutrients in order to achieve a healthy culture with high biomass productivity. These nutrients include macronutrients, vitamins and trace elements. There is still much debate on what the ideal levels of these nutrients are. In most cultures the macronutrients required are nitrogen, and phosphorus at the ratio of 16N: 1P (6). In most cultures, however nutrients are often added in excess in order to minimize nutrient limitation. Typically, the trace metals used are chelated salts of iron, manganese, selenium zinc, cobalt, and nickel (7).

2.2.1.5 Gas Exchange

Approximately 45-50% of algae cells are made up of carbon, thus algae require continual intake of carbon (8). If a supplementary carbon source is not supplied cell growth will quickly become limited. In autotrophic cultures, carbon is most commonly added in the form of CO$_2$, which is usually bubbled though sparging stones or perforated pipes but in some cases CO$_2$ can be supplemented through floating gas exchangers or hollow fiber membranes.

During photosynthesis, CO$_2$ is consumed and O$_2$ is released into the liquid. High O$_2$ concentrations will cause photo-oxidative damage to chlorophyll which will inhibit photosynthesis and reduces productivity (9). In systems, such as open ponds, this is a non-issue due to the large surface area for O$_2$ mass transfer to occur but in systems, such as closed photobioreactors, there are additional compartments known as gas exchange chambers that aid gas exchange to reduce the dissolved O$_2$ levels.

CO$_2$ is also often used to maintain a steady pH in the culture system. As CO$_2$ is absorbed into the liquid phase it is converted into carbonic acid, which effectively lowers the pH. As algae
consume the carbonic acid the pH rises. By controlling CO₂ levels, the pH can be controlled very effectively.

2.2.2 Open Pond Culture Systems

Open ponds is the oldest system for mass cultivation of microalgae. In this system, the pond is usually between 1-100cm deep. The most common open pond culture system consists of a pond in the shape of a raceway and the liquid is circulated around the pond by a paddle wheel. This system mimics the way algae grow in their natural environment. The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner. These raceways vary from a few feet in length to thousands of meters. Due to scalability and low cost of building these systems, they are the most popular cultivation system (10).

In open ponds, temperature is very difficult to control and usually fluctuates in a diurnal cycle. Temperature is also dependent on the season. One other issue with open ponds is evaporative water loss can be significant. Because of significant losses to atmosphere, CO₂ utilization is also much less efficient than in photobioreactors. The biomass concentration generally remains low between 0.1-1.5 g L⁻¹. This is mainly because raceways are poorly mixed and optimum light intensity cannot be achieved (11).

In addition to the low cell density, open ponds are often subjected to significant contamination by native species, and thus, are suitable for only a small number of algae species. Generally, those species can tolerate extreme environments (such as high saline or alkaline) so invasive species cannot outcompete the desired species (12).

Raceways are not the only type of open-pond system. Circular ponds are also used to cultivate algae. This cultivation system is more popular in wastewater treatment. These ponds
have a centrally located rotating arm that mixes the algae (13). There are also unmixed open ponds for algal culture. Unmixed ponds allow the algae to settle to the bottom of the reactor vs. a mixed pond that keeps the algae suspended. These ponds are usually used to grow specific species of algae and generally have very low productivities (14).

2.2.3 Closed Photobioreactors

Closed photobioreactors (PBR) were established to overcome the major issues associated with open-pond cultures, including low cell densities, contamination issues, evaporation, environment regulation and high land requirements. PBRs are very versatile and can be located both indoors with artificial light and outdoors with natural light. PBRs are very attractive due to the previously stated benefits, however compared to an open-pond system PBR’s require higher capital investments and have issues with scalability. Many unique PBRs have been developed; each has advantages and disadvantages. The most popular of these systems will be discussed further.

2.2.3.1 Tubular PBR

The most widely used PBR is of tubular design, which has a number of clear transparent tubes, composed of either glass or plastic (11). The culture is circulated through the tubes where it is exposed to light for photosynthesis, and then returns to a reservoir. The tubes are usually 10 cm or less in diameter, which allows for sufficient sunlight penetration. The algal biomass is prevented from settling by maintaining highly turbulent flow within the reactor with either a mechanical pump or an airlift pump (11). These tubular reactors can be run either vertically or horizontally. Many of these systems require a gas exchange chamber to reduce the elevated dissolved O₂ levels in the liquid.
2.2.3.2 Helical PBR

Helical PBRs are composed of parallel transparent tubes coiled around a cylinder. The helical shape is effective in increasing the surface area sunlight is able to reach. This in turn can increase productivity. These systems increase productivity compared to tubular PBR’s but due to the unique shape and increased cost, it has not been as popular. (15)

2.2.3.3 Airlift PBR

An airlift PBR can be a simple vertical cylinder made out of transparent glass or plastic. On the bottom of the tube is an air inlet. This air inlet bubbles air through the column, which provides mixing and gas exchange. These systems have aerial productivity compared to algae grown in a similar tubular reactor.

2.2.3.4 Flat Panel (Flat Plate) PBR

Flat panels, also known as flat plate PBRs, are essentially rectangular boxes composed of translucent glass or plastic. Air is bubbled from the bottom, which provides sufficient mixing and gas transfer. These reactors can have baffles running horizontally inside the reactor to aid mixing and gas exchange efficiencies. Because of the increased surface area for light to reach algae cells, flat panel PBRs can have significantly higher productivity than an open-pond system. For example, in one study, cell densities were compared between a flat panel PBR and an open pond using the algae *S. platensis*; the flat panel PBR produced 2.15 g L$^{-1}$ d$^{-1}$), and under similar conditions in an open pond produced 0.15 g L$^{-1}$ d$^{-1}$ (15).
2.2.4 Heterotrophic Culture Systems

The majority of algae gets energy from light and is strictly phototrophic. Some algae species are able to utilize organic substrates as an energy source. Generally, heterotrophic algae cultivation is cultivated in fermentators where a high degree of culture manipulation can be performed. This cultivation method can have many benefits; for example, it can utilize well-established fermentation technologies, high degree of process control, good production repeatability, elimination of light limitation, and lower harvesting costs. (16)

In most cases, heterotrophically grown algae can contain a much higher lipid concentration than phototrophically grown algae of the same species. One study showed that *Chlorella* sp. cells accumulated 55.2% lipids when heterotrophically grown and only 14.6% when phototrophically grown (17). Although heterotrophic algae culture usually results in algal biomass with higher lipid content, the fermentation vessels are usually very expensive. For this reason, heterotrophically grown algae has not been considered feasible for biodiesel production but has shown great promise in producing high value products such as omega-3 fatty acids (18).

2.2.5 Attached/biofilm-Based Culture Systems

An attachment/biofilm-based algae culture system is very different than other cultivation systems. In most cultivation systems, such as open ponds or PBRs, microalgae cells are suspended in the liquid and the cultivation system is designed to keep these cells from attaching or settling on surfaces. Biofilm-based culture systems are the complete opposite. These systems encourage algae to settle or attach on a desired surface. By allowing cells to attach, algae naturally concentrates and are easily harvested. Thus, the costly and energy intensive harvesting practices can be removed. Algal biomass harvested from an attached surface has water content...
similar to that of algae following centrifugation (19). Compared with either open ponds or photobioreactors, attached growth systems have not been well studied.

2.2.6 Harvesting and Dewatering Systems

Cultivation of algae always requires the downstream processing steps of harvesting and dewatering. This is because the standard algae culture has approximately 1 g of algae in 5,000 g of water. So the standard algae cultivation has approximately 99.98% water and only 0.02% algae (20). Due to the high water content, harvesting and dewatering algae can be very expensive and can account for 30% of total production costs (21). In general, the higher the starting concentration of algae, the lower the harvesting cost.

