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Bio-Refinery of Wastewater in Presence of Carbon Dioxide Utilizing Microalgae

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by

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Abstract

In this research, three sets of experiments were conducted to assess the ability of *Chlorella Vulgaris* (*Chlorella V.*) to grow in different physical conditions such as light intensity, pH, temperature and mixing by measuring oxygen evolution. *Chlorella V.* thrives if the medium (Bold's Basal Media) has a pH range of 7-8, temperature range of 25-30 ^{o}C, light intensity of 4755 LUX, and 5% CO_2 is introduced into the culture.

Chlorella V. has illustrated the same growth rate in both sterile and non-sterile wastewater media under optimal physical conditions. It demonstrated uptake of 100% of dissolved nitrogen compounds and almost 30% of dissolved phosphorus compounds presented in the medium. This ability nominates *Chlorella V.* for potential tertiary treatment of wastewater.

Finally, the ability of this specie for CO_2 uptake was determined. *Chlorella V.* could sequester 20% CO_2 in the total gas volume of the photobioreactor headspace in optimal physical conditions. The CO_2 fixation rate was higher in the middle of the exponential growth phase compared to other growth phases.

Acknowledgements

"In the Name of Allah, the Most Beneficent, the Most Merciful"

I would like to thank my supervisor, Professor Angus Chu, for the patient guidance, encouragement, and advice he has provided throughout my time as his student. I have been extremely fortunate to have a supervisor who cared so much about my work and who promptly responded to my questions and queries. I would also like to thank all the staff members at the University of Calgary who helped me directly or indirectly.

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Dedication

To my lovely supportive family

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
ABS	Absorbance
aq	Aqueous
BBM	Bold's Basal Media
BBM-a	Bold's Basal Media - amended
BOD	Biochemical oxygen demand
C	Carbon
^{o}C	Degree of centigrade
C_c	Average carbon content
CH_4	Methane
cm	Centimeter
COD	Chemical oxygen demand
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
Chlorella V.	Chlorella Vulgaris
CPCC	Canadian Phycological Culture Center
d	day
DO	Dissolved oxygen
g	gram
GHG	Greenhouse gases
H^+	Hydrogen ion
HCL	Hydrochloric acid
HCO_3^-	Bicarbonate
H_2CO_3	Carbonic acid
H_2S	Hydrogen sulphide
$H_2PO_4^-$	Dihydrogen phosphate
HPO_4^{2-}	Hydrogen phosphate

hr	Hour
I	Final light intensity
I_o	Initial light intensity
IRGA	Infrared gas analyzer
L	Litre
mg	Milgram
mL	Millilitre
min	Minute
M_{CO2}	CO_2 molecular weight
M_C	Carbon molecular weight
N	Nitrogen
n	Number of moles
nm	Nanometer
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NH_4^+	Ammonium
O_2	Oxygen
O_3	Ozone
OD	Optical density
P	Phosphorus
pH	Power of hydrogen ion
R^2	Regression coefficient
R_{CO2}	Fixation rate
rpm	Rounds per minute
TIC	Total inorganic carbon
TN	Total nitrogen
TSS	Total suspended solids

UV	Ultraviolet
µm	Micrometer
μ_L	Volumetric growth
WW-n	Wastewater - Non sterile
WW-s	Wastewater - Sterile

1 Chapter one: Introduction

1.1 Background

An increase in population has lead to a rise in the global demand for energy and waste treatment, and this demand has contributed to global warming. Fossil fuels are the primary source of energy all around the world, and their increasing consumption will lead to higher emissions of greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4), ozone (O_3) and water vapour. GHG reflect incoming radiation from the sun and trap outgoing infrared radiation [1]. In this GHG crisis, CO_2 plays a vital role, which has raised concerns in recent years. The main industrial sources responsible for CO_2 emissions are coal-fired power plants [2].

Coal-fired power plants have historically been Alberta's inexpensive supply of electricity. Sixteen percent of Alberta GHG emissions come from the electricity sector; this amount is higher compared to other Canadian provinces. According to the latest federal regulations, all coal-fired power plants should phaseout once they become fifty years old [3].

Further, municipal wastewater has many pollutants that should be removed before the effluent returns to the water body. Calgary has three wastewater treatment facilities: Fish Creek, Pine Creek, and Bonnybrook wastewater treatment plants. Together they meet the wastewater and sewage needs of more than one million Calgarians each day [4].

Wastewater is an appropriate, renewable, economical medium, which contains enough nutrients, such as nitrogen and phosphorus, for algae to grow. By utilizing algae to treat wastewater, different issues can be solved such as the removal of coliform bacteria, nitrogen, phosphorus, and heavy metals. Moreover, microalgae can reduce both chemical and biochemical oxygen demands (COD and BOD), and algae can treat sewage biologically through photosynthesis. Using the sun's energy, algae utilize CO_2 to produce useful biomasses while absorbing nutrients such as nitrogen and phosphorus. Since an excess of nutrients may lead to eutrophication in the environment in the long term, algae prevents eutrophication from occurring because it absorbs the nutrients. The abilities of microalgae are promising because of the potential for the production of several added-value products [5].

The November 20, 2016, Government of Alberta's Climate Leadership Plan focused on reducing carbon emissions. They implemented a $20/tonne carbon price to all GHG emitters on January 1, 2017. Beginning in 2018 (Figure 1.1)this price rose to $30/tonne carbon and will increase by 2% every year [6]. The new regulations necessitate innovative technologies to mitigate CO_2 to meet Alberta's aggressive targets while remaining leaders in oil production.

1.2 Research purpose

1.2.1 Community

The increasing rate of CO_2 emissions has prompted environmental scientists to find a solution for this hazard. Algae strains are aquatic plants that are available at low cost, and they are able to fix CO_2 through photosynthesis thereby reducing the amount of CO_2 in the atmosphere. Further, wastewater is a renewable, low-cost medium that contains sufficient amounts of nutrients, such as nitrogen and phosphorus, for algae to grow. Thus, the result is a reduction of CO_2 in atmosphere and cleaner, cheaper wastewater treatment process.

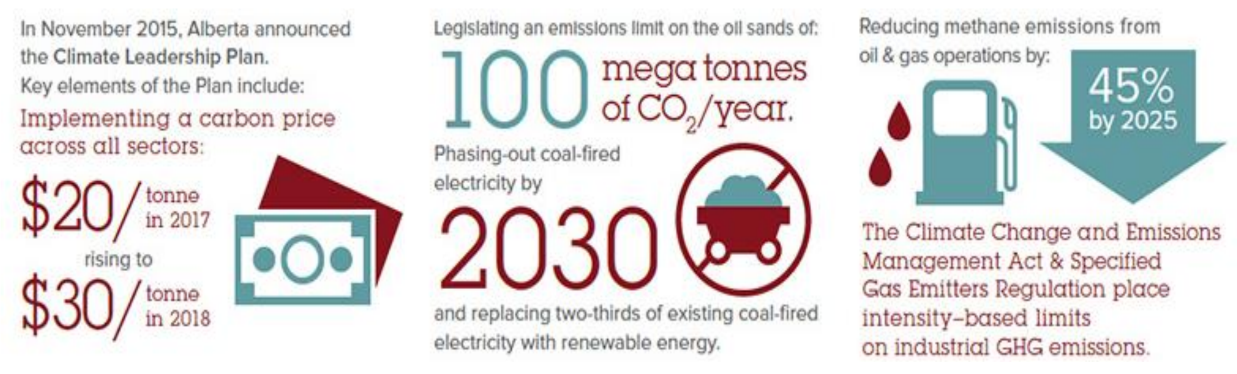


Figure 1.1 Alberta Climate Leadership Plan [6]

1.2.2 Academic

It is hypothesized that algae can grow under different environmental conditions such as nutrients concentration, temperature, pH, mixing and light intensity. One of the purposes of this study is to find the optimum point to have a maximum growth rate for the chosen species.

Moreover, since the wastewater is a source of microorganism nutrient, microalgae has the potential to uptake this nutrient to thrive faster while sequestering CO_2 from any provided source such as powerplant released CO_2.

1.3. Research outline: This dissertation is composed of six chapters. Chapter 1 summarizes the experiments presented in this research and provides a brief peer review of similar studies followed by the research purpose and research outline. Chapter 2 reviews the literature on this topic. In Chapter 3, the optimized growth condition of *Chlorella Vulgaris* is presented. In Chapter 4, the nutrient uptake ability of *Chlorella Vulgaris* is measured in real wastewater effluent. In Chapter 5, the process of CO_2 consumption and O_2 production of *Chlorella Vulgaris* is discussed. Chapter 6 presents the conclusions from this research and recommendations for future research and development.

2 Literature review

2.1 Microalgae

As an aquatic plant, algae lack roots, leaves, and stems and have the ability to conduct photosynthesis similar to terrestrial plants. *Chlorella Vulgaris* is a well-known green eukaryotic microalgae in the *Chlorella* species [7]. Microalgae can be found everywhere in the world and grow in either fresh or saline water; they can tolerate the most severe environmental conditions [8]. As a result of the photosynthesis process, energy is stored in the produced biomass. In general, the maximum possible photosynthesis efficiency is determined by the photosynthesis equation as follows:

$$CO_2 + H_2O + nh\upsilon = \frac{1}{6} C_6H_{12}O_6 + O_2$$

where $h\upsilon$ is one quantum of light frequency υ, h is Planck's constant, and n is the quantum demand for the evolution of one mole of O_2 [9].

In recent decades, microalgae have been used to produce high-value products, such as pharmaceuticals, cosmetics, and food supplements, biodiesel as engine fuels, and biomass as livestock food.

2.1.1 Impact of different parameters on microalgae growth

2.1.1.1 Light intensity

The presence of light plays an essential role in the photosynthetic growth and productivity of microalgae. The amount of light absorbed by an algal cell in a photobioreactor depends on many factors, including the position of the cell, the density of the culture, and the agitation condition of the culture [12]. Sufficient light energy must be provided to be utilized by algae to result in higher biomass productivity [13]. However, exposure of photosynthetic cells to an excessive amount of light could lead to photoinhibition and, as a result, a decrease in the growth rate [14]. The intensity, wavelength, and duration of the light affect the growth of the culture. Also, microalgae perform best with a photoperiod of 18 hours light and six hours dark because the light/dark cycle enhances the photosynthesis process of the algae [15]. In countries with a lower light period in the year, such as Canada, it would be very costly to use artificial light to grow algae. As a result, the feasibility of using microalgae for wastewater treatment will be reduced due to high operational costs. Furthermore, some operational matters will affect the light penetration in the microalgae culture such as the depth of the culture and the self-shading of microalgae cells.

2.1.1.2 Temperature

Microalgae species have a wide range of temperature tolerance. Every species has an ideal temperature; however, they are resilient to a specific high and low heat. Increasing temperature can promote algal growth until it reaches the optimum temperature [16]. Certain species do not grow fast in non-ideal temperatures but still stay alive, such as *Chlorella Vulgaris*. Some microalgae can survive cold weather, such as blue-green algae. In addition, changes in temperature influence other factors that help the microalgae to grow. For instance, high temperatures lead to pH changes because of the release of CO_2 from the medium, and this CO_2 impacts the nutrient uptake of microalgae strains by affecting their metabolism.

2.1.2 Microalgae growth process

2.1.2.1 Nutrient uptake

Nutrient uptake is one of the principal purposes of utilizing microalgae to eliminate nutrients such as nitrogen and phosphorus. Nutrients are incorporated into the algae biomass for metabolic maintenance or growth [26]. Figure 2.1 shows the correlation between nutrient uptake and growth

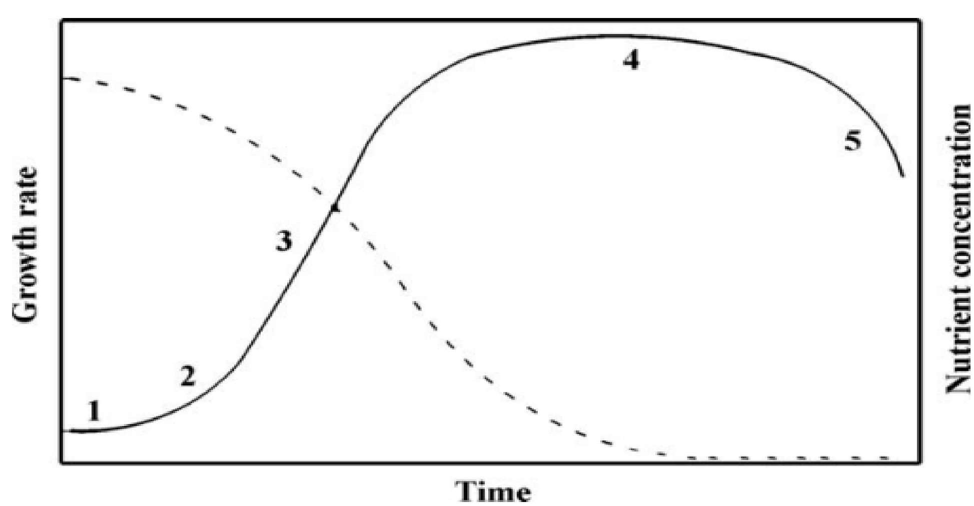


Figure 2.1 Schematic representation of algae growth rate in batch culture: 1. Lag phase 2. Exponential phase 3. Declining relative growth phase 4. Stationary phase 5. Death phase (solid line) and nutrients concentration (dashed line) [27]

rate.

As a novel technology, environmental science can benefit from the advantages of microalgae nutrient removal in the following ways:

1. Low cost if the sun is used as a light source
2. Sequestration of CO_2
3. Generation of O_2 and discharge of O_2 to a waterbody
4. Harvest added value products such as biomass, which contains biodiesel and biogas

There are two methods used to remove nutrients from microalgae cultures: direct nutrient uptake by algae and physical precipitation as an indirect method. In the direct method, nutrients diffuse through the microalgae cell wall, which has a direct correlation with the thickness of the exterior layer of water surrounding the cell. Proper agitation and hydraulic retention time play an essential role in this phenomenon [27],[16]. As mentioned in section 2.3, the concentration of nutrients present in a culture directly affects the nutrient absorption rate. In the indirect method, it is possible to change a culture's chemistry. For instance, by deliberately increasing the pH, the phosphate species precipitates out. Since microalgae consume CO_2 for photosynthesis, by replacing CO_2 with bicarbonate (HCO^-_3) according to Figure 2.5, the pH level rises. Thus, phosphate compounds react with cations and precipitate out. Moreover, a high pH helps to strip ammonia compounds from a culture.

2.1.3 Oxygen production and CO_2 sequestration

Photosynthetic organisms, such as microalgae, have adopted the CO_2 sequestration process to absorb inorganic carbon and store it as biomass for a long time even after their death [28]. Using sunlight as an energy source, microalgae absorbs CO_2 through photosynthesis from a prepared carbon source for the biomass and O_2 production according to the following stoichiometric formula:

$$6\ H_2O + 6\ CO_2 + \text{light} \longrightarrow C_6\ H_{12}O_6 + 6\ O_2$$

Schenk et al. [21] reported that 1.57 g of CO_2 produces 1 g of glucose. Produced O_2 has an important role in aerating the growth culture as well as supplying the required oxygen for bacteria to degrade organic compounds in the wastewater. There are two sources of oxygen to treat wastewater with microalgae technology: photosynthetically produced oxygen and the absorption of atmospheric oxygen through the interface of gas and liquid [29]. The absorption of atmospheric oxygen strongly relates to mechanical aeration, and it can account for 50% of the wastewater treatment cost [20]. In contrast, photosynthetic oxygen may lower operational costs. Further, algae need to be mechanically agitated to obtain the optimized nutrient uptake. Mechanically agitating the microorganisms allows them to access the proper amount of oxygen; therefore, treated wastewater should have a low BOD and biogenic nutrient content.

2.1.4 Biomass, biodiesel, biogas

2.1.4.1 Biomass

All algae strains produce biomass under different environmental conditions; this varies environmental conditions depending on the algae strain. Some strains can double their biomass within 24 hours (Table 2-1). Produced biomass is high in protein and lipid content. Although wastewater is not used as a typical medium for the culture commercial-scale of algae [30], using wastewater will affect the quality of biomass and, consequently, biodiesel and biogas. According to Montingelli [31], approximately half of the dry weight of microalgal biomass is carbon, which is derived from CO_2 assimilation through photosynthesis. As a result, producing 100 tonnes of algal biomass fixes roughly 183 tonnes of CO_2. This promising potential of microalgae could lead to the use of some of the CO_2 that is released by burning fossil fuels.

Harvesting is the next important process of treating wastewater with algae. According to the literature, the method of harvesting impacts the quantity of biomass. The harvesting process can account for 30% of the total microalgae biomass production cost [32], [33].

Table 2-1 Biomass productivity, lipid content, and lipid productivity of 30 microalgal strains cultivated in 250 mL flasks [35]

Microalgae	Biomass productivity (g/L/day)	Lipid content (% biomass)	Lipid productivity (mg/L/day)
Marine strains			
Porphyridium cruentum	0.37	9.5	34.8
Tetraselmis suecica F&M-M33	0.32	8.5	27.0
Tetraselmis sp. F&M-M34	0.30	14.7	43.4
Tetraselmis suecica F&M-M35	0.28	12.9	36.4
Phaeodactylum tricornutum F&M-M40	0.24	18.7	44.8
Nannochloropsis sp. F&M-M26	0.21	29.6	61.0
Nannochloropsis sp. F&M-M27	0.20	24.4	48.2
Nannochloropsis sp. F&M-M24	0.18	30.9	54.8
Nannochloropsis sp. F&M-M29	0.17	21.6	37.6
Ellipsoidion sp. F&M-M31	0.17	27.4	47.3
Nannochloropsis sp. F&M-M28	0.17	35.7	60.9
Nannochloropsis CS 246	0.17	29.2	49.7
Isochrysis sp. (T-ISO) CS 177	0.17	22.4	37.7
Pavlova salina CS 49	0.16	30.9	49.4
Pavlova lutheri CS 182	0.14	35.5	50.2
Isochrysis sp. F&M-M37	0.14	27.4	37.8
Skeletonema sp. CS 252	0.09	31.8	27.3
Thalassiosira pseudonana CS 173	0.08	20.6	17.4
Skeletonema costatum CS 181	0.08	21.1	17.4
Chaetoceros muelleri F&M-M43	0.07	33.6	21.8
Chaetoceros calcitrans CS 178	0.04	39.8	17.6
Freshwater strains			
Chlorococcum sp. UMACC 112	0.28	19.3	53.7
Scenedesmus sp. DM	0.26	21.1	53.9
Chlorella sorokiniana IAM-212	0.23	19.3	44.7
Chlorella sp. F&M-M48	0.23	18.7	42.1
Scenedesmus sp. F&M-M19	0.21	19.6	40.8
Chlorella vulgaris F&M-M49	0.20	18.4	36.9
Scenedesmus quadricauda	0.19	18.4	35.1
Monodus subterraneus UTEX 151	0.19	16.1	30.4
Chlorella vulgaris CCAP 211/11b	0.17	19.2	32.6

There are several harvesting methods that can be used:

1. **Gravity sedimentation:** This method is the simplest, cheapest, and most commonly used in wastewater treatment processes. However, the method is a prolonged process and is not applicable to species smaller than 70 µm [34],[35].
2. **Flotation:** This method is suitable for algae species that float (flocculants) [34]. However, it is not a proper way to harvest algae in the saline medium [35].
3. **Flocculation:** In this method, with the assist of flocculant, the algae cells will clump, and thus the harvesting is easier and more efficient than other harvesting methods. However, the use of a flocculant can be costly and harmful to the environment [34],[35].
4. **Centrifuge:** This method has the highest harvesting result, but it is expensive [34],[35]. Moreover, this method could damage some algae species' cells. However, damaged cells are preferred to make high-value products.
5. **Filtration:** This method is recommended for low concentrations of algae and algae cells smaller than 30µm [35],[36]. In high density growing cultures, there is a chance of blockage, and pressure filtration is required, which leads to higher operational costs [34].

2.1.4.2 Biodiesel

Due to the fast-growing characteristic of algae, more than 50% of the dry weight of algae can potentially be lipid content [37]. *Chlorella Vulgaris* grown in an artificial medium can produce a lipid content of about 20-42% of its dry biomass weight [38]. Microalgae lipid content contains several compounds, such as triglyceride, which is vital to make biodiesel. Some possible biodiesel extraction processes are the transesterification reaction; thermal cracking (or pyrolysis), which involves thermal decomposition; and cleavage of triglycerides [36] (Figure 2.2).

