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Magnetic Stimulation on the Growth of the Microalga Nannochloropsis oculata

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A thesis submitted in partial fulfillment of the requirements for the Master of Engineering
Science degree in Chemical and Biochemical Engineering

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ABSTRACT

Fossil fuels, our principal sources of energy supply, are non-renewable and research is needed on alternatives that are renewable and potentially more environmentally friendly. Microalgae have been investigated as a future feedstock alternative to petroleum but the technology is still expensive and improvements are needed. Reduction in costs might be achieved by increasing algal biomass and lipid productivity. The lipids can be used to produce biofuels such as biodiesel and biojet fuel. The marine microalga *Nannochloropsis oculata* grows well and can accumulate high lipid content. In this study, the effects of static magnetic field stimulation (SMF) of 0 (control), 5, 10 and 15 mT were investigated in terms of growth and biochemical composition of this microalga. In comparison to the control, the cells grown at 10 mT had the highest increase in biomass productivity (45%) and lipid productivity (57%) in addition to increase in other co-product yields. Some of the co-products could potentially be used for high value-added applications, thus helping to offset costs even further. The use of magnetic field stimulation on microalgae is a promising technique to enhance growth and productivity, and *Nannochloropsis oculata* was shown in this study to be a suitable microalgae species to be considered for biodiesel applications.

Keywords: *Nannochloropsis oculata*, microalgae, static magnetic fields, biomass, lipid, co-products, productivity

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LIST OF ABBREVIATIONS

AC	alternating current
AV	acid value
COD	chemical oxygen demand
DC	direct current
DDW	double distilled water
DHA	docosaheptaenoic acid
DNA	deoxyribonucleic acid
DO	dissolved oxygen concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDX	energy-dispersive X-ray spectroscopy
EMF	electromagnetic field
EPA	eicosapentaenoic acid
Fe-EDTA	ethylenediaminetetraacetic acid iron(III) sodium salt
G	Gauss
GHG	green house gas
HPS	high-pressure sodium
ICNIRP	International Commission on Non-Ionizing Radiation Protection
ICP-MS	inductively coupled plasma mass spectrometer
IV	iodine value
LR	London resin
MAA	mycosporine-like amino acid
MRI	magnetic resonance imaging
NO _x	nitrogen oxides
PAR	photosynthetically active radiation
PBR	photobioreactor
PBS	phosphate-buffered saline
POER	photosynthetic oxygen evolution rate
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
SEM	scanning electron microscopy
SMF	static magnetic field
SO _x	sulfur oxides
T	Tesla
TAG	triglyceride
TEM	transmission electron microscopy
TN	total nitrogen
TP	total phosphorous
UV	ultraviolet
UV-Vis	ultraviolet-visible
v/v	volume/volume
vvm	volume of gas per volume of cell suspension per minute
w/w	weight/weight

CHAPTER 1 – Introduction

It is estimated that the world's oil reserves will be depleted in less than 50 years [1]. Although the exact time for exhaustion is speculative, it is well known that petroleum-derived fuels are non-renewable and will end nonetheless. In addition, combustion of petroleum-derived fuels generates green house gases (GHG), primarily carbon dioxide, which have been associated with global warming and climate change [2, 3]. As a result, demands for a decrease in our carbon footprint, particularly via carbon dioxide reduction, and the search for cleaner and renewable fuels is increasing [1, 4, 5].

In order to sustain global economic development, renewable plant based fuel feedstocks such as crops and microalgae have been investigated as a future replacement for petroleum [3], especially in the transportation sector which has the second highest demand for energy in the world [6]. In 2016, the U.S Energy Information Administration reported that the majority of petroleum consumption came from the transportation sector (Figure 1.1), (due to rounding, data might not add up exactly to 100%) mainly in the form of gasoline, diesel and jet fuel [7, 8].

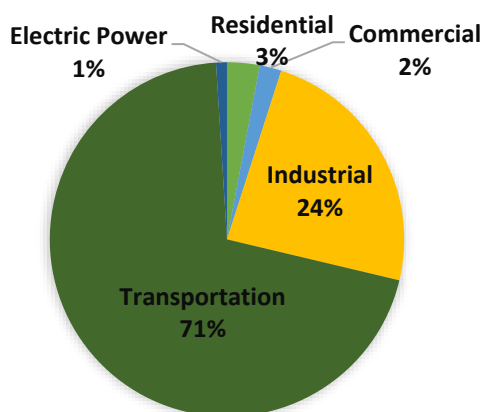


Figure 1.1: U.S petroleum consumption by sector (2016).

The most common liquid biofuels sought as an alternative for petroleum-derived fuels in the transportation sector are bioethanol and biodiesel. Typically, sources utilized for bioethanol production are sugarcane and corn [9], with sugarcane being the most productive feedstock, while

biodiesel usually comes from oil crops (oilseed plants) like soybeans (15-20% oil content) and palm oil (30-60% oil content) [10, 6].

However, the current oil crops utilized for both bioethanol and biodiesel occupy a considerable amount of land space. It is estimated that if all petroleum-derived fuels were substituted by oil crops in the United States, then 61% of all agricultural land would need to be required [10], which not only would compete with crops that could be used for human consumption but would also impact the biological diversity on Earth if more forests were destroyed to be replaced with oil crops [3]. Therefore, the practice would be unsustainable.

Microalgae have been studied as a promising feedstock for biofuels due to their high photosynthetic efficiency, removing CO₂ from the environment while growing fast and accumulating high biomass in addition to occupying less land space than crops. Additionally, microalgae have the ability to accumulate high lipid content, commonly in the range 20-50% and with some species being able to accumulate more than 80% of their biomass weight in lipids [5].

In addition to lipids, it is also desirable to look into other co-products produced by microalgae such as proteins, carbohydrates and pigments due to their added value to the biomass helping offset biofuels production costs [4]. These co-products can be used in different applications, for example, as food, energy (biodiesel, bioethanol and jet fuel), pharmaceuticals/nutraceuticals (beauty products, anti-aging), biodegradable plastics and non-toxic paint [11].

When investigating microalgae for fuel applications, it is important to look at species selection for their growth ability and product accumulation [12]. Moreover, it is also important to increase microalgae production in order to increase the cost competitiveness with respect to petroleum-derived fuels. Growth and accumulation of products are commonly enhanced by different types of stress conditions in the microalgae's environment such as changes in nutrients, salinity and temperature [13]. Some research has investigated the addition of magnetic fields as an external stressor [14, 15, 16, 17, 18, 19, 20, 21].

This work focuses on the marine microalga *Nannochloropsis oculata* as a promising feedstock for biofuel and additional co-products production through growth stimulation (enhancing biomass production) using an external static magnetic field (SMF). From the product optimization side, in addition to maximizing lipid (fuels product) yields, the high value products are usually found in smaller percentages of the biomass but can be significantly more valuable and could further enhance the production economics. This technology has a great opportunity to become economically feasible for biofuels production competitively in the future [22].

1.1 Research Objectives

The objectives of this research are to: (1) Design and implement a system to grow and expose *N. oculata* to static magnetic field stimulation; (2) Study the kinetics of the microalgae growth process in terms of biomass yield as a function of magnetic field intensities (0, 5, 10, 15 mT); (3) Analyze cell morphology and ultrastructure; (4) Characterize the algae biomass composition to determine the effect of magnetic field in the biomass content of lipids and high value co-products.

1.2 Thesis Overview

This thesis consists of six chapters. Chapter 1 gives a brief introduction about microalgae and the research motivation and focus. Chapter 2 contains a literature review about microalgae cultivation and applications, species commonly used for biodiesel applications, including the selected species for this work, growth stimulation techniques and how static magnetic fields have been used in research, followed by how the research in this thesis relates to the literature. Chapter 3 contains the materials and methods for the experiment set up and analysis. Chapter 4 covers the results obtained. Chapter 5 contains a discussion about different results obtained in this work for each magnetic field intensity and also how they relate to the literature. Chapter 6 consists of the conclusions that can be drawn based on the results and discussion of the work in addition to future work recommendations.

CHAPTER 2 - Background and Literature Review

Different microorganisms such as yeasts, bacteria and microalgae have been investigated in the broad field of biotechnology, which explores the use of living organisms for a variety of commercial purposes. Microalgae are very versatile microorganisms managing to survive and thrive on Earth for billions of years despite of adverse weather and environmental changes. In addition, microalgae can be used for more than one application at the same time, for example, for both carbon dioxide sequestration and production of food and fuels. Sustainability and versatility combined make microalgal biotechnology a powerful tool to continue sustaining our current and future high standard of living while potentially offering lower environmental impacts than current technologies such as petroleum extraction and use.

2.1 Microalgae

Algae (singular alga) have been around for about 2 billion years [23] and are a very diverse group of organisms having a simpler structure in comparison with land plants. There is a large variation of algae in terms of habitats, morphology and size ranges, for example, which typically go from 0.2-2.0 μm in diameter for picoplankton to 60 m in length for giant kelps. They are classified into two broad categories: macroalgae, which are multicellular large species, and microalgae, which are unicellular, microscopic in size and generally not visible by the human eye [24]. It is estimated that the number of algal species are in between one and ten million, with the majority of the species being microalgae [25].

Microalgae, like other types of algae, live in places where water is present (even in small quantities) and are typically photosynthetic organisms, converting carbon dioxide (CO_2) and water (H_2O) into sugars ($\text{C}_6\text{H}_{12}\text{O}_6$) and oxygen (O_2) by utilizing energy from the sun (Figure 2.1). They also use nutrients found in their environment, such as phosphorous and nitrogen, to produce substances like lipids and proteins. Carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorous (P) and sulfur (S) are the major macronutrients required for microalgae growth but other elements, such as iron, manganese and other trace metals are important and need to be provided to the media since they are used in photosynthesis, nitrate assimilation and other enzymatic processes in the cell [26]. They

occupy less land space, can be grown in salt, brackish or waste water and have higher light conversion efficiency in comparison with oil crops since they can accumulate more biomass more quickly per hectare [27].

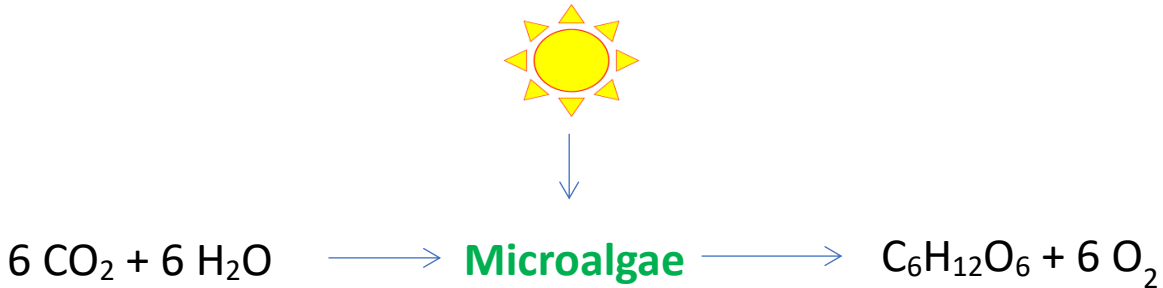


Figure 2.1: Photosynthesis.

2.1.1 Cultivation

In nature, microalgae are found in different ecological habitats and therefore, each species will have a different requirement for growth unless the species selected thrive in very similar environment. The growth will depend on factors such as light, temperature, salinity, pH and nutrients (which can be essential such as nitrogen, phosphorous and iron, and in trace quantities like cobalt and magnesium) [5, 28].

When growing microalgae in a laboratory setting, it is important to initially mimic the microalgae's natural growth environment. If the purpose is to keep seed (starting) cultures alive and at minimum nutritional requirements, the quantity of major nutrients can be estimated by using the approximate microalgae biomass molecular formula $\text{C}_{100}\text{O}_{48}\text{H}_{183}\text{N}_{11}\text{P}_1$ [5]. Recipes for some species, including optimized ones (depending on the application), can also be found in the literature [29, 30, 31, 32]. Growth conditions in a laboratory setting are performed aseptically and are more controlled to allow for better investigation and inference of how different parameters affect the cells. Since microalgae are very adaptable, once the culture is well established and the cells are growing well, the conditions can be changed for different experiments such as to observe growth or biochemical composition changes.

In addition, initial culture and experiments are performed in Erlenmeyer flasks while mass cultivation of microalgae (large scale) is performed in three main types of systems: open, closed or a combination of both (Figure 2.2). Open systems include open ponds and tanks. They are simple and low cost, however not as efficient as bioreactors due to the limited growth conditions, water evaporation, low biomass productivities and easy contamination by foreign species. Closed systems like photobioreactors (PBRs) are found in various types of design and are built to optimize growth, giving higher biomass productivities, and make it easier to control growth conditions, which make them best suitable for applications where species purity is required, however they are perceived to be more expensive although some claim that since the microalgae productivity is higher, this helps offset any additional costs to build and maintain the PBRs [33, 5].

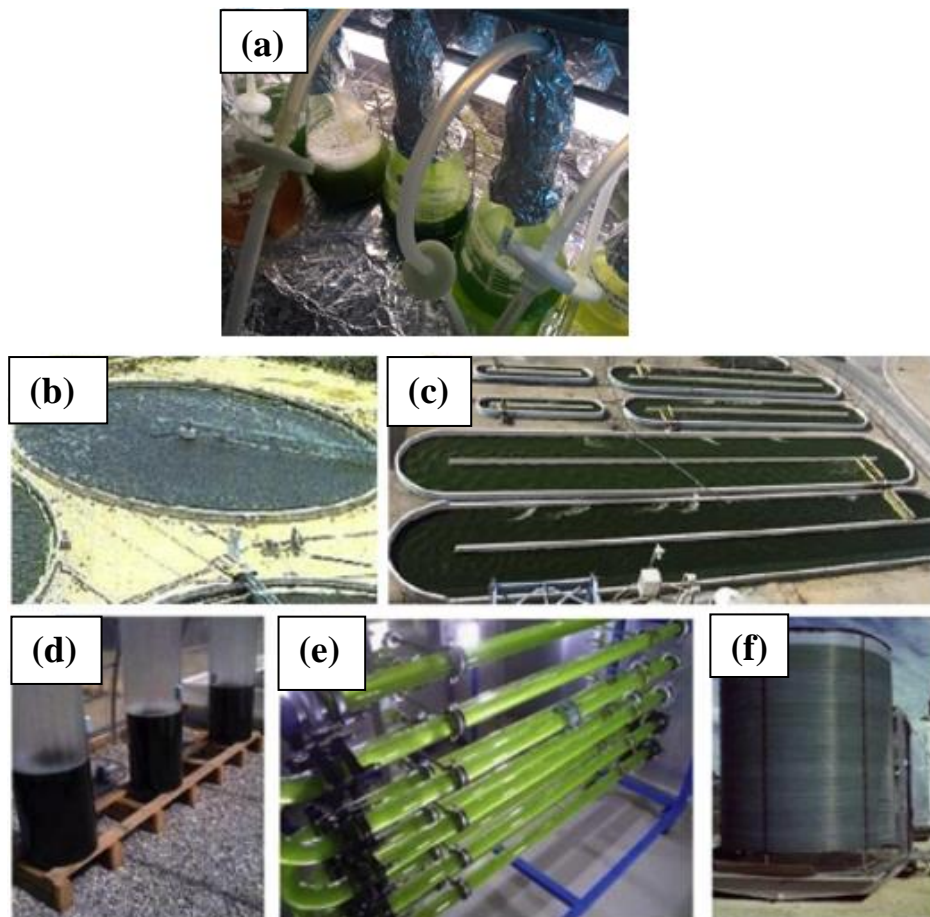


Figure 2.2: Microalgae cultivation systems: (a) Erlenmeyer flasks, (b) circular pond, (c) raceway pond, (d) vertical tank, (e) horizontal tubular photobioreactor, (f) helical tubular photobioreactor.