Most commonly, harvesting is done in two steps. These are concentrated to 1-5% solids by flocculation and sedimentation techniques. The cells are further concentrated to 10 to 25% via filtration and centrifugation.

2.2.6.1 Flocculation

Flocculation is the agglomeration of algae cells. This can cause cells to fall out of suspension. An important aspect of a well maintained culture system is to not allow this to happen unless the cells need to be harvested. In general, algae cells carry negative charges on the surface, which can cause the cells to form large clumps or flocs when neutralized (22). These flocs can be more readily separated from the growth medium. Many different means have been investigated to induce flocculation of algal cells.

One method is chemical flocculation. Inorganic molecules, such as aluminum sulfate, ferric sulfide or lime, can neutralize the cells charge or reduce it and thus, the cell form clumps
However, the use of these chemicals leads to buildup of undesirable compounds in the harvested cells which leads to issues in using the algae as animal or human food. One other method of chemical flocculation is the use of highly charged organic molecules known as polyelectrolytes. In addition to neutralizing the charge these polyelectrolytes can also physically link cells together, which helps form very stable flocs (24). They also do not have the toxicity that many of the inorganic molecules cause. This makes them a much more attractive chemical option for flocculation.

Electroflocculation is the process of forming flocs by introducing an electric current into the culture. This method is very effective resulting in very high separation efficiencies. Electroflocculation can also be done in very large cultures without consuming a high amount of electricity. This has made this method increasingly popular.

Bioflocculation is accomplished without adding any external material or force to the culture. This can be stimulated by limiting nitrogen or altering pH and dissolved O₂ levels (25). Although this method does not need any additional cost for flocculants, it generally requires a longer time and is unreliable.

2.2.6.2 Dissolved Air Flotation

Dissolved air flotation (DAF) was developed for sewage treatment and was adapted by the algae industry. DAF is often coupled with some form of flocculation treatment. The cells are usually flocculated first and then air is bubbled through the liquid causing the flocs to float to the surface for easier harvesting. The bubble geometry can play a large role in the efficiency of DAF (26).
2.2.6.3 Filtration

Filtration is the action of flowing particles onto a screen, which separates substances according to particle and screen pore size. Filtration can be an effective method for harvesting larger strains of algae. The algae *Spirulina sp.* can be simply filtered from the culture medium. Although filtration can be inexpensive and an effective method for harvesting algae, it has the major issue of filter fouling and clogging, which limit its application in large-scale cultivation.

2.2.6.4 Centrifugation

Centrifugation is a widely used separation method used in many liquid-solid separations. This technology utilizes centrifugal forces to separate substances of different densities. Separation efficiency is dependent upon the size of desired algal species. Although very effective, centrifugation is considered unfeasible in large-scale algae culture facilities due to the high capital and operational costs.

2.2.6.5 Attachment/Biofilm-Based Systems

In the recent years, several new cultivation technologies have been developed. Various systems have been developed to solve various issues in different stages of algae cultivation. One system that has been developed to address the issue of high harvesting costs is the attachment/biofilm-based system. In these culture systems, algae is encouraged to attach to a substrate. Once attached, cell proliferation occurs and a biofilm forms. This biofilm is ideal for harvesting because it is already held together and can be easily scraped from the substrate and separated from the culture medium. In one study, harvested cells had only 90% water content after harvesting (19). The water content was similar to the water content post-centrifugation.
(80%-95%). Thus, the biofilm-based system could skip the costly harvesting techniques currently used.

2.3 Biofuels from Microalgae

2.3.1 Biodiesel

The most prevalent way of making biofuel from microalgae is to produce biodiesel from algal lipids (oil) through transesterificaiton (11). Compared to plant-based oils, algae oil has relatively high carbon and hydrogen contents and low oxygen content. These characteristics make algae an attractive biodiesel feedstock because they lead to a fuel with high energy content, low viscosity, and low density. In most cases, biodiesel can be directly combusted in a standard diesel engine without the issue of blending with regular diesel. Transesterification of alcohols and lipids are the chemical reaction required to produce biodiesel, with glycerol being produced as a byproduct (43).

Total lipid levels are commonly the most important factor in considering the applicability of algae biomass for biodiesel production. However, considering only the total lipid content is misleading as only the neutral lipids are converted to biodiesel during transesterificaiton. The best algal feedstock for biodiesel production is a biomass high in neutral lipids.

2.3.2 Ethanol

Certain strains of algae are capable of producing high levels of carbohydrates, such as starch, which is an ideal fermentative substrate. High ethanol productivity can be accomplished from fermentation of algae biomass (33). The production of ethanol from algae is done by
breaking the cells via mechanical means followed by dissolving carbohydrates with either water or solvent. Once the carbohydrates are extracted, traditional methods, such as scarification with enzymes and fermentation with bacteria or yeasts, are used to produce ethanol. Finally, a distillation step concentrates the ethanol.

In addition to producing ethanol via fermentation of algal starch, ethanol can be also be produced directly by algae. This process includes genetically modifying an algae species so it secretes ethanol into the medium. This process has the advantage of not having to harvest the actual algae cells but rather separating the ethanol from the culture medium. This technique has been adopted by the algae company Algenol. Algenol projects that their technology can produce 9,000 gallons of ethanol per acre per year. In comparison, corn ethanol produces approximately 890 gallons per acre per year. They project the production cost to be only $0.85 per gallon.

2.3.3 Biogas

Anaerobic digestion is widely used to treat various carbonaceous feedstocks in an effort to extract value and energy from otherwise difficult to utilize wastes. The product of anaerobic digestion is biogas, which is composed of methane and CO$_2$. Methane can be used to produce heat and electricity. The CO$_2$ can be recycled back into an algae culture system.

The use of raw algal biomass for biogas production can avoid the expensive biomass-harvesting and oil-extraction processes used in algal biodiesel production, and significantly reduce production costs associated with algae processing. Anaerobic digestion of the cell residues after lipid extraction can be a viable use of the residual biomass.

Although anaerobically digesting algae biomass is a low-cost process overall, it does not produce a very high value product. In addition, previous research shows that only 50-70% of
algae biomass can be converted to biogas (35). Due to these realities anaerobic digestion is only considered to be a viable secondary treatment option of algae biomass after lipid extraction or other processing.

2.3.4 Bio-Oil and Syngas

Algae biomass can be converted into bio-oil or syngas through thermochemical processing methods. Depending on temperature, pressure and oxygen content, algae biomass can be converted into various solid, liquid, and gas products. The three most common thermochemical processing techniques are gasification, pyrolysis, and hydrothermal liquefaction (48).

In the gasification process, algae biomass is converted into a gaseous mixture called syngas (49). The major compositions of syngas are CO₂, CO, CH₄, H₂ and small hydrocarbons. This syngas mixture can be combusted to produce heat or electricity or be either biologically upgraded (syngas fermentation) or catalytically upgraded (Fischer-Tropsch process) into various liquid biofuels. Gasification usually occurs at temperatures between 750-900 °C and oxygen starved conditions. A major issue in the gasification of algae biomass is the high moisture content in the algae cells which will increase the energy requirement of the process.

Pyrolysis is the process of heating biomass at temperatures between 400-500°C in the absence of oxygen and ambient pressures. The products of pyrolysis are primarily bio-oil and char. Bio-oil can be directly combusted or be upgraded into various transportation fuels. Char can be used as a fertilizer on agricultural fields. Pyrolysis also requires dry feedstocks.