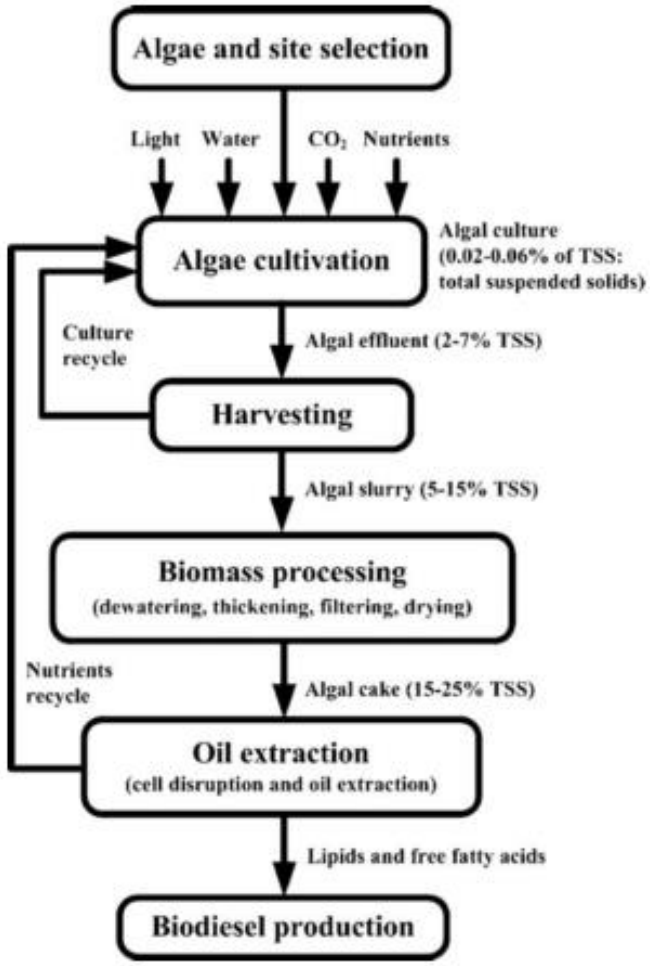


Figure 2.2 Microalgae biodiesel value chain stages [27]

2.1.4.3 Biogas

Through anaerobic digestion, algae biomass is decomposed into biogas, a mixture of CH_4 and CO_2, and mineralized nutrients; later it is utilized as recycled nutrients to sustain the algae growth. Table 2-2 shows the biogas production from different species. If the mineralized nutrients do not have pathogens, they can be used as livestock feed or topsoil. Anaerobic digestion is a combination of sequenced biological processes that biodegrade microorganisms and break down the microorganisms to primary material in the absence of oxygen. One of the products is biogas. Biogas can be combusted to generate electricity and heat. This process is currently being used at the Pine Creek facility, Calgary, Alberta. Moreover, biogas can be processed into renewable natural gas and transportation fuels.

Table 2-2 Methane production at different organic loading rates (OLR) and algal species [32]

Algae	Temp (°C)	HRT (Days)	Drying	OLR	Methane
Ulva	35	15	–	1.7 g VS L^{-1} d^{-1}	203 mL g^{-1} VS^{-1}
Laminaria hyperborea	35	24	–	1.65 g VS L^{-1} d^{-1}	280 mL g^{-1} VS^{-1}
Ascophyllum nodosum	35	24	–	1.75 g VS L^{-1} d^{-1}	110 mL g^{-1} VS^{-1}
Ulva lactuca	53	15	YES	4.4 g VS L^{-1} d^{-1}	16 mL g^{-1} $feed^{-1}$
Ulva lactuca	50	22	–	0.3 g VS L^{-1} d^{-1}	157.6 mL g^{-1} VS^{-1}
Laminaria sp.	50	22	–	1.2 g VS L^{-1} d^{-1}	185.7 mL g^{-1} VS^{-1}
Laminaria sp.	35	22	–	1.2 g VS L^{-1} d^{-1}	139 mL g^{-1} VS^{-1}
Chlorella vulgaris	35	28	–	1 g COD d^{-1} L^{-1}	174 mL L^{-1} d^{-1}
Chlorella sp.	25	3	–	7 g VS	100 mL L^{-1} d^{-1}

2.2 Algae and wastewater

Growing algae requires significant quantities of energy and water and a source of organic or inorganic carbon. The key purpose of using wastewater to grow algae is because it is a cheap and an accessible source of nutrients such as nitrogen and phosphorus. The advantages of using algae make it ideal to simultaneously prepare for aggressive CO_2 emission targets in Alberta while providing tertiary wastewater treatment. Current wastewater plants meet the regulations, and their discharge is acceptable to be added to the water body; however, there are some inorganic materials containing nitrogen and phosphorus that cause eutrophication in the long term.

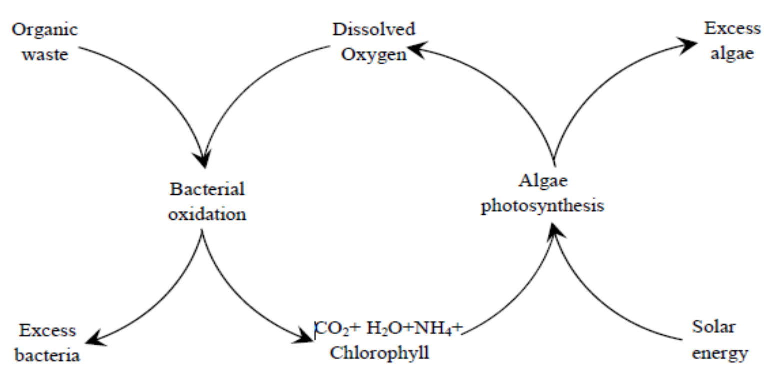


Figure 2.3 Schematic process of wastewater treatment with algae [10]

Figure 2.3 illustrates the overall process of utilizing microalgae to treat wastewater. In this cycle, organic matters as wastewater are degraded with microorganism by consuming O_2. Simultaneously, microalgae uptakes CO_2 and nutrients from organic matters and produce chlorophyll pigments.

Microalgae tertiary bio-treatment can eliminate the harmful residue and convert the inorganic materials to valuable products such as biodiesel, biomass and biogas. The ability of microalgae to sequester CO_2 is not only promising for environmental practitioners, but it also could be profitable in some developed countries such as Canada. Wastewater has a significant amount of nutrients, such as nitrogen and phosphorus, which are necessary to grow algae. In contrast, it is not economical to use fertilizers to promote the growth of the large-scale culture. Using wastewater as a culture medium can significantly lower the overall cost [10]. Wastewater is a renewable, abundant, and low-cost culture medium. The increasing human population will lead to more wastewater treatment requirements. Figure 2.4 shows recent statistics predict population growth in Calgary to be 1.5 million by 2033 [11].

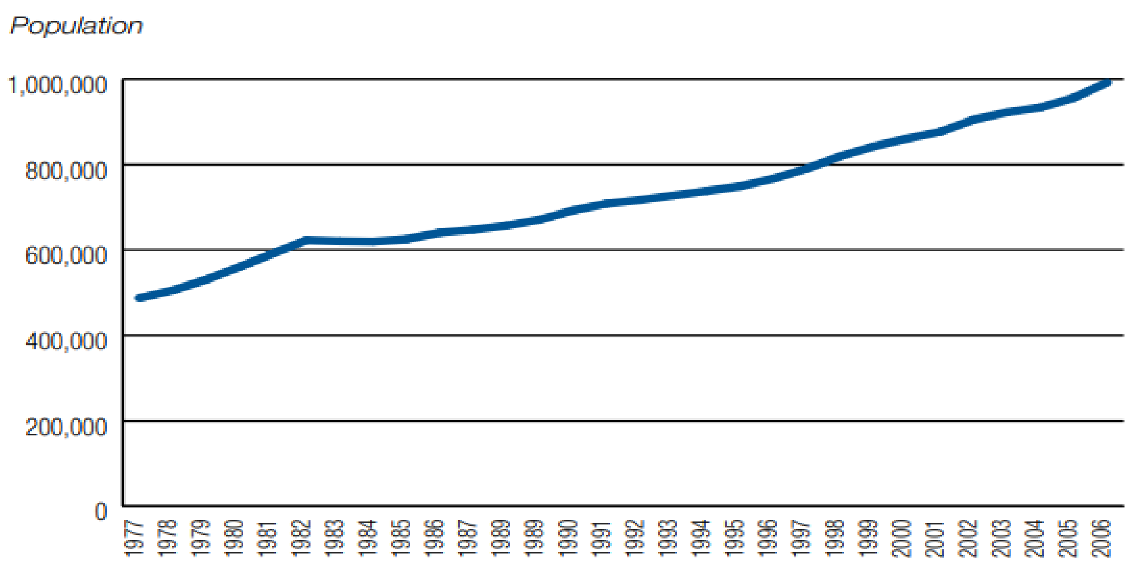


Figure 2.4 Population growth in Calgary, 1969 to 2006 [11]

2.3 Nutrient and inorganic carbon

2.3.1 Nitrogen

Nitrogen is one of the significant components of chlorophyll, which plants utilize to convert sunlight energy, water, and CO_2 into sugars. Therefore, it is an important element for the growth of plants such as microalgae. Microalgae are capable of absorbing nitrogen in the form of nitrate, ammonia, or other organic sources of nitrogen such as urea [17]. The total nitrogen (TN) in wastewater refers to the sum of organic, ammonia, and nitrate concentrations in wastewater. Although the core purpose of absorbing nitrogen using algae is to avoid eutrophication in the water source, a lack of nitrogen also leads to a significant increase in lipid content production [18]. The presence and assimilation of nitrate and ammonia are linked to the pH of the culture since nitrogen absorption changes the pH. The assimilation of ammonia, in the case of being the only source of nitrogen, decreases the pH of the culture [17].

2.3.2 Phosphorus

Another element essential to life processes and the growth of algae is phosphorus. Phosphorus is usually consumed in the form of either dihydrogen phosphate ($H_2PO_4^-$) or hydrogen phosphate (HPO_4^{2-}) [17]. Generally, 1% of the dry weight of microalgae is comprised of phosphorous compounds [19]. Microalgae assimilate phosphorus through the accumulation and synthesis of polyphosphate in their cells [20]. Therefore, the growth rate of microalgae does not significantly change in the absence of a phosphorus source. Technically, a C:N:P ratio of 106:10:1 is recommended to have the proper amount of nutrients for microalgae growth. According to the nutrient concentrations of typical wastewater, phosphorus was found to rarely affect microalgae growth, while nitrogen was found to have a complex impact [20].

2.3.3 Inorganic carbon – CO_2

As a requirement for photosynthesis, microalgae need light, nutrients, and preferably an inorganic carbon source. Moreover, CO_2 in water appears as follows:

$$CO_2\ (g) \rightarrow CO_2\ (aq)$$

$$CO_2\ (aq) + H_2O \rightarrow H_2CO_3\ (aq)$$

$$H_2CO_3\ (aq) \rightarrow H^+\ (aq) + HCO_3^-\ (aq)$$

$$HCO_3^-\ (aq) \rightarrow H^+\ (aq) + CO_3^{2-}\ (aq)$$

CO_2 dissolves in water through an interface with the atmosphere and the biological processes of inorganic carbon absorption and photosynthesis. The presence of CO_2 in water strongly relates to the pH of the culture. Figure 2.5 illustrates that for a pH below 6, inorganic carbon in the culture exists as CO_2, while for a high pH, dissolved CO_2 appears as a carbonate (CO_3^{2-}), which is difficult for microalgae to absorb [16]. A neutral pH is desired for the optimal growth of microalgae; thus, based on Figure 2.5, most of the inorganic carbon is assimilated in the form of bicarbonate.

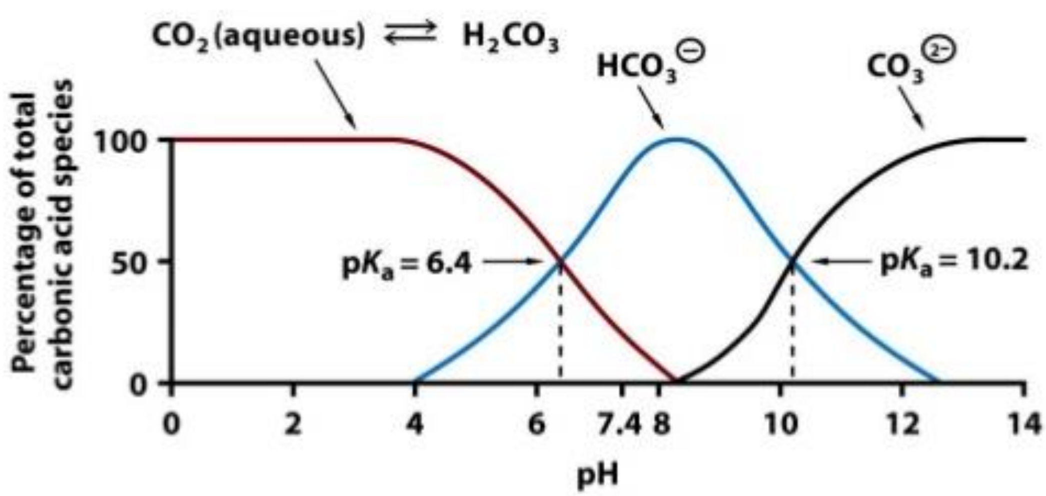


Figure 2.5 Distribution of total carbon dioxide, bicarbonate, carbonate vs. pH

2.4 pH

Similar to temperature, every microalgae species has the desired pH range at which they can function and thrive properly. However, most microalgae are capable of growing below or above a neutral pH. Changes in pH not only affect the function of microalgae, but they also influence the physical characteristics of the medium. For example, a change in pH can instantly alter the solubility of oxygen, carbon dioxide, and the mineral salts in the wastewater. In a microalgae culture, if CO_2 is deprived, it may lead to the precipitation of minerals such as phosphate. Moreover, extra CO_2 will acidify the culture as microalgae absorb substrates such as ammonium [17]. Using a buffer solution, such as a hydrogen ion buffer, in culture can control the pH level. Other buffers may cause toxicity, while hydrogen ion has no effect on a culture [21].

2.5 Aeration and agitation

In conventional wastewater treatments, the purpose of aeration is to provide the proper amount of oxygen so that the biological treatment of microorganisms naturally degrades wastewater contaminants. Another objective of aeration is to mix a culture, which avoids the self-shading effect of microalgae. In dense cultures, aeration also allows microalgae to absorb nutrients easily. However, aeration with oxygen may lead to photo-oxidation in microalgae cells, which causes photo-inhibition and a reduction in productivity [20]. As a solution, although it might be costly, mechanical agitation is a better option compared to aeration. For agitation, the instrumentation should be chosen carefully since some devices, such as pumps, can damage and shear microalgae cells (Table 2-3). The hydrodynamic stress of a culture is dependent on the geometry of the photobioreactor, type of the pump, the morphology of the microalgae cells, and physiological condition of the microalgae [22].

Table 2-3 Culture pumping and cell fragility [22]

Types of pumps	Algae species				
	Porphyridium reuentum	Chlamydomonas reinhardi	Chlamydomonas reinhardi (cell wall less mutant)	Dunaliella bardawill	Haematococcus pluvialis
Centrifuge Pump Eccentric pump Screw pump Trilobs pump		Good growth	No growth		
Cell fragility	Low	Low	High (no cell wall)	High	High

2.6 Impact of the presence of bacteria

Microalgae can be affected by many types of bacteria present in the wastewater based on the high sensitivity of microalgae to ecological changes [23]. In the presence of a proper amount of organic materials, bacteria use dissolved oxygen through aerobic respiration. In algae-dense cultures, if the nutrients are used up, algae start to die. The microorganism decomposition process depletes the oxygen concentration. Also, self-shading in algae-dense cultures decreases the photosynthesis rate, which leads to lower oxygen production. Consequently, rapid oxygen depletion leads to algae growth inhibition. Furthermore, the metabolism of bacteria could lower the pH by producing acids or oxidating existing compounds such as hydrogen sulphide (H_2S) and ammonium (NH_4^+) [24]. Lastly, bacteria may cause competition among algae and bacteria regarding absorbing essential nutrients such as nitrogen and phosphorus.

Although bacteria can inhibit microalgae growth in several ways, there are methods to prevent these issues. Making the pH lower than 5 for a short period of time eliminates most rotifers and protozoa. Also, exposing the culture to a high concentration of ammonia removes zooplankton [25]. Finally, providing a short period in an anaerobic environment prevents fungi from growing in culture [16].

2.7 Choice of appropriate species

Before starting research in this field, the appropriate species should be chosen. Preferably, this species should have high nutrient uptake and high biomass and, consequently, a high biodiesel yield. In addition, these species should be able to tolerate a wide range of pH levels and temperatures. Another critical parameter is the accessibility of information about a strain. Many strains lack the proper documentation and background research. Thus, it is risky to choose a strain if there is not enough information to determine the optimal growth conditions for that strain. Moreover, the lack of sufficient background data would make it difficult to validate the results of the project [39]. Finally, the preferred species is one that is reasonable in price and readily available.

As shown in Table 2-1, there are species such as *Nannochloropsis sp.* that have a high lipid production rate of 61 mg/L/day. However, these species are not cheap or easy to find. Also, these species need to grow in a brackish medium with specific salinity, which makes experimenting more complicated to conduct. However, *Chlorella sp.* has high lipid productivity, and there is a sufficient amount of information on the species. Further, *Chlorella sp*. is economical and is readily available in almost every city in Canada.

Chlorella V. is a genus of the uni-celled green algae belonging to the division Chlorophyta. Its spherical shape ranges from about 2 to 10 μm in diameter, and through photosynthesis, it multiplies rapidly [40] (Figure 2.6).

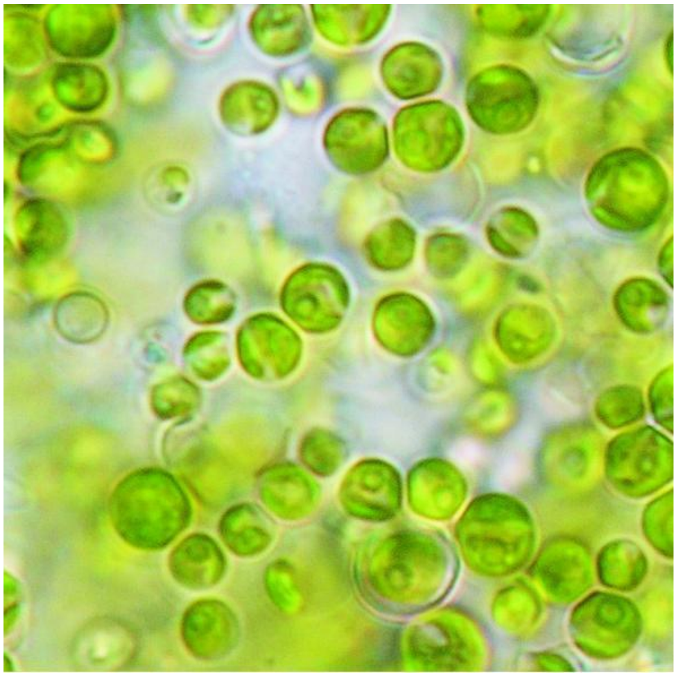


Figure 2.6 Chlorella Vulgaris

2.8 Microalgae wastewater treatment culturing system

For the microalgae growth process, two culturing methods currently exist, open and closed systems (photobioreactors). One of the most common systems to cultivate algae is the open pond system. They are used commercially to produce nutritional products and treat wastewater. The open pond is exposed to the environment, so natural sunlight penetrates the culture to produce biomass. However, closed systems are usually used in laboratories, and the artificial light illuminates the surfaces of the photobioreactors.

2.8.1 Open pond systems

The open pond system is typical because of its simple construction and easy operation. In nature, natural open systems can be found such as lakes, lagoons, and ponds. Besides the natural open ponds, human-made artificial open ponds as well. Constructed open pond systems are usually about one foot deep and could be several acres in size. They are usually built close to power plants or heavy industries that discharge a large amount of CO_2 [7]. While designing open pond systems, it should be considered that increasing the algae concentration in a pond decreases the exposure of the cells to solar energy. A mechanical mixing system should be prepared using a paddle wheel to resolve the microalgae self-shading problem and to ensure the proper mixing of nutrients [17]. Also, an open pond should have an optimal depth of 15–50 cm [7]. Different seasons may impact the operation and production of open systems due to changes in temperature and lack of sunlight in winter. Evaporation is another disadvantage of an open system. In hot regions, the rate of evaporation can increase to such an extent that salt concentration rises in the medium. However, in tropical areas that experience heavy rainfall, can cause dilution in the medium and, consequently, poor biomass production [17]. Moreover, weak diffusion of CO_2 from the atmosphere to the water phase slows algae production. Finally, contamination, in the form of algae, mould, fungi, yeast and bacteria, may occur in open systems with large-scale microalgal cultivation [41]. The pond can be covered with a plastic layer to prevent contamination.

Environmental regulatory agencies can impose limitations to avoid the risk of pollution. Water evaporation, contaminants, and the risk of growth of other algae species are the main concerns regarding open pond systems [7].

Figure 2.7 are pictures of a raceway pond, a circular pond, and a large pond (unstirred), which are all standard open systems.