Partially reprinted from [28], Pages 483-499, with permission. Copyright (2015) Elsevier.

2.1.2 Applications and Commercial Interest

While microalgae have been known and used as a food source for thousands of years, the cultivation of microalgae and commercialization are only a few decades old [34]. The research on microalgae has been motivated by some moments of crises in our history and has developed from there due to new discoveries on microalgae applications and drives for technologies that are more environmentally friendly. The first interest on microalgae dates back to 1950 when algal biomass was investigated as a potential solution to attend the protein demand of the increasing world's population. Still in the 1950s, additional research on microalgae and microalgal products began. Furthermore, the USA had been experimenting with microalgae for wastewater treatment and subsequent fermentation of the algal biomass as a renewable energy source for production of methane. The oil crisis in 1970 increased the research on microalgae for energy applications [34].

Most of the commercial work has been focused, but not exclusively, on food, cosmetics and nutraceutical/pharmaceutical applications [34, 35]. Commercial cultivation in large scale begin in 1960 with *Chlorella* in Japan, and various companies followed after that in locations such as USA, Mexico, India, Australia, China, Myanmar and Germany. Another common type of microalga called *Spirulina* (or *Arthrospira*) has been typically produced in USA and China. It is considered safe and used as a dietary supplement or colour additive [36].

As microalgae grow, they produce and accumulate in their bodies substances such as lipids, carbohydrates, proteins, pigments, antioxidants and polyunsaturated fatty acids (PUFAs). Research on microalgae has been broad but not all applications have resulted in large scale production and commercialization. Figure 2.3 shows a summary of potential applications of microalgae co-products whether they have already been implemented in large scale or not. Various applications can be combined in a single plant for a biorefinery approach which can have different designs depending on the best species for a targeted product (or products), culture conditions (to stimulate production of a targeted product) and design of an efficient and economical cultivation system (or photobioreactors) [37].

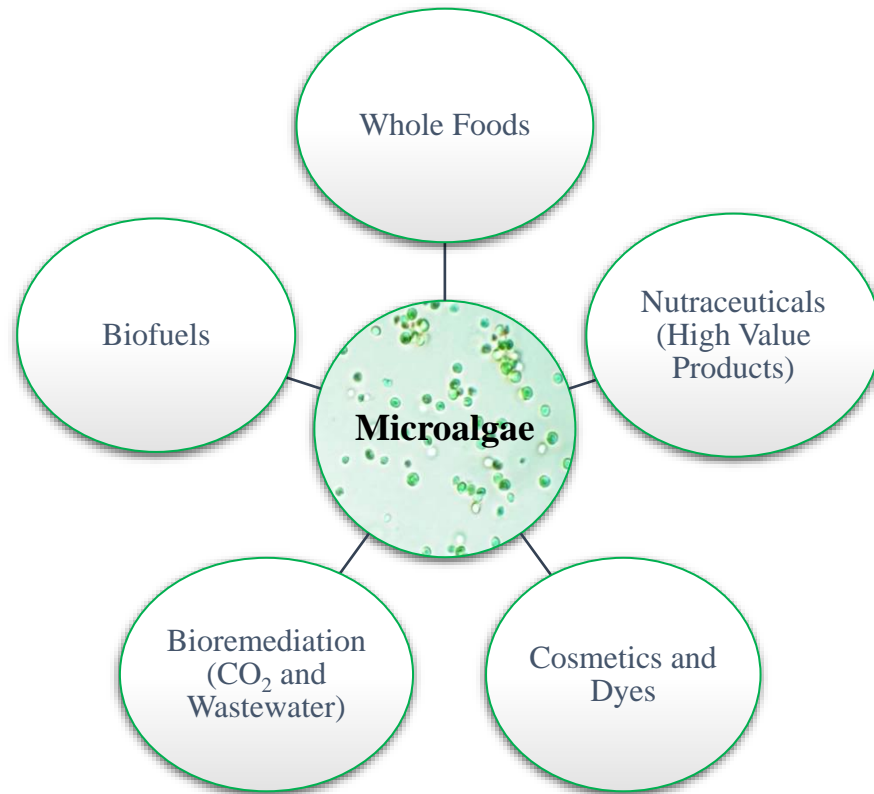


Figure 2.3: Microalgae applications.

2.1.2.1 Whole Foods

In the food industry, microalgal biomass can be used as is for human and animal nutrition. Species commercially utilized as food sources are *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* and *Spirulina sp.* [35]. As whole foods, microalgae are a great nutritional source for both human and animals because of their nutritional content in terms of good fatty acids (omega-3 and omega-6), proteins, antioxidants, vitamins (A, C, E, B₁₂) and minerals (magnesium, calcium) [35, 37]. Microalgae's natural and powerful antioxidants help fight against aging and diseases such as cardiovascular disorders and cancer [35, 38].

They are also a very attractive choice for vegans. Microalgae have advantage in comparison to fish since they can accumulate more of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per mass, which our bodies need for proper cell function and signal

transduction [39], and fish have often problems with heavy metal contamination [40]. In fact, microalgae are also a better protein source than common protein sources used in the vegan diet (which are richer in carbohydrates than anything else). Figure 2.4 shows a comparison of typical biochemical content of chickpeas (similar to other legumes like beans) and the microalga *Chlorella regularis*:

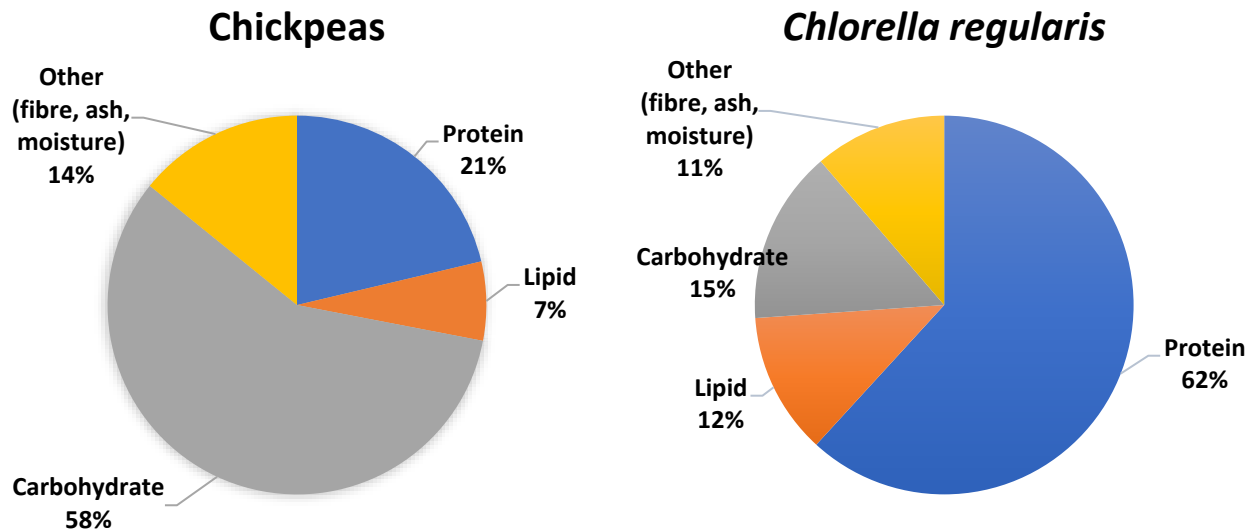


Figure 2.4: Biochemical composition of chickpeas [41] and the microalga *Chlorella regularis* [23].

In aquaculture, omega-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) are fed to salmon to increase its nutritional content and the advertised price for consumers [42]. In addition, in nature, fish, shrimp, lobster and flamingo birds develop their pink-red colour due to eating other small aquatic animals and microalgae that are rich in the red pigment astaxanthin [43].

2.1.2.2 Nutraceuticals (High Value Products)

Isolated high value products from microalgae that are commonly explored by the nutraceutical industry are omega-3 fatty acids and carotenoids, which can be sold as dietary supplements in the form of capsules, tablets or powder. The omega-3 fatty acids of most commercial interest are EPA and DHA. As mentioned in section 2.1.2.1, they are needed by the human body, and

supplementation might be beneficial for individuals who cannot obtain enough of them through diet. In fact, the estimated omega-6 to omega-3 ratio in the current Western diet is, respectively, 15:1 while the optimal ratio is supposed to be 1:1 [44], therefore most people do not get enough omega-3s in their diets. Although our bodies need both omega-3 and 6, they also compete for the same enzymes [44] and in order to have optimal health, a balance is desirable. Additionally, omega-3 fatty acids (like EPA and DHA) help with brain development and fight oxidative stress and inflammation in people's bodies. Their consumption has been associated with enhanced longevity and better health [39].

Carotenoids are pigments and help cells harvest light from the sun but some carotenoids have additional antioxidant properties and nutritional value like the carotenoids astaxanthin and β -carotene, a precursor of vitamin A. Astaxanthin can be both produced synthetically (not approved for human consumption except for aquaculture) or found naturally in microalgae (major source due to easy growth, high content), yeast or crustaceans. As a powerful antioxidant, astaxanthin protects the body against free radicals which are formed due to physiological stress, smoking and ultraviolet (UV) light exposure. The free radicals can damage the DNA, proteins and lipid membranes and are linked to aging, cancer and heart disease [45, 46]. The estimated production cost for synthetic astaxanthin is around US\$1,000/kg and the market value is above US\$2,000/kg. For natural astaxanthin, the estimated production cost is above US\$3,000/kg [46] and the estimated market value is above US\$7,000/kg [37].

The microalga *Dunaliella salina* has been typically used for the production of β -carotene in commercial scale because it grows in water with high salinity (less risk for contamination), and also due to being able to accumulate the highest β -carotene content (up to 14% of its dry weight) of any organism (and plants). The price for β -carotene is approximately US\$ 300-3,000/kg [37, 42, 34]. Natural β -carotene is superior in quality to the synthetic ones since it comes naturally mixed with other antioxidants and nutrients, bringing additional health benefits [44, 47].

2.1.2.3 Cosmetics and Dyes

Microalgae are usually used in cosmetics as natural colorants (pigments) in creams or for protecting against the sun and promoting healthy hair and skin. Mycosporine-like amino acids (MAAs) from microalgae are water soluble secondary metabolites (formed as part of normal metabolism reactions) and protect the microalgae cells from sun damage [48], absorb ultraviolet (UV) light and can work on human skin as natural sunscreens. Other microalgae solutions (rich in antioxidants, polysaccharides, proteins and minerals extracted from microalgae) can be used for anti-aging effects or as anti-irritant in peelers and even to provide a feeling of freshness on the skin and face [49, 42]. Extracts from *Chlorella vulgaris* are claimed to stimulate collagen production, helping to minimize the appearance of wrinkles, and *Nannochloropsis oculata* extracts are claimed to have excellent skin-tightening properties even in the long-term [34].

Microalgae also produce compounds (as part of biomass or excreted) with antimicrobial properties such as some polysaccharides, sterols and peptides [50, 51], which can help keep skin clean and protected against harmful microbes. For example, cell extracts and extracts from the growth media of microalgae such as *Chlorella vulgaris* and *Hapalosiphon hibernicus* have also shown antibacterial and antifungal activity, respectively [52, 35].

As dyes, various species of microalgae look green, however some species will develop different colours depending on the amount of yellow, red or blue pigments that they accumulate (Figure 2.5). Although techniques to improve stability of pigments (to stop them from leaching from foods, fabrics etc.) are still under research, there is a drive to use microalgae pigments as a source for natural dyes because they are non-toxic unlike some synthetic dyes and they come from a sustainable source that has no negative impacts in the environment [47].



Figure 2.5: Freeze dried biomass of blue-green *Spirulina platensis*, yellow *Synura uvella*, green *Nannochloropsis oculata*.

The most common pigments used (since they are found in larger quantities in microalgae) as dyes are carotenoids (yellow-orange-red tones, from *Haematococcus pluvialis* and *Dunaliella salina*), phycocyanin (blue and unique to microalgae, from *Spirulina sp.*) and phycoerythrin (red and unique to microalgae, usually from *Porphyridium*) that can be used for giving natural colours to pills, cosmetics or foods like yellow margarine and enhanced yellow egg yolks [42, 35], orange juice, blue ice cream, candies and beverages [34, 47].

2.1.2.4 Bioremediation (CO₂ and Wastewater)

At the same time as producing products of interest, microalgae can be used to help remediate carbon dioxide emissions and also to clean wastewater. Microalgae are very promising microorganisms for CO₂ mitigation because they can remove 10 to 50 times more CO₂ than terrestrial plants [53]. It is estimated that one kilogram of microalgae (in dry weight) uptakes 1.83 kg of CO₂ [40].

Due to the concern with climate changes because of carbon dioxide (CO₂) emissions, several techniques for CO₂ removal have been investigated (chemical and biological) with biological CO₂ fixation becoming attractive since it has the benefit of generating products which can, in turn, generate profit [54].

In Canada, the energy sector (which includes stationary combustion, transport and fugitive emissions from equipment leaks) accounted for 81% of the Greenhouse Gas (GHG) emissions in the country in 2013, with the stationary sector (power plants, industrial plants) being responsible for 45% of the emissions [55]. The CO₂ emissions could be fed to microalgae for production of fuels and other co-products while reducing our dependency on non-renewable sources.

It is not certain if biodiesel production from microalgae would cause a net reduction in CO₂ already accumulated in the environment due to fossil fuel activities [10], especially because electricity used for running equipment such as pumping and mixing emit GHG [56]. However, assessment based on pilot-scale plants have shown that algae-derived fuel and systems have the potential to produce lower GHG than petroleum fuels [57], with up to 78% reduction in CO₂ emissions in comparison with petro-diesel [58], which means that the system will potentially be more environmentally friendly overall.

Microalgae have also been investigated for wastewater remediation since they can consume nutrients from the wastewater and CO₂ produced by bacteria to release oxygen (O₂) in the water, via photosynthesis, which contributes to microbial growth and organic matter degradation [23]. Wastewater rich in phosphates and nitrates is the best for microalgae growth and some wastewater generated by piggeries are high in ammonia and phosphorous, which can be suitable for microalgae applications. Domestic wastewater also has been tested for this purpose [53]. Some species have been investigated for these applications including both CO₂ and wastewater remediation together. For example, *Nannochloropsis sp.* has been successfully grown in a mix of 50:50 municipal sewage and sea water medium with 15% (v/v) CO₂, a typical concentration found in flue gas [59]. Some other research with microalgae has observed that the stage of the wastewater in the treatment process is an important factor to be considered when growing microalgae and concluded that the effluent from after primary settling is the best to stimulate growth and lipid yield [16].