Hydrothermal Liquefaction is the process of heating moist biomass to temperatures of approximately 300°C and pressures of 10 MPa. If an alkali or alkaline catalyst is added to the
mixture, it is known as solvolysis. Although processing at high pressures require more specialized equipment hydrothermal liquefaction has the benefit of using wet biomass.

2.4 Non-Fuel Products from Microalgae

2.4.1 Animal Feed

In the United States, a majority of livestock feed is derived from corn or soybean. In recent years, algae has received increased attention as an alternative feed source that can reduce the demand for these two crops. Microalgae have the benefit compared to conventional feed crops in that it can be grown on non-fertile land. Another benefit is that many species have protein content that exceeds that of both soybean- and corn-based products.

In 2007, approximately 30% of all cultivated algae were grown for animal feed (38). In the coming years if large-scale algae cultivation for biofuels is successful, a massive quantity of lipid-extracted algal residue must find a market. The most obvious market is animal feed applications. The most promising algal feed applications are poultry and aquaculture (47). There is also interest in using it as a cattle, swine, pet, and various other animal feeds.

World aquaculture is expected to dramatically increase in the upcoming decade due to shortages in wild fish populations. As aquaculture becomes more popular, the need for feed will also increase. Algae can serve as an optimal feed for various forms of aquaculture including fish, crustaceans, and molluscs (47). A common provider of both protein and lipid for aquaculture is fish meal which price is heavily dependent on capture fisheries and wild fish populations. Algae can provide an alternative protein and lipid source for these animals.
2.4.2 Human Consumption

Microalgae were first consumed by the Chinese nearly 2000 years ago to survive during famine (37). In modern times, humans can consume whole cell algae in tablets, capsules, liquids, and incorporated into various other products, including pastas, drinks, gums and snack foods. The commercial applications are dominated by a few genus of algae. *Spirulina* and *Chlorella* dominate the world's production in the consumption of whole cell algae (37). The largest microalgae human food production site is owned by Earthrise Farms. This facility is located in California, USA and stretches over an area of 440,000 m².

A majority of the algal products that humans consume are produced from a specific portion of algae, not whole cells. These products are commonly taken as a supplement in a pill or capsule. The most common algal compounds consumed are omega-3 fatty acids and β-carotenes, both of which are produced by special algae at very specific growth conditions. *Dunaliella salina* is the most common species to produce β-carotene because of its ability to produce up to 14% dw of this product. The most common species for producing the omega-3 fatty acids, eicosapentaenoic acid (EPA), are *Nannochloropsis*, *Phaeodactylum*, and *Nitzschia* (18).

2.4.3 Fertilizer

Algae can be grown for a specific purposes such as high lipid content for biodiesel production. Once the lipids are extracted the remaining residual biomass can be further processed for higher value products or it could be simply used as fertilizer (46). Algae residual biomass is commonly high in nitrogen, phosphorus, and other compounds that benefit terrestrial plant growth. This makes it very attractive as a fertilizer.
2.5 Pollution Control

2.5.1 Water Pollution Control

Algae can be grown in municipal sewage treatment plants along with various bacteria species (45). Organic sewage that is broken down by bacteria is used by algae in their photosynthesis and growth. Algal growth rate can be very high and commonly algae must be removed from the treatment plant periodically. In addition to degrading the organic compounds, algae have also been used to consume various heavy metals (39).

In addition to municipal sewage treatment plants, algae can be used to treat agricultural wastewater. In uncontrolled situations where agricultural wastewater high in N and P is allowed to enter natural water supplies, it causes massive algal blooms. These blooms will lead to utilization of all CO$_2$ in the water supply, then as algae die off bacteria digest the algae and consume all of O$_2$ in the water; this is known as hypoxia (42). This event is what causes the hypoxia in the United States’ Gulf Coast that leads to massive loss of aquatic life. In controlled systems, agricultural wastewater can be stored in wastewater storage ponds and algae can be grown there to consume large quantities of N and P before the water leaves for natural water supplies (42).

Microalgae can also be used for pollution control in many other ways (40). For example, algae growth can be used as an indicator to assess the pollution level in a water stream. In one example, algae was grown in a water stream coming out of an aluminum coating plant. Studies were done to develop a correlation between algae growth and toxicity of the pollutants being
expelled from the plant. Similar methods have been developed by oil and pharmaceutical companies. Each method showed an inexpensive way to monitor pollution levels being expelled.

2.5.2 Air Pollution Control

Microalgae can serve as a valuable asset to remove unwanted compounds from the air. The most common compound that algae can remove from the air is CO\textsubscript{2}, which is an important greenhouse gas. Algae consume CO\textsubscript{2} and release O\textsubscript{2} during photosynthesis. Microalgae consume CO\textsubscript{2} more efficiently than any other group of organisms in the world (44). Because of their high photosynthetic rates, microalgae have been extensively explored to consume CO\textsubscript{2} from various production plants such as coal power plants and corn ethanol plants. In some cases, an algae production facility has been co-located with a waste CO\textsubscript{2} producer to utilize the waste CO\textsubscript{2} stream (41). In the coming years if governments issue credits to lower CO\textsubscript{2} emissions, microalgae could be one of the frontrunners for CO\textsubscript{2} removal.

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CHAPTER 3: DEVELOPMENT OF A BIOFILM-BASED MICROALGAE CULTURE SYSTEM FOR EFFICIENT BIOMASS HARVESTING

Abstract

Microalgae has been mainly cultivated in open ponds or closed photobioreactors in which algal cells were grown in suspension and harvested through sedimentation, flocculation, filtration and/or centrifugation. The objective of the present research is to develop an attached algal growth system to facilitate biomass harvest with improved biomass yield. In the attached system, algal cells were grown on the surface of a material rotating between nutrient-rich liquid phase and carbon dioxide rich gaseous phase. Using *Chlorella vulgaris* as the model algal species, 16 materials were tested for suitability as attachment materials for algal growth. Cotton duct resulted in the best cell attachment in terms of durability, attachment and economy. The algal cells from the attached growth system were harvested by simply scraping off the algal biofilm.

The operation conditions of the attached growth system were optimized for improved biomass productivity. A harvesting frequency of 7 days with a rotation speed of 4 rpm resulted in the highest cell productivity. CO₂ content changing from atmospheric CO₂ level (~300ppm) to 3000 ppm did not result in a significant growth performance change. The attached growth system resulted in a biomass productivity of 10.5 g m⁻² day⁻¹. The biomass harvested from the attached system had higher carbohydrate content, but lower lipid content compared to the suspension culture system.
3.1 Introduction

Microalgae are a promising feedstock for biofuels and various other bio-based products (Chisti, 2007; Hu et al., 2008). They are an ideal feedstock for liquid transportation fuel production due to their high biomass productivity coupled with the ability to store a high concentration of lipids within their cells. Typical algae culture can produce 150-400 barrels of oil per acre per year, which is 30 times greater than what can be produced by typical land-based oil crops (Baum, 1994). The algal lipids can be easily converted into biodiesel (Mata et al., 2010).

In addition to algae’s potential as a biodiesel feedstock, it shows potential as a feedstock in various other biofuel applications. These applications include ethanol production via fermentation (Wang et al., 2011), methane production by anaerobic digestion (Gunaseelan, 1997) and crude bio-oil and syngas production from various thermochemical processing methods (Yang et al., 2004). In addition to the diverse applicability in biofuels, microalgae can also be used as a valuable feedstock for fertilizers, nutraceuticals, and livestock or aquacultural feed (Mulbry et al., 2008).

Despite the numerous applications for algae biomass, current large-scale algae cultivation is not commercially viable due to the high capital and operating costs. In 2008, the US Department of Energy released the National Algal Biofuels Technology Roadmap that outlined three main bottlenecks that have slowed the commercial production of algae biomass. One major issue identified was the high costs associated with harvesting and dewatering algae biomass. Algae harvest and dewatering contribute up to 30% of total costs (Molina Grima et al., 2003).