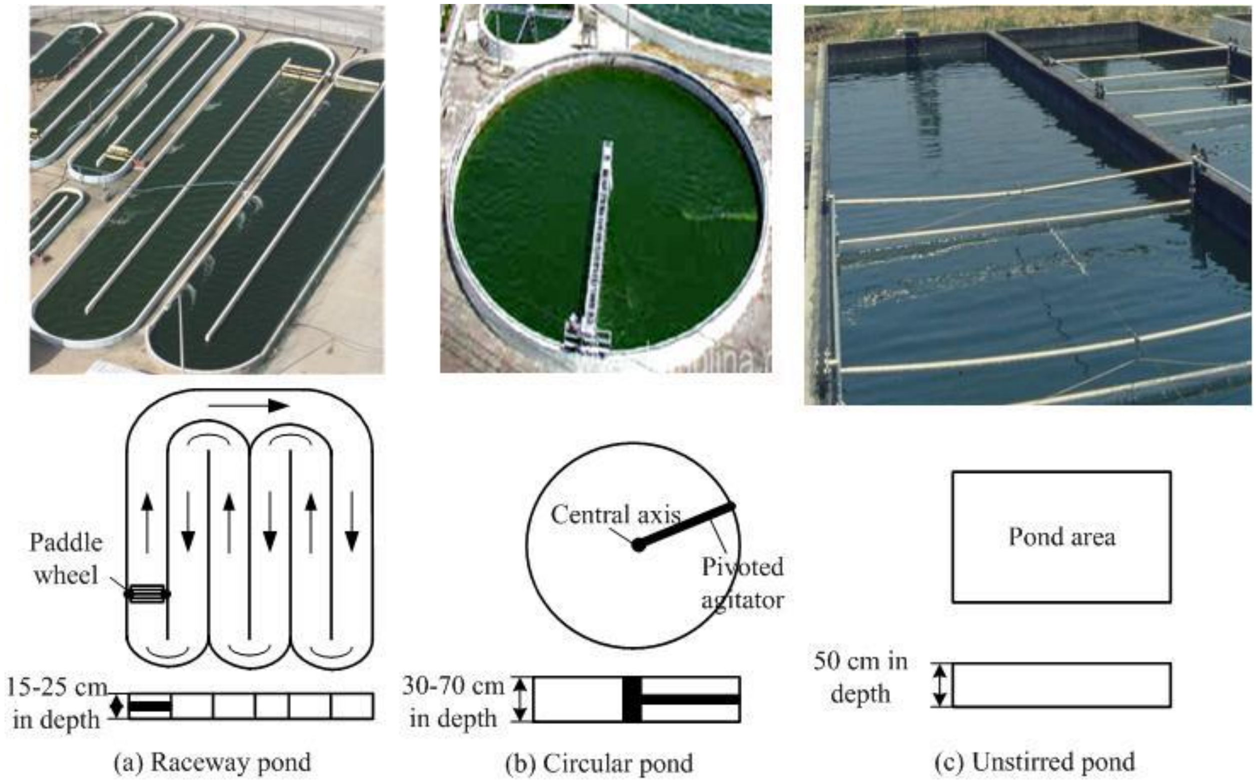


Figure 2.7 Three different designs of open pond systems [43]

2.8.2 Closed systems

Closed systems are called photobioreactors. Photobioreactors are usually made of acrylic pipes so light rays can penetrate through to the growing culture. Different types of closed systems currently exist such as flat-plate photobioreactors, tubular photobioreactors, and column photobioreactors (figure 2.8) [7]. The benefits of closed systems compared to open systems are lower evaporation, less chance of contamination in the photobioreactor, and pH, temperature, and agitation of the culture are more easily controlled. However, two disadvantages of these systems are that they are difficult to construct and operate, and they are costly [42]. A further disadvantage is an accumulation of produced oxygen because the system is air-tight. An excess amount of oxygen can be toxic to the culture. All plants must consume inorganic carbon, of which the most accessible type is CO_2. Therefore, plants make organic compounds such as sugars and carbohydrates. The enzyme that aids this process is ribulose bisphosphate carboxylase-oxygenase (or RuBisCO). This enzyme can fix either CO_2 or O_2. Hence, the enzyme's name is carboxylase-oxygenase. Plants only need to fix CO_2 and when an O_2 molecule bonds with RuBisCO it results in photorespiration, which is a waste of energy. Therefore, plants, despite gaining energy through photosynthesis, lose energy according to O_2 fixation [43].

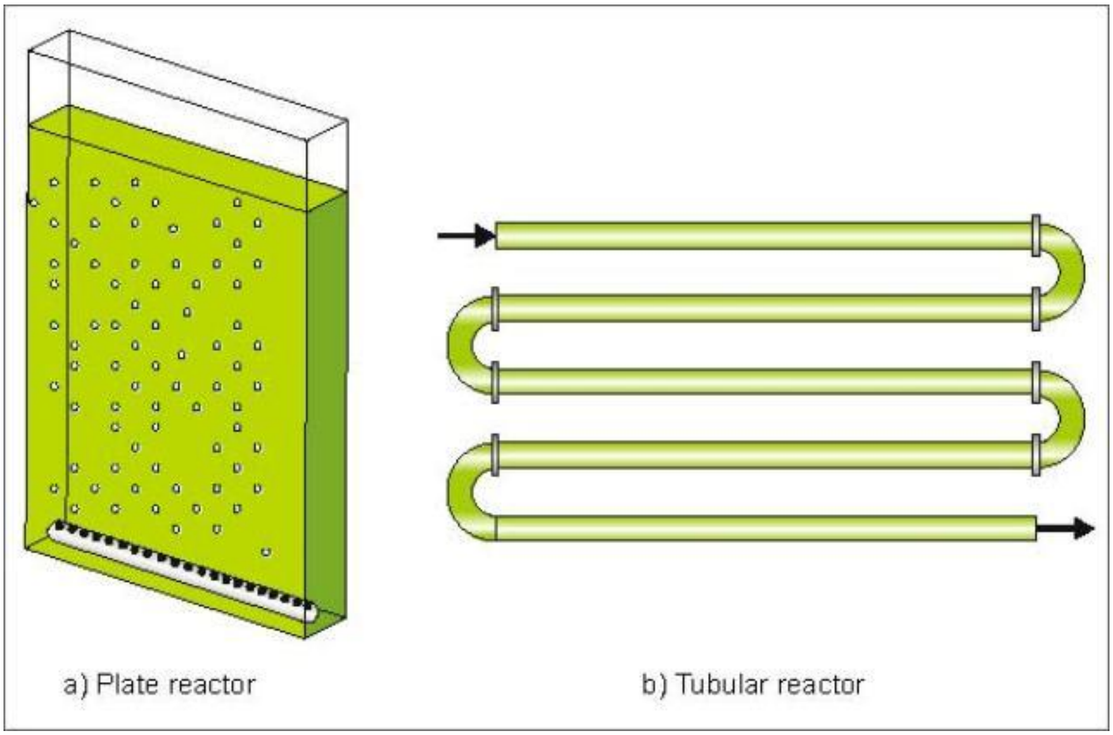


Figure 2.8 Schematic image of the two basic types of reactors [46]

Autotrophic: An autotroph is a culture system in which microalgae can produce complex organic compounds, such as sugar, from simple substances present in the culture. Using light energy and inorganic chemicals makes this system more appropriate for sensitive strains that cannot thrive and grow in a harsh environment. Aeration of the biomass with CO_2 through the reactor tubes provides an inorganic carbon source. If there is insufficient natural sunlight, artificial lights can be used.

Heterotrophic: In a heterotroph system, microalgae is not able to synthesize its food, so it is dependent on complex organic substances for nutrition**.** This system does not require light, and the biomass is fed with an organic carbon source. *Chlorella V.* can use glucose as the organic carbon source [7].

Growing autotrophic algae have several disadvantages. In this system, the photobioreactor must have a large surface area and shallow depth to have the proper light distribution in the culture. The needed space and continuous required light make autotrophic algae cultivation an expensive process. Growing algae heterotrophically is more beneficial financially. In this system, the algae can grow denser and produce a greater biomass yield without the self-shading problem and hassle of proper light distribution. Also, the heterotrophic system occupies a smaller area compared to an autotrophic system; this smaller area helps to decrease the cost of the overall project [44]. Kong believes [45] using an organic carbon source, such as glucose, as an alternative source of energy is significantly less expensive than providing algae with light. Further, glucose is a complex carbon substrate that produces microalgal biomass and biochemical components of the algae such as lipids [45].

Mixotrophic: One of the unique abilities of *Chlorella V.* is being able to thrive under both autotrophic and heterotrophic conditions by performing photosynthesis and consuming organic materials such as glucose. As a result, the cells can rely on light or an organic source to grow. The main advantages of a mixotrophic culture are a decrease in biomass loss during dark respiration and an increase in the absorption of organic substrates used for increasing the biomass [46].

3 Impact of environmental factors on the growth of *Chlorella Vulgaris*

3.1 Introduction

According to Table 2-1, the only strain for which sufficient information was available is *Chlorella V*. This species can quickly reproduce and is very cheap compared to other strains. Another advantage of *Chlorella V.* is its endurance in contact with contaminants and different medium conditions [15]. This algae strain is extensively studied, which makes the interpretation of outcomes simple which could be a great support to achieve this research objective faster. Proven high added value products and all the above reasons make this speicie an appropriate choice for this study. Algae do oxygenic photosynthesis to survive thus measuring oxygen evolution is a suitable method to trach the growth of algae in different conditions [47].

3.2 Material and method

3.2.1 Impact of pH on the growth of *Chlorella V.*

Chlorella V. was obtained from the Canadian Phycological Culture Center (CPCC) located at the University of Waterloo in Ontario. The medium used in this experiment was Bold's Basal Medium (BBM). CPCC recommended this modified medium to ensure the optimal growth of *Chlorella V.* because BBM contains macro and micronutrients necessary for the growth of microalgae. The medium was prepared by dissolving the stock solution obtained from the CPCC in distilled water. The prepared medium, only for the first scale-up to provide enough stock solution, was sterilized in an autoclave at a temperature of 121°C and a pressure of 15 to 17 psi for 60 minutes. Since the goal is to use wastewater to grow algae, the BBM was not sterilized afterwards for scaling up to higher volumes nor for use in the follow-up experiments. The first sterilization kept the algae in a contaminant-free environment to let them thrive faster. After the sterilized medium became cooler, it was added to our bench-scale bioreactors.

In the initial scale-up in autoclaved 250 mL flasks, 100 mL of BBM was added to 20 mL of live algae that had been kept in an incubator at 4 °C. Glucose glutamic acid (GGA) was added once at the beginning to stimulate the organic component and help algae growth. Next, 5% of CO_2 in the gas mixture (Air-CO_2) was diffused into every flask, utilizing stone diffusers in the presence of light at an ambient temperature. The hood in the biological safety cabinet was running over the course of the experiment. Every other day, a volume medium that had evaporated was replaced with a new medium to provide enough nutrients for the growing algae biomass. Agitation was provided by aerating the flasks. All aeration pipes were disinfected with ethanol, and the flasks were sealed with sterile cotton (Figure 3.3).

Table 3-1 Bold's Basal Medium (modified)

Stock	**Stock solution**	**mL/L**
1. KH_2PO_4	8.75 g/500 mL	10 mL
2. $CaCl_2$•$2H_2O$	12.5 g/500 mL	1 mL
3. $MgSO_4$•$7H_2O$	37.5 g/500 mL	1 mL
4. $NaNO_3$	125 g/500 mL	1 mL
5. K_2HPO_4	37.5 g/500 mL	1 mL
6. NaCl	12.5 g/500 mL	1 mL
7. Na_2EDTA•$2H_2O$	10 g/L	1 mL
KOH	6.2 g/L	-
8. $FeSO_4$•$7H_2O$	4.98 g/L	1 mL
H_2SO_4 (concentrated)	1 ml/L	-
9. Trace Metal Solution	See below	1 mL
10. H_3BO_3	5.75 g/500 mL	0.7 mL
11. F/2 Vitamin Solution (optional)	See below	1 mL

Table 3-1, 3-2 and 3-3illustrate the details substances of BBM.

It should be mentioned that all experiments conducted in this research had at least two replicas and if the answers were not very different the average is reported.

Table 3-2 Trace metal solution

Substance	Primary stock (g/L)	Concentrated stock (g/L)	Final concentration in medium (mg/L)
Vitamin-B12 (Cyanocobalamin)	0.001	0.0001	0.001
Biotin	0.001	0.0001	0.001
Thiamine	0.2	0.02	0.2

Table 3-3 F/2 vitamin solution

Substance	Gram/ L
1. H_3BO_3	2.86 g
2. $MnCl_2{\bullet}4H_2O$	1.81 g
3. $ZnSO_4{\bullet}7H_2O$	0.222 g
4. $Na_2MoO_4{\bullet}2H_2O$	0.390 g
5. $CuSO_4{\bullet}5H_2O$	0.079 g
6. $Co(NO_3)_2{\bullet}6H_2O$	0.0494 g

The purpose of this experiment was to measure the dissolved oxygen (DO) production rate in the presence of light at different pH levels of *Chlorella V.* solutions. The sample was taken from a closed photobioreactor with an initial pH of 8.5, an optical density (680 nm) of 0.4285, and at 26.1 ^{o}C and kept at ambient temperature (20-22 ^{o}C) . One meter of white ABI cool light LED light with 120 bulbs and 2231 LUX wrapped around two 300 ml BOD bottles was prepared for the test. Since the initial pH was high and the designed pH levels for the experiments were 5 and 6, 10% diluted hydrochloric acid (HCL) was used, dropwise, to lower the pH to the desired range (5 and 6). The HCL was 30%, with a density of 8.2 molar. DO and the temperature were measured every 30 minutes until the DO meter read “over range” or until the DO production decreased.

For pH values of 7 and 8, all conditions were the same as the previous experiment, except for the initial pH. Since the initial pH was 6.6, we used sodium bicarbonate ($NaHCO_3$) to increase the pH of the samples, and we had to adjust for a pH of 9 and 10 in the experiment to raise the pH. Also, because the solubility of sodium bicarbonate in 20° C is 96 g/L, sodium bicarbonate did not dissolve after a certain amount was added to the solution, and therefore, the pH did not change. To solve this issue, we used sodium hydroxide (NaOH). We prepared a 10% diluted solution of sodium hydroxide and added the solution dropwise to the samples to increase the pH to the desired range. To measure the DO and temperature of the BOD bottles, we used a YSI 52CE as the dissolved oxygen meter. Before each bench test, the membrane was cleaned and refilled with DO electrolyte. Moreover, the HI 9828 (HACH) probe was used to adjust the pH of samples for the first pH experiment. This probe was calibrated before each experiment with three different HANNA buffers, 4, 7, and 10.

3.2.2 Impact of light intensity on the growth of *Chlorella V.*

In this experiment, the impact of exposure of the microalgae culture to different light intensities was measured to determine the impact of light on dissolved oxygen (mg/L) in *Chlorella V.* solutions. Two BOD bottles were filled with algae from the photobioreactor (Figure 3.1). The initial pH, temperature, and optical density in 680 nm wavelength were 8.5, 26.5° C, and 0.49, respectively.

Since it was not possible to measure the light intensity inside the BOD bottles, we decided to count the LED bulbs on the LED strips. Hence, the first bottle had 27 LED bulbs equal to 2231 LUX, and the second bottle had 54 bulbs equivalent to 4755 LUX, which was almost double the luminosity of the first bottle. DO and the temperature were measured every 30 minutes with the YSI DO meter. The samples were placed in a safety cabinet at ambient temperature.

Figure 3.1 BOD bottles with 54 light bulbs and 27 bulbs

3.2.3 Impact of temperature and CO_2 saturation on the growth of *Chlorella V.*

In this experiment, 4 BOD bottles were prepared. Two BOD bottles were wrapped with aluminum foil to avoid any light penetration. From the other two bottles, one was wrapped with 54 LED and the other with 27 LED. The purpose was to measure the oxygen depletion of algae samples over time. The initial pH and temperature of the samples before aerating pure CO_2 into the reactor were 6.6 and 26.2 °C, respectively. Hence, two BOD bottles with algae solutions were collected before

CO_2 saturation occurred, and they were kept in the dark incubator at ambient temperature to avoid any oxygen production in the incubator. Next, the photobioreactor was aerated with 100 % CO_2 for one hour. Then, two samples were collected after the bioreactor became saturated with CO_2. Interestingly, the pH and temperature did not change drastically as they were 6.5 and 26.1 ^{o} C. Thus, we had two samples without CO_2 and two samples that were CO_2 saturated; one sample from each group was kept in the dark, and the other sample from each group was kept in the light. To provide the same luminosity as previous experiments, we used a meter of LED strips with 120 LED bulbs equal to 2231 LUX. All samples were kept in the safety cabinet in ambient temperature. Also, a regular fan was provided to keep the samples cool in case the temperature increased due to the LED lights.

3.2.4 Oxygen production in non-mixed and mixed environments

The purpose of this experiment was to determine the impact of agitation on DO production (mg/L) for *Chlorella V.* in the presence of light. Two BOD bottles were filled with samples from the closed photobioreactor. Their initial pH, temperature, optical density (680nm), and DO were 6.65, 26.7 ^{o} C, 0.59, and 1.41 mg/L, respectively. The DO was kept low for the CO_2 saturation experiment. Both BOD bottles were placed inside a cardboard cube with a one-meter LED light strip, which had 120 bulbs equal to 2231 LUX, wrapped inside. To make a mixing environment, we placed one of the BOD bottles on the magnetic stirring instrument (Thermo Scientific SP194715 Cimarec) set at 100 rpm. DO and temperature were measured every hour. Samples were placed in a safety cabinet at ambient temperature.

To conduct further investigations on the impact of agitation, we prepared four replicas of the unmixed and mixed environment. In the previous experiment, the pH and DO were lower than in this experiment because of the CO_2 saturation experiment. For this experiment, the samples had an initial pH of 8.5 and DO of 5.56 mg/L. The BOD bottles were placed in the cardboard cube with an LED strip (2231 LUX) wrapped inside to provide the same luminosity as in the previous experiment. The samples were placed in the safety cabinet at ambient temperature.

In addition to above experiment, to gain a better understanding of the impact of agitation on the oxygen production rate of *Chlorella V.*, three samples were tested at 0 rpm, 1100 rpm, and 1200 rpm using a magnetic mixer. All three samples were normalized and had an optical density (400 nm) of 0.2 and were kept in the safety cabinet at ambient temperature. For the light intensity, the cardboard cube with 120 LED (2231 LUX) bulbs were used.

The recent experiments on the impact of agitation did not undeniably lead to a correlation between the mixing effect and growth of *Chlorella V.;* thus, a further set of tests were designed.

In the next experiment, eight 200 ml bottles of *Chlorella V.* samples were blended in a jar blender (WARING Bar Blender WPB05) for different periods of time (Figure 3.2). This blender has two options: low speed and high speed. The VWR digital tachometer model 23609-212 showed 18,780 rpm for the low speed and 22,364 rpm for the high speed. The blender impeller in this experiment worked at low speed to agitate microalga(18,780 rpm).

Next, 10 mL from every eight sample after blending were collected

Using a hemocytometer, the cell density (cell/mL) and the viability of the samples were measured. Hemocytometers are used to calculate cell densities of algal cultures for cells ~5-50 µm in size. The device has two chambers, which are divided into nine large squares measuring 1 mm x 1 mm (1 mm^2) each. The four corner squares are subdivided into 16 medium squares, and the central square is subdivided into 25 medium squares measuring 0.2 mm x 0.2 mm (0.04 mm^2) each; these 25 medium squares are further divided into 16 smaller squares. A sample is placed into the hemocytometer, and the cells are counted to determine the average concentration of viable and nonviable cells within the sample [48].

To work with a hemocytometer, it should be loaded as per the instructions below:

- Ensure the coverslip and hemocytometer are clean and grease-free (use alcohol).
- Insert the cell suspension into one chamber of the hemocytometer by placing the pipette tip at the "V" edge and slowly allowing it to fill by capillary action. The chamber should not be over/underfilled.
- Count the number of cells (total and viable):

  • View the cells under a microscope at 100 X magnification.

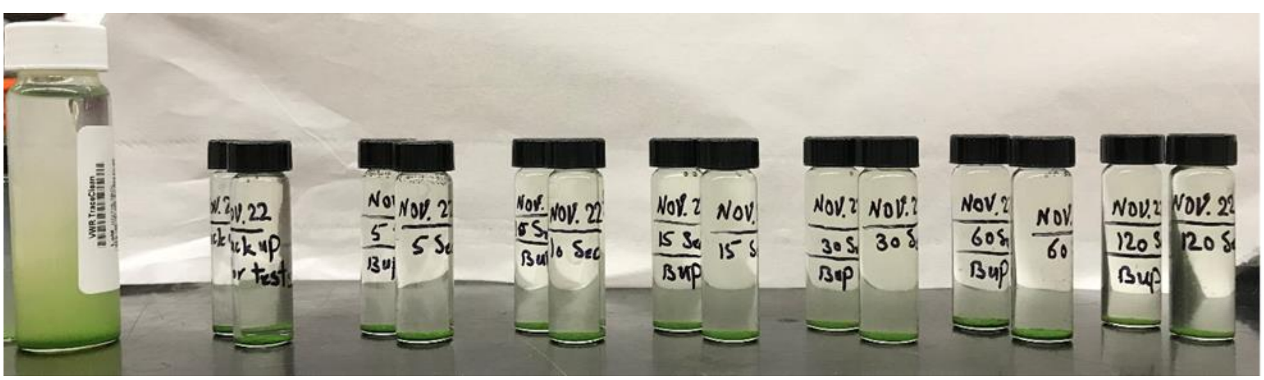


Figure 3.2 Eight samples of blended for different time periods

• Focus the microscope on one of the four outer squares of the grid. This grid should contain 16 smaller squares. Count all the cells in the four outer 1mm corner squares or count random four squares within the centre square as cell size allows.

• Count both the viable and non-viable cells separately.

• For an accurate determination, the total number of cells overlying 1 mm^2 should be between 15 and 50. If the number of cells exceeds 50, dilute the sample and count again. If the number of cells is less than 15, use a less diluted sample.

• Include the cells that lie on the top and left boundaries. Do not include the cells touching the bottom and right boundaries.

- Determine the number of total and viable cells:

$$\%\, Cell\, Viability\ = \frac{Total\, Viable\, Cells}{Total\, Cells\, (viable\ +\ non - viable)} * 100$$

• If more than 25% of the cells are non-viable, the culture is not being maintained in the appropriate amount of medium. Adjust cultures as required to account for the proper amount of medium.

- Determine the number of viable cells per mL:

• Each large square (corner squares) represents a total volume of 0.1 mm^3 (10^{-4} cm^3). Since 1 cm^3 is equal to approximately one ml, each large square corner, identified in the log sheet as orange, represents a total volume of 10^{-4} mL, and each smaller square, identified in the log sheet as blue, represents a total volume of 4 x 10^{-6} mL.

• Average viable cell count per square = Total # of viable cells in six squares divided by six.

• Viable cells per mL = Average viable cells per square x dilution factor x 10^{-4}.

or viable cells per mL = Average viable cells per square x dilution factor x 4 x 10^{-6}.