2.1.2.5 Biofuels

Most of the focus on current microalgae research has been on the production of microalgal lipids as a renewable alternative for fossil fuels since it seems to be the only feedstock with the ability to partially or fully replace fossil diesel in a global scale [60, 5].



Figure 2.6: Extraction of algal lipids.

Microalgae minimally compete with current space used for food crops, can be grown in salt or brackish water and on non-arable land, and can use sunlight energy to efficiently convert CO₂ and water into high biomass and lipid yield per-acre [27]. Biodiesel production from microalgae is estimated to be up to 300 times higher than traditional oil crops such as soybean crops and that is because microalgae can grow more quickly than oil crops, with some species being able to double their biomass in a day and having shorter harvest cycles (days) in comparison to higher plants (once to twice harvest/year) [27]. Biomass yields have a great effect on the economics for biodiesel production and several species have been identified and proposed as a suitable feedstock for biofuel production, with marine microalgae having an advantage to freshwater species since they do not require freshwater to grow and can be cultured on nonagricultural coastal areas [60].

Table 2.1 shows the chemical and physical properties of soy biodiesel (most common feedstock for biodiesel in the U.S.), microalgae biodiesel and fossil diesel, and the requirements in the EN 14214 Biodiesel Standard. Microalgal biodiesel can be used in current transportation systems without major modifications in current technologies and fuel pipelines [40].

The main properties looked at when working with biofuels are the acid value, iodine value, density, kinematic viscosity, flash point, pour point, heating value and cetane number. The acid value (AV) means how corrosive a fuel is while the iodine value (IV) shows how unsaturated the oil is, and the higher the degree of unsaturation, the less stable the oil is (it will oxidize more easily). Flash point is the lowest temperature when the fuel will start to form vapour than can be ignited. Pour point is the lowest temperature when the liquid will start to turn into solid (or semisolid), not being able to flow as well anymore. Kinematic viscosity also measures the fluid's resistance to flow, which affects the operation of fuel injection equipment [61]. The density and heating values (energy released when combusted) are more for comparison in efficiency when comparing with fossil diesel [40].

Table 2.1: Properties of soy biodiesel, microalgae (mixed culture) biodiesel and fossil diesel.

#	Fuel Property	Soy Biodiesel	Microalgae Biodiesel	Fossil Diesel	EN 14214 Biodiesel Standard
1	Acid Value (mg KOH/g oil)	0.14 ^a	0.42 ^d	-	0.5 max ^f
2	Iodine Value (g I/100g of oil)	128 ^a	19 ^d	0 ^f	120 max ^f
3	Density (g/cm ³)	0.867 ^c	0.85 ^d	0.83 to 0.84 ^e	0.86 to 0.9 ^f
4	Kinematic viscosity (mm ² /s)	4.2 ^a	5.2 ^e	1.2 to 3.5 ^e	3.5 to 5 ^f
5	High Heating Value (MJ/kg)	39.7 to 40.1 ^b	41 ^e	45.9 ^e	-
6	Flash Point (°C)	171 ^a	>130 ^d	60 to 80 ^e	>101 ^f
7	Pour Point (°C)	-1 to 1 ^b	-6 ^d	-35 to -15 ^e	-
8	Cetane Number	49 ^a	46 ^d	51 ^e	≥51 ^f

References: ^a [62], ^b [63], ^c [64], ^d [40], ^e [58], ^f [61]

It is estimated that biodiesel production from microalgae biomass can only have the potential to be competitive when areal biomass productions have a minimum annual average of 30 g dry weight m⁻² d⁻¹, with at least 30% lipid content, and if other major biochemical coproducts are marketed [53, 65]. Chisti (2007) estimated that the cost for biomass with 30% oil content would be \$1.40 in photobioreactors to provide a liter of oil with a total cost of \$2.80/L to have the final biodiesel product produced (considering an areal production of 72 g dry weight m⁻² d⁻¹). In April 2016, the USA reported that the price for biodiesel, still mainly produced from soy [66], and petrodiesel were \$0.74/L (\$2.81/gal) and \$0.56/L (\$2.13/gal), respectively, with biodiesel costing \$0.18 more to

produce per liter [67]. The differences in prices are still similar to comparison performed by Chisti (2007). Considering most current prices, the cost of biodiesel would need to be \$0.55/L to be currently cost competitive with petrodiesel. In addition, to completely substitute petroleum, the price of algal oil would need to be sold at roughly the price of crude oil. Taking the fact that biodiesel has roughly 80% the energy content of petrodiesel and that current crude oil costs approximately \$43/barrel [68], using the formula proposed by Chisti (2007) in order for microalgae to completely substitute petroleum as a feedstock, the price for the microalgal oil would need to be \$0.297/L [5].

2.2 Microalgae Selection for Biodiesel Applications

When considering species for biodiesel production, selecting appropriate microalgae species is important for the overall success of the process. It is desired to look for species that grow easily and have high lipid productivities (which is a combination of biomass productivity and lipid yield). Additional considerations such as fatty acid profile and adaptability to various growth conditions are also important but information is not always readily available.

2.2.1 Selected Microalgae - Biomass Productivities, Lipid Content and Lipid Productivities

Although there are thousands of microalgae species known, there are less than 20 genera investigated for biofuel production since they are better known and documented, which makes it easier to use them for experiments [69]. Table 2.2 shows the most common genera (general classification) investigated for biofuel applications [69], however *Prochlorococcus* was removed from the list due to difficulty in finding reliable data from laboratory experiments. The microalgae type (marine or freshwater) and average values of lipid content and productivities from laboratory scale are additionally shown in the table. If data for more than one species for a certain genus was provided (eg.: *N. oculata* and *N. salina* for *Nannochloropsis*), the average was calculated including all the species reported in the genus. Where not provided directly, lipid content, biomass productivity or lipid productivity for Table 2.2 were calculated from data provided in the articles. For example, lipid productivity was calculated as the product of biomass productivity and lipid content and converted to the appropriate units.

Table 2.2: Common microalgae genera investigated for biofuels with their type, biomass productivity, lipid content and lipid productivity determined in laboratory experiments from the literature.

#	Genus	Common Type ^a	Biomass Productivity (g/L/d)	Lipid Content (% w/w)	Volumetric Lipid Productivity (mg/L/d)	References
1	<i>Botryococcus</i>	F	0.13	27.93	40.73	[70], [71], [72], [73], [74]
2	<i>Chlamydomonas</i>	F	0.27	28.23	60.30	[70], [75], [76], [77], [78], [79]
3	<i>Chlorella</i>	F/M	0.13	20.80	27.76	[80], [70], [81], [82], [83], [71], [73], [79]
4	<i>Chlorococcum</i>	F	0.10	15.45	18.43	[81], [84], [85], [86], [87]
5	<i>Dunaliella</i>	M	0.12	45.69	79.48	[75], [88], [89], [90]
6	<i>Isochrysis</i>	M	0.32	22.45	41.83	[81], [91], [92], [93], [94]
7	<i>Nannochloris</i>	M/F	0.39	32.50	124.05	[95], [96]
8	<i>Nannochloropsis</i>	M	0.21	26.76	45.02	[81], [97], [98], [59], [93]
9	<i>Neochloris (Ettlia)</i>	F	0.25	26.25	64.44	[95], [93], [99], [100]
10	<i>Pavlova</i>	M	0.09	16.22	19.63	[81], [101], [93], [94]
11	<i>Phaeodactylum</i>	M	0.30	20.12	50.16	[102], [93], [103]
12	<i>Scenedesmus</i>	F	0.12	23.50	22.57	[82], [81], [70], [71], [73], [77], [79]
13	<i>Synechococcus</i>	M	0.26	19.98	37.28	[102], [104], [105], [106]
14	<i>Synechocystis</i>	F/M	0.20	18.58	37.10	[98], [107], [108], [109]
15	<i>Tetraselmis</i>	M	0.30	13.27	35.99	[81], [93], [110], [111]
16	<i>Thalassiosira</i>	M	0.03	29.43	8.24	[101], [112]

^aCommon Type: F (mainly or all freshwater), M (mainly or all marine), M/F (Some marine or freshwater)

It is worth to keep in mind that the values in Table 2.2 are not absolute ones which makes comparisons challenging, however, the information provided in Table 2.2 is helpful in providing an insight on which genera are most attractive for biodiesel applications. Variations in biomass productivities, lipid content, and therefore lipid productivities, in the literature can occur for multiple reasons [113, 102, 81, 58].

Lipid productivity is considered a more useful parameter for selection since it takes into account both biomass productivity and lipid content [58]. From Table 2.2, it can be observed that the marine species show greater lipid productivity. Overall, *Nannochloris*, *Dunaliella*, *Neochloris (Ettlia)*, *Chlamydomonas*, *Phaeodactylum* and *Nannochloropsis* seem to be the most suitable microalgae for biodiesel production in terms of lipid productivity.

2.2.2 Additional Considerations

Biomass yield, lipid content and lipid productivity are important but are not the only factors to be considered for a successful investment in microalgae culture.

It further helps to save on costs and resource utilization (eg.: land space, energy input) if species can resist contamination, are easy to harvest (eg.: bigger and heavier cells, to decrease energy and cost with harvesting) and tolerate to shear and changes in growth conditions better than others. Unfortunately, there is limited information for all the species to this regard in the literature [102].

Furthermore, it is also desirable if the strain selected has a lipid profile that gives a biodiesel that meets more closely the desirable regulations, which vary slightly depending on the location (European standard is commonly used). Microalgae can store lipids as reserve bodies (neutral) or functional (polar), in the cell membrane, for example. When considering lipid types in microalgae, although all lipids can be converted into biodiesel, the higher the quantity of neutral lipids, in the form of triglycerides (TAGs), the better since they give higher biodiesel yield when converted to biodiesel via transesterification [114]. The degree of saturation is also important. It is reported that fuels that better meet fuel regulations, and thus have better quality, are richer in monounsaturated

fatty acids, such as palmitoleic and oleic, and lower in both saturated and polyunsaturated fatty acids [115, 116].

It appears that no single species meets all the requirements in terms of fatty acid profile for ideal biodiesel properties. In this respect, it is advised to have a blend of biodiesel from different species in order to meet the standard requirements for a top-quality biodiesel or to have further improvements in culture conditions or strain manipulation (metabolic engineering or genetics) to manipulate the microalgae to produce a fatty acid profile that gives the best properties for biodiesel [116, 117].

2.2.3 *Nannochloropsis oculata*

Nannochloropsis oculata is a marine microalgae species of the genus *Nannochloropsis* that is well known and has been widely used in aquaculture to feed aquatic animals such as rotifers, fish and mollusks because of its high content of total lipids, fatty acids and also because of its lipid profile, which contains considerable amounts of polyunsaturated fatty acids (PUFAs), especially the omega-3 EPA, helping to increase aquatic food's nutrition and value [23, 118, 119]. When looking at biodiesel applications, appreciable amounts of PUFAs such as the fatty acid EPA are not very desirable, however EPA can be separated to give final biodiesel a better quality and be sold separately as a supplement to increase profit [120].

Nannochloropsis oculata is a unicellular green microalga with a simple morphology and ellipsoid to round shape (Figure 2.7). It used to be called marine *Chlorella* and placed in the *Chlorophyceae* class due to the similarity of the cell shapes with *Chlorella* species until further research identified more differences structurally (such as continuum connection of chloroplast and nucleus unlike *Chlorella*) and biochemically (such as different pigments and fatty acid profiles). Therefore, *Nannochloropsis* was placed in a different class (Eustigmatophyceae). *N. oculata* is typically 2-5 μm in diameter, which can vary depending on growth conditions. Its main pigments are chlorophyll a (and absence of chlorophyll b and c unlike *Chlorella* species), carotene, violaxanthin and vaucherxanthin ester [119, 28].

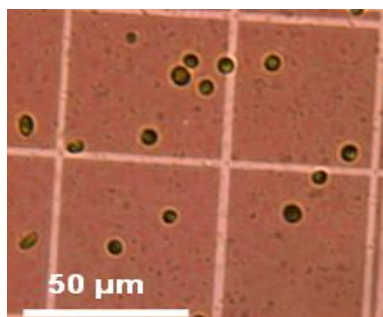


Figure 2.7: *Nannochloropsis oculata* cells.

N. oculata has been considered a great candidate for biodiesel applications since it can have high lipid and triglyceride (TAG) content, for example up to 50% w/w of lipids with 43% w/w corresponding to the TAG content [121]. The same fatty acids (eg.: EPA) can be present as part of both neutral lipids (in the form of triglycerides) or polar [122]. Under standard culture conditions, the main fatty acids present in *N. oculata* are myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n-7) and eicosapentaenoic (C20:5n-3) acids as part of the total lipids with the neutral lipids mostly made out of palmitoleic (C16:1n-7) and palmitic (C16:0) [121]. The microalga has also resistance to mixing (shear stress) and contamination [123] and has wide salinity tolerance (10-35 ppt) [122]. In addition, commercial scale in ponds has been established for decades with predictable year-round productivities [118] and it has been shown to achieve productivities in ponds larger than $22 \text{ g m}^{-2} \text{ d}^{-1}$ during summer months. Contamination with blue-green algae has been reported in outdoor cultures but *Nannochloropsis* species have reduced sensitivity to some types of herbicides and antibiotics that can be used to selectively kill the invading algae or other microorganisms when needed [122]. Alternatively, photobioreactors can be used to increase productivity and maintain aseptic cultures. In addition, changes in growth conditions can be used to cause further increase in biomass production and products formation.

2.3 Growth and Products Stimulation

There are different ways to stimulate biomass and products formation. Some techniques work for both increasing biomass productivity (g/L/d) and product content (% w/w) per cell while others offer a trade-off or are not necessarily correlated. For example, microalgae can be stimulated to have a higher lipid content per cell, but this does not mean that the total lipid productivity (g/L/d)

will be high since total biomass productivity might decrease giving an overall reduction in lipid yield or productivity [58]. Techniques used for stimulation of microalgae, whether it is in biomass or product's enhancement, can be found in Figure 2.8. Since one of the main drives for microalgae research is for biodiesel applications, the focus of this section will be on growth and lipid production. Examples of parameters investigated for growth stimulation are found on Figure 2.8. It is worth to note that the effects and best conditions, even for the same stimulus, generally vary from species to species since they have different requirements and sensitivities. Usually, a microalgae species and a parameter are chosen, then the parameter is changed to find optimal conditions in terms of biomass or products productivity, or both if possible.