Current algal cultivation has been mainly performed in open ponds or closed photobioreactors in which algal cells are suspended in liquid and harvested through
sedimentation, flocculation, filtration and/or centrifugation devices. Due to the issue with separating large volumes of water from algae, there is a growing interest in developing biofilm-based cultivation methods that allow algae to attach to a substrate and form a biofilm. Attachment-based cultivation systems have been less studied than suspended algae systems even though they have received increased interest in the past five years. Examples of these attachment-based systems are outlined in Table 1. One major benefit of these attachment-based systems is that harvested biomass typically contain 80-95% of water, much higher than that in suspension culture systems (Johnson and Wen, 2010).

We have developed a Revolving Algal Biofilm Photobioreactor (RABP). In this system, algal cells attach to a material that is rotating between nutrient-rich liquid phase and a CO\textsubscript{2}-rich gaseous phase for alternating absorption of nutrients and CO\textsubscript{2}. The system keeps the algal cells fixed in place and brings the nutrients to the cells, rather than suspending the algae in a culture medium. The algal biomass can be harvested by simply scrapping the biofilm from the attached surface with a biofilm harvesting squeegee. Compared to suspended culture systems, the proposed system has several advantages: (i) the biomass can be in-situ harvested during the culture process, rather than using an additional sedimentation, flocculation filtration or centrifugation unit; (ii) the culture can enhance the mass transfer by directly contacting algal cells with CO\textsubscript{2} molecules in gaseous phase, while traditional suspended culture systems rely on the diffusion of CO\textsubscript{2} from the gas phase to the liquid phase, which is often limited by gas-liquid mass transfer; (iii) the culture system needs a small amount of water and land area by utilizing the unique design that only requires the bottom of the reactor system to be placed in the liquid, which allows for algae cultivation to maximize area by growing vertically vs horizontally. In
addition, this reactor system is adaptable and can retrofit a standard raceway system to use existing infrastructure to cultivate algae.

The aim of this work was to optimize the RABP system for improved biomass productivity and to accomplish this a thorough evaluation of the RABP performance was performed. The major parameters evaluated included type of attachment material, harvest frequency, rotational speed, and CO₂ concentration. A compositional analysis (lipid, protein, carbohydrate, ash) was conducted to compare the biofilm based system and standard suspended cultivation system. Fatty acid and amino acid analyses were also conducted.

Table 1 Comparison of Various Attachment-Based Cultivation Systems.

<table>
<thead>
<tr>
<th>Cultivation Method</th>
<th>Biomass Productivity (g m⁻² day⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>****</td>
<td></td>
</tr>
<tr>
<td>Carrageenan and alginate matrix</td>
<td>NA</td>
<td>(Hameed and Ebrahim, 2007)</td>
</tr>
<tr>
<td>Rotating disks of aluminum</td>
<td>NA</td>
<td>(Torpey et al., 1971)</td>
</tr>
<tr>
<td>Rotating polystyrene</td>
<td>2.2</td>
<td>(Przytocka-Jusiak et al., 1984)</td>
</tr>
<tr>
<td>Algal Turf Scrubber</td>
<td>5.0-20.0</td>
<td>(Adey et al., 1993)</td>
</tr>
<tr>
<td>Rocking polystyrene</td>
<td>2.59</td>
<td>(Johnson and Wen, 2010)</td>
</tr>
<tr>
<td>Rotating algae system</td>
<td>20-31</td>
<td>(Christianson and Sims, 2012)</td>
</tr>
<tr>
<td>Polycarbonate slides</td>
<td>8.7</td>
<td>(Bruno et al., 2012)</td>
</tr>
</tbody>
</table>

3.2 Materials and Methods

3.2.1 Cell Strain and Subculture

The microalgae *Chlorella vulgaris* (UTEX 265) was used. The algal cells were maintained in an agar slant and transferred to liquid Bold's Basal Medium (Bischoff and Bold, 1963) in 250-mL Erlenmeyer flasks, each containing 100 mL medium. The media was sterilized with an autoclave prior to use at 121°C for 15 min. The flasks were incubated at 25°C in an orbital shaker set to 150 rpm. The light intensity was adjusted to 100 µmol s⁻¹ m⁻² with
continuous illumination. The subcultured cells were used as inoculum in the following experiments.

3.2.2 Evaluation of Attachment Material

Sixteen materials were evaluated as attachment materials for algae (Table 2). An apparatus based on a rocker-shaking mechanism was used for testing the cell attachment performance. The detailed description of the system was reported previously (Johnson and Wen, 2010). In short, the material were cut into a 4”×4” sheet and fixed on the bottom of a growth chamber (6”×6”) made of plexiglas. The chamber was incubated with 250 ml algal cell suspension (from the subculture flasks) with an initial concentration of 4 g/L (DW). The chamber was placed on a rocker shaker with rocking motion at 30 tips per minute. The surface of the supporting material was alternately submerged into the culture medium that provided nutrients for algal growth and then exposed to illumination that provide light for algal photosynthesis. The growth chamber was continuously illuminated with cool white fluorescent lights at 100 μmol s⁻¹ m⁻². The culture temperature was 25°C. This process was repeated until all 16 materials were analyzed. The best material was selected based on algae attachment, cost, and durability.
3.2.3 RABP System Design and Optimization

3.2.3.1 RABP Set-Up and Operation

The best attaching material (in terms of biofilm growth, durability, and cost) screened from the rocker shaking apparatus was further used when fabricating the Revolving Algal Biofilm Photobioreactor (RABP). As shown in Figure 1, the attaching material was designed in a triangle configuration and rotated at a certain speed. One end of the triangle was submerged into the culture medium, while the rest of the surface was exposed to air. Algal cells attached to the material alternatingly absorbed nutrients from the liquid medium and CO₂ and sunlight from the gas phase. The algal biomass was harvested by scraping the biofilm from the revolving attached surface with a harvesting squeegee. Three identical RABP systems were used. Each system had a growth medium reservoir with a 1 L capacity and a revolving fabric with a surface length of 58 cm and a width of 15 cm for a total surface area of 870 cm².

Figure 1 Basic RABP Diagram
The RABP system was placed in an airtight plexiglass chamber to control CO\(_2\) concentration in the gas phase. An artificial light source with a light intensity of 100 \(\mu\)mol s\(^{-1}\) m\(^{-2}\) was used for continuous illumination (24 hr/day). The temperature was maintained at 25\(^{\circ}\)C. A gas flow rate of 250 mL/min was constantly pumped through the chamber to ensure fresh air was available continuously. To initiate attached algal growth, the reservoir was inoculated with a 1-L suspended algal cell culture with 2 g/L cell density. The fresh material was constantly rotated through the liquid culture and suspended cells gradually attached to the surface of the material. The reservoir was then run in continuous mode at 5-day hydraulic retention time (200 mL of medium was replaced daily) for 14 days. The cells were then harvested, and this was considered initial harvest, and later harvests were considered regrowth.

The biofilm was harvested by scraping with a rubber blade. After harvesting, algae cells remaining on the attachment material provided inoculum for the next cycle of cell regrowth. The RABP system was run at different operational conditions (harvest frequency, rotating speed, and CO\(_2\) concentration) and was repeated until three consecutive harvests resulted in constant biomass yield (steady state), and was then switched to the next condition. The harvested biomass was freeze-dried and stored for later analysis.