• Total viable cells/sample = Viable cells per mL x original volume of fluid that

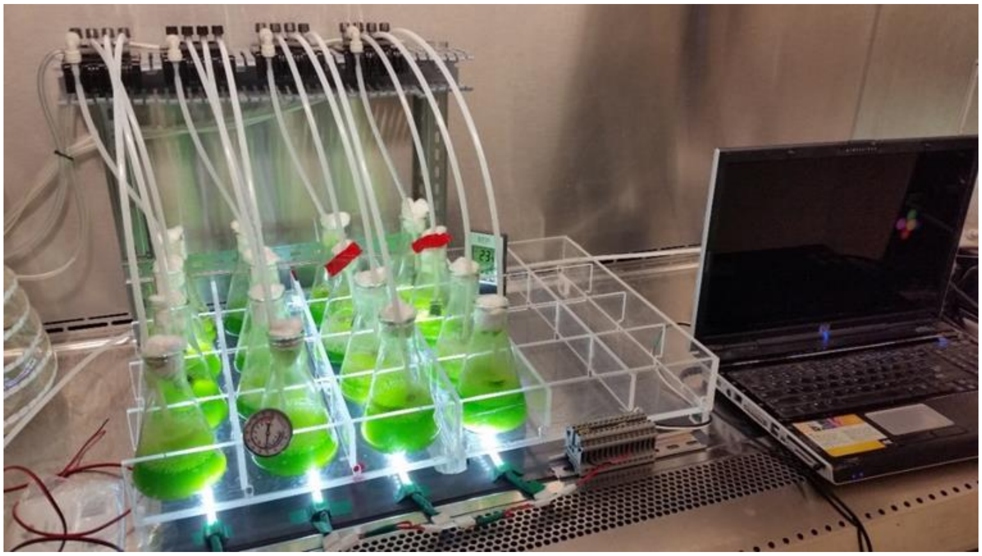


Figure 3.3 First scale-up experiment

was sampled.

• Volume of medium needed = # of cells needed/total # of viable cells x 100.

The final experiment to test cell fragility of *Chlorella V.* used a centrifuge. Amini recommended [17] not to centrifuge an algae culture for more than 10 minutes nor at a higher rate than 3500 rpm. In this experiment, *Chlorella V.* was centrifuged for 15 minutes at 6000 rpm.

3.3 *Chlorella V.* Concentration measurement method

One of the significant parameters to be measured is biomass concentration since it indicates the productivity of a microalgae culture system. There are three methods used to test biomass concentration: optical density (OD), total suspended solids (TSS), and the hemocytometer.

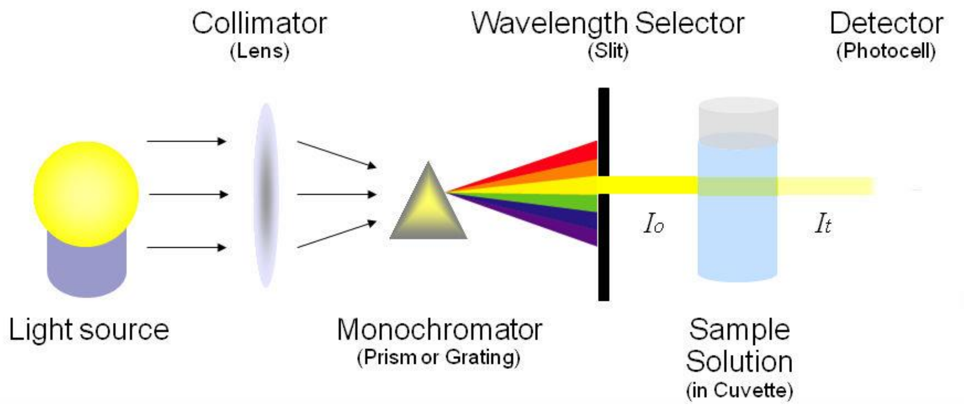


Figure 3.4 Optical density process [58]

OD measures the ratio of the light illuminating the material and the resultant light transmitted through the material (Figure 3.4). According to the below equation, OD is unitless:

$$OD = \log I_o/I = \text{Absorbance}$$

where $I_\circ$ is the initial light intensity, and I is the intensity of light that passes through the sample.

Spectrophotometry is a popular method to estimate concentration by measuring the absorbance, turbidity, or fluorescence of the culture suspension [49].

In the literature, researchers have used a variety of wavelengths to determine the concentration of *Chlorella V.* such as 400 nm, 540 nm, 600 nm, 680 nm and 750 nm. Consequently, there is no certain wavelength that has been studied that measures the optimum concentration of *Chlorella V.* Our first set of experiments determined the proper light wavelength is passing through the dry mass that precisely measured the concentration of biomass. To start this experiment, we required two samples from the same flask in 25 mL spectrophotometer vial cells. Using HACH DR/2000 Spectrophotometer, we measured the OD of the two samples. Starting with a wavelength of 540 nm, we added 10 nm at equal intervals up to 800 nm.

3.4 Results and discussion

3.4.1 Impact of pH on the growth of *Chlorella V.*

In this experiment (Figure 3.5), comparing the results of each dissolved oxygen concentration indicated that a pH of 7 was the optimum value for algae growth and biomass production. Interestingly, although the dissolved oxygen production at a higher pH was high compared to the pH's, it provided the desired environment for contamination to thrive and dominate the microalgae [50]. As the pH value of the algae medium increased, aeration, using a constant and proper amount of CO_2, decreased the pH to the optimum level, and the aeration was followed by an increase in the carbon supply; this result led to an increase in the algae biomass production [17]. The *Chlorella V.* supplier recommended keeping the pH at 6.8 for better health and productivity of *Chlorella V.*

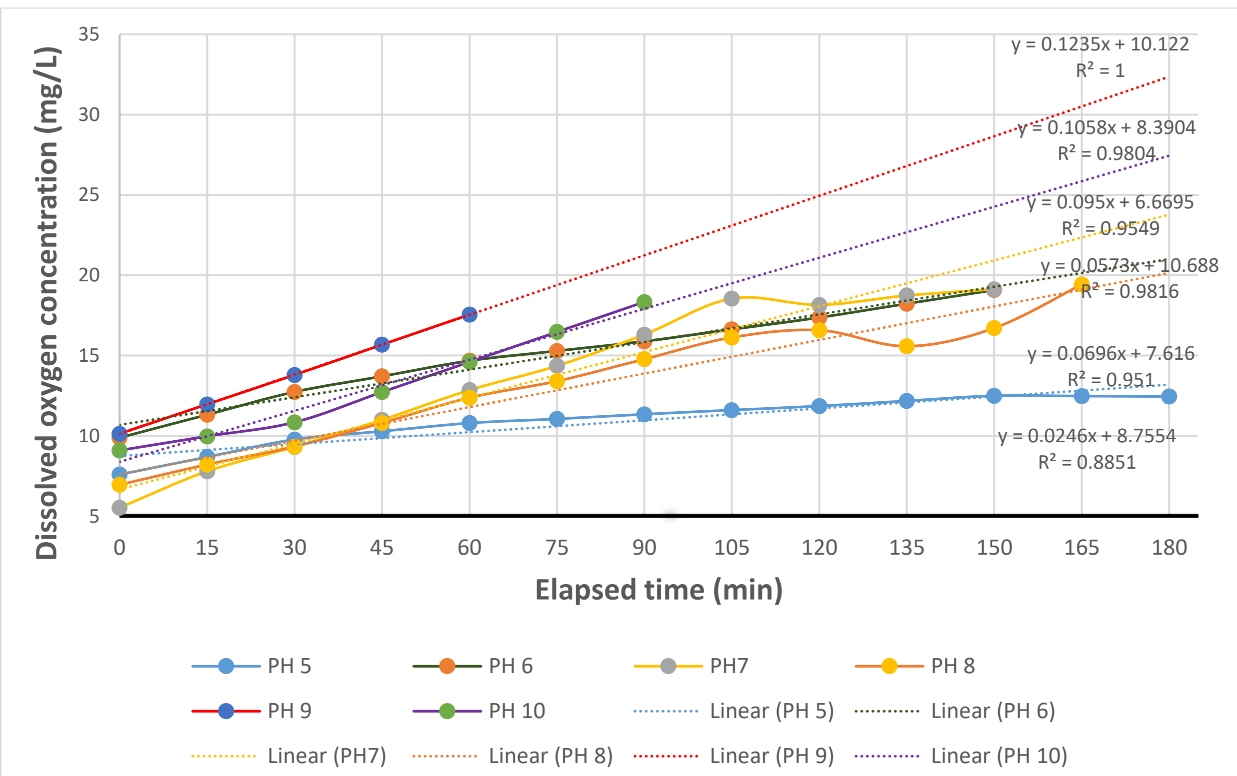


Figure 3.5 Impact of pH, temperature and light intensity on dissolved oxygen concentration

3.4.2 Impact of light intensity on the growth of *Chlorella V.*

Light is another critical factor for microalgae growth. It is an energy source for phototrophic cells such as microalgae. Maximum growth occurs when the light is saturated, which depends on how efficient light spreads through a medium; however, an excess amount of light can lead to photoinhibition. Thus, dark periods are necessary for microalgae cells to allow chemoautotrophic growth [51]. Based on the literature, 10% of light is converted to chemical energy and the rest escapes as heat [52]. For light, light intensity, wavelength, and duration are essential [53]. In this experiment (Figure 3.6), the BOD bottle with 54 LED (4755 LUX) bulbs had 1.64 times higher oxygen production rate compared to the BOD bottle with 27 LED (2231 LUX) bulbs even though it had twice as many bulbs.

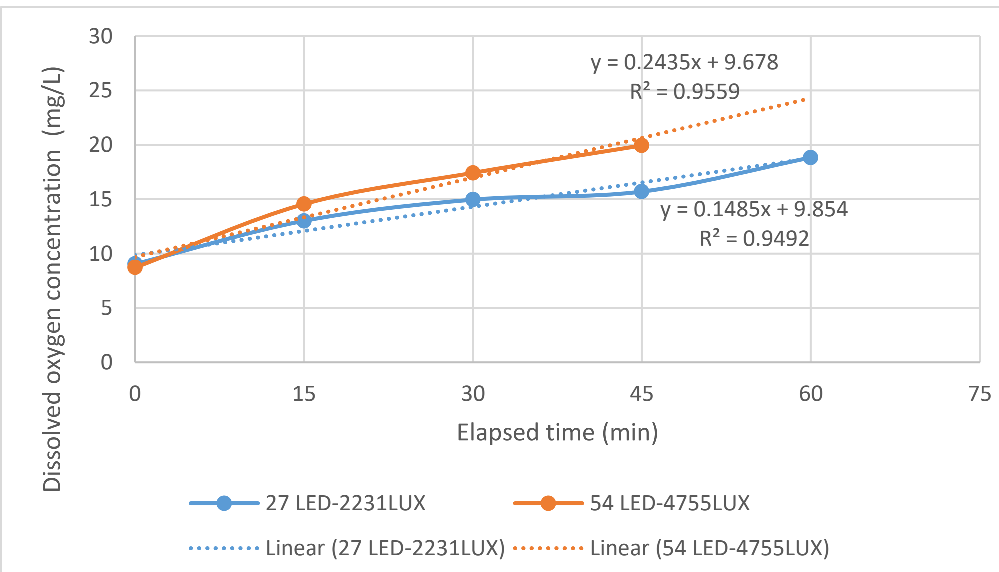


Figure 3.6 Impact of light intensity on oxygen production of Chlorella V.

3.4.3 Impact of temperature and CO_2 saturation on the growth of *Chlorella V.*

As Figure 3.7 illustrates, comparing two samples were kept in the light, the oxygen production rates for the samples of algae without CO_2 and CO_2 saturated that were kept in light were 5.33 and 4.1 mg/L/hr, for first four hours respectively. As shown, the sample without CO_2 had a 1.3 times higher rate of oxygen production. This result shows the inhibiting effect of pH on photosynthesis process by lower oxygen production rate. The oxygen production increased until the temperature passed 30.8 °C. Also, this result shows that high temperature can be limiting for algae growth. Increasing the temperature promoted algal growth until it reached the optimum temperature [16],[54]. Next, a fan was added to the experiment to cool down the LED lights 10 hours after beginning of the experiment. After cooling, oxygen production started as soon as the temperature went below 30°C. This experiment showed that although the CO_2 saturated sample had a lower oxygen production rate compared to the unsaturated sample, it still had a high rate of oxygen production. Also, both samples which were kept in the dark had oxygen depletion in 6 hours.

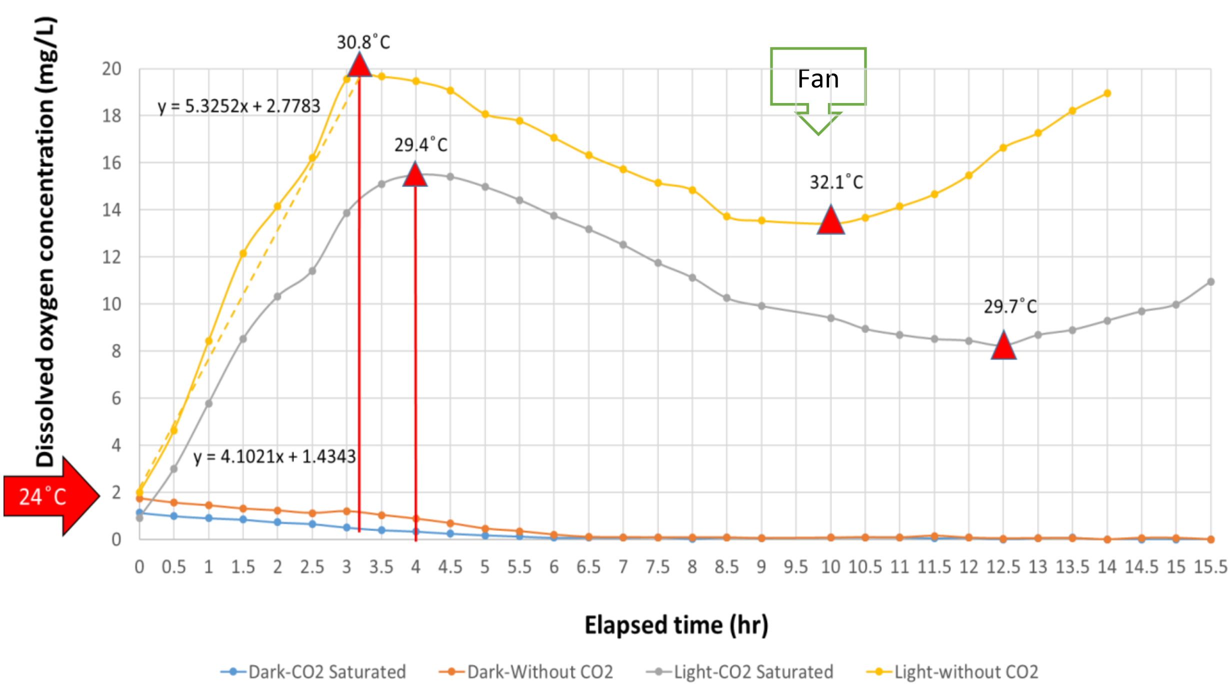


Figure 3.7 Impact of CO2 saturation culture and temperature on dissolved oxygen concentration

3.4.4 Oxygen production in non-mixed and mixed environments

Figure 3.8 shows the results of mixed and unmixed *Chlorella V.* cultures and their dissolved oxygen production (mg/L). The mixed culture had a growth rate of 0.0281 mg/L/hr, and the unmixed culture had a growth rate of 0.0457 mg/L/hr. Consequently, agitation did not have a positive result.

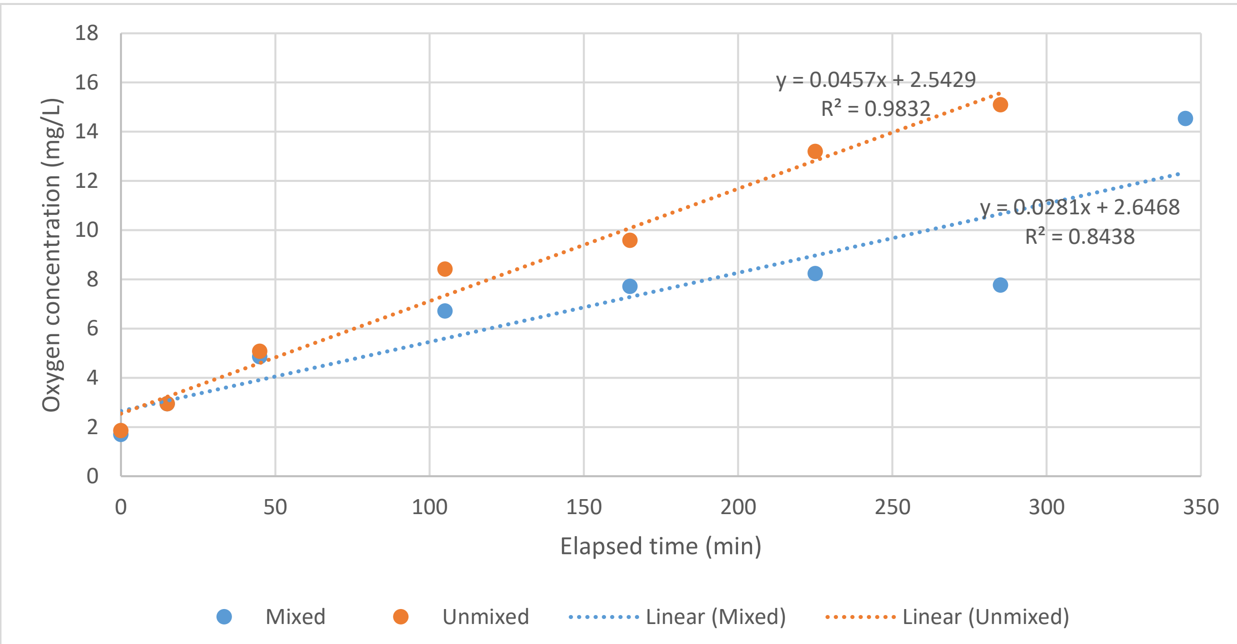


Figure 3.8 Impact of agitation on dissolved oxygen concentration

In the experiment with three alternative agitation speeds (Figure 3.9), the data for the mixed and unmixed environments were not significantly different. The experiment did not provide a concrete result for the impact of agitation. Based on the literature review, turbulent flow leads to damaged and sheared cells [55],[56].

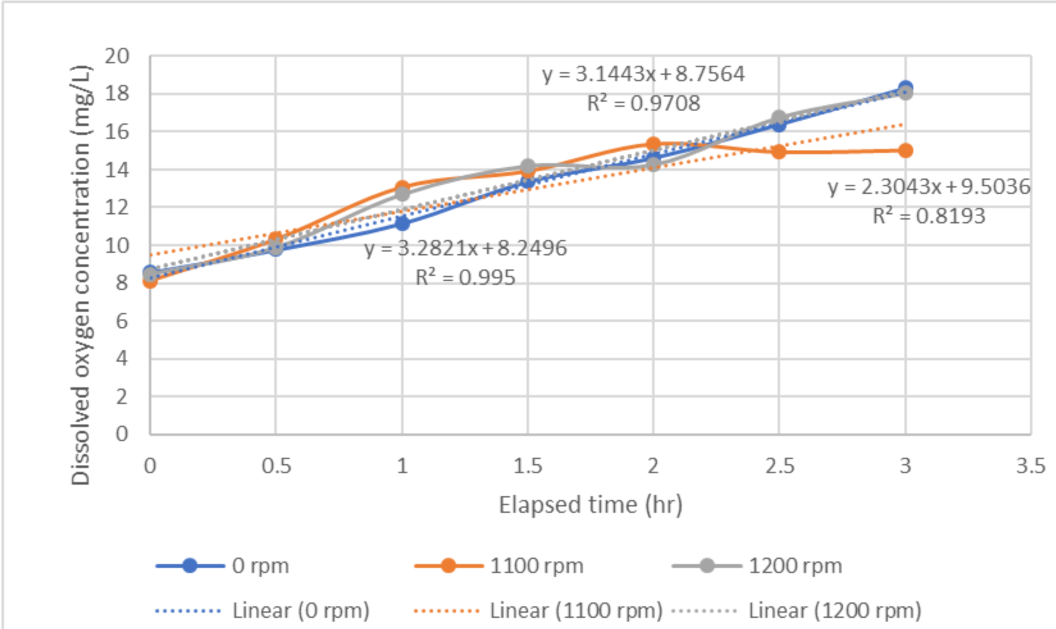


Figure 3.9 Impact of agitation with different rpms in the culture of Chlorella V.

Therefore, given that the photobioreactor creates high turbulence inside each section of the bioreactor, the photobioreactor could be harmful to some algae species. Calculations cannot always indicate the type of flow regime inside the photobioreactor, but a simple experiment can also illustrate the type of flow (Figure 3.10). Blue food dye was injected using a syringe into the pilot photobioreactor in Banner shop while the photobioreactor tower was running, and, consequently, the nature of the flow regime was visible due to the dispersion of the dye; the dispersion indicated turbulence.