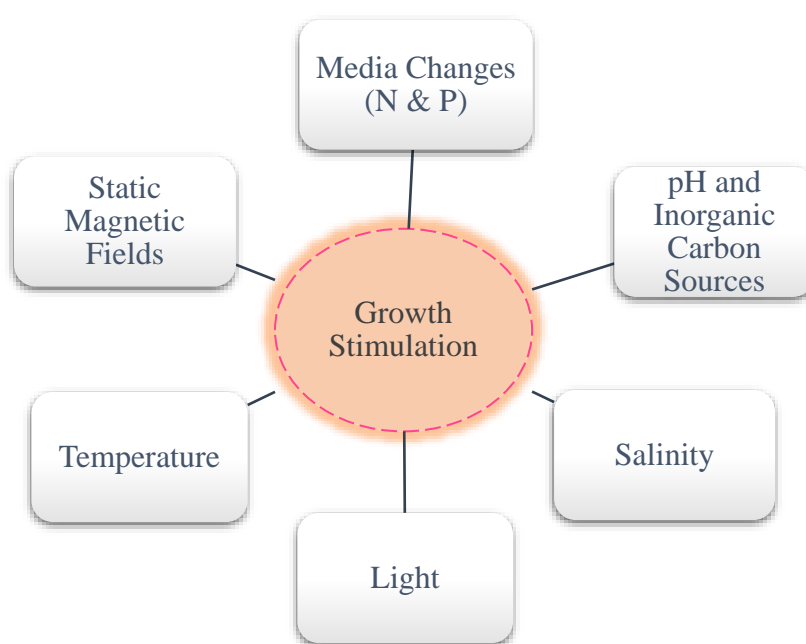


Figure 2.8: Common techniques used to stimulate growth in microalgae.

2.3.1 Media Changes (N & P)

Nitrogen (as nitrate, ammonium or urea) and phosphorous (phosphate salts) levels are the ones commonly changed in the media [124]. Growing microalgae in media with nitrogen limitation or starvation seems to be the most effective media change to stimulate increase in lipid content (10-20%) in microalgae cells in comparison with normal conditions. It is believed that under nitrogen limitation, proteins are converted into higher and long-term energy storage (lipids). However, although this strategy has been shown to increase lipid content per cell, it typically slows down the

growth significantly (biomass productivity) causing a decrease in lipid productivity (product of biomass productivity and lipid content) [40] although it has been reported that in some exceptions, productivity can continue increasing and that hypothetically, it has to do with the fact that some cells might be able to consume chlorophyll (as a source of nitrogen) to support cell growth [124].

It has been reported that microalgae under phosphorous limitation might also accumulate more lipids. Xin et al., 2010 [125] studied *Scenedesmus sp.* under either nitrogen or phosphorous limitation and concluded that although the lipid content was the highest for this species under either condition, the biomass productivity decreased, therefore lipid productivity was not enhanced in comparison to the nutrient replete conditions (57% decrease in lipid productivity for 2.5 mg/L vs. 25 mg/L total nitrogen and 5% decrease in lipid productivity for 0.1 mg/L vs. 2 mg/L total phosphorus). *Nannochloropsis oculata* has been shown to increase in both lipid content (twofold) and lipid productivity (64%), despite lowered biomass productivity, when the cells are exposed to a 75% decrease in nitrogen source (from 0.3 g/L sodium nitrate, NaNO_3 , to 0.075 g/L) [83].

2.3.2 pH and Inorganic Carbon Sources

Usually changes in pH occur as a consequence of other changes in the microalgae's environment such as CO_2 levels or some other media changes. Aqueous CO_2 is found in the three different forms (dynamic equilibrium): as dissolved CO_2 and as the carbonate ionic forms bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) [60]. Feeding additional CO_2 to the media is beneficial and stimulates growth however it might increase acidity of the media, dropping the pH (pH = 5.0 or lower) and shifting the equilibrium towards bicarbonate [40]. Although microalgae show the ability to utilize different inorganic carbon forms for growth, the preference and utilization efficiency is species-dependent [126, 127, 128, 129]. Since pH and inorganic carbon sources are related, a change in either of these parameters typically have a combined effect on microalgae growth and lipid productivity.

The marine species *Nannochloropsis salina* was grown in pH 5, 6, 7, 8, 9 and 10 adjusted by the use of different chemical buffers. It was found that although pH did not have a significant effect on lipid accumulation, overall, pH 8 was the best for the combined effects of maximum growth rate, cell densities, mean lipid accumulation (thus higher lipid productivity) and reduction in

contamination by undesirable organisms such as other competitive algae or algae predators [130]. The microalgae *Desmodesmus sp.* was studied under the effect of high pH stress adjusted by the addition of sodium bicarbonate to the media with or without nitrogen deficiency (proximate values of resulting pH investigated: 7.5, 8.9 and 10.5). In batch cultures grown for 15 days, although total biomass density was affected negatively (27-55% reduction) when sodium bicarbonate (high pH) was present in the media in comparison with the control, the cells accumulated 60-84% more lipids. The highest total lipid productivity in comparison with the control occurred when the cells were grown in nitrogen sufficient conditions with bicarbonate added (17% increase, pH = ~10.5). In additional experiments (outdoor bioreactors), it was concluded that both biomass and lipid content increased when cultures were grown in two stages (first until nitrogen was about to be depleted, then bicarbonate was added) [131].

Increase in CO₂ concentrations has been shown beneficial to stimulate accumulation of total lipids in microalgae. For example, *Chlorella pyrenoidosa* and *Scenedesmus obliquus*, commonly found in freshwater, tolerated and managed to grow with CO₂ levels up to 50% and had a 28-61% increase in lipid content in comparison with the control (CO₂ only provided via air), however biomass production decreased. Best compromise was found for 10% CO₂ for *C. pyrenoidosa* and 10% for *S. obliquus*, where both biomass productivity and lipid content increased resulting in 2.6 and 2.4-fold increase in lipid productivity, respectively [132]. Some species such as *Tetraselmis suecica* and *Chlorella sp.* have also shown high lipid productivities in comparison with a control when grown in pH controlled conditions by the addition of CO₂ or untreated flue gas from coal-fired power plants, with no significant differences between both carbon sources [133].

2.3.3 Salinity

Salinity is a parameter more looked at when working with marine species, and different salt concentrations can stimulate the production of products of interest such as lipids [40]. While some species of microalgae can produce metabolites to protect them from salt damages and osmotic stresses, too much salt cause higher osmotic stress to the cells causing inactivation of photosynthetic enzymes (Rubisco) and decrease in photosynthesis [23]. The marine species *Isochrysis sp.* and

Nannochloropsis oculata have shown highest lipid productivity when salinity level is 30-35 g/L [134, 135].

Research on salinity stress has also been performed on freshwater species. *Scenedesmus sp.*, a freshwater species, accumulated significantly higher lipid concentrations with sodium chloride exposure (salinity) in a single-stage cultivation. At the salinity level of 23.4 g/L (400 mM) NaCl, the lipid content of *Scenedesmus sp.* was higher (33.13% vs. 18.98% for the control), but the biomass productivity was lower (4.75 mg/L/d for 400 mM NaCl vs. 22.72 mg/L/d for the control), affecting lipid productivity negatively when salt was added. However, the problem was minimized by growing *Scenedesmus sp.* in a two-stage process, where the microalga was grown in freshwater first then exposed to salinity after a few days, with the three-day stress (400 mM added to the media after three days) offering best results (higher lipid content and marginal reduction in biomass productivity with overall higher lipid productivity than the control) [136].

2.3.4 Light

For photoautotrophic algae, light is the source of energy utilized for cell growth and it can come from the sun or artificial lights. Critical factors include light source (and the wavelength range they cover) and light intensity [40]. Sunlight, for example, covers a wide spectrum of wavelengths but the wavelengths utilized by microalgae for photosynthesis (photosynthetically active radiation (PAR)) is in the range 400-700 nm, which corresponds to about 50% of the sunlight [124]. Additionally, the effect of light intensity on the growth can be divided into three main phases: light limitation, light saturation and light inhibition (Figure 2.9). Light limitation typically occurs when the culture is grown at low light intensities, and light inhibition is when the cells are exposed to too much light, which generates reactive oxygen species (ROS) that damage the cells. It is advised to have the entire culture under light saturation levels in order to maximize biomass production, however it is difficult to achieve that all the time since cells can shield each other when their concentration increases over time. Poor mixing or biofilm formation can also cause some cells to be more exposed to light than others [124, 23, 40, 28].

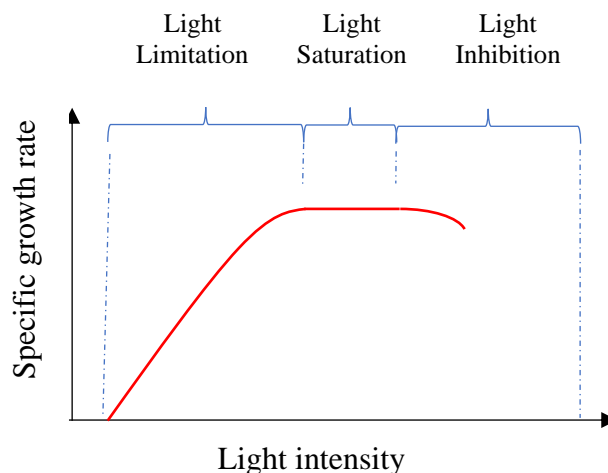


Figure 2.9: Effect of light on microalgal growth rate (redrawn and adapted from [40, 23]).

The freshwater species *Chlorella vulgaris* was tested under 24, 60 and 120 $\mu\text{mol photons/m}^2\text{s}$ and had largest lipid content and biomass productivity at 60 $\mu\text{mol photons/m}^2\text{s}$ (twofold larger lipid productivity than at 120 $\mu\text{mol photons/m}^2\text{s}$) while light intensities below or above 60 caused lower biomass productivity and lipid content, suggesting that at 24 $\mu\text{mol photons/m}^2\text{s}$, the cells were under light limitation and at 120 $\mu\text{mol photons/m}^2\text{s}$, cells were under photoinhibition (too much light) [137]. Research was also performed on a marine species (*Nannochloropsis oculata*) under three light intensities (100, 300 and 500 $\mu\text{mol photons/m}^2\text{s}$). The highest lipid productivity occurred at 500 $\mu\text{mol photons/m}^2\text{s}$ (threefold larger than at 100 $\mu\text{mol photons/m}^2\text{s}$) [135].

2.3.5 Temperature

Temperature is one of the most important parameters that regulate cell morphology, physiology and products production, and the optimal temperature is different depending on the species. Typically, higher temperatures accelerate cell metabolism while lower ones inhibit growth. In order to have higher biomass accumulation, higher temperatures are preferred during the day to stimulate photosynthesis and less preferred at night since they stimulate respiration rate, which causes a reduction in cellular mass in the form of released CO_2 [40].

Chlorella vulgaris, a freshwater species, is estimated to have best growth conditions at temperature 30 °C. By changing the temperature from 25 to 38 °C, it was found that the species had the highest

lipid productivity at the lowest temperature tested in the study (25 °C), which was 2.5-fold higher in comparison with 30 °C. Temperatures above 30 °C were harmful to the cells decreasing both growth and lipid productivity (in comparison with 25 °C). Therefore, lipid productivity for *Chlorella vulgaris* is best at 25 °C. Experiments also have been performed with the marine species *Nannochloropsis oculata* under three different temperatures (15, 20 (optimal) and 35 °C). While specific growth rate decreased when the microalga was grown below and above the optimal temperature, the lipid content increased in both cases, giving similar lipid productivities (around 10 mg/L/d) regardless of the temperature used. It could be concluded that this species can tolerate a range of temperatures without having a negative impact on lipid productivity [83].

2.3.7 Static Magnetic Fields

Electromagnetic fields (EMFs), sometimes referred as radiation, can be found everywhere. They are produced naturally, such as by the Earth, and artificially, such as by man-made electrical power and devices. Overall, electromagnetic fields can be divided into two broad categories: non-ionizing and ionizing [138]. Figure 2.10 shows the electromagnetic spectrum with an approximate range of frequencies and classification:

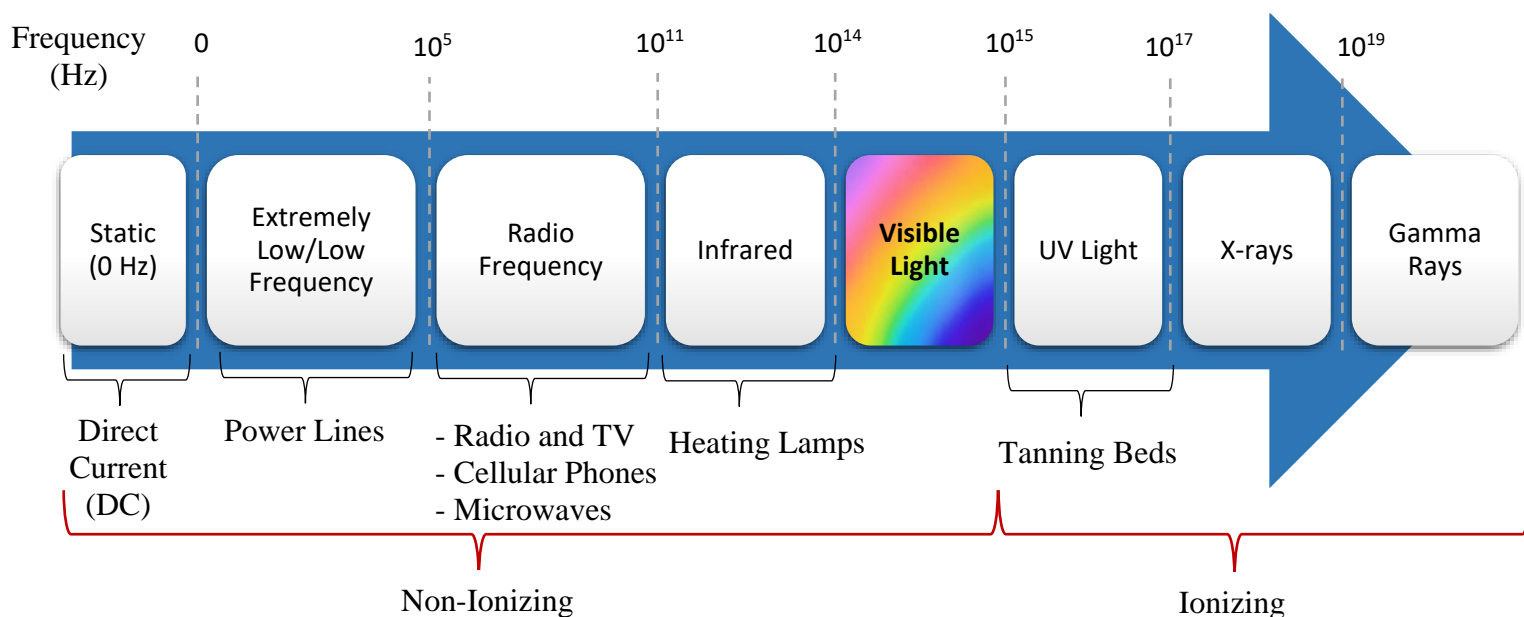


Figure 2.10: Electromagnetic Spectrum (Adapted from [138] [139] [140] [141]).

Non-ionizing electromagnetic fields are generally perceived as harmless to humans (considering the typical exposure in people's daily lives and considering that microwaves and other devices are used according to their proper instructions and intended use) but studies suggest that they might interact with biological systems by generating currents and/or heating [139, 138], therefore regulations exist to limit the exposure. The maximum human exposure range recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) on electromagnetic fields is from 0-300 GHz [142], which include static, extremely low, low frequencies and some radio frequencies (such as cellphones) in the electromagnetic spectrum.