3.2.3.2 Optimization of RABP Operational Parameters.

To achieve higher biomass productivity from the RABP system, the effects of harvest frequency, rotation speed and CO\(_2\) concentration in the growth chamber were investigated. In the investigation of optimal harvest frequency, the biofilm was harvested after 3, 5, 7, and 10 days at 0.667 rpm rotation speed and atmospheric CO\(_2\) concentration. In the assessment of optimal rotational speed on RABP productivity, the RABP was rotated at 0.33, 0.67, 2, 4, and 6 rpm with
7-day harvest frequency and atmospheric CO$_2$. In the investigation of optimal CO$_2$ concentration on RABP productivity the CO$_2$ content varied from atmospheric level (0.038%) to 1, 2, and 3% with a 7-day harvest frequency and 4 rpm rotational speed. ANOVA analysis was conducted to identify significance.

3.2.4 Analyses

To determine moisture content, the harvested biomass was directly measured for its wet weight and then freeze-dried for its dry weight. The ash content of the biomass was quantified by heating the biomass in a furnace to 550$^{\circ}$C for 6 h. The remaining biomass after heating was considered ash. The lipid from the freeze-dried biomass was extracted and quantified according to the Bligh and Dyer method (Bligh and Dyer, 1959). To determine the fatty acid composition, the procedure for fatty acid methyl esters (FAME) preparation was followed using previous protocols (Pyle et al., 2008). The FAME were then analyzed by using a Varian GC-450 gas chromatograph equipped with a flame ionization detector and a SGE SolGel-Wax column (30 m $\times$ 0.25 mm $\times$ 0.25 $\mu$m). The fatty acids were identified by comparing the retention times with fatty acid standards and quantified by comparing their peak areas with that of the internal standard (C17:0) (Liang et al., 2011). The total nitrogen content of the biomass was determined using the Dumas method using a Rapid NII Nitrogen Analyzer (Elementar Americas Mt. Laurel, NJ, USA) (AOAC, 1995). The crude protein content was estimated by multiplying TKN by the conversion factor of 6.25. The amino acid profile was determined according to the method described previously (Pyle et al., 2008). The carbohydrate will be estimated by subtracting the total lipid, total protein and ash contents from the total weight of dried biomass.
Biomass samples were also collected from the 16-L flat panel photobioreactor running at day 5 hydraulic retention time (HRT), light intensity 100 µmol s\(^{-1}\) m\(^{-2}\), temperature 25\(^{0}\)C and aeration rate of 1 L/min with 2.50% CO\(_2\), the chemical composition from this suspended culture were also analyzed for proximate comparison.

### 3.3 Results and Discussion

#### 3.3.1 Screening of Attachment Materials

Table 2 summarizes the results from the attachment material test. This test qualitatively analyzed 16 different materials that were believed to be suitable for our RABP cultivation system. These materials were analyzed by using three criteria (algae attachment performance, material durability, and cost). As shown in Table 2, the best cell attachment occurred with armid fiberglas, chamois, and cotton duct. Armid fiberglass achieved good cell growth and durability but was prohibitively expensive to be considered for further research. Chamous also achieved good growth but began to degrade after 14 days of cultivation. Thus, cotton duct was selected due to its good cell growth, good durability, and low cost.
Table 2  Evaluation of Different Materials for Attached Growth

<table>
<thead>
<tr>
<th>Material</th>
<th>Attachment</th>
<th>Durability</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muslin Cheesecloth</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Armid Fiberglass</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>PTFE Coated Fiberglass</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Chamous</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Microfiber</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Synthetic Chamous</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Fiberglass</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Burlap</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Cotton</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Velvet</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tyvek</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Poly-Lactic Acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Abrased Poly-Lactic Acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vinyl Laminated Nylon</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Polyester</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

The attachment was defined as: +++ completely covered with biofilm, ++ partially covered with biofilm, + no biofilm attachment. Durability (how well material resists deterioration during cultivation): +++ lasts > 2 months before deterioration starts, ++ lasts 1-2 months before deterioration starts, + lasts < 1 month before deterioration starts. Cost (per square foot): +++ <$0.50, ++ $0.50-1.50, + >$1.50).

Christianson and Sims (2012) showed that cotton (rope) was the best material used in their biofilm-based system. We speculate that there was an attraction between the algae cell wall and the cotton fibers. This attraction could be that the negative charge associated with the algae cell wall is drawn to the cotton fabric, which has a high surface energy.

Christianson and Sims (2012) also found cell attachment can be affected by material thread count. This led us to do additional material tests on four different cotton materials to identify optimal cotton fabric. These cotton fabrics were cotton duct, cotton rag, cotton denim, and cotton corduroy. Each cotton fabric was run on the RABP system. The cultures were run for 14 days and the best material was selected based on algae attachment and durability. Cotton duct showed the best productivity and had exceptional durability compared to cotton rag, denim and
corduroy. Based on these results cotton duct was selected as the material for further optimization of the RABP system (Table 3).

### Table 3 Cotton Attachment Material Test

<table>
<thead>
<tr>
<th>Material</th>
<th>Biomass Productivity (g m(^{-2}) day(^{-1}))</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton duct</td>
<td>1.08</td>
<td>Best growth and durability</td>
</tr>
<tr>
<td>Cotton rag</td>
<td>0.7</td>
<td>Good growth but very flimsy</td>
</tr>
<tr>
<td>Cotton denim</td>
<td>0.09</td>
<td>Poor growth</td>
</tr>
<tr>
<td>Cotton corduroy</td>
<td>0.08</td>
<td>Poor growth</td>
</tr>
</tbody>
</table>

#### 3.3.2 Operation of Lab-scale RABP Systems

*3.3.2.1 Effect of Harvest Frequency.*

Harvest frequency is a critical parameter that needs to be considered for optimal biomass productivity. If the biofilm becomes too thick, the algae on the interior may not receive enough sunlight and/or nutrients to survive. In contrast, if it is harvested too soon, the algae may not be able to retain sufficient biofilm for regrowth. Figure 2 shows both total biomass yield and daily productivity after different harvest frequencies. These data show that 7 day harvest frequency results in the highest biomass productivity. We speculate that at this frequency the biofilm is allowed to grow rapidly without over-shading and limiting nutrients to the underlying cells.

A statistical one-way ANOVA was conducted to identify if the there was a significant difference between various harvest frequencies. The P-value was 0.24, thus the results did not show significant differences in productivity; however, 7 days was selected to be the baseline harvest frequency for the remainder of experiments because it allowed the convenience of harvesting on the same day once per week which could be beneficial in large-scale cultivation.
Figure 2 Effects of harvest frequency on biomass yield (left) and daily biomass productivity (right). Data not significantly different (P-value >0.05)

3.3.2.2 Effects of Rotational Speed

Figure 3 shows that rotational speed of the RABP system was a contributor to fluctuations in biomass yield and productivity and 4 rpm (3.86 cm/s) rotational speed was the ideal speed. ANOVA confirmed that 4 rpm resulted in a significantly higher productivity than other speeds tested (P-value 0.007). We speculate that rotational speed lower than 4 rpm will result in the material spending too much time without contact with the medium, which could lead to the biofilm drying out. On the contrary, if the material rotates too fast the biofilm could be sheared off while rotating though the liquid. Future studies should be conducted to identify not only the ideal rotational speed but also the optimal ratio of time in medium phase vs. time in the gas phase.
3.3.2.3 Effect of the Carbon Dioxide Concentration