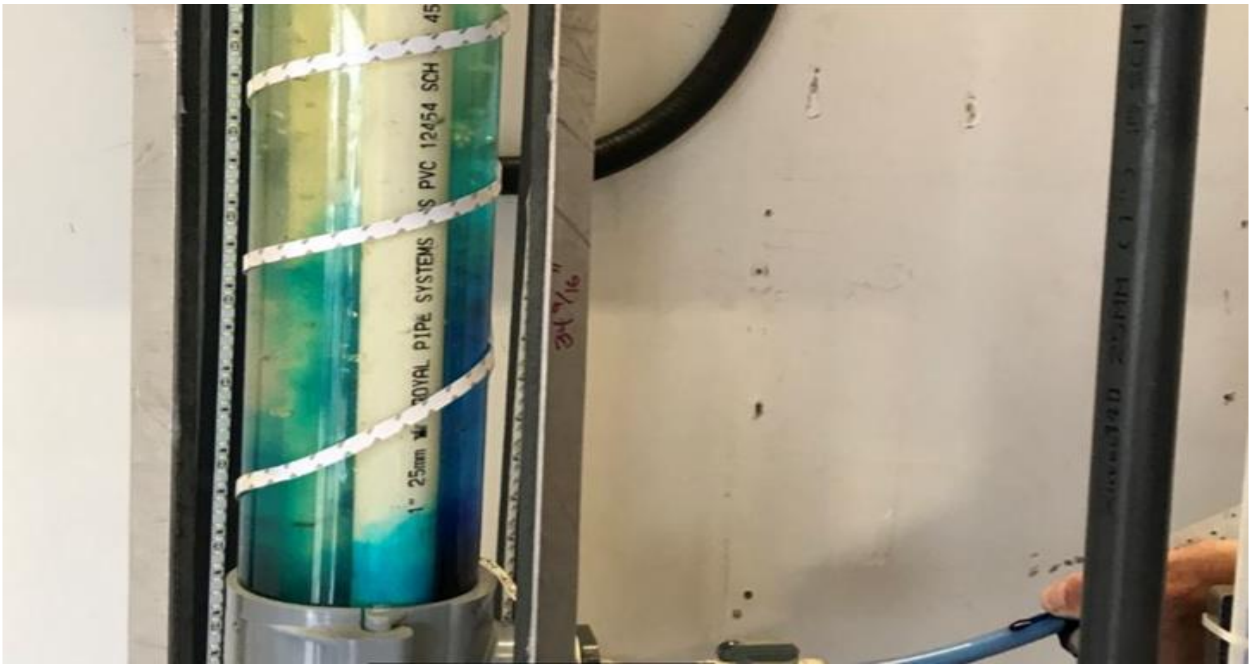


Figure 3.10 Dispersion of blue dye in the photobioreactor showing the turbulence

The recent experiments on the impact of agitation did not strongly indicate any correlation between mixing effect and growth of *Chlorella V.*; thus, we designed another set of tests, and the results are presented below.

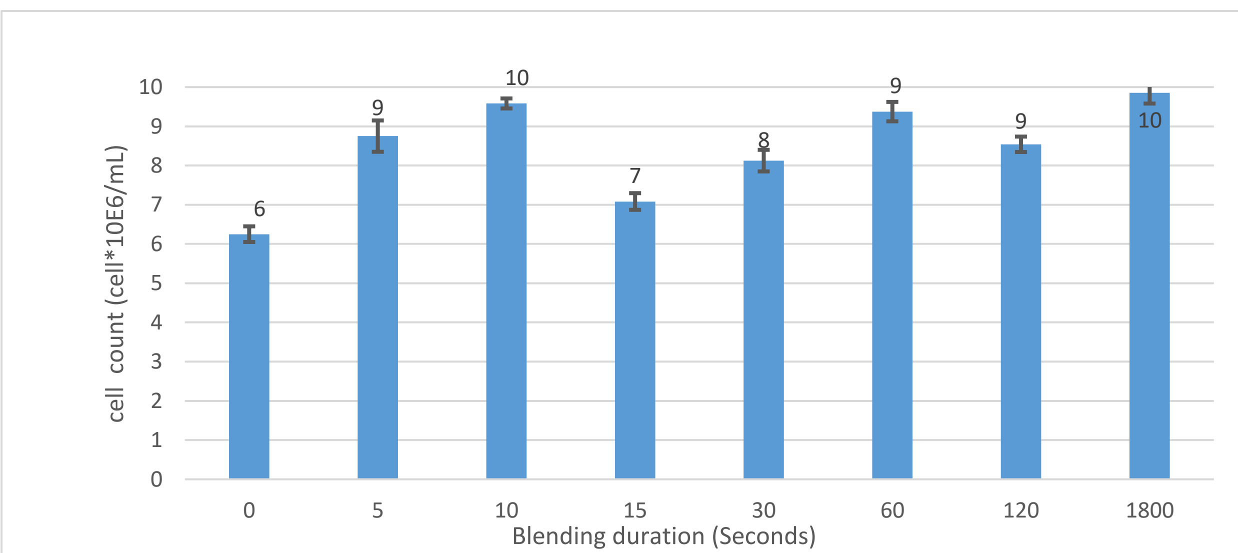


Figure 3.11 Cell densities for eight samples with different agitation durations

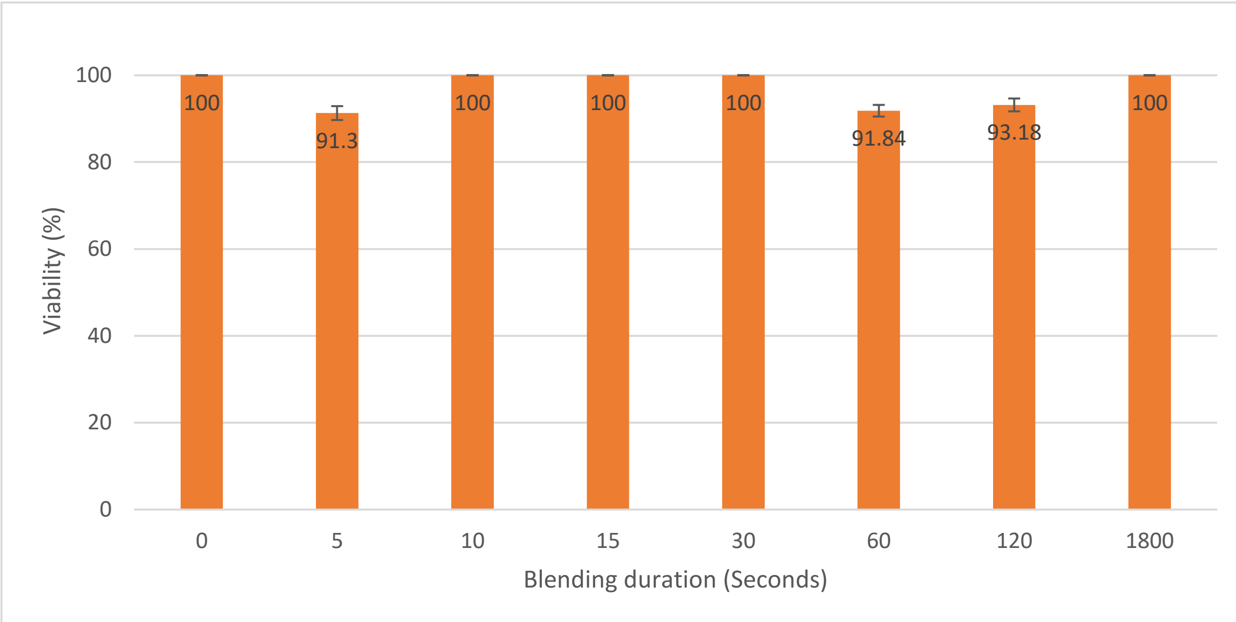
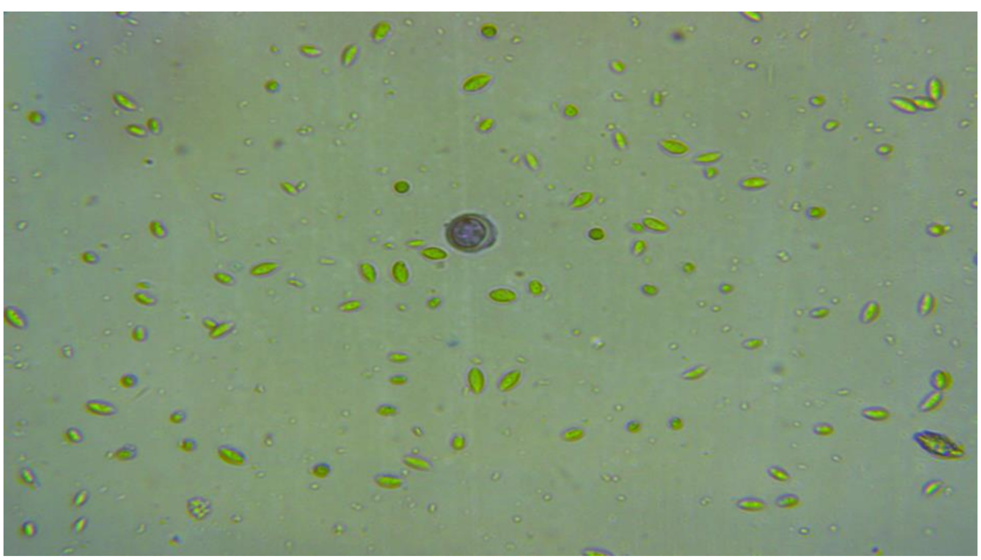


Figure 3.12 Chlorella V. viability after half an hour (1800 seconds) of blending

According to the graphs above (Figure 3.11 , Figure 3.12), no significant changes in cell density nor viability of *Chlorella V.* were seen, which indicated that this species had a high tolerance for mechanical agitation. Also, Figure 3.13 shows that the initial spherical shape of *Chlorella V.* changed to elliptical.

Figure 3.13 The elliptical shape Chlorella V. after blending



Before the high-speed centrifuge experiment results are discussed, it should be mentioned that *Chlorella V.* has a spherical shape and an approximate diameter of 5-6 μm. Having such a shape and size leads to a smaller impact from shear stress and a remarkably high hydrodynamic stress capacity [56]. The results presented in the above cell fragility experiments were initial, short-term results. However, interpreting the overall hydrodynamic impact on photosynthesis in *Chlorella V.* requires a long-term set of analyses of shear stress. The turbulent flow of the culture is necessary to achieve good mass transfers and good light penetration. However, the algae suspension must not be considered as a simple mineral suspension but as living cells [22].

As shown in Figure 3.14, *Chlorella V.* is live with more than 90% viability. The hemocytometer picture indicates that this species is very flexible even in such a high-speed centrifuge.

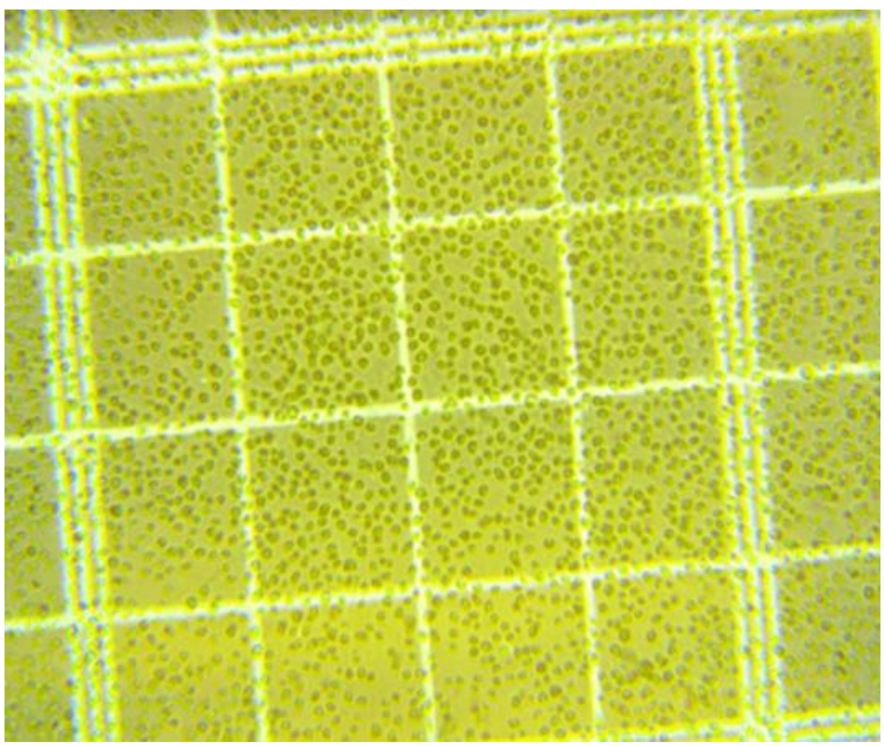


Figure 3.14 Spherical shape and after centrifuging with 6000 rpm

3.4.5 *Chlorella V.* concentration measurement method

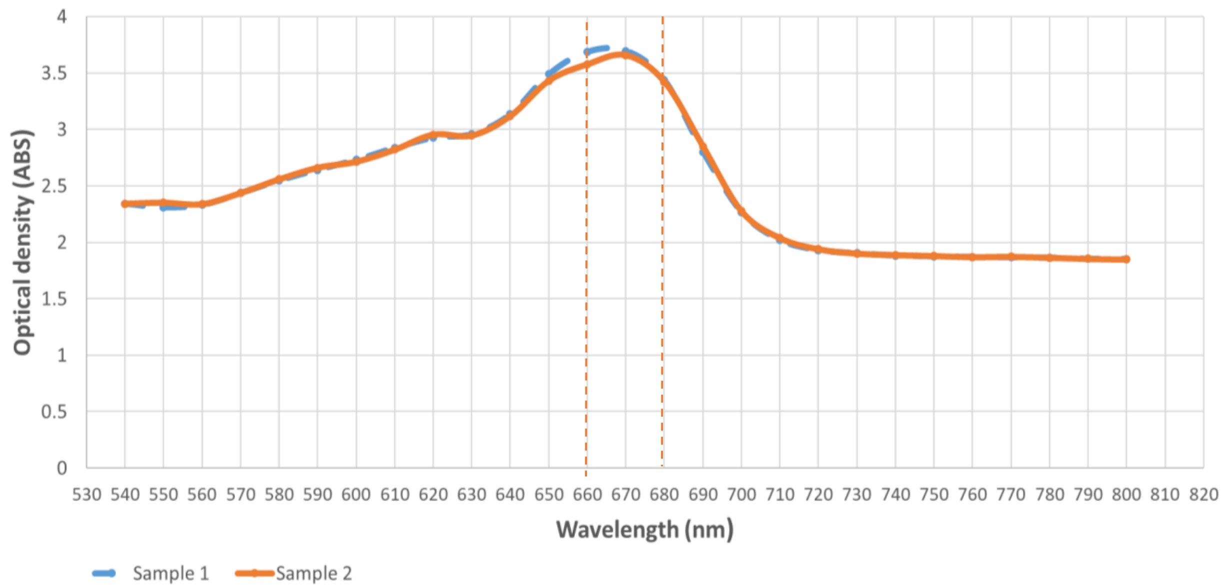


Figure 3.15 Optical density (ABS) vs. Wavelength (nm)

As expected, the two graphs perfectly match which approve the accuracy of ASB measurement (Figure 3.15). The significance of this graph is a single pick in the vicinity of 670 nm.

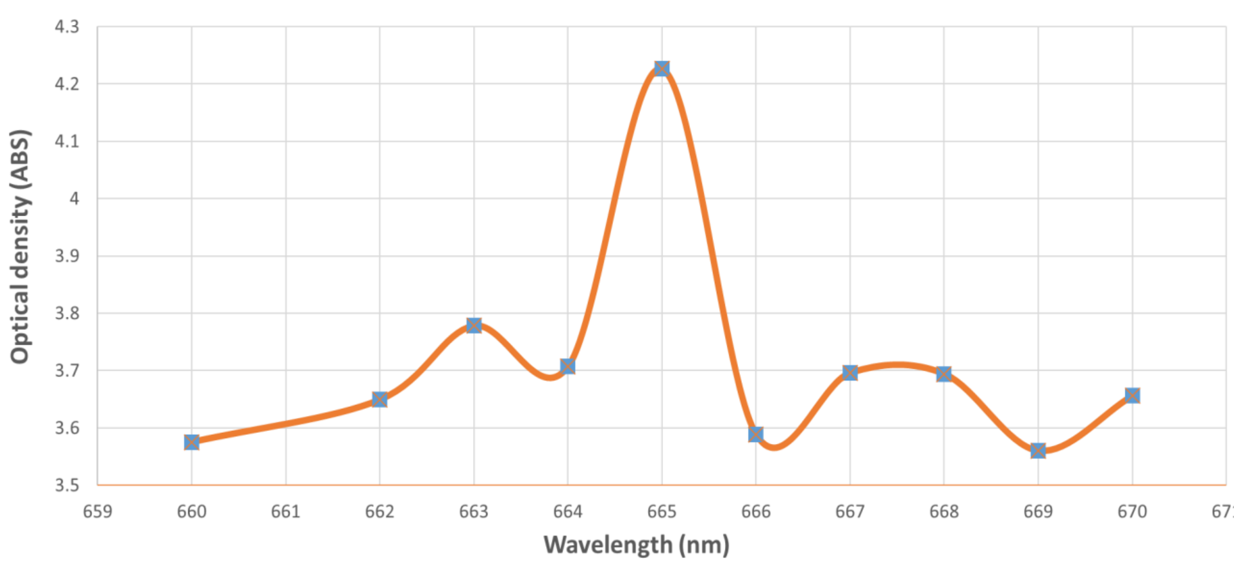


Figure 3.16 Highest optical density for Chlorella V.

Closing the wavelength intervals near to where the pick occurred and conducting further investigations results in the highest OD for *Chlorella V.*, which is 665 nm as illustrated in Figure 3.16. However, Fulleringer et al. [39] reported OD_{625} to be optimum wavelength for *Chlorella V.* concentration measurement.

Next, the correlation of OD and TSS was found using five collected samples. Since the samples had the same OD at 680 nm wavelength, they were diluted with distilled water from 100% to 60% of initial OD_{680}, so the result was five samples with different OD_{680}. For the next step, the five samples were moved to centrifuge vials. The samples were centrifuged using HERMLR Z 206 A for 10 min at 3500 rpm (Figure 3.17).

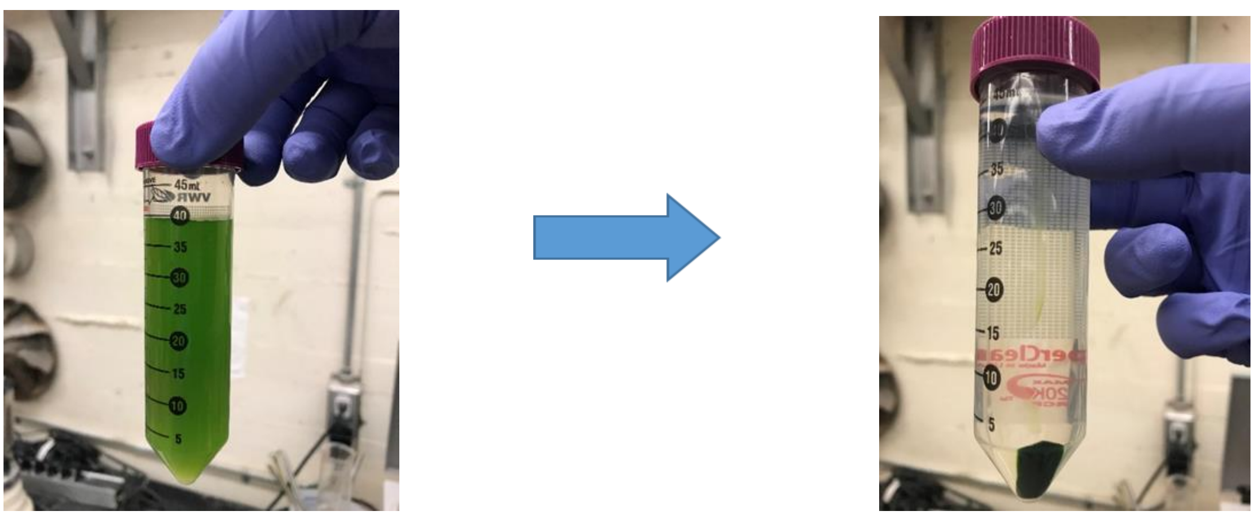


Figure 3.17 A sample of Chlorella V. before and after centrifuge

Next, the supernatant and the dense biomass at the bottom of centrifuge vial were filtered through 0.45 µm pre-dried filters (Figure 3.18). The filters were placed in the oven at the 105 ^{o}C for 24 hours to obtain the actual dry mass of the biomass. After, the samples were placed in the desiccant incubator for an hour to dry the filters.