Static magnetic fields (SMF) are generated by permanent magnets (due to the spin of electrons inside the material itself) or by a steady flow of electricity (direct current, DC) such as in the case of appliances or solenoids using DC currents [143]. SMF remain constant over time, not changing in intensity or direction, and therefore have a frequency (number of oscillations or cycles per second) of 0 Hz. They typically do not induce currents like alternating current (AC) systems but exceptions to the general rule occur at the moment of activation and deactivation of the field or when there is movement of biological systems inside the field [140]. The common units used for magnetic field measurements are Gauss (G) or Teslas (T). Static magnetic fields are similar to the natural magnetic fields found in our environment such as the Earth's magnetic field ($\sim 50 \mu\text{T}$) [141] [144]. Based on scientific research, ICNIRP regulations have established that for the general public, the recommended limit of human exposure (any part of the body) to static magnetic fields is 400 mT, while occupational exposures are limited up to 2 T for head and trunk and 8 T for limbs (such as when needed in operation of equipment like MRI), if the environment is controlled to reduce effects related to movement [144, 139, 142].

Static magnetic fields have been shown to interact with biological systems. However, it is worth to mention that biological effect is different than health effect in the sense that it can be a normal reaction of the body and not necessarily harmful. For example, pupils constrict when exposed to light but this biological change is a normal response and not harmful [141].

2.3.7.1 Static Magnetic Fields and Microalgae

Electro and magnetic fields are everywhere from power lines to appliances at home, kitchen magnets and medical devices such as MRI. With increasing concerns of the population regarding their effects on human health, research on various living organisms have been performed. Although most research available seems to be on power lines, MRIs and cell phones due to greater concern from the population, since static magnetic fields are one of the most common fields found in our day-by-day lives, some of the research has also been performed with human cells, animals, plants and bacteria [143, 139, 140, 142, 144, 145, 146, 147]. The curiosity has extended to microalgae but there are only a few studies in this area, and the number is even smaller when looking into lipid productivity. Table 2.3 shows a summary of studies regarding static magnetic fields on microalgae.

From all studies, the *Chlorella* and *Spirulina* genus have been the most investigated and perhaps because cultivation of the species in commercial scale has already been established for other applications. For the purpose of this section, control (0 mT) means that the culture was only exposed to the natural Earth's magnetic field (eg.: no additional magnetic field was applied to the samples). All studies had a control. Lipid productivity is typically measured as the product of biomass productivity and lipid content.

The microalga *Chlorella pyrenoidosa* was grown outdoors in a closed photobioreactor with supernatant of campus sewage (after 2 h sedimentation) and natural (sun) light, under 3 h daily application of 500 mT SMF. The experiment was performed in both batch and semi-batch (where fresh SMF pre-treated wastewater was added to bioreactor after 5 days) to study the effects of SMF on biomass productivity, lipid content, lipid productivity and wastewater treatment efficiency. At day 5, in comparison with the control, 500 mT SMF increased chlorophyll-a production by 10.5% and although lipid content did not change, biomass productivity increased (by 12.3%), and therefore lipid productivity increased as a result (by 10%). In addition, SMF treatment reduced chemical oxygen demand (COD), ammonia nitrogen, total nitrogen (TN) and total phosphorous (TP) during the first 5 days, then remained stable with time (with semi-batch growth). It also reduced turbidity of wastewater and increased bacteria numbers, which possibly had a symbiotic relationship with the microalgae by consuming O₂ produced by microalgae and producing CO₂ and potentially growth-promoting factors, which in turn could be used by microalgae for growth [14].

Table 2.3: Summary of SMF on microalgae (tests, parameters and lipid productivity¹).

Species	Type	SMF Tested (mT)	Length of exposure	Optimal (mT)	Parameters Measured
<i>Chlorella pyrenoidosa</i> ^a	Freshwater (grown in wastewater)	0 and 500	3 h/d	500	Biomass, Lipids, Chlorophyll a, Wastewater treatment ability
<i>Chlorella fusca</i> ^b	Freshwater	0, 30 and 60	1h/d and continuous (24h/d)	30 for 1h/d	Biomass, Growth Rate, Proteins, Carbohydrates, Lipids
<i>Scenedesmus obliquus</i> ^c	Freshwater (grown in wastewater)	0, 50, 100, 200, 300, 400 and 500	2 h (once, every day or log phase), then 0.5, 1, 2, 4 h at the optimal mT and exposure type (log phase)	100 for 0.5h in log phase	Oxygen Production, Dissolved oxygen, Chlorophyll a
<i>Chlorella kessleri</i> ^d	Freshwater	0, 5, 10, 15	Continuous (24 h/d)	10	Biomass, growth rate, production rate, Carbohydrates, Proteins, Lipids, Ash, Chlorophyll a and b, Carotenoids, Antioxidants, Metal Analysis, Fatty Acids, Photosynthetic Capacity, Cell Morphology, Transient Effect of SMF
<i>Chlorella vulgaris</i> ^e	Freshwater	0, 5, 10, 15, 25, 35, 45, 50	12 h	10	Growth rate, Carotenoids, DPPH Radical Scavenging Activity, Lipid Peroxidation, Antioxidant Enzyme Activities
<i>Spirulina platensis</i> ^f	Freshwater	0, 100, 250, 400, 550	Continuous (24 h/d)	250	Biomass, Amino Acids, Metal Content, Chlorophyll a, Changes in pH, Carbon, Nitrogen and Phosphorous Uptake
<i>Spirulina platensis</i> ^g	Freshwater	0, 5, 10, 20, 35, 40, 70	Continuous (24 h/d)	10	Biomass, Growth rate, Oxygen Production, Sugar content, Pigments, Glyceroglycolipid
<i>Dunaliella Salina</i> ^h	Salt water (marine)	0 and ~ 10, 23, 50, 130, 230	N/A	10	Biomass, β -carotene, Metal Content Change with Time

References: ^a [14], ^b [15], ^c [16], ^d [21], ^e [17], ^f [18], ^g [19], ^h [20]

¹Lipid productivity (g/L/d) increase at optimal SMF in comparison with the control: *C. pyrenoidosa* (10% increase)^a, *C. fusca* (not significant)^b, *C. kessleri* (47%)^d, Not available: *S. obliquus*^c, *C. vulgaris*^e, *S. platensis*^f, *D. salina*^h

The microalga *Chlorella fusca* was grown in a photobioreactor and the influences of SMF intensities 30 and 60 mT (with either 1 h/d or continuous exposure) on biomass concentration (g/L), maximum growth rate (d^{-1}), maximum biomass productivity (g/L/d), pH and contents of proteins, carbohydrates and lipids were investigated. Deamici et al., 2016 emphasized that 60 mT for 24 h/d offered the best result in terms of biomass concentration (g/L) and carbohydrate content. However, for lipid productivity, 60 mT had a decrease in lipid content and a negative impact on lipid productivity. For lipid productivity, no significant increase can be concluded for the magnetic field intensities studied. The best-case scenario (eg.: not a decrease) would be 30 mT for 1h/d or no magnetic field (control) [15].

Tu et al., 2015 focused on treatment of wastewater by using microalgae under SMF rather than investigating biomass and lipid productivity. *Scenedesmus obliquus* was grown in the supernatant of municipal wastewater (after 1 h sedimentation) and measured the dissolved oxygen concentration (DO), the algal photosynthetic oxygen evolution rate (POER), the oxygen production rate (O) and chlorophyll-a concentration, in addition to investigating the effects of different intensities (0-500 mT), intervention timing (once in early cultivation, every day and log phase) and treatment time (0.5-4 h). From some of the results such as chlorophyll-a content and oxygen production (indicative of growth), it was concluded that *S. obliquus* had the highest growth at 100 mT. Although some of the parameters tested for intervention and treatment time were not much significant from each other (eg.: higher or longer exposure did not offer much better results), considering savings in energy use, 100 mT exposure for 0.5 h during the log phase offered the best results for microalgae growth and oxygen production [16].

Small et al., 2012 grew *Chlorella kessleri* in Erlenmeyer flasks under SMF 0 (control), 5, 10 and 15 mT to determine the optimal magnetic field intensity. From growth rate results, it was determined that 10 mT was the optimal SMF intensity. Subsequent experiments were performed in an open small-scale pond at 10 mT to determine total biomass production, production rate, composition of cells (carbohydrate, protein, lipid, ash, chlorophyll a and b, carotenoids and antioxidants), metal content, fatty acid composition and photosynthetic capacity. Although lipid content did not change with SMF treatment, biomass productivity increased, and therefore lipid

productivity was higher for 10 mT. Based on the other measurements, the biomass was also more nutritious at 10 mT [21].

Chlorella vulgaris was the subject of interest on how SMF affect microalgae growth. Biomass and lipid productivity were not the focus of the study. Wang et al, 2008 exposed the microalgae (grown in flasks) for 12 h to different SMF (0-50 mT) and then, calculated carotenoid content and investigated other SMF effects on the cells by measuring DPPH radical scavenging ability, lipid peroxidation and enzyme activity. The growth rate was also measured but only 72 h after the SMF exposure had been removed. *C. vulgaris* had the highest growth at 10 mT and based on other results, from 10-35 mT the cells showed an efficient regulation of their antioxidant defense system to protect them against any harmful effects (eg.: from radical formation). No significant changes in carotenoids were observed among the SMF tested [17].

Li et al., 2007 cultivated *Spirulina platensis* in a photobioreactor under 0 (control), 100, 250, 400 and 550 mT and the culture was analyzed for biomass dry weight daily to determine optimal magnetic field intensity for biomass production, which was determined to be 250 mT. Then, the amino acid, mineral (metal content) and chlorophyll-a content for 250 mT were measured. The consumption of nutrients (carbon, phosphorous and nitrogen) was also investigated at 250 mT. Lipid productivity was not the focus of the work. 250 mT was found to be the optimal magnetic field intensity to increase biomass, chlorophyll-a and nutritional content of *S. platensis* (greater essential amino acids and minerals). In comparison with the control, the pH did not change significantly with magnetic field exposure, however it was observed that there was higher uptake of nutrients (inorganic carbon, phosphorous and nitrogen) at 250 mT [18].

Hirano et al., 1998 cultivated *Spirulina platensis* in both autotrophic (with light) and heterotrophic (in dark, with 0.3% glucose) conditions and exposed the cells to SMF 0 (control), 5, 10, 20, 35, 40 and 70 mT for the autotrophic growth and 0 (control), 10 and 70 mT for the heterotrophic growth. The growth rate, cell concentration, photosynthetic O₂ evolution, and the cell content of glyceroglycolipid (main membrane lipid), pigments (phycocyanin, chlorophyll, β -carotene) and sugars were measured. It was found that the magnetic field had no effect on the heterotrophic culture while for autotrophic cultures, 10 mT was the optimal intensity to enhance biomass (growth

rate and concentration), photosynthesis and sugar and phycocyanin content. Total lipids and lipid productivity were not the focus of the work and a decrease in glyceroglycolipid occurred with SMF exposure, especially at higher SMF intensities [19].

The effect of magnetic field was also studied with the microalga *Dunaliella salina* using intensities of 0 (control) and approximately 10, 23, 50, 130, 230 mT (extracted from figures) together with Fe-EDTA (0.1 (common in media/control), and added 1 and 10 mg/L). The biomass concentration and the β -carotene content were measured in addition to the cell's temporal change in metal content. Total lipids and lipid productivity were not the focus of the work. It was concluded that 10 mT was the optimal strength to stimulate biomass and β -carotene content, especially when 1 mg/L of Fe-EDTA was present in the media (10 mg/L was worse than with no added EDTA). From 10-120 mT after 10 h, the Cu, Cd, Co and Ni content increased slightly in the cells while the Mn and Zn content decreased slightly [20].

2.4 Thesis Relationship with the Literature Review

The work on this thesis has been motivated by the need to find a substitute feedstock for petrodiesel since current fossil fuel sources are non-renewable. Microalgae are claimed to be the only feedstock with the potential to substitute fossil-diesel completely [5]. Microalgae are also very adaptable microorganisms and have the ability to use solar energy for high lipid production while occupying less land space than other fuel alternatives (eg.: crop feedstocks). Furthermore, they have the potential to be more environmentally friendly due to requiring carbon dioxide for growth. It is worth to note that since it has been shown that it is not feasible to produce microalgae exclusively for lipid production, this work also investigated the microalgal content of other co-products such as proteins, carbohydrates and pigments.

The microalga *Nannochloropsis oculata* was chosen for this study because it is well known, has high lipid content, grows well and is already commercialized for the fish industry. The technique in this thesis can possibly improve even further the production in large scale. Although *Nannochloropsis oculata* is known to accumulate appreciable amounts of eicosapentaenoic acid (EPA) depending on growth conditions (which can make the biodiesel more susceptible to

oxidation), the EPA is a valuable supplement (for both humans and aquaculture), therefore even if the lipids produced have EPA, it can be separated so that the biodiesel produced from *N. oculata* lipids can meet regulations more closely while at the same time the EPA can be sold for additional profit [120].

Since *N. oculata* is a marine species, it can be grown in natural salt water (usually enriched with additional nutrients such as nitrates and phosphates) [148] or a mixture of natural salt water and wastewater (with no need for enrichment) [59] which would not only lessen the competition with land crops, that could be used as food, but also freshwater, that could be used for drinking. Artificial microalgae media is also typically inexpensive, if needed [5]. In Ontario, this technology could become applicable by the use of reactors or in open systems with proper nutrients provided. In Canada, the marine microalgae used in my studies could be grown in sea water, in Atlantic and Pacific oceans.

Furthermore, for this study, static magnetic field stimulation has been chosen as the (only) stressor on the microalga since not many studies have been performed in this area with microalgae especially regarding lipid productivity. Although different microalgae react differently to the same type of stress, from the 8 articles mentioned in the review, 4 used 10 mT with 3 out of 4 reporting positive growth at 10 mT. Therefore, a range of SMF intensities was chosen for this study to find out if 10 mT was the optimal for *N. oculata*. Additionally, to the best of my knowledge, no studies have been reported on *N. oculata* and SMF stimulation. This report aims to aid the research on microalgae biomass, lipids and co-products productivity with the potential of using magnetic field stimulation on the microalga *N. oculata* as a candidate for biodiesel feedstock. By increasing productivity, the possibility of utilizing microalgae as a future replacement of fossil diesel becomes closer to reality.

CHAPTER 3 - Materials and Methods

3.1 Microalgae Strain and Seed Culture Conditions

Nannochloropsis oculata (UTEX LB2164) was the selected species. *N. oculata* was cultivated in a 1 L Erlenmeyer flask with 0.5 L of autoclaved Modified Seawater Medium (Table 3.1) made with double distilled water (DDW). All chemicals were analytical grade and obtained from Sigma-Aldrich (Oakville, ON, Canada).

Table 3.1: Modified Seawater Medium composition.

Composition	In 1 L of distilled water (g)
NaCl	30
KCl	1.105
MgSO ₄ ·7H ₂ O	11.09
Tris	1.21
CaCl ₂ ·2H ₂ O	1.83
NaHCO ₃	0.25
NaNO ₃	1.87
NaH ₂ PO ₄	0.054
Ferric-EDTA	0.0109
Trace Metal Solution (A5) ^a	3 mL/L
Vitamin Solution ^b	1 mL/L

^aTrace Metal Solution (A5) (g/L): H₃BO₃ (2.86), MnCl₂·4H₂O (1.81), ZnSO₄·7H₂O (0.222),

CuSO₄·5H₂O (0.079), Co(NO₃)₂·6H₂O (49.4); ^bVitamin Solution (g/L): cyanocobalamin (0.135), biotin (0.025), thiamine (1.1), HEPES (12).