Photosynthetic algae can use CO₂ during cell growth to attain carbon for normal cell growth and reproduction. In a suspended algae cultivation system, CO₂ is dissolved into the liquid in the form of carbonic acid, which is then consumed by algae. This process is limited by the low mass transfer efficiency of CO₂ into the liquid medium. This results in a major obstacle for suspended algae culture systems. In our RABP system, the algae was removed from the liquid and entered into the CO₂-rich gas phase to enable more efficient gas mass transfer. Figure 4 shows that even by enhancing the CO₂ atmosphere from 0.03% (atmospheric level) to 3% the biomass productivity did not increase. ANOVA analysis was conducted, which confirmed there was no significant difference in productivities with varying CO₂ concentration from 0.03% to 3% (P-value 0.57). We speculate to be due to the algae biofilm getting sufficient amount of CO₂ when it rotates out of the nutrients into the gas phase. This gives evidence that a commercial-scale RABP cultivation system does not require to be built at a location that has an external CO₂ supply, which could be a huge advantage.
Effects of CO₂ concentrations on biomass yield (left) and daily biomass productivity (right)

### 3.3.3 Characterization of Algal Biomass from RABP System

Table 4 shows a compositional analysis of the algae biofilm grown on the RABP system compared to algae grown in a suspended algae system. Algae grown using the RABP system had 14% less lipid, 5% more protein, and 9% more carbohydrate than the suspended culture of the same species of algae. The decreased lipid content in algae grown in the RABP system is not attractive for producing algal oil-based fuels; however, the increased protein content is promising for animal/aquacultural feeds and the increased carbohydrates are promising for using the algae biomass for fermentation into ethanol. One possible explanation for the increased carbohydrate content is that the algae cells grown in the RABP system were exposed to higher light intensity than in suspension-based systems. This was attributed because the cells revolving out of the water are in direct contact with light instead of the light having to go through water first. When algae are exposed to high light intensities, higher carbohydrate content is developed (Brańyikova, 2010).
Table 4: Compositional Analysis of RABP Culture vs. Suspended Culture

<table>
<thead>
<tr>
<th>Dry Biomass (mg)</th>
<th>RABP Culture</th>
<th>Suspended Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Biomass</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lipid*</td>
<td>7.72± 1.21</td>
<td>22.3± 2.99</td>
</tr>
<tr>
<td>Protein*</td>
<td>37.7 ± 0.54</td>
<td>32.9± 1.66</td>
</tr>
<tr>
<td>Carbohydrate*</td>
<td>48.5± 0.98</td>
<td>38.8± 1.31</td>
</tr>
<tr>
<td>Ash</td>
<td>6.03± 0.08</td>
<td>5.99± 0.01</td>
</tr>
</tbody>
</table>

*p-value < 0.05

3.3.3.2 Amino Acid Analysis

The amino acid analysis results are shown in Figure 5. Of the nine essential amino acids, the RABP system produced a higher concentration of five essential AAs (threonine, valine, isoleucine, leucine, phenylalanine), two essential AAs were not significantly different (methionine, histidine), and two essential AAs (lysine, tryptophan) were lower in concentration than for the suspended culture. These results are promising to using algal biomass produced by the RABP system in animal feed applications. The difference in specific AAs between systems could have been due to the difference in required structural proteins that are necessary for the algae cells to grow in the very different cultivation environments.
3.3.3 Fatty Acid

Although the lipid content of algae grown in the RABP system was lower than for the suspended culture system, the fatty acid compositions of the total lipid were similar. The only significant difference in fatty acid content between the two culture systems was for C18:1. The RABP system produced more cis C18:1 and the suspended system produced more trans C18:1 (Fig. 6).
Figure 6  Fatty acid composition comparison between *C. vulgaris* cells grown in RABP system vs. cells grown in suspended culture.

### 3.4 Conclusions

The RABP system was an effective cultivation system for growing the microalgae *Chlorella vulgaris* for high biomass production with simple harvesting. The optimal operational parameters were: 7 days harvest frequency, 0.03% CO₂ concentration and 4 rpm rotational speed. These operational parameters resulted in 10.5 g m⁻² day⁻¹ biomass productivity. Growth in the RABP system resulted in a higher carbohydrate content than algae grown in a suspended system. Further development and understanding of biofilm based algae culture systems is necessary to continue development and optimization of this design.

### 3.5 References


CHAPTER 4: DEVELOPMENT AND OPTIMIZATION OF A NOVEL MICROGRAVITY SIMULATING MICROALGAE PHOTOBIOREACTOR

Abstract

The microalga *Chlorella vulgaris* was grown under simulated microgravity conditions to evaluate CO\(_2\) consumption and O\(_2\) generation rates and the effects of hydraulic retention time, gas flow rate, and CO\(_2\) concentration on algal growth were investigated. All three factors significantly influenced CO\(_2\) consumption and O\(_2\) generation rates. A statistical response surface design was used to optimize these two parameters. The optimal conditions for CO\(_2\) consumption and O\(_2\) generation were 6.59 days hydraulic retention time, 0.153 vvm gas flow rate and 0.80% CO\(_2\) concentration. Algae growth and CO\(_2\) consumption rates in microgravity were not significantly different than growth at earth (1 g) gravity. A novel hollow fibre membrane photobioreactor was also developed which led to enhanced CO\(_2\) consumption rates.

4.1 Introduction

Developing a sustainable life support system in a space environment is crucial for human space exploration. During space missions, astronauts currently exclusively rely on physical and chemical means for oxygen generation. This process has proved simple, effective, and reliable for short term space missions. For long duration missions a continuous renewable supply of oxygen is necessary. Therefore, it is cost-effective and practical to develop a controlled ecological life support system (CELSS) to remove CO\(_2\) while replenishing O\(_2\) (Hu et al., 2010).
Higher plants, photosynthetic bacteria, and algae are candidates to be used in CELSS. Higher plants as a photosynthetic life support system have some drawbacks due to their low CO₂ consumption and O₂ generation efficiencies. Based on the estimation that 20-30 g m⁻² day⁻¹ of O₂ can be produced by plant canopies, a total of 40 m² of plant canopies would be required for maintaining adequate oxygen level for each astronaut (Lehto et al., 2006). Photosynthetic bacteria are also not a good candidates for CELSS as many of them can produce harmful toxins. Compared with higher plants and photosynthetic bacteria, microalgae can have higher photosynthetic efficiency and, in most cases, do not generate toxins, therefore, they can be implemented in the CELSS for CO₂ removal and O₂ replenishing.

Wang et al., (2004) investigated the growth of *Nostoc sphaeroides* in the spacecraft SHENZHOU-2. The algae exhibited a better growth performance in space than it did in regular gravity conditions. In another study using *Anabaena siamensis*, the algae grew slower in space than in earth gravity (Wang et al., 2006). These results, although somewhat conflicting, confirm that algae can be used as a supplement to the physicochemical based CELSS to absorb CO₂ and generate significant amounts of O₂.

Although microalgae can be an effective CO₂ consumer and O₂ generator, some factors of algal culture need to be fully studied before it is applied in the field. The aim of the present work was to optimize CO₂ consumption and O₂ generation rates of *Chlorella vulgaris* under microgravity conditions by using a microgravity mimicking revolving photobioreactor and evaluate the use of a hollow fiber membrane for enhanced gas exchange rate.
4.2 Materials and Methods

4.2.1 Microorganism, Medium, and Culture Conditions

The microalgae *Chlorella vulgaris* UTEX#265 was used for this work. The growth medium used was Bolds Basal Medium (Bischoff and Bold, 1963). Throughout the duration of the work temperature was maintained at 25°C and 100 μmol s⁻¹ m⁻² average light intensity was maintained 24 h/day.