Figure 3.18 Filtering the supernatant and centrate of Chlorella V. samples

The correlation of OD_{680} and TSS was graphed as shown in Figure 3.19 :

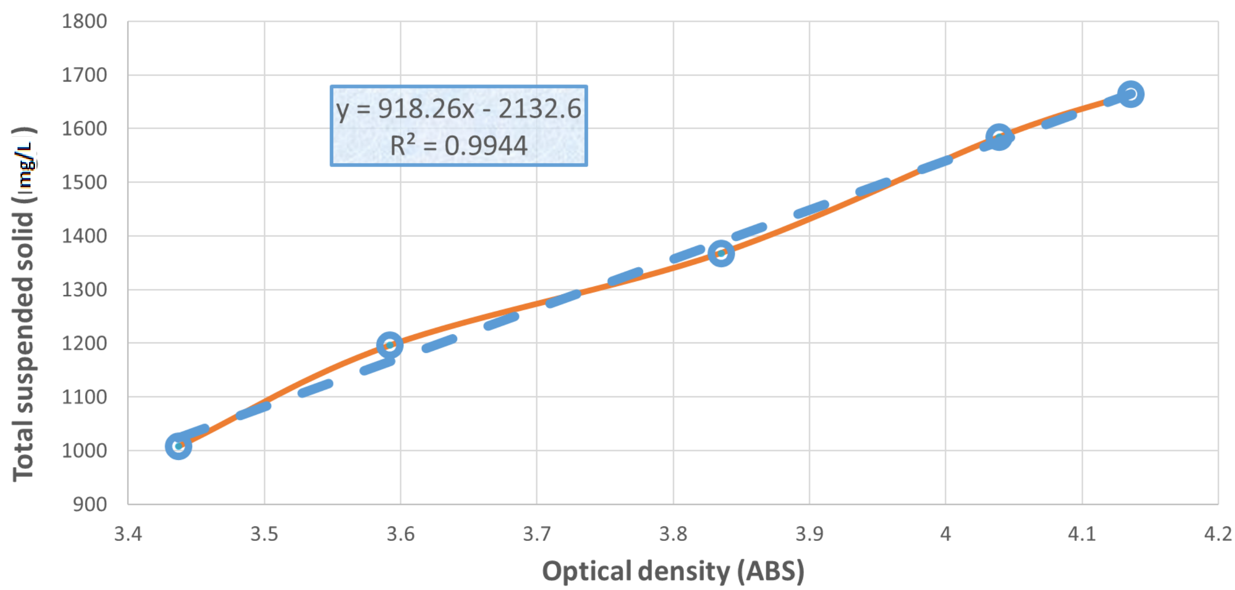


Figure 3.19 OD and TSS correlation (680 nm)

The coefficient of determination known as R^2 between OD_{680} and TSS is 0.9944 which indicated a strong linear correlation between OD and TSS. The only problem with this correlation is that it does not cover ODs lower than 3.5 ABS. According to Lee [57], the OD equation is valid for cell concentrations only up to OD units of about 0.5. As a result, to use this linear correlation to calculate the biomass concentration from a measurement of culture OD, the OD reading must be in the linear range, which is nearly 0-0.5. If cell concentrations are high, the culture must be diluted to the measurement of OD to make the OD lower than 0.5 ABS. The corresponding biomass concentration is then multiplied by the dilution factor to obtain the actual biomass concentration in the culture. Following the dilution instructions below, the new OD_{680} and TSS correlation is

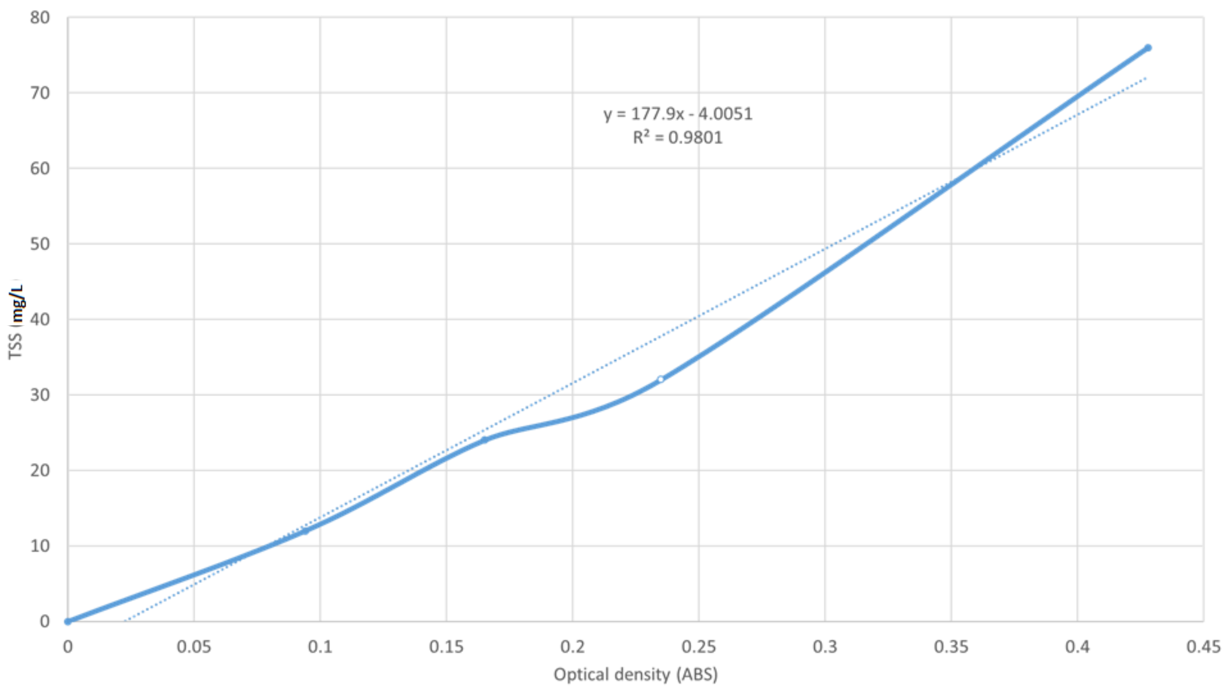


Figure 3.20 OD_{680} correlation with TSSOD680 correlation with TSS

presented, which is used in the concentration calculation (Figure 3.20).

The correlation is as follows:

Y=177.9 X - 4.0051

where X represents the OD_{680} and Y is TSS (mg/L) with regression of 0.9801.

3.5 Conclusion

The critical environmental growth elements, temperature, light intensity, and pH for *Chlorella V.*, were analyzed in these experiments using batch tests. The optimum growth conditions were 4755 LUX, 25-30 °C, and a pH of 7-8, using the BBM.

Although agitation is required to have a homogenized culture, it could be harmful to individual species. In this set of experiments, *Chlorella V.* had a high tolerance of harsh agitation in the short-term. Several articles expressed that long-term investigations are required to determine the behaviour of *Chlorella V.* under mixing.

Also, to measure the concentration of *Chlorella V.*, we investigated whether a sample should be diluted lower than OD_{680} of 0.5; by knowing the OD_{680}, we can determine the cell density (mg/L).

4 CO_2 sequestration of *Chlorella V.*

4.1 Introduction

Historically, scientists presumed that the atmospheric CO_2 concentration was high during the ancient geological periods [58]. Also, it was discussed in Chapter 2 that Ru**b**isCO is an enzyme that aquatic photosynthetic microorganisms, specifically eukaryotic microalgae, cyanobacteria, and non-oxygen-evolving photosynthetic bacteria utilize to fix CO_2 during photosynthesis. However, RuBisCO reacts only with dissolved CO_2 and not bicarbonate or carbonate ions. There are two ways to supply CO_2: through externally diffusing CO_2 and through converting bicarbonates. However, these processes are prolonged and can be limiting for photosynthetic process [58].

In this section, since Bhola et al. [59] reported the best CO_2 concentration to grow *Chlorella V.* is 4% we decided to scrutinize the ability of *Chlorella V.* to grow under constant 3% CO_2 in a total volume of gas passed through a pre-disinfection effluent culture. Moreover, the behaviour of *Chlorella V.* is investigated in the closed system with 20% CO_2 in the total volume of photobioreactor headspace. Finally, the ability of *Chlorella V.* to absorb nutrients in the pre-disinfection effluent from the treatment of wastewater is measured.

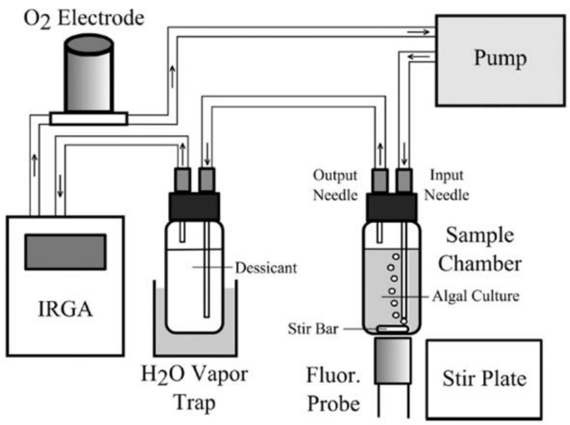
4.2 Material and method

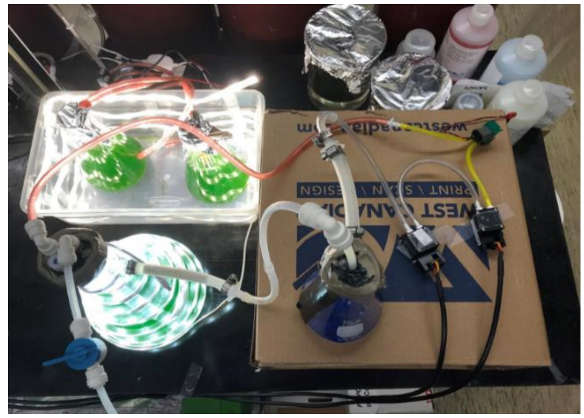
4.2.1 Algae culture

The microalgae strain used in this study was *Chlorella Vulgaris.* More details are provided in section 2.4.4. The medium used to cultivate and grow *Chlorella V.* was BBM. After growing the microalgae in three 500 mL Erlenmeyer flasks, the total volume of 1.5 L was transferred to the 2 L vacuum flask for this experiment.

4.2.2 Apparatus

To carry out this experiment, we required the photobioreactor to have a closed, airtight system. A closed system makes controlling and measuring the gas concentrations easier. The CO_2 and O_2 flux are determined by measuring their concentrations in the gas inlet through a stone diffuser in microalgal culture in a closed loop. Infrared gas analysis (IRGA) had a variety of uses in the photosynthetic assessment of terrestrial plants. IRGA systems, which determine photosynthetic or respiratory gas flux, may be run in open, closed, or compensating configurations [60]. In a closed system, the gas in the headspace of the photobioreactor is continuously recirculated throughout the *Chlorella V.* culture in a loop, passing through the gas sensors. Consequently, gas concentrations changes, due to photosynthesis and respiration of *Chlorella V.*, can be easily monitored in real time. Moreover, using such a configuration is financially beneficial since it avoids the necessity of having more sensors for the gas inlet and outlet simultaneously (Figure 4.1).





1 2

Figure 4.1 Diagram of the described apparatus. 1. Gas flow direction indicated by arrows [63] 2. Lab configuration of apparatus

COZIR-WR is a low power, a high-performance CO_2 sensor that can measure up to 100% CO_2 levels. It is suitable for measuring high concentrations of CO_2 in closed-loop sampling applications or battery-operated portable sampling instruments. Based on infrared LED technology, the COZIR combines the accuracy of non-dispersive infrared technology (NDIR) with a wide range of CO_2 measurements in a package. The accuracy of this sensor is ±70 ppm ± 5% of readings, and it can also operate between 0°C to 50°C and 950 mBar to 10 mBar [61].

In addition, the UV Flux 25% oxygen sensor measures ambient O_2 levels using the principle of fluorescence quenching by oxygen. This unique, patented process creates an oxygen sensor that benefits from the low power requirements traditionally associated with electrochemical sensing devices while providing an extended life due to the non-depleting sensing principle. Moreover, the UV Flux is internally pressured and temperature compensated, enabling accurate operation in a wide range of environments without the need for additional system components. It measures O_2

concentrations in the range of 0.1-25%, can sample as low as 1 sample/sec, and can work under 500 to 1200 mBar.

For non-stop recirculation of gas from the headspace into the algae culture, a pump was necessary. A micro diaphragm pump for gas sampling sensors was also purchased from CO_2METER. The micro gas sampling pump was designed to provide a constant flow of gas across sensors with appropriate tubing. The pump was a perfect option to bench test the sensors for closed-loop sampling applications. Micro diaphragm pumps are based on the principle of the oscillating displacement pump. The motor causes an internal axle to move back and forth, and this action is transferred to the elastic diaphragm, which, in cooperation with the non-return valve, produces the pumping effect (Figure 4.2-2).

One of the key concerns related to these sensors is moisture. They do not function properly if their membrane becomes wet and excess moisture is not removed from the sensors because moisture in the gas may damage the sensor. A water trap, consisting of a 250 mL Erlenmeyer flask that was half full of a desiccant, was placed between the gas and sensors to avoid this issue. The desiccant beads were bought from SIGMA-ALDRICH Canada. The pump then pushed the gas across a CO_2 sensor with a tube-cap that created a sealed system (Figure 4.2-1). Finally, the gas entered the O_2 sensor and returned to the original closed-loop photobioreactor. The water trap and sensors were placed in the proper position so the gas flow could easily pass through the pipes.

CO_2METER offers specific software named GasLab for its sensors. It is possible to setup and calibrate the sensors using GasLab and also perform data logging and real-time data analysis. GasLab prepares logged data to be exported as a CSV file that can be imported into any industry-standard software or spreadsheet such as Excel.

1 2

Figure 4.2 Water trap configuration to avoid moisture reaching the sensors. Headspace recirculation micro diaphragm pump

4.2.3 Experiment setup

As mentioned in the introduction to Chapter 3, *Chlorella V.* was cultivated in BBM in three 500 ml Erlenmeyer flasks to have enough stock culture. A 2 L vacuum flask was chosen as a photobioreactor. The volume of the vacuum flask was checked with a graduated cylinder. One and a half litres of the flask was occupied by microalgae culture to measure the volume of the headspace. Using a graduated cylinder, we filled the rest of flask with water up to the rubber stopper, which airtight the flask the volume of the headspace was calculated as follows:

2000 mL (total volume of flask) - 1500 mL (algae culture) - 16 mL (stopper volume) = 484 mL

According to Nielsen [62], an appropriate CO_2 supply for the saturation of microalgal growth is approximately 5% of the unicellular green algae *Chlorella*. If there is an excess amount of CO_2, the pH can drop, and the growth of microalgae can be inhibited. For this experiment, 20% of the headspace was occupied by CO_2. Since we calculated the volume of headspace, we were able to feed the proper amount of CO_2 to the microalgae culture while not inhibiting growth.

Headspace volume = 484 mL

If the gas in the headspace is a combination of ambient air plus 20% CO_2, then the following applies:

0.484 L x 0.2 = 0.0968 L (the portion of CO_2 fed to headspace directly from CO_2 canister)

0.484 L – 0.0968 L= 0.3872 L (the portion of CO_2 comes from the air in the headspace)

Air has a small amount of CO_2, and this amount can be added to the 20% of pure CO_2.

44 grams of CO_2 occupies 22.4 litres so 0.0968 litres has 0.19 gram of CO_2

Also, the concentration of CO_2 in the air is 400 mg/L so 0.387 litre of air has 0.155 gram of CO_2

Therefore, the total CO_2 mass in the headspace is 0.35 gram.

Knowing that for 0.35 g of CO_2 is equal to 20% in the CO_2 sensor detector, the initial mass of CO_2 in four experiments can be easily measured.

Applying the same method above the mass of oxygen in the headspace was found to be 0.58 g for 15.58% of oxygen detected by the O_2 sensor.

Based on the growth of microalgae, which is similar to other living microorganisms, they have different growth phases, as shown in Figure *2.6*. In this experiment, the photobioreactor was filled with *Chlorella V.*, which was in various growth phases, to monitor the ability of microalgae to fix CO_2 in different phases. Four OD_{680} were chosen to test the CO_2 mitigation ability of *Chlorella V.* (Table 4-1).

Table 4-1 Different concentrations of Chlorella V. for the CO2 fixing test

Test number	Chosen OD_{680}
1	0.490
2	0.650
3	0.900
4	1.030

The exponential growth phase of microalgae has the most rapid growth possible under the conditions presented in this batch system. Three optical densities were chosen from the exponential phase to conduct this experiment. For the first test, an OD was selected from the beginning of the exponential growth phase; the second test used an average OD from the entire exponential phase. The third test used an OD that was chosen from just before the end of the exponential growth phase, and finally, the fourth OD was selected from the end of the exponential growth phase and the beginning of the stationary phase. The vacuum flask was wrapped with white LED lights, which provided a light intensity of 2200 LUX. GasLab was set up to log CO_2 and O_2 concentrations every 1 hour. Since this experiment was non-stop, an operator was connected to a laptop, which recorded data using Teamviewer software.

Before the main experiment began, 20% CO_2 was fed into the photobioreactor, which had only water in to monitor the behaviour of CO_2 as the CO_2 contacted the water. As shown in Figure 4.5, 8% of CO_2 depleted over 12 hours, after which the depletion slowed down. This result implied that a portion of the CO_2 might have dissolved in the water; further, when the CO_2 in the gas phase reached equilibrium with the CO_2 in the aqueous phase, the depletion rate slowed down.

4.3 Results and discussion

Figure 4.3 shows the depletion of CO_2 in the photobioreactor that had 1.5 L of water. Most of the CO_2 that was depleted dissolved in the water. The result of dissolving CO_2 in the water is a decrease in pH; the initial pH was 7.2, and at the end of the experiment, the pH was 5.8. Moreover, the solubility of gases had a correlation to the pressure and the temperature of the gas, and the solvent. For example, the solubility of CO_2 at 25°C and 1 atm was 1.45 g/L. This meant that after a certain amount of depletion, the CO_2 concentration was constant, as shown in Figure 4.3.

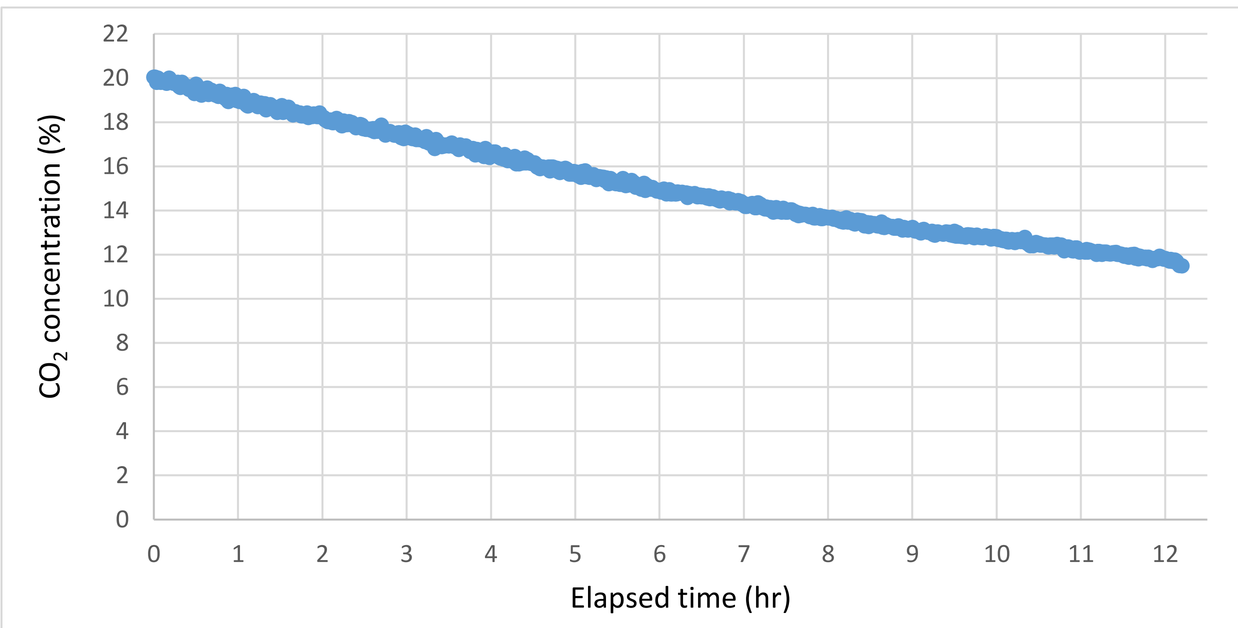


Figure 4.3 The CO_2 solution in the water

In Figure 4.4, the same trend of CO_2 depletion can be seen similar to Figure 4.3. The difference is that in Figure 4.4, the CO_2 concentration approaches zero. Since the system is airtight and has a fixed solubility of CO_2 in water, the removed CO_2 only can be sequestered by algae cells in the photobioreactor. To prove the photosynthesis process, the potential oxygen production was measured at the same time of CO_2 depletion.