The seed culture was kept at 23.5 ± 1.5 °C and was manually shaken everyday to minimize microalgae sticking to the inner wall of the flask. Evaporation inside the flasks was controlled by daily addition of autoclaved DDW in order to keep the volume constant at 0.5 L. Air provided to the flasks was first passed through a glass wool trap and then through a water glass trap. Aeration was then provided to the seed flask by 1 µm filter sterilized air through a sparger. The sparger was held in place inside the flask with glass wool placed at the opening of the flask and covered externally with aluminum foil. The entire flask assembly was autoclaved for sterilization (along with the media) before connecting it to the air supply. The culture was kept under a light intensity of 100 µmol photons/m²·s measured on the outer surface of the flask with a Li-Cor LI190 quantum sensor (Lincoln, NE, USA), provided by a 1:1 mix of 40 W GE Ecolux (#80188, GE Lighting, USA) and 40W GE Plant and Aquarium Ecolux (#49893, GE Lighting, USA) fluorescent tubes.

The light:dark cycle was chosen to be 16:8 h, which is closest to a natural cycle. The growth curve of the seed culture was performed in duplicates and can be found in Figure A.1, Appendix A.

3.2 Experimental Set Up and Magnetic Field Exposure

The picture of the magnetic field experimental setup is shown in Figure 3.1. Triplicates were performed unless otherwise stated. *N. oculata* was studied at four static magnetic field intensities: 0, 5, 10 and 15 mT. The control (0 mT) was performed with the solenoid turned off and the cells were exposed only to Earth's magnetic field. Each set of experiments lasted about 30 days in addition to two sets of 10 day experiments for the control and 5 mT. Three 1 L Erlenmeyer flasks with an effective volume of 0.5 L were used each run to generate three independent trials per magnetic field strength. The media used for the experiments was the same as the seed culture. Autoclaved DDW was added daily to the flasks to keep the volumes constant and avoid inconsistencies with the measurements (Figure A.2, Appendix A). All experimental conditions were kept the same for all runs; the only difference was the magnetic field intensities.

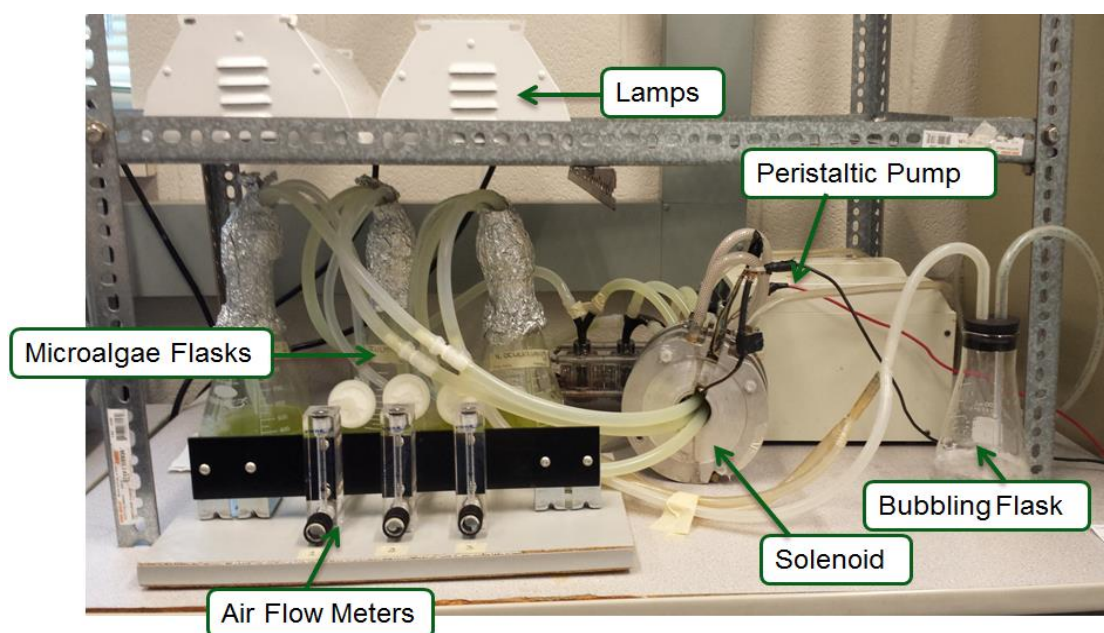


Figure 3.1: Experimental set up.

A 10% inoculum from the *N. oculata* seed culture was used to inoculate each of the 1 L flasks used in this experiment for the first run (0 mT), which was equivalent to an initial cell concentration of $1.12 \pm 0.27 \times 10^6$ cells/mL (haemocytometer counting). All runs were inoculated with the same number of cells. The inoculum for each experiment was collected from the seed culture on the same day of growth (Day 7). The carbon source for photosynthesis came from CO₂ via 1 µm filter sterilized and humidified air (passed through a water containing flask – bubbling flask in Figure 3.1) containing approximately 400 ppm of CO₂ [149] at an aeration rate of 2 vvm (volume of gas per volume of cell suspension per minute). Two 150 W high-pressure sodium lamps (150 HPS 900490, Sun System) were used as energy source for a light-dark ratio of 16:8 h. A Li-Cor LI190 quantum sensor (Lincoln, NE, USA) was used to measure photosynthetically active radiation (PAR), which was kept at 600 ± 30 µmol photons/m²·s externally on the flasks surface. The temperature inside the flasks was 29 ± 0.5 °C.

A peristaltic pump was used to pump the culture from the flasks through the solenoid and back to the flasks at a rate of 50 mL/min, with a cell residence time of 6 s inside the solenoid via silicone rubber tubing with an inner diameter of 0.313 in. The tubing was secured inside the flask along with a sparger with glass wool at the opening of the flask and covered externally with aluminum foil, and then the entire flask assembly was autoclaved before use.

The entire volume of each flask had an exposure of 1% (v/v) at any instant to a water-cooled solenoid (electromagnet), which had no significant heating [21], and the total flask volume passed through the magnet every 10 min. The SMF produced by the solenoid was uniform. The temporal and spatial homogeneity of the SMF was measured with a three-axis Hall-Probe (Hall Probe A for F3A Magnetic Transducers, Senis AG, Baar, Switzerland) and varied by less than 10% for each magnetic field intensity used. The solenoid inner diameter was 3.6 cm with a length of 8.6 cm. The static magnetic field (SMF) generated by the solenoid was controlled by the current provided from a DC power supply (DCR40-10A, Sorensen Power Supplies, San Diego, CA, USA). The calibration curve of the solenoid can be found in Figure A.3, Appendix A.

3.3 Analysis

3.3.1 pH Measurements

The pH of the samples was measured daily with pH strips (pH Hydrion papers, range 1-12) every other day.

3.3.2 Growth Curves (Biomass Concentration vs. Time)

Samples for the growth curves were collected every day. The lights and solenoid were turned off during sample collection for safety reasons. The peristaltic pump was turned to maximum setting (for short improved mixing), the tubing was manually pinched for 10 min and then flasks were shaken once before collecting samples. This was performed everyday during the experiments to reduce microalgae sedimentation on the tubing and ensure better consistency of magnetic field exposure for all cells and better mixed samples. Then, a sample was collected from each flask for cell density determination.

Growth curves were generated based on turbidity measurements of samples at 750 nm by using an ultra-violet (UV-Vis) spectrophotometer (DU-520, Beckman-Coulter, Brea, CA) and based on the calibration curve obtained for each set of experiments. A wavelength of 750 nm was chosen for turbidity measurements to avoid interference by pigment absorption such as chlorophyll. The calibration (standard) curve of concentration vs. turbidity, relating biomass concentration with turbidity, was obtained on Day 7 of growth (Figure A.4, Appendix A). In order to generate the calibration curve, a sample was collected from the flask, and turbidity measurements of serial dilutions were taken. Another sample of known volume was centrifuged (Sorvall RC-5C) at 12,000 rpm for 5 min and washed three times with warm distilled water (DDW) to remove excess salt from the media (same time and rpm). The pellet was then remixed with a little bit of water and dried in a vacuum oven at 50 °C for 24 h, then the biomass was weighed. The actual biomass concentration (based on gravimetric results) could then be related to turbidity measurements. The equation obtained from the plot's straight line (concentration vs. turbidity) was used to determine unknown biomass concentrations from known turbidity values during the growth experiments.

3.3.2.1 Growth Models

Additionally, it was attempted to fit the growth curves to kinetic models with MATLAB (MathWorks R2011b), more specifically to the Monod model (Equation 3.1) and some modification of Monod (Equation 3.2). The modification of Monod's model (Equation 3.2) was based on the idea that growth rate is more closely related to internal nutrient concentrations rather than external and growth rate can drop to zero when there is still external nutrient available [150, 151].

$$\text{Monod: } \mu = \frac{\mu_{\max} * S}{K_S + S} \quad (3.1)$$

Where μ is the specific growth rate (d^{-1}): $\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$ (where X is the biomass concentration in g/L and t is time (d), measured at two different time points), μ_{\max} is the maximum specific growth rate (d^{-1}), S is the substrate concentration (g/L) and K_S is the saturation constant (g/L, value of S when μ is half of μ_{\max}).

$$\text{Modified Monod: } \mu = \frac{\mu_{\max} * (s - s_0)}{k_s + (s - s_0)} \quad (3.2)$$

Where μ is the specific growth rate (d^{-1}): $\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$ (where X is the biomass concentration in g/L and t is time (d), measured at two different time points), μ_{\max} is the maximum specific growth rate (d^{-1}), s (g/L) is the external available substrate concentration at time t, s_0 (g/L) is the value of s when μ (growth rate is zero) and K_s (g/L) is the saturation constant.

The substrate concentration s at time t was calculated from the yield coefficient (Y) (Equation 3.3).

$$Y = \frac{\text{mass of cells produced}}{\text{mass of substrate consumed}} = \frac{dX}{dS} \quad (3.3)$$

3.3.3 Final Biomass Concentration

At the end of the run, the experiments were stopped and the volume of each flask was measured with a graduated cylinder. Then, the biomass concentration was obtained gravimetrically by washing the biomass in a centrifuge (Sorvall RC-5C) at 12,000 rpm three times with warm DDW for 5 min each time, to remove excess salt from the media, freezing overnight and then immediately lyophilizing the concentrated biomass. All values reported are the average of triplicates. The initial biomass concentration (Day 0) for all runs was approximately zero. The final biomass concentration (C_{bmF}) was determined by dividing final mass (m_F) by final volume (V_F) using the formula:

$$C_{bmF} = \frac{m_F}{V_F} \quad (3.4)$$

3.3.4 Biochemical Analysis

For each experiment, lyophilized biomass (method described in section 3.3.3) was first ground with a mortar and pestle, then sonicated at 75 W using a Misonix XL-2000 sonication probe (Newtown, CT, USA).

Lipids were extracted by sonicating biomass in 2:1 chloroform/methanol (v/v) for 3 min, then by using the Soxhlet extraction method. The system was run until the solvent going to the bottom flask looked clear in colour. The final lipid solution collected at the bottom flask was poured in a weighing boat and waited until all the solvent had evaporated. Lipid content was then determined gravimetrically [152].

Carbohydrates were extracted by sonicating biomass in 1 M acetic acid for 5 min. The samples were then used for the phenol sulphuric acid method and absorbance at 490 nm was measured in order to determine the carbohydrate content of each sample. Glucose was used as a standard for carbohydrate measurements [153, 23].

Protein content was determined by elemental analysis (LECO CNS-1000, Leco Corporation, St. Joseph, MI, USA). 50 mg samples (triplicates) were combusted at 1,200 °C in a furnace coupled with an infrared detector, in an oxygen rich atmosphere. The nitrogen content determined by the equipment was then converted to protein content by the nitrogen-to-protein conversion factor of 4.44 [154].

Pigments were extracted by sonicating the biomass in 80% (v/v) aqueous acetone for 3 min and the supernatant was used for measurements. Chlorophyll a and carotenoids were determined by UV-Vis spectrometry with optical density (OD) absorbance measurements at 663, 645 and 480 nm and the equations below were used to determine chlorophyll a and carotenoid content from a known biomass sample concentration [21]:

$$\text{Chlorophyll a } \left(\frac{\text{mg}}{\text{L}}\right) = 12.7 * \text{OD}_{663} - 2.69 * \text{OD}_{645} \quad [155] \quad (3.5)$$

$$\text{Carotenoids } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{(\text{OD}_{480} + 0.114 * \text{OD}_{663} - 0.638 * \text{OD}_{645}) * V}{112.5 * \text{wt}} * 537 \quad (3.6)$$

Where V is the extraction volume in millilitres and wt is the weight of the biomass in milligrams (based on [156, 157, 158]).

Ash was obtained gravimetrically by burning 200 mg of biomass samples in a furnace at 550 °C for 4 hours [21]. Samples were placed in a desiccator under vacuum afterwards to avoid water vapour condensing on samples and affecting results (increasing mass), and left to cool overnight before weight measurements.

Metals were determined by nitric acid digestion then quantified using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700 ICP-MS, Agilent Technologies, Santa Clara, CA, USA). 20 mg of each biomass sample was mixed with 5 mL of concentrated nitric acid in a glass vial with a plastic screw top and autoclaved for 2 hours. After cooling, each sample was further diluted with 3% nitric acid to a concentration of 1 mg/mL biomass. Next, the solution was filtered to remove any undissolved matter, and the clear yellowish final solution was then used for analysis [159]. Control experiments were performed the same way with separate vials without the

biomass, in order to eliminate any interference of the vials themselves. All experiments were performed in duplicates and the average value was used.

3.3.5 Biomass, Lipids and Co-Products Productivity

Biomass productivity (P), here on a volumetric basis (g/L/d) is given by (Equation 3.7):

$$P = \frac{C_{bmF}}{t} \quad (3.7)$$

Where C_{bmF} is the final biomass concentration (g/L) and t is the total number of cultivation days (d).

The productivities of lipids and other co-products such as carbohydrates, proteins and pigments (chlorophyll a and carotenoids) were also determined. Product productivity (PP) was calculated based on the product content (PC) in the biomass and biomass productivity (P) (Equation 3.8).

$$PP = PC * P \quad (3.8)$$

Where PP is in volumetric basis (g/L/d), PC is in w/w and P is in (g/L/d).

3.3.6 Cell Size Determination and Cell Ultrastructure

3.3.6.1 Cell Size

Cells were imaged on Day 7 with a haemocytometer (Brightline 3100, Hausser Scientific, Horsham, PA). The images of 100 cells for each run (0,5, 10 and 15 mT) were used to calculate cell size by manually measuring the short and long lengths (radius) of the ellipsoid-shaped cells using ImageJ 1.50i software for Windows [160]. Since cells had an ellipse shape in 2d view, they were assumed to be ellipsoid in shape (Figure 3.2).