4.2.2 Development of Photobioreactors to Simulate Microgravity Conditions

4.2.2.1 Rotating-Wall Vessel for Microgravity and 1G Gravity

To simulate microgravity a Rotating-Wall Vessel Photobioreactor (RWVP) was constructed to simulate a design developed by NASA researchers at Johnson Space Center (Nickerson et al., 2003). As shown in Figure 1, the RWVP was a 12 L, cylindrical, horizontally rotating bioreactor in which cells were maintained in a fluid orbit in a low-shear, low-turbulence environment. This unique design created a simulated microgravity environment inside the RWVP (Wolf and Schwarz, 1991). Gas is diffused through a tube on the ventral side of the RWVP. A reactor identical to the RWVP but not horizontally rotating was used to grow algae in earth gravity (1 g) conditions (Fig. 1).
4.2.2.2 Hollow-Fiber Membrane Rotating Wall Vessel Photobioreactor (HFM-RWVP)

Using a hollow-fiber membrane (HFM) can enhance gas mass transfer from gas phase to liquid phase (Hwang, 1999). A HFM-based rotating reactor (HFM-RWVP) was developed to determine if an HFM module can improve CO$_2$ consumption by *C. vulgaris* in microgravity. As shown in Figure 2, a HFM module was connected to the existing RWVP. The tube that was originally used for bubbling gas on the ventral side of the reactor was removed. Instead, the medium circulated from the growth vessel through a hollow-fiber membrane module with a 2.4 m$^2$ surface area (MedArray, Permselect, Ann Arbor, MI) and back into the growth vessel. The HFM had gas flowing inside the lumen of the membrane and medium flowing over the outside of the membrane. This caused the high concentration of CO$_2$ in the gaseous phase to diffuse into the liquid phase and the high concentration of O$_2$ in the liquid phase to diffuse across the membrane into the gaseous phase (Fig. 2).

**Figure 1** (left) RWVP simulating microgravity with the vessel rotating on its horizontal axis. (right) Photobioreactor in normal gravity (1 g) with identical vessel to RWVP but not rotating.
4.2.3 Analytical Methods

Optical density (OD) was used to analyze algal cell concentration at the wavelength of 680 nm by using a UV/Vis spectrophotometer. The OD value was then converted to cell density based on a linear relationship. Light intensity was recorded by using a photometer (LICOR model LI-250A). To identify CO₂ consumption of the system, a CO₂ gas analyzer (Dejay Technology, 5 Gas Analyzer) was used. CO₂ concentration of both gas inflow and exhaust were recorded. The volumetric CO₂ consumption rate was determined as follows:

\[ \text{CO}_2 \text{ consumed} = \text{Total Gas Flow Rate} \times (\text{CO}_2 \% \text{ Inflow} - \text{CO}_2 \% \text{ Exhaust}) \]  (1)

The specific photosynthetic quotient (PQ) for *Chlorella sp.* was used to estimate the amount of O₂ generated by the algae. The PQ value gives the ratio of CO₂ consumed to O₂ generated by a specific organism. The PQ value ratio for *Chlorella sp.* is 1:0.90 (CO₂ vs. O₂) (Ammann and Lynch, 1965). Thus, the O₂ production rate was calculated as follows.

\[ \text{O}_2 \text{ Generated} = \text{PQ value} \times \text{CO}_2 \text{ Consumed} \]  (2)
4.2.4 Experimental Design and Data Analysis

A central composite design was used to obtain the optimal conditions (hydraulic retention time, gas flow rate, and CO₂ concentration) for CO₂ consumption by *C. vulgaris*. The design matrix was a $2^3$ factorial design combined with four central points and six axial points where one variable was set at an extreme level ($±1.68$) while the other variables were set at their central points (Table 1). The coding unit of variable $i$ was done as follows:

$$x_i = \frac{(X_i - X_{cp})}{\Delta X_i}, \quad i = 1, 2, 3$$

where $x_i$ was the coded level, $X_i$ the true value, $X_{cp}$ the true value at central point, and $\Delta X_i$ the step change of variable $i$. The true values of the variables are given in Table 1.

The activity of CO₂ consumption can be written as a function of the independent variables by second-polynomial, i.e.,

$$Y = a_0 + \sum a_{ii}x_i + \sum a_{ii}x_i^2 + \sum a_{ij}x_i x_j$$

where $Y$ was the predicted response (CO₂ consumption), $a$ the coefficients of the equation, and $x_i$ and $x_j$ the coded levels of variables $i$ and $j$.

After CO₂ consumption activity for each run was obtained (Table 1), the response and variables (in coded unit) were correlated by the “Response Surface Analysis” function of the JMP Pro Software to obtain the coefficients of Eq. (3). Only the estimates of coefficients with significant levels higher than 95% (i.e., $p < 0.05$) were included in the final model. The significance of the model was evaluated by $F$-test. (Wen et al., 2005)
The central point (run 15) was repeated four times. Runs 1-14 were all replicated in duplicates to increase the power of the results.

### Table 1 Central Composite Design and Results

<table>
<thead>
<tr>
<th>Run</th>
<th>Variables</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coded unit</td>
<td>True value</td>
</tr>
<tr>
<td></td>
<td>HRT F CO₂%</td>
<td>HRT F (VVM)</td>
</tr>
<tr>
<td>1</td>
<td>-1 -1 -1</td>
<td>10.00 0.042 2.50</td>
</tr>
<tr>
<td>2</td>
<td>-1 -1 1</td>
<td>10.00 0.042 7.50</td>
</tr>
<tr>
<td>3</td>
<td>-1 1 -1</td>
<td>10.00 0.125 2.50</td>
</tr>
<tr>
<td>4</td>
<td>-1 1 1</td>
<td>10.00 0.125 7.50</td>
</tr>
<tr>
<td>5</td>
<td>1 -1 -1</td>
<td>5.00 0.042 2.50</td>
</tr>
<tr>
<td>6</td>
<td>1 -1 1</td>
<td>5.00 0.042 7.50</td>
</tr>
<tr>
<td>7</td>
<td>1 1 -1</td>
<td>5.00 0.125 2.50</td>
</tr>
<tr>
<td>8</td>
<td>1 1 1</td>
<td>5.00 0.125 7.50</td>
</tr>
<tr>
<td>9</td>
<td>-1.68 0 0</td>
<td>15.20 0.083 5.00</td>
</tr>
<tr>
<td>10</td>
<td>1.68 0 0</td>
<td>4.29 0.083 5.00</td>
</tr>
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<td>11</td>
<td>0 1.68 0</td>
<td>6.67 0.013 5.00</td>
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<td>12</td>
<td>0 1.68 0</td>
<td>6.67 0.153 5.00</td>
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<td>0 0 -1.68</td>
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<td>0 0 1.68</td>
<td>6.67 0.083 9.20</td>
</tr>
<tr>
<td>15</td>
<td>0 0 0</td>
<td>6.67 0.083 5.00</td>
</tr>
</tbody>
</table>

* Runs 1-14 were performed in duplicate, while run 15 (central point) was performed in quadruplicate.