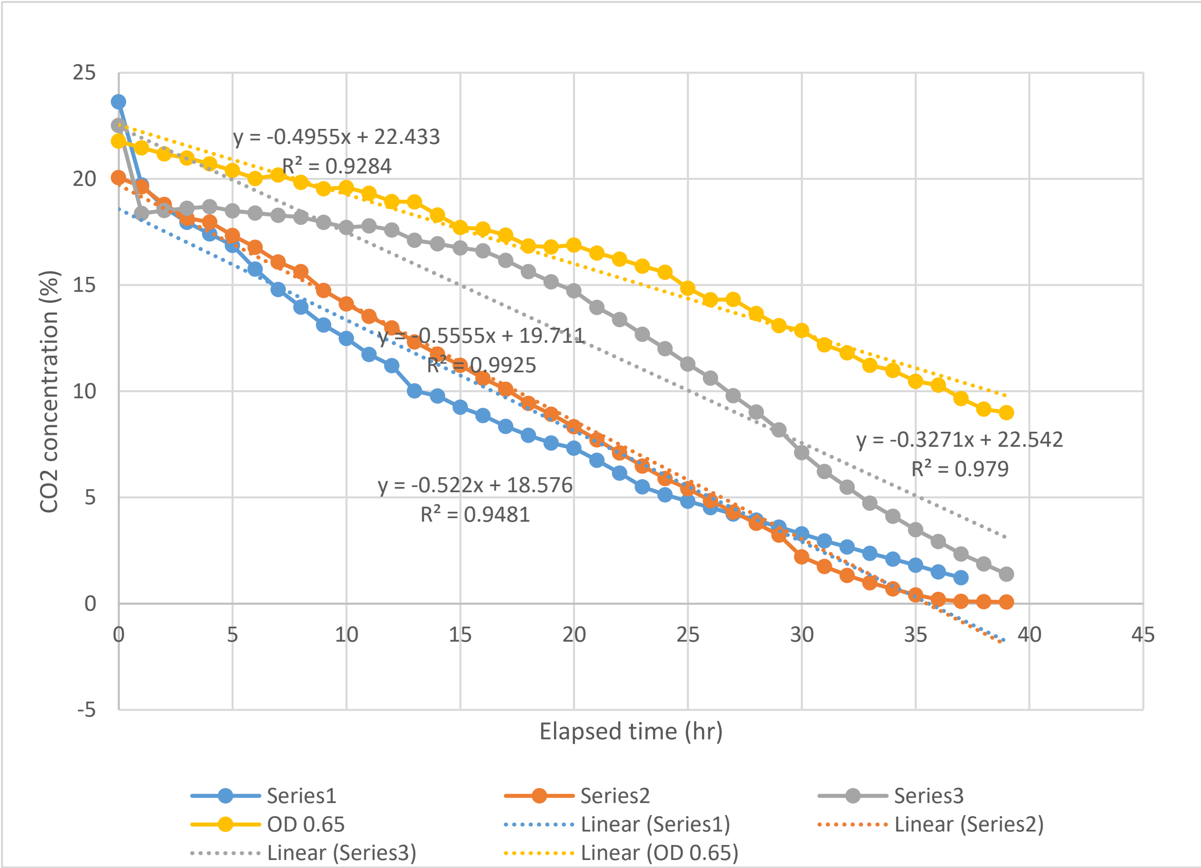


Figure 4.4 CO_2 (%) sequestration with 20% CO_2 in the headspace

Figure 4.5 shows the increase of oxygen in the headspace of the photobioreactor. As previously

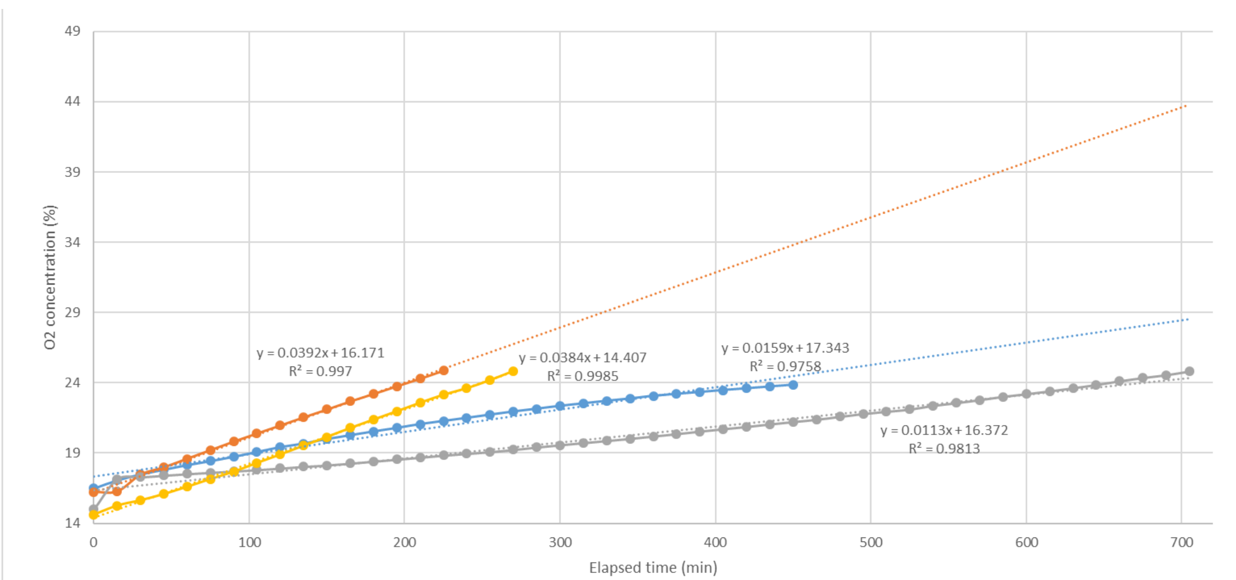


Figure 4.5 O_2 production by Chlorella V. fixing 20% CO_2 in the headspace

discussed, four algae cultures with varying initial ODs were added to the photobioreactor. Figure 4.5 illustrates that an OD_{680} of 0.650, which is at the beginning of the exponential growth phase, had a higher oxygen production rate compared to other tested optical densities.

The Table 4-2Chlorella *V*. presents the results of applying the same method as described in Chapter 3 to determine the produced biomass concentration. Also, the concentration of biomass has converted to the mass of biomass per litre of the bioreactor.

Table 4-2 The initial and final mass of Chlorella V. for every test

OD initial (680 nm)	**Initial CO_2 mass (g)**	**CO_2 mass after 35 hrs (g)**	**Consumed CO2 mass (g) after 35 hrs**
0.493	0.263	0.041	0.222
0.650	0.231	0.122	0.109
0.900	0.234	0.005	0.229
1.030	0.276	0.021	0.255

Table 4-2 The initial and final mass of *Chlorella V.* for every test illustrates the rate of CO_2 consumption per produced biomass in a period of 35 hours. Hu et al. [63] reported that most microalgae strains need to uptake 1.7 kg of CO_2 to produce 1 kg of dry weight biomass. However, the reported rate belongs to open pond raceway with constant

Table 4-3 Consumption of CO2 and biomass production over 35 hours

OD initial (680 nm)	**OD Final (680 nm)**	**Algae initial concentration (g)**	**Algae final concentration (g)**	**Produced algae (g)**	**Consumed CO_2 (g)**	**CO_2 consumed / Produced algae (g CO_2 / g biomass)**	**CO_2 consumed / Produced algae / hour (g CO_2 / g biomass/ hr)**
0.493	0.977	0.056	0.113	0.057	0.222	3.90	0.11
0.650	1.072	0.074	0.124	0.050	0.109	2.18	0.06
0.900	1.032	0.104	0.120	0.016	0.229	14.31	0.42
1.030	1.115	0.119	0.130	0.010	0.255	25.5	0.72

nutrient feeding. Clearly, in such a closed system such as our experiment, this rate would be way lower because of lack of nutrient and carbon substrate over the time. In the table above, the result shows that in OD of 0.493, both algae production and CO_2 uptake are high values compared to other values. Moreover, in OD of 1.030, although the algae production is the lowest, the CO_2 uptake is the highest value compared to the other OD's. This is because of the presence of high concentration of biomass which existed in the photobioreactor from the beginning of the experiment. Moreover, Vaiciulyte et al. [64] reported that they could achieve the highest biomass productivity of Chlorella V. at 0.3 g/L/d with a constant 2% CO_2 flow into the culture. This result is because of the presence of an adequate amount of nutrients and carbon substrate. However, the above experiment conducted over two days without adding any nutrients or carbon substrate. Based on the above experiments, the highest productivity of biomass in 24 hours is 0.039 g/L/d for OD of 0.4

Table 4-4 Biomass produced by microalgae species at a CO2 concentration in literature

Amount of CO_2 provided (%)	**Produced biomass (g/L/day)**	**References**
0.03	0.226	Bhola et al. [59]
4	1.222	Bhola et al. [59]
6	0.21	Chinnasamy et al. [65]

The above Table 4-4 summarizes the existing literature on the amount of provided CO_2 and produced biomass for *Chlorella V*. While providing a basis for comparison, differing experimental conditions between this thesis and the presented literature should be considered. Specifically, the bioreactors presented in the literature are roughly one third the size of our bioreactor as well as using different mediums and 15 days duration.

One of the reasons for low biomass productivity is because the photobioreactor was air-tight. To maintain the system air-tight we could not top nutrient and definitely, after a certain period, the nutrient became limiting for algae growth. Also, Bhola et al. [59] stated that in the similar experiment 15% of CO_2 in the photobioreactor led to a three-time lower yield of biomass compared to the presence of 4% CO_2. This is because of the toxic effect of CO_2 by converting CO_2 to carbonic acid. Produced carbonic acid decreases pH and making an acidic environment for algae (Table 4-5).

Table 4-5 Provided CO_2 and produced biomass based on the different initial concentration

Initial OD (680 nm)	Amount of CO_2 provided (%)	Produced biomass (g/L/day)
0.493	20	0.039
0.650	20	0.034
0.900	20	0.011
1.030	20	0.007

The shows the ratio of produced O_2 mass and consumed CO_2 mass in the period of four hours. The reason for four hours measurement is because the O_2 sensor cannot detect the O_2 concentration above 25% and the O_2 concentration in all four experiments reached the highest point the sensor could read in four hours. Finding corresponding CO_2 uptake in four hours we measured the ratio of produced O_2 and consumed CO_2. Based on the photosynthesis equation stoichiometry, for every CO_2 molecular mass (44 g) which is uptaken by *Chlorella V.* one O_2 molecular mass (32 g) should be produced (ratio=0.73). In this experiment, the ratio in the table above is lower than 0.73 for all four sets of experiments. Comparing the exponential growth phase, the beginning, for its high rate of biomass production and end of the exponential growth phase, for its high accumulate biomass concentration have a greater ratio compared to the middle of the exponential growth phase (Table 4-6).

Table 4-6 The O_2 production of Chlorella V. in the presence of 20% CO_2 after 4 hours

OD initial (680 nm)	**Initial O_2 (g)**	**Final O_2 (g)**	**Produced O_2 (g)**	
0.493	0.650	0.710	0.150	
0.650	0.544	0.879	0.335	
0.900	0.604	0.931	0.327	**Produced O_2 per consumed CO_2, (g O_2/g CO_2)**
1.030	0.614	0.800	0.186	0.45
				0.08
OD initial (680 nm)	**Initial CO_2 (g)**	**Final CO_2 (g)**	**Consumed CO_2 (g)**	0.14
0.493	0.394	0.327	0.067	0.60
0.650	0.381	0.353	0.028	
0.900	0.351	0.304	0.047	
1.030	0.414	0.305	0.109	

4.4 Conclusion

The closed loop is an easy and direct measurement of CO_2 sequestration with minimal sample preparation and without any pre-measurement. Moreover, an additional benefit of this method is simultaneous oxygen production measurements.

As shown in Figure 4.4 and Figure 4.5, *Chlorella V.* has a higher CO_2 sequestration rate and biomass production rate during the beginning of the exponential phase compared to other parts of the exponential phase and stationary phase. During the exponential growth phase, the rate of increase of cells in the culture is correlated to the number of cells present at any time. As a result, if more algae cells are present, then more CO_2 will be fixed.

Overall, the efficiency of CO_2 fixation in a closed cultivation system depends on the microalgae specie, CO_2 concentration, photobioreactor design, and operating conditions. Klinthong [42] states that *Chlorella V.* has a maximum CO_2 removal efficiency of 55.3% at 0.15% CO_2 in a membrane photobioreactor while this experiment shows almost 100% of CO_2 removed at 20% of total volume in 36 hours. Yun [66] calculates the CO_2 removal rate as follows:

$$R_{CO2} = C_c \times \mu_L \times \frac{M\ CO2}{M\ c}$$

where R_{CO2} and μ_L are the fixation rate (g CO_2/m^3 h) and the volumetric growth rate (g dry weight/m^3 h), respectively, and M_{CO2} and M_C are the molecular weights of CO_2 and C, respectively. C_C is the average carbon content as measured by elemental analysis.

5 Nutrient uptake of *Chlorella V.*

5.1 Introduction

In Chapter 3, the growth of microalgae in the BBM and under different environmental conditions was studied measuring oxygen production. In this chapter, we investigate the potential use of microalgae for tertiary wastewater treatment. Also, the effect of the presence of bacteria on the growth of *Chlorella V.* and *Chlorella V.'s* ability to absorb nutrients is analyzed. Also, to keep algae healthy and growing, CO_2 addition was provided to optimize microalgae growth and to keep the system active.

5.2 Material and methods

Wastewater samples were taken from the Bonnybrook wastewater treatment plant (Calgary, AB). The samples were taken from secondary effluent discharge right before the disinfection unit. In this study, wastewater samples were used directly after collection and filtration with 0.45 µm. Also, after filtration, half of the samples were autoclaved to monitor the nutrient uptake of microalgae without the presence of bacteria. Experiment 1 is investigation the ability of nutrient uptake of algae in sterile and non-sterile wastewater effluent medium while experiment 2 is same experiments with an excess amount of nutrient. The characteristics of the collected secondary effluent are listed in Table 5-1:

Table 5-1 Pre-disinfection effluent properties

Properties	**Secondary effluent (Non-sterile) mg/L**	**Secondary effluent (Sterile) mg/L**	**BBM (mg/L)**	**Algae seed (mg/L)**
Total nitrogen	56.642	57.486	145.358	72.48
Total phosphorus	0.673	0.223	139.31	83.739
Total inorganic carbon	0.5184	0.307	0.788	3.136

As mentioned in Chapter 3 in more detail, the algae strain in this experiment is *Chlorella Vulgaris.* Initially, it was cultured in the BBM to maintain the proper amount of stock cultures to carry out the tests.

In this batch of experiments, three 250 mL sterilized Erlenmeyer flasks, shown in Figure 5.1, were chosen. Also, Figure 5.2 shows the growth of algae after three days. The Erlenmeyer flasks were incubated at room temperature, 22 ± 2°C, in the lab. Continuous dimmable LED strip lighting was provided for the batch at an intensity of 1700 LUX and a distance of 10 cm behind and around the flasks. Samples were taken daily to analyze optical density (680 nm), pH, temperature, phosphorus, and total nitrogen. The pH and temperature were monitored by the PINPOINT pH monitor. The experimental period was six days. All measurements had two replicates, and the average values are reported in the figures. The wastewater and microalgae culture was maintained in suspension by aeration. Constant aeration was provided to the three flasks using flowmeters. Also, the total inorganic carbon (TIC) was measured.

Aeration was achieved through air bubbling provided by aeration stones placed on the bottom of the Erlenmeyer flasks. The flasks were covered with aluminum foil to minimize evaporation; the effect of the minimal amount of evaporation was considered by calculating the daily amount of sample that was lost. The experiments were carried out without the addition of nutrients or any chemicals for pH adjustment. Three flasks were seeded with 40 mL of microalgae seed inoculated in 200 mL of wastewater samples. The first sample had a medium of non-sterile wastewater (WWn); the second sample contained sterile wastewater (WW-s), and the third flask used BBM as the medium.

Figure 5.1 Initial inoculation of microalgae

Figure 5.2 The growth of microalgae (exponential phase)

In the second set of experiments, 200 mg/L of ammonium nitrate and 30 mg/L of potassium dihydrogen phosphate were added to the three flasks. Thus, BBM with added nutrient is called amended BBM (BBM-a). Besides all three samples, a normal sample with BBM medium also was prepared as a control sample. The purpose of the second experiment was to increase the amount of nutrients deliberately in the culture and monitor the nutrient absorbing behaviour of *Chlorella V.*

5.2.1 Chemical analyses

The characteristics of the cultures were analyzed every day after taking liquid samples from the reactors using a pipette during the experiment period. The collected samples were filtered through a 0.45 μm syringe filter and then through another 0.45 μm filter using a vacuum pump. Before filtration, the temperature of the sample, PH, and OD_{680} were measured. After filtration, the supernatant samples were immediately analyzed for TN and P with an IC machine. Nitrate, nitrite, sulphate, and phosphate were analyzed using 930 Compact Flex Ion Chromatography, Metrohm, equipped with a Metrosep A Supp 5 – 100/4.0 column, an Rp2 Guard/3.5 column and a 913 autosampler. An eluent containing 3.2 mmol/L Na_2CO_3 + 1 mmol/L $NaHCO_3$ was used. The eluent flow was set at 0.8 mL/min. The detection limit was 1.0 mg/L. Also, Total organic compound (TOC) and Total inorganic compound (TIC) were determined using Shimadzu TOC-LCPH equipped with an ASI-L autosampler. The combustion temperature for TOC and TIC was 680 °C, and the detection limit was 0.5 mg/L.

5.3 Results and discussion

The results of growth experiments and growth with added nutrients are listed below. As previously mentioned, microalgae growth was monitored by taking two daily measurements for pH, OD_{680}, temperature, (TSS), and nutrients. The lag phase lasted for one day, which indicated that the microalgae growth was extended due to the new environmental conditions in both experiments. After the lag phase, during the second day, a sudden increase of biomass occurred, implying the exponential growth phase of microalgae. The microalgae biomass started to increase until the fourth day of inoculation, at which point the stationary phase began (Figure 5.3).

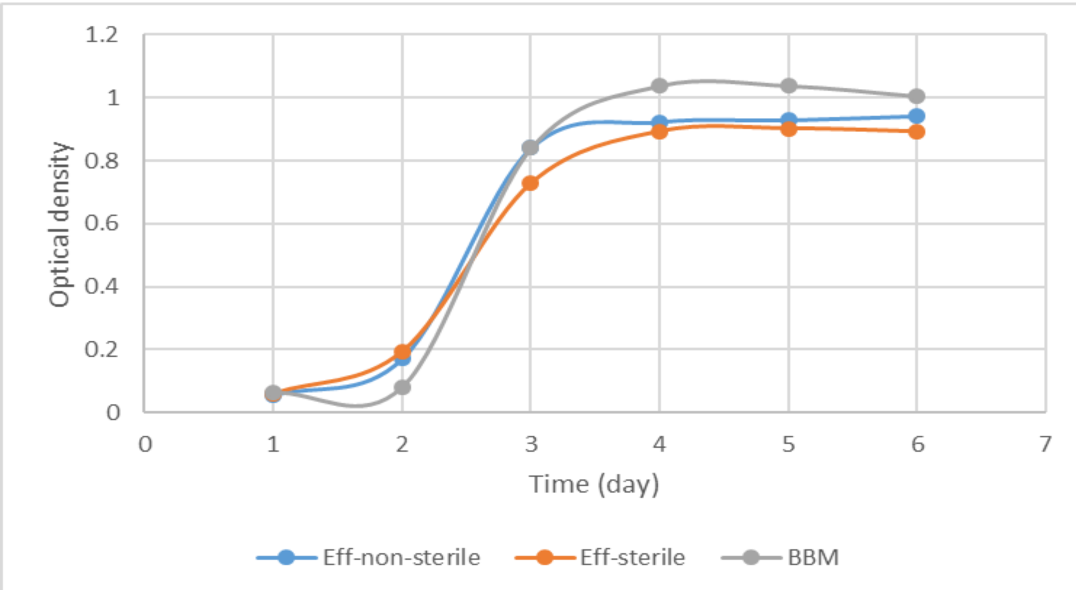


Figure 5.3 Growth in biomass of Chlorella V. in experiment 1

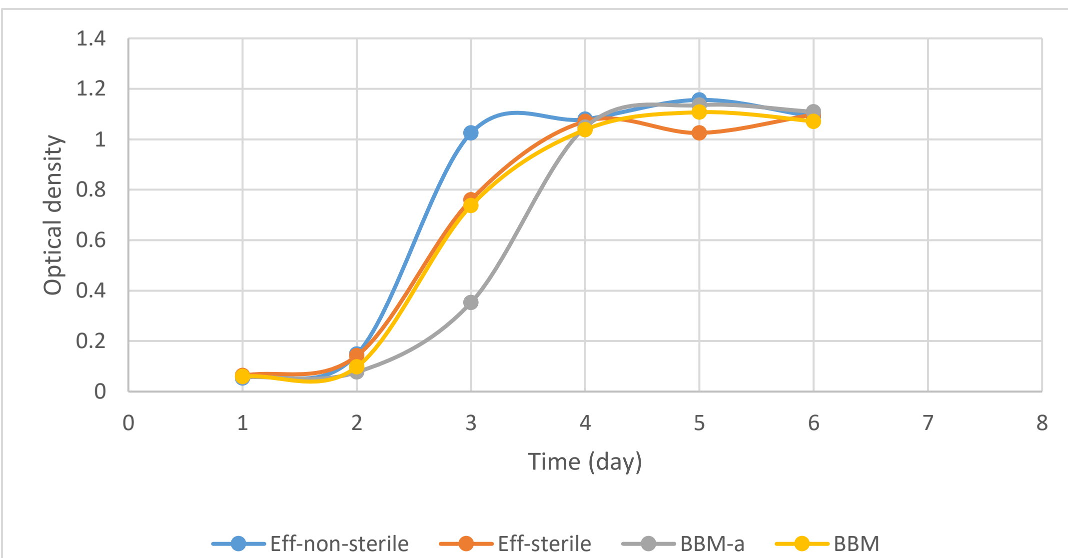


Figure 5.4 Growth of biomass of Chlorella V. in experiment 2 with added nutrients

In the second experiment with added nutrients, the same trend of growth was observed in all experiments excepting the sample of the BBM culture with added nutrients (Figure 5.4).