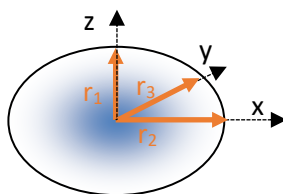


Figure 3.2: Cell Shape.

For the calculation of the volume, since only two lengths could be identified by the optical microscope, the ellipsoid was assumed to contain two shorter radii ($r_1 = r_3$) and one long radius (r_2). Volume of an ellipsoid [161]:

$$V = \frac{4}{3}\pi r_1 r_2 r_3 \therefore V = \frac{4}{3}\pi r_1^2 r_2 \quad (3.9)$$

Approximate surface area of the ellipsoid was calculated as:

$$S = 4\pi \left(\frac{(r_2 r_3)^{1.6} + (r_2 r_1)^{1.6} + (r_3 r_1)^{1.6}}{3} \right)^{\frac{1}{1.6}} \therefore S = 4\pi \left(\frac{2(r_1 r_2)^{1.6} + r_1^{3.2}}{3} \right)^{\frac{1}{1.6}} \quad (3.10)$$

And the surface area to volume ratio ($r_{S/V}$) was calculated as:

$$r_{S/V} = \frac{S}{V} \quad (3.11)$$

3.3.6.2 Effect of 10 mT on Cell Ultrastructure

In order to investigate changes in cell ultrastructure, samples of the control (0 mT) and 10 mT runs were collected on day 10 to be fixed, stained and further observed with transmission electron microscopy (TEM). First, samples were fixed in 2% (v/v) glutaraldehyde solution, in 0.1 M phosphate-buffered saline (PBS buffer), for 1 h, washed with PBS buffer, and left in buffer at 4 °C overnight. Second, the fixed cells were stained with 2% (w/v) osmium tetroxide in phosphate buffer (0.025 M), left at 4 °C for 1 h, then centrifuged to remove the stain solution and washed with phosphate buffer (0.025 M) three times. Third, 2% agarose was added to the biomass pellets and the mixture was left to solidify.

Thin sections of the agarose-biomass samples (1 mm thick) were subjected to a dehydration series with ethanol (25%, 50%, 75% and 100% v/v) and then embedded in London Resin (LR) White hard acrylic resin (L9774, Sigma-Aldrich), molded in gelatin capsules, placed in the oven at 55 °C for 48 h to polymerize, ultrathin sectioned (~70 nm) using a Reichert Ultracut E ultramicrotome (701701, Buffalo, NY), then collected on Formvar carbon-coated 100 mesh copper grids (FCF100-Cu, Electron Microscopy Sciences, Hatfield, PA). Finally, post-staining was done with 2% (w/v)

uranyl acetate, followed by Reynold's lead for 10 min each. The grids were washed after each post-stain with DDW. A Philips CM-10 transmission electron microscope (New York, NY, USA) was used for imaging with an accelerating voltage of 60 kV. Pictures were taken with a Hamamatsu digital camera (C9100-03, Bridgewater, NJ) [21]. The samples were also investigated with Scanning Electron Microscopy (SEM) (LEO (Zeiss) 1540XB FIB/SEM).

3.4 Statistical Analysis

Statistical Analysis was performed with one-way ANOVA with the Tukey's test for multiple comparisons using the program GraphPad Prism 6 (La Jolla, CA, USA) for Windows. Multiple comparisons of the means were performed with the significant difference test ($p \leq 0.05$). A P-value of 0.05 or less is considered significant and results are reported as the mean \pm standard deviation.

CHAPTER 4 – Results

For this section, an effort was made to separate the results into different sections although in Chapter 5, some of them will be referred to each other since they are related and together might help understand better the results. Here, cell measurements were placed in a separate section.

4.1 Growth

4.1.1 pH Levels

The pH of all runs started at approximately 8 and ended at approximately 7. Therefore, no changes in pH of the suspension occurred due to the magnetic field effect.

4.1.2 Growth Curves

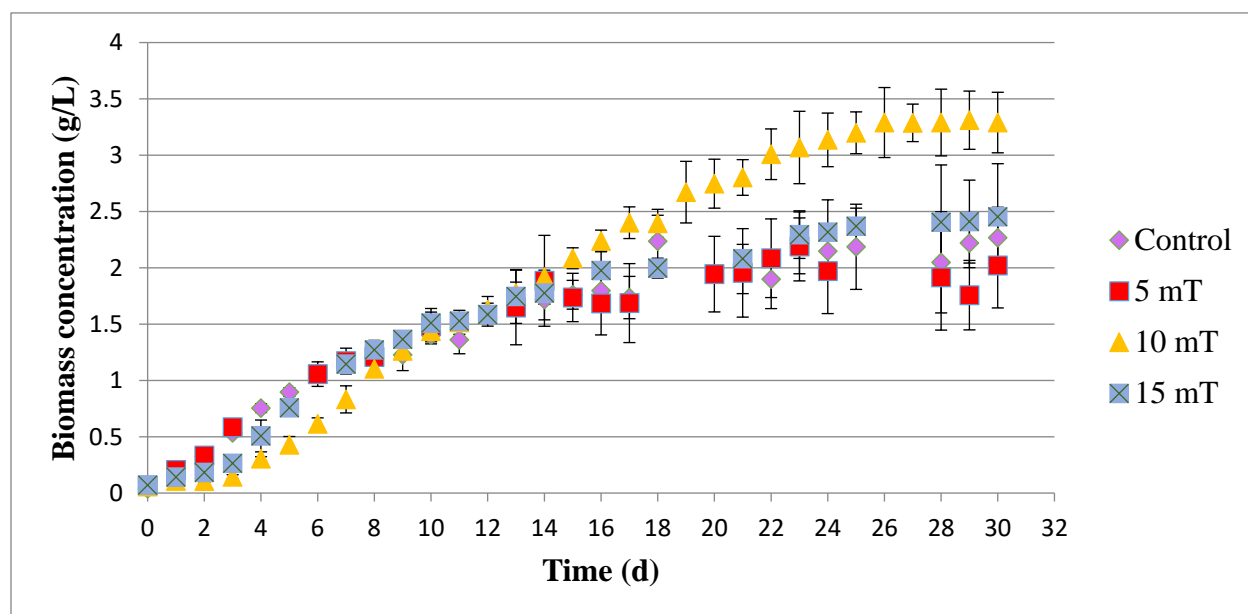


Figure 4.1: Biomass concentration vs. time for cells exposed only to the Earth's magnetic field (control) and cells exposed to 5, 10 and 15 mT SMF. Mean \pm standard deviation is shown (n=3).

The calibration curves for the growth curves in Figure 4.1 can be found in Figure A.4, Appendix A. From Figure 4.1, it can be seen that when the cells were grown for the same time point, the only significant difference in biomass concentration was observed for 10 mT at the end of the 30 days.

The curve fitting results and more details can be found in Appendix B. The substrate chosen for the curve fitting was the phosphate (proposed to be the limiting substrate). By inspection of the growth curves (Figure 4.1), there is not a visible difference in growth rate among the control and SMF treatments. Table B.1, Appendix B from Monod shows a difference in growth rates which does not seem to agree with the experimental data. Additionally, the curve of the specific growth rate vs. the substrate concentration does not match with the experimental data (Figure B.1 and B.2). Since it was observed that the specific growth rate was near zero when there was still substrate available (Figure B.2), a modified version of Monod was attempted. Table B.2 shows that the predicted growth rates for the control and SMF treatments varied which does not seem to agree with the experimental data, therefore the modified version did not offer too much of an improvement although the predicted curve (Figure B.3) was closer to the experimental curve (Figure B.2). Based on these results and conditions chosen for the fitting, both Monod and the Modified Monod curve were not good models to represent the experimental data. Since the growth of the cells in this work was mostly linear, the fact that the specific growth rate was calculated based on an exponential equation could have affected the fitting or these equations were simply not a good fit because the cells were not phosphate limited and could be limited by a different parameter such as light.

4.1.3 Final Biomass Concentration

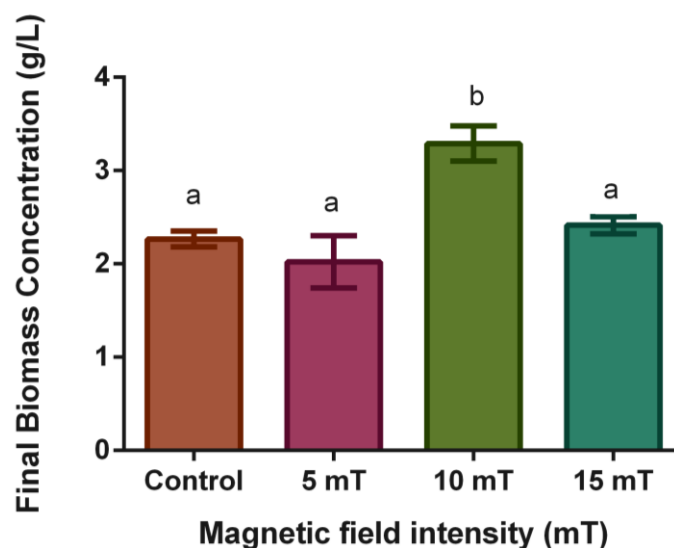


Figure 4.2: Final biomass concentration for the 0 (control), 5, 10 and 15 mT when grown for 30 days. Mean \pm standard deviation is shown (n=3). Different letters mean statistically significant differences at $p < 0.05$.

The 10 mT treatment also showed the highest biomass accumulation at the end of the experiment while the other treatments did not show significant differences.

4.2 Biochemical Analysis

4.2.1 Biomass and Pigment Composition - Control, 5, 10 and 15 mT (30-Day Runs)

At the end of the 30-day experiments, the lyophilized biomass was analyzed for lipid, carbohydrate, protein, ash, chlorophyll a and carotenoid content (Figure 4.3).

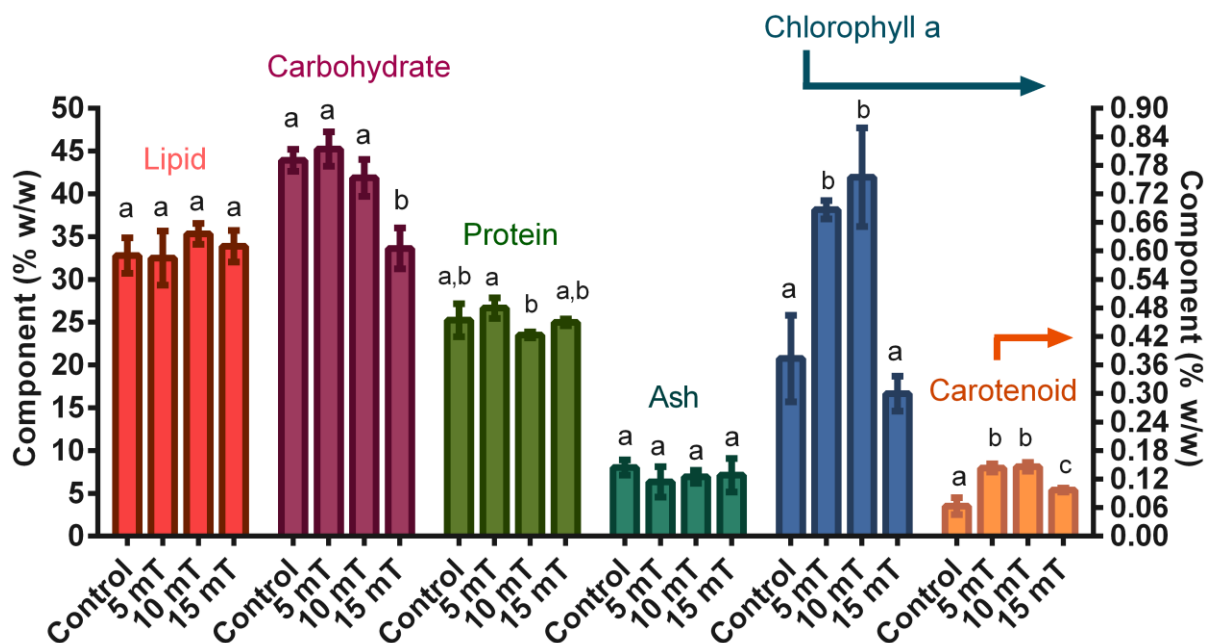


Figure 4.3: Biomass and pigment composition for the 0 (control), 5, 10 and 15 mT when grown for 30 days. Mean \pm standard deviation is shown (n=3). Different letters in the same group indicate statistically significant differences at $p < 0.05$.

One-way ANOVA was used for each component separately to compare differences among the 0 (control), 5, 10 and 15 mT treatments. Lipid and ash content were not affected by SMF treatment. Carbohydrate content was affected negatively at 15 mT and the levels of pigments were highest for 5 and 10 mT treatments although carotenoid accumulation was stimulated for all SMF intensities studied.

4.2.2 Metal Content - Control, 5, 10 and 15 mT (30-Day Runs)

The metal analysis was performed in duplicates. One-way ANOVA was used for each metal separately to compare differences among the 0 (control), 5, 10 and 15 mT treatments.

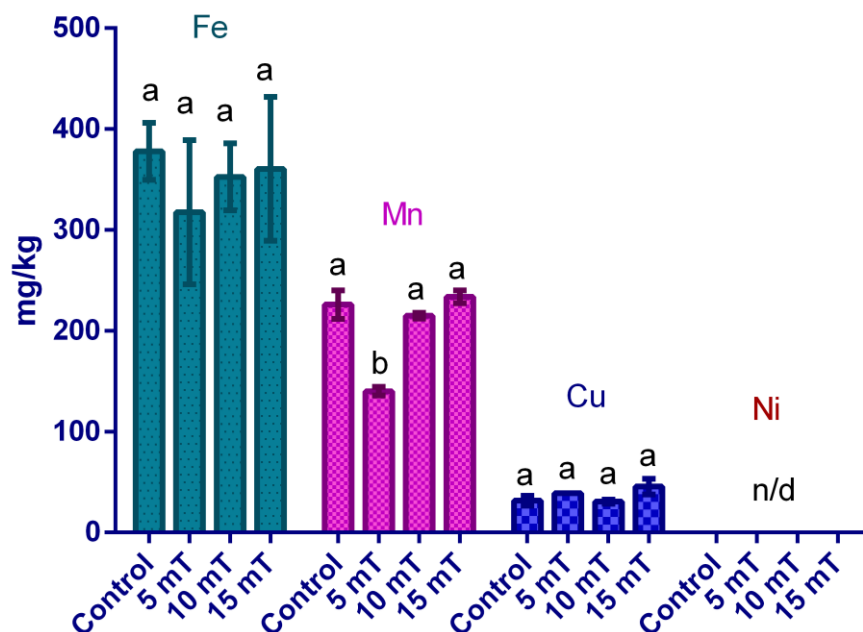


Figure 4.4: Biomass metal content for the 0 (control), 5, 10 and 15 mT when grown for 30 days. Mean \pm standard deviation is shown (n=2). Different letters in the same group indicate statistically significant differences at $p < 0.05$. N/d means non-detected.

No significant differences were observed for the metals tested except for manganese content at 5 mT, which was the lowest. Nickel levels were investigated but nickel content was not large enough to be detected reliably by ICP-MS.