### 4.3 Results

#### 4.3.1 Optimization of CO₂ Consumption in Microgravity in RWVP

A central composite design was used for optimization. This experimental method is an efficient experimental design for fermentation related optimization processes (Haaland, 1989). As shown in Table 1, CO₂ consumption was monitored in runs 1-14, which were performed in duplicate and the center point (run 15) was run in quadruplicate.
The responses and variables in Table 1 were correlated by using a second-order polynomial model (Eq. 3). Table 2 lists the estimates of coefficients and associated t-values and significant levels. In the present work, only interactions greater than 95% \((p < 0.05)\) were considered significant and included in the final model. Thus, the reduced model describing \(\text{CO}_2\) consumption as a function of only significant variables was as follows:

\[
\text{CO}_2 \text{ Consumed (mL/min)} = -0.73 + [\text{Gas Flow Rate}] \times 0.02 + [\text{CO}_2 \text{ Percentage}] \times 0.23 + [\text{Gas Flow Rate}] \times [\text{CO}_2 \text{ Percentage}] \times 0.25 + [\text{HRT}]^2 \times 0.24 + [\text{Gas Flow Rate}]^2 \times 0.32
\] (4)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>(t)-Value</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.738</td>
<td>-5.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hydraulic Retention Time</td>
<td>0.022</td>
<td>0.53</td>
<td>0.598</td>
</tr>
<tr>
<td>Gas Flow Rate</td>
<td>1.38</td>
<td>27.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO(_2) Percentage</td>
<td>0.235</td>
<td>23.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hydraulic Retention Time (*) Hydraulic Retention Time</td>
<td>-0.241</td>
<td>-2.31</td>
<td>0.0078</td>
</tr>
<tr>
<td>Hydraulic Retention Time (*) Gas Flow Rate</td>
<td>0.092</td>
<td>0.84</td>
<td>0.4045</td>
</tr>
<tr>
<td>Gas Flow Rate (*) Gas Flow Rate</td>
<td>0.328</td>
<td>2.67</td>
<td>0.0111</td>
</tr>
<tr>
<td>Hydraulic Retention Time (*) CO(_2) Percentage</td>
<td>-0.0092</td>
<td>-0.42</td>
<td>0.6765</td>
</tr>
<tr>
<td>Gas Flow Rate (*) CO(_2) Percentage</td>
<td>0.254</td>
<td>9.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO(_2) Percentage (*) CO(_2) Percentage</td>
<td>0.008</td>
<td>1.74</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Significant interactions <0.05

Eq. (4) was then used to derive the optimal values of hydraulic retention time, gas flow rate and \(\text{CO}_2\) percentage. The optimal \(\text{CO}_2\) concentration identified by this equation was 9.2%; however, this value was much too high and without an additional step of concentration it is not attainable in an outer space environment. In the international space station, the \(\text{CO}_2\) concentration is 0.80%, so this value was inserted into Eq. (4) to obtain the optimal results (Table 3). The predicted maximum \(\text{CO}_2\) consumption was 1.52 mL/min. To validate this experimental design, an experiment was conducted using the optimal conditions identified by Eq.
The CO\(_2\) consumption in this experiment was 1.49 mL/min. This result was within the 95% confidence interval identified by the experimental design and was thus verified.

| Table 3 Summary of optimal conditions for CO\(_2\) production by \textit{C. vulgaris} |
|---------------------------------|------------------|
| Parameter                      | Optimal Value    |
| Hydraulic Retention Time       | 6.59 days        |
| Gas Flow Rate                  | 0.153 VVM        |
| CO\(_2\) Concentration         | 0.80%            |

### 4.3.2 Comparison of CO\(_2\) Consumption between RWVP and HFM-RWVP

A comparison of CO\(_2\) consumption between a RWVP and a HFM-RWVP was conducted to determine whether the hollow-fiber module enhanced the CO\(_2\) consumption by microalgae due to enhanced mass transfer. A comparison was conducted at four different conditions. In all four of these comparisons, the HFM-RWVP outperformed the RWVP; however, an ANOVA analysis showed only two of the four conditions had a significant difference (Fig. 3).
**4.3.3 Comparison of CO₂ Consumption between Simulated Microgravity and 1g Gravity**

Experiments were conducted to identify whether CO₂ consumption in 1 g gravity is significantly different than CO₂ consumption in microgravity. A comparison was conducted at four different experimental parameters. In all four of these comparisons, ANOVA analysis showed there was no significant difference in CO₂ consumption (Figure 4).

![Comparison of HFM-RWVP and RWVP](image-url)
Comparison of Microgravity & Regular (1g) Gravity

![Graph showing comparison between microgravity and regular (1g) gravity](image)

**Figure 4** Comparison between microgravity and earth (1 g) gravity

### 4.4 Discussion

Microalgae can serve as a suitable living organism for CELSS. This research also gives a roadmap on how to optimize the O$_2$ generation rates of microalgae. The information will be useful in further optimization work in developing a CELSS using microalgae. Although this research shows promise, it also presents many challenges to implementation.

Using algae as a CELSS for long-duration space travel has many technical challenges. The most prevalent is bringing water into space. The cost of a space launch is directly correlated to the weight of material that needs to be launched. According to the optimal O$_2$ generation rates identified in this research 2,314 L of algae are required to sustain life for one astronaut. That is approximately 2314 kg. If algae are used as a life support system, the volume of water must be reduced by increasing the algae cell density.
In order to increase algal density, one approach is to increase light intensity. In the present study 100 μmol s\(^{-1}\) m\(^{-2}\) average intensity was used. In space, this value will be much higher. A significant amount of research shows that increasing light intensity directly increased cell density and photosynthetic rate. Both of which increased O\(_2\) generation rates. Although increasing light intensity generally increased growth, photosynthesis becomes saturated at a certain point and increased light intensities damages the cell. It will be very important to identify the optimal light intensity to sustain the densest cell culture.

In the present research, only one strain of algae was tested. An important phase of future research will be to identify a strain that can produce a high level of O\(_2\). When selecting a strain it will be important to ensure the strain is very robust and can withstand cultivation in high light intensities and temperatures similar to the space stations. Another consideration of species selection must be its ability not only to recycle O\(_2\) and CO\(_2\) but also other nutrients that are required for both human and algae growth such as nitrogen phosphorus and potassium.

The final consideration of using microalgae as a CELSS is the photobioreactor system used for cultivation. This system must minimize water usage, maximize cell density and provide an efficient gas exchange system. One possible design is to utilize an algae biofilm-based system that minimizes the water requirement. The final issue that needs to be considered when developing the photobioreactor is construction material. The material must be translucent but it must be able to screen out harmful wavelengths of light that are much more prevalent in space.
4.5 Conclusions

The present work showed that *C. vulgaris* can effectively consume CO$_2$ in simulated microgravity conditions at a rate of 1.49 mL/min. A hollow fiber membrane could be a viable candidate to replace simple bubble diffusion to increase CO$_2$ consumption by microalgae. There was no significant difference between algal CO$_2$ consumption in microgravity and CO$_2$ consumption in earth (1 g) gravity. Thus, algae culture does not need to be in microgravity to identify optimal conditions for growth in space.

4.6 References


CHAPTER 5: CONCLUSION

Microalgae has been identified as a feedstock for a diversity of applications such as biofuels, feed, and fertilizer. However major engineering obstacles are hindering its large-scale production. Once these obstacles are overcome algae could become a very useful crop which can be grown worldwide.

The RABP system was an effective cultivation system for growing the microalgae *C. vulgaris* for high biomass production with simple harvesting. The optimal operational parameters were: 7 days harvest frequency, 0.03% CO$_2$ concentration and 4 rpm rotational speed. These operational parameters resulted in 10.5 g m$^{-2}$ day$^{-1}$ biomass productivity. Growth in the RABP system resulted in a higher carbohydrate content than algae grown in a suspended system. Further development and understanding of biofilm based algae culture systems is necessary to continue development and optimization of this design.

The present work showed that *C. vulgaris* can effectively consume CO$_2$ in simulated microgravity conditions at a rate of 1.49 mL/min. A hollow fiber membrane could be a viable candidate to replace simple bubble diffusion to increase CO$_2$ consumption by microalgae. There was no significant difference between algal CO$_2$ consumption in microgravity and CO$_2$ consumption in earth (1 g) gravity. Thus, algae culture does not need to be in microgravity to identify optimal conditions for growth in space.