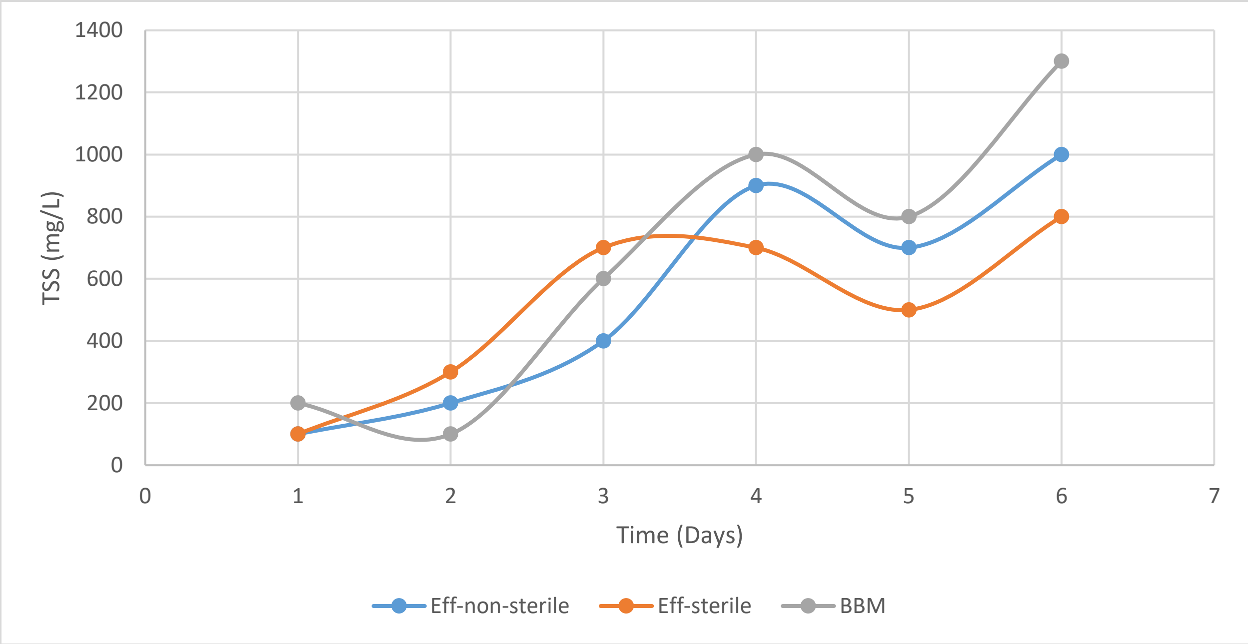


Figure 5.5 Total suspended solid changes in experiment 1

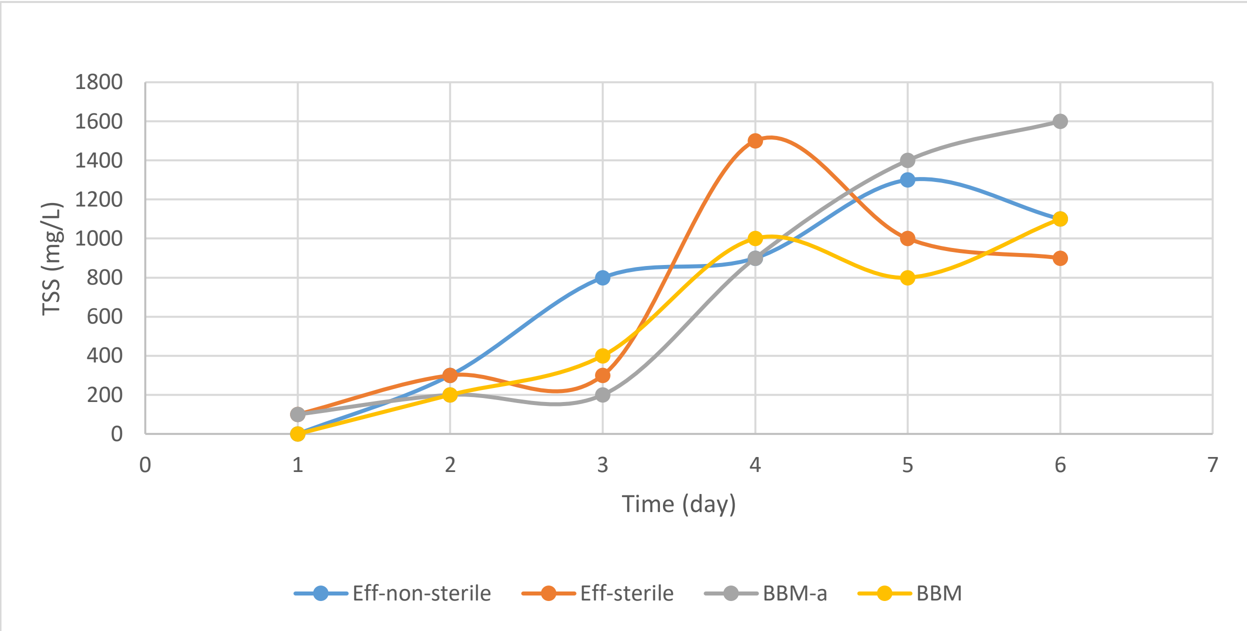


Figure 5.6 Total suspended solid changes in experiment 2 with added nutrients

Using TSS measurements to monitor the microalgae growth rate, we found a drastic increase in TSS from the beginning to the end of the experiment. The mean growth rate of TSS for the six days was 150 $mg/L.d^{-1}$, 116.67 $mg/L.d^{-1}$, and 183.33 $mg/L.d^{-1}$ for WW-n, WW-s, and BBM, respectively. These values show that BBM is an optimal medium for microalgae to thrive in wastewater (Figure 5.5, Figure 5.6).

Further, for experiment 2, the BBM medium with 200 mg/L of ammonium nitrate and 30 mg/L of potassium dihydrogen phosphate had the highest growth rate, 250 $mg/L.d^{-1}$; WW-n and BBM-n both had the next highest growth rate at 183.33 $mg/L.d^{-1}$. WW-s had the lowest growth rate of the samples, 133.3 $mg/L.d^{-1}$. This result again clearly states that BBM is an optimal medium to grow algae compared to wastewater.

5.3.1 Change of pH

The pH value in both experiments stayed almost constant with an average value of 7.12 and 7.19 in experiments 1 and 2, respectively. The pH increased as the dissolved CO_2 was removed from the water through photosynthesis. According to the equilibrium equation is as follows:

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O$$

H^+ is consumed during the conversion of HCO_3^- to CO_2, and CO_2 is ultimately fixed by Rubisco during photosynthesis. Thus, reducing H^+ in the culture medium unavoidably leads to an increased pH. However, constant aeration of 3% CO_2 into the culture provided an adequate amount of an inorganic source for microalgae uptake.

5.3.2 Removal of total nitrogen

The presence of nitrogen is essential for microorganisms and plants to live and grow. Microalgae are capable of consuming dissolved nitrogen. In Figure 5.7 and Figure 5.8, the amount of total nitrogen decreased over the six days of the experiment.

In experiment 1, the total dissolved nitrogen decreased to zero after three days, and, in experiment 2, after five days. In both experiments, WW-n and WW-s showed the same TN depletion rate. In experiment 1, although the concentration of TN in the BBM culture was two times higher than in WW-n and WW-s cultures, the TN was consumed in the same amount of time, which indicated that TN was consumed two times faster in BBM. Consequently, BBM is a better culture environment for microalgae. The same trend was seen in experiment 2. In experiment 2, for the BBM culture with added nutrients, a small peak occurred, which implied a slight increase in TN. This increase may have occurred because of the nitrification of the ammonium portion of ammonium nitrate. The total removal of TN by *Chlorella V.* is a promising result in wastewater treatment.

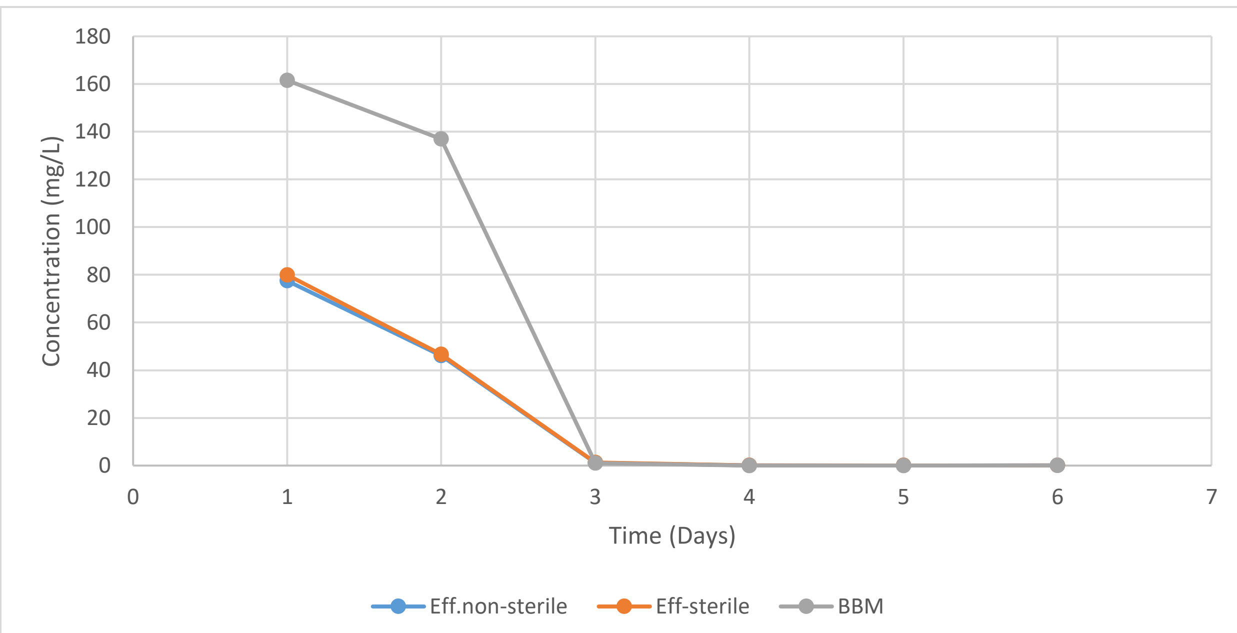


Figure 5.7 Dissolved total nitrogen concentration depletion in experiment 1

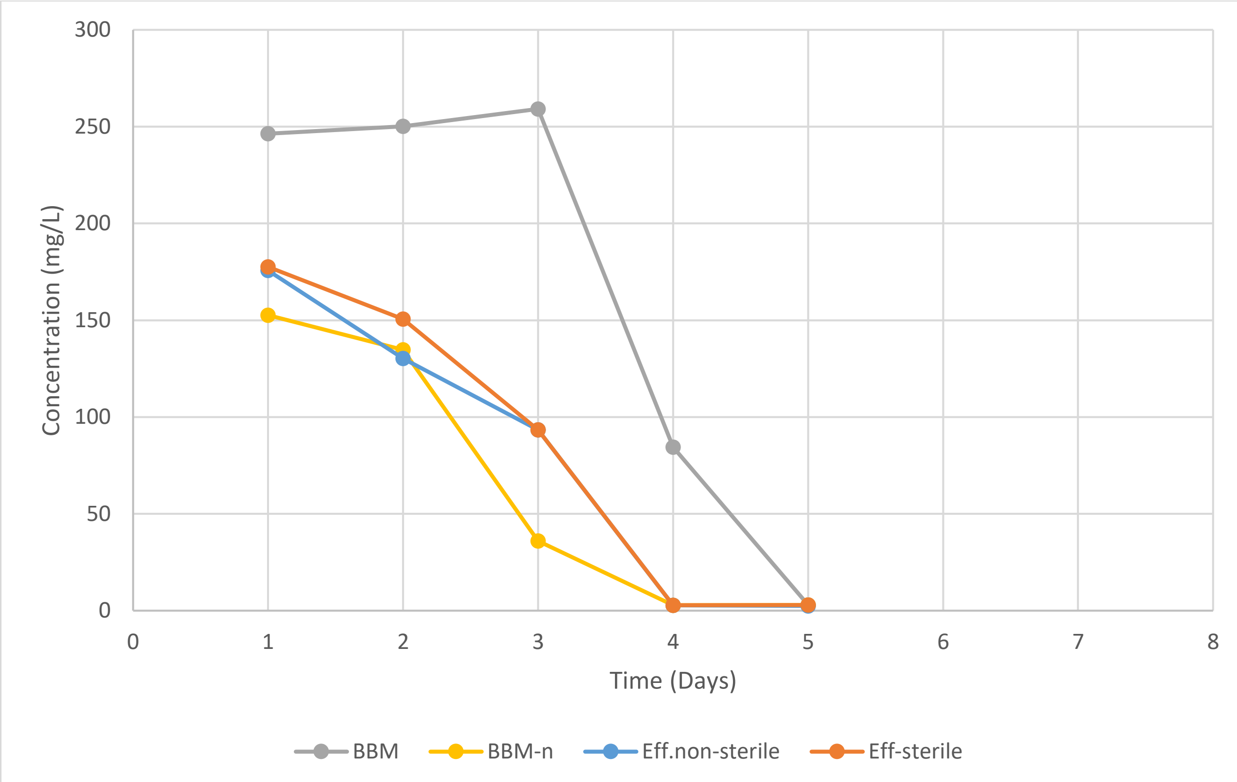


Figure 5.8 Dissolved total nitrogen concentration depletion in experiment with added nutrients

5.3.3 Removal of phosphorus

Phosphorus is another required nutrient for the growth of microalgae. The consumption of

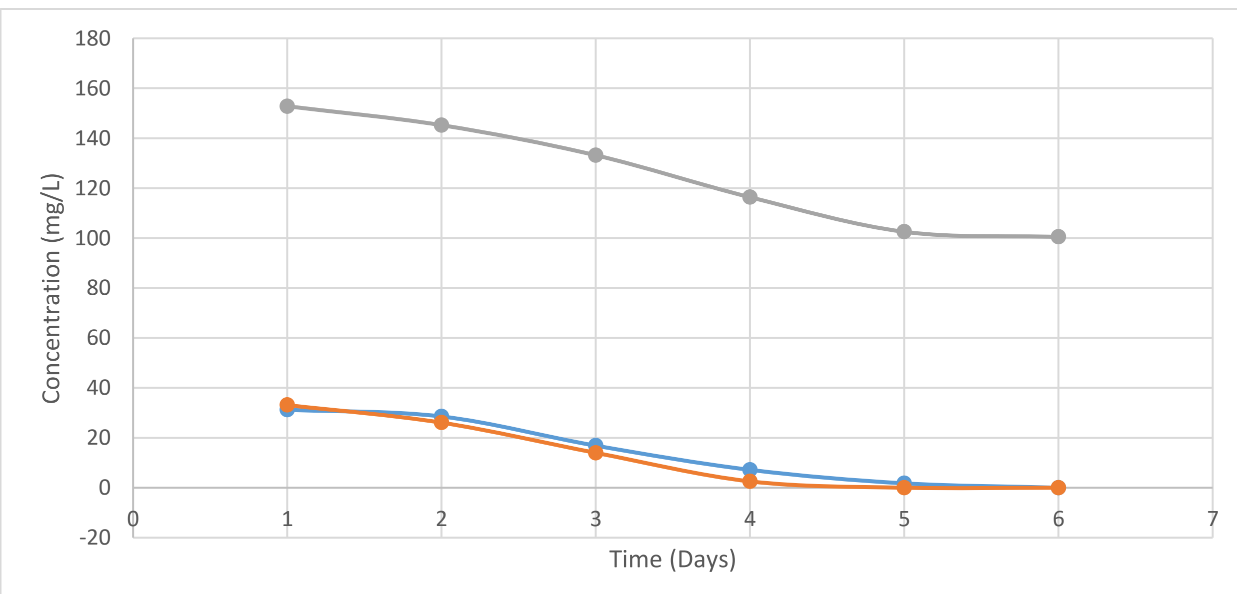


Figure 5.9 Phosphorus concentration changes during experiment 1

phosphorus is illustrated in Figure 5.9and Figure 5.10 for experiment 1 and 2, respectively.

In experiment 1, the initial concentrations of phosphorus were 31.22 mg/L and 33.1 mg/L for WW-n and WW-s, respectively, and this amount reached zero on the fourth day, which indicated 100% uptake of the phosphorous in the wastewater. The initial concentration of phosphorus in BBM was 152.78 mg/L; the concentration after six days was 100.44 mg/L, which indicated a 34.3% reduction.

In experiment 2 with added nutrients, the initial concentrations of phosphorus were 36.51 mg/L and 35.62 mg/L for WW-n and WW-s, respectively, and this amount reached zero on the fourth day of the experiment, which indicated 100% uptake of the phosphorus in the wastewater. The initial concentration of phosphorus in the BBM was 139.36 mg/L; the concentration after six days was 123.97 mg/L, which indicated an 11% reduction. The initial concentration of phosphorus in BBM-n was 140.16 mg/L, which, after an 18.2% reduction, reached 138.9 mg/L.

This experiment shows that *Chlorella V.* is capable of absorbing low concentrations of phosphorus, approximately 30 mg/L up to 100%. However, for higher concentrations, about 140 mg/L, the consumption of phosphorus drops 10%.

Conducting this experiment illustrates that nitrogen can limit the growth of *Chlorella V.*, while *Chlorella V.* may absorb phosphorus up to a certain amount.

Also, *Chlorella V.* can adjust the uptake process of nitrogen and phosphorus concentration in their biomass based on existing nitrogen and phosphorus supply in the wastewater, which is important to have simultaneous removal of both nitrogen and phosphorus in wastewaters. According to Beuckels et al. [67], microalgae can accumulate more P when N concentrations are high. In contrast, our study showed that *Chlorella V.* phosphorus uptake in high nitrogen medium did not significantly change compared to lower nitrogen concentration medium.

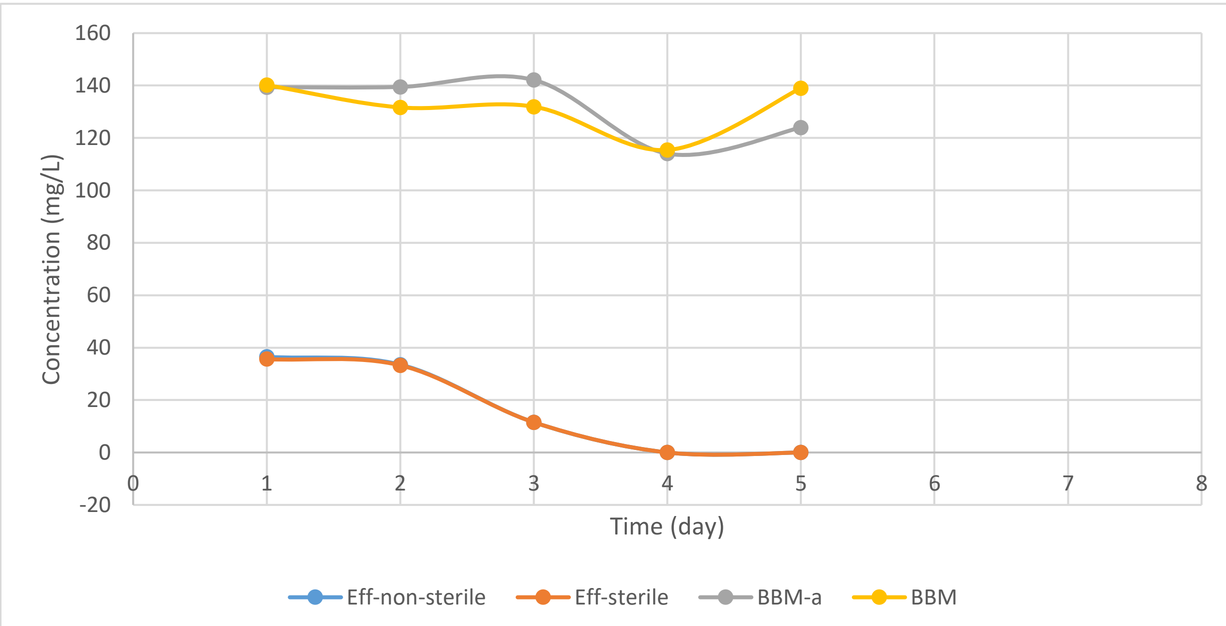


Figure 5.10 Phosphorus concentration changes during experiment 2

5.4 Conclusion

In this set of experiments, the possibility of improving tertiary wastewater treatment with microalgae as an axillary process was investigated. Absorbing nutrients from sterile and non-sterile wastewater effluents were identical. Over these six days of treatment, *Chlorella V.* successfully removed nitrogen and phosphorous from the wastewater effluent. For WW-n and WW-s, 100% of nitrogen and almost 30% of phosphorus was removed from the wastewater effluent.

This study illustrated that microalgae strains, such as *Chlorella V.*, are capable of removing nutrients. This promising characteristic of microalgae allows microalgae to be used as a tertiary treatment or side treatment at wastewater treatment plants for a reasonable cost. Also, this study showed that the presence of bacteria and pathogens would not interrupt the nutrient removal ability of microalgae.

Microalgae can facilitate the wastewater treatment process, and it also generates high-valued products, such as biomass, that can be harvested and used to make biodiesel and biogas, cheap by-products of the overall process.

6 Conclusion

The purpose of this thesis was to research the growth of *Chlorella Vulgaris* for the tertiary treatment of domestic wastewater and the sequestration of CO_2 to meet the Alberta Climate Leadership Plan which is ending pollution from coal-generated electricity and developing more renewable energy.

Chlorella Vulgaris will grow fast under certain physical conditions in BBM such as pH range of 7-8, temperature range of 25-30°C, light intensity of 4755 LUX, while 5% CO_2 is introduced into the culture.

The presence of inorganic materials in effluent containing nitrogen and phosphorus cause eutrophication in the long term. The ability of microalgae to photosynthesize similar to other plants lets microalgae thrive in wastewater effluent as a medium. In this research, *Chlorella V.* was able to remove 100% of the dissolved nitrogen and more than 30% of the dissolved phosphorus in the culture.

Moreover, a correlation between optical density in a wavelength of 665 nm and cell density (mg/L) was found, which allowed for an easier measurement of the concentrations produced by the microalgae.

In addition to the literature reviews on the cell fragility of *Chlorella V.*, a variety of experiments were conducted to determine the behaviour of *Chlorella V.* under different, harsh physical conditions. It was found that *Chlorella V.* survived vigorous blending with more than 90% cell viability. In addition to blending, *Chlorella V.* lasted a long time under intensive centrifuge.

Microalgae's power to sequester CO_2 and eliminate harmful residues found in effluent and convert CO_2 to valuable products, such as biomass, biodiesel, and biogas, is promising for environmental science and is profitable for developed countries such as Canada. In this research, *Chlorella V.* was able to consume CO_2 and produce O_2 through photosynthesis as high as 20% in total gas volume. This result is significant since no study performed to sequester CO_2 higher than 15% of CO_2 in total gas volume. Microalgae at the beginning of the growth phase had a high potential for fixing CO_2. Keeping the culture in the exponential phase by harvesting microalgae more frequently and adding a fresh medium can promote an even higher CO_2 sequestration rate. The production of oxygen from this method can potentially be applied to the tertiary wastewater treatment. Produced oxygen can be used for the biodegradation process, which can reduce the price of aeration in wastewater treatment plants.

6.1 Future study

- Determine the physical properties of the cells under environmental stress conditions
- Focus on increasing the concentration of microalgae and optimizing harvesting methods
- Conduct experimental analyses to decrease the cost of biofuel production compared to conventional fossil fuel production

- Find the optimum medium for a higher growth rate
- Investigate the impact of mixed-culture strains
- Research microalgae growth in the Alberta climate

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