4.2.3 Time Effect on Biomass and Pigment Composition – Control and 5 mT (10-Day and 30-Day Runs)

Additional experiments were performed with the control and 5 mT at different time points to determine any time dependent biochemical composition variation of the biomass produced. The growth curves of the 10-d and 30-d experiments can be found in Figure A.5, Appendix A. Figure 4.5 shows biochemical analysis of the control and 5 mT when grown for both 10 and 30 days.

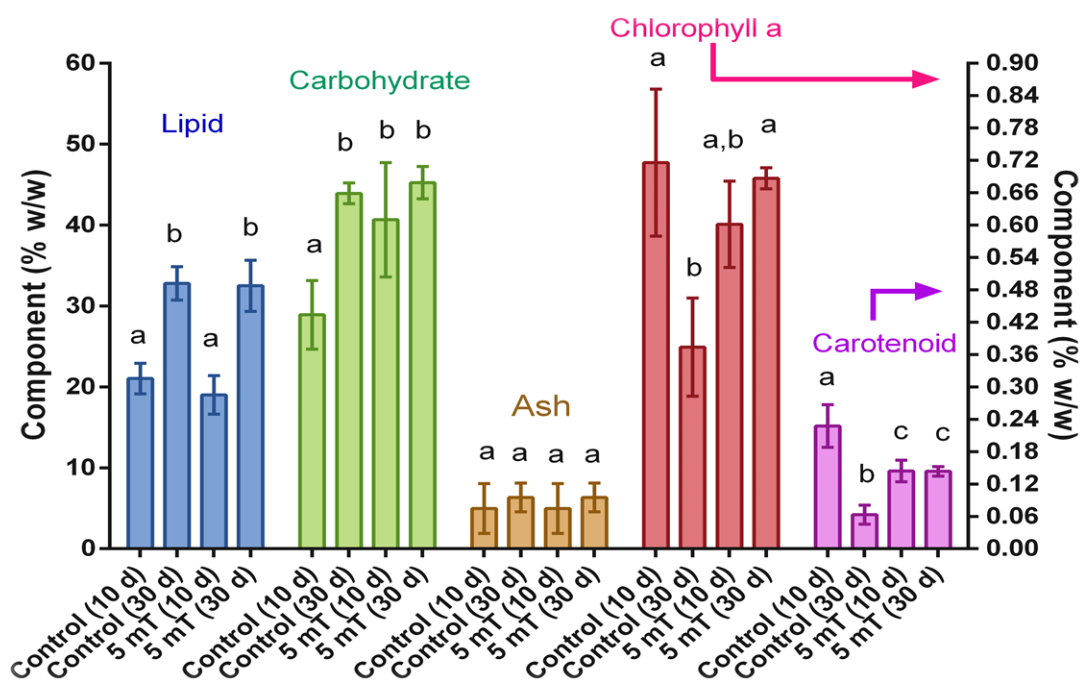


Figure 4.5: Biomass and pigment composition for the 0 (control) and 5 mT when grown for 10 and 30 days. Mean \pm standard deviation is shown ($n=3$). Different letters in the same group indicate statistically significant differences at $p < 0.05$.

One-way ANOVA was performed for each component separately. Protein was not analyzed due to lack of additional biomass for the 10-day runs. The control and 5 mT treatment showed similar growth patterns when grown for either 10 or 30 days. Lipid content increased equally for both control and 5 mT treatment over time while carbohydrate production was stimulated since the beginning for the 5 mT treatment and remained constant over time. The control cells consumed more pigments over time while the levels remained nearly constant for the 5 mT treatment. Ash content was the same irrespective of SMF treatment or time.

4.3 Biomass and Product Productivity

The maximum biomass and product productivity of the cells when grown for 30 days are presented in Table 4.1.

Table 4.1: Biomass and product productivity for the 0 (control), 5, 10 and 15 mT SMF treatments when grown for 30 days. Mean \pm standard deviation is shown (n=3). Different letters in the same column indicate statistically significant differences at $p < 0.05$.

SMF (mT)	Biomass Productivity (g/L/d)	Lipid Productivity (mg/L/d)	Carbohydrate Productivity (mg/L/d)	Protein Productivity (mg/L/d)	Chlorophyll a (mg/L/d)	Carotenoid (mg/L/d)
0	0.076 \pm 0.003 ^a	24.76 \pm 1.44 ^a	33.21 \pm 2.07 ^a	18.49 \pm 0.91 ^a	0.28 \pm 0.07 ^a	0.05 \pm 0.01 ^a
5	0.067 \pm 0.009 ^a	22.07 \pm 4.90 ^a	30.53 \pm 4.99 ^a	17.42 \pm 2.44 ^a	0.46 \pm 0.06 ^a	0.10 \pm 0.01 ^b
10	0.110 \pm 0.006 ^b	38.77 \pm 3.59 ^b	45.91 \pm 3.45 ^b	25.59 \pm 1.85 ^b	0.83 \pm 0.16 ^b	0.16 \pm 0.01 ^c
15	0.080 \pm 0.003 ^a	27.26 \pm 2.12 ^a	27.02 \pm 1.36 ^a	19.91 \pm 0.53 ^a	0.24 \pm 0.04 ^a	0.08 \pm 0.01 ^b

Carotenoid productivity increased for all SMF tested in comparison to the control (Table 4.1) because of the higher carotenoid content in all cells exposed to SMF stimulation (Figure 4.3). No other increase was observed for 5 and 15 mT (Table 4.1) since the biomass accumulation and biochemical content were similar to the control. Although 15 mT had approximately 23% lower carbohydrate content, since the biomass was slightly higher ($\sim 4\%$) at 15 mT (2.4 ± 0.1 g/L) in comparison with the control (2.3 ± 0.1 g/L), the product of biomass concentration and carbohydrate content (carbohydrate productivity) was not statistically significant. Cell exposed to 10 mT had an increase in productivity for all components, mainly because of its higher biomass accumulation over the same period (Table 4.1).

4.4 Cell Size and Cell Ultrastructure

4.4.1 Cell Size

In addition to the radii measurements for the cells in all runs, the surface area to volume ratio was also calculated. A picture of the cells (taken with an optical microscope, in a haemocytometer chamber) at the day of the measurements is also provided (Figure 4.6).

Table 4.2: Cell lengths of the 0 (control), 5, 10 and 15 mT treatments on Day 7 of growth (rounded to two significant digits). Mean \pm standard deviation is shown (n=100). Rows with different letters indicate statistically significant differences at $p < 0.05$ for the row values. Columns with different letters indicate statistically significant differences at $p < 0.05$ for column values in comparison with the control. If more than one letter is provided for a value, the first letter refers to the row and the second letter to the respective column.

	Shorter Radius (μm), r_1	Longer Radius (μm), r_2
Control	2.0 ± 0.5^a	2.4 ± 0.6^b
5 mT	2.4 ± 0.6^b	3.3 ± 0.6^c
10 mT	2.1 ± 0.5^a	$2.3 \pm 0.5^{a,b}$
15 mT	2.0 ± 0.5^a	2.6 ± 1.0^b

Table 4.3: Surface area to volume ratio ($r_{S/V}$) of the 0 (control), 5, 10 and 15 mT treatments on Day 7 of growth (rounded to two significant digits). Mean \pm standard deviation is shown (n=100). Different letters indicate statistically significant differences at $p < 0.05$.

	$r_{S/V} (\mu\text{m})^{0.2}$
Control	1.5 ± 0.3^a
5 mT	1.2 ± 0.2^b
10 mT	1.5 ± 0.3^a
15 mT	1.5 ± 0.3^a

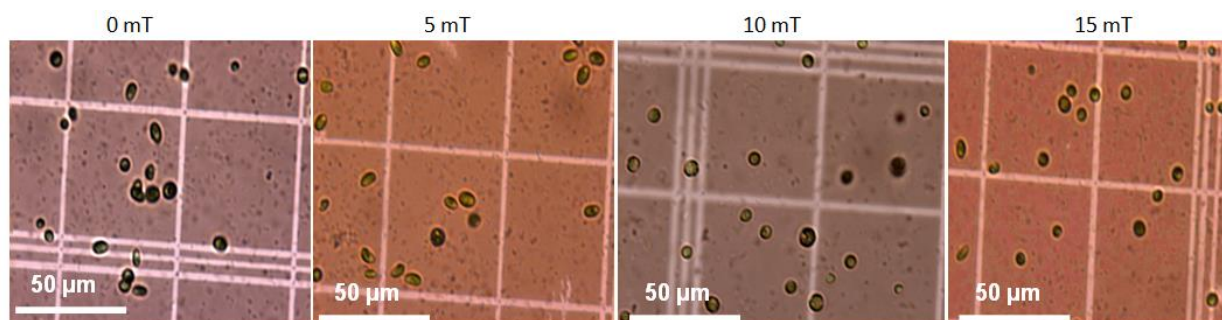


Figure 4.6: Pictures of *N. oculata* cells when cell size was calculated (Day 7).

The *N. oculata* cells in the majority of the runs had statistically different radii values (shorter and longer) indicating that the shape of the cells was most likely an ellipsoid (Table 4.2), which was visually observed when looking at the cells (Figure 4.6). The cells for all runs had similar measurements except for 5 mT that seemed bigger overall and had a smaller surface area to volume ratio (Table 4.3).

4.4.2 Cell Ultrastructure – Control vs. 10 mT Cells

Since 10 mT showed best results, TEM images of the control and 10 mT were taken (Figure 4.7).

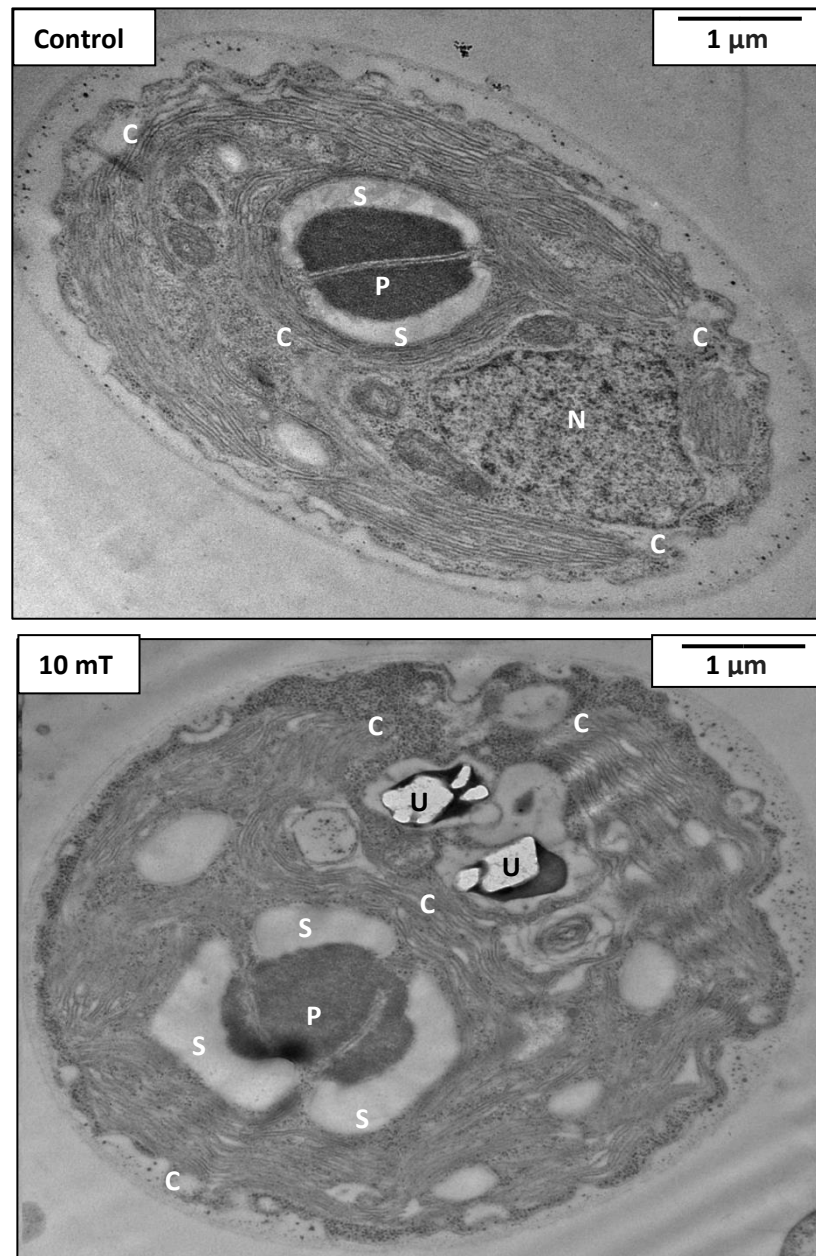


Figure 4.7: TEM images of a cell exposed only at the Earth's magnetic field (top) and a cell exposed to 10 mT magnetic field intensity (bottom). The photos were taken on the same day of growth. C = Chloroplast (four corners are shown), P = Pyrenoid, S = Pyrenoid Starch Plate, N = nucleus, U = unidentified.

The *Nannochloropsis oculata* cells showed common structures for both control and 10 mT treatment which are highlighted in the pictures. However, only the 10 mT treated cells showed an unknown structure (marked U as unidentified in Figure 4.7) which could not be characterized by energy-dispersive X-ray spectroscopy (EDX) from a scanning electron microscope (SEM). A couple of SEM photos and a sample of the EDX result can be found in Appendix C.

Additionally, although multiple pictures were taken (see Appendix C for more samples), it was challenging to get the same position of the cells in space for comparison. It could also be observed that there was some shrinkage of the cell. This could be caused by the higher salt concentration in the buffer used. The osmotic pressure induced water loss from the cell leading to cell shrinkage and cell membrane detachment in the process. Therefore, no measurements of organelles were taken.

CHAPTER 5 – Discussion

This chapter provides a more detailed explanation about the results obtained in this study, how they compare to each other and also how they compare to the literature. Where not provided explicitly in papers cited and where possible, some data was calculated here based on additional information present in the papers. Additionally, cell growth can either mean that the cell is physically growing in size (eg.: increasing cell material/components) or when the cell divides. In most cases, the term cell growth is referred to cell division and this will be the main meaning in this section. It is also worth to note that although there was an effort to separate the results into main sections, since all the analysis are related and help explain cell behaviour, some sections might have paragraphs that include a combination of more than one type of result.

5.1 Growth

5.1.1 Seed Culture and Control Culture

Cell growth is typically controlled by nutrient uptake, synthesis of molecules and organelle assembly. When nutrients (or light) are limited, this will cause a secondary reduction in molecule synthesis and then in assembly which will prevent cells from replicating and dividing [162]. Cell will then grow bigger by converting and accumulating any available nutrients or sugars into other components (such as carbohydrates and lipids) as a possible future energy source when cells need it for survival and until conditions are favourable again for cell replication such as in a nutrient and light replete environment. Growth curves, even when in terms of biomass concentration vs. time, relate to cell division and can be used to provide an idea of how the cell population is changing with time.

All growth curves (Figure 4.1) showed similar stages of growth including the seed culture (Figure A.1, Appendix A). When there is plenty of light and nutrient in the media, the cells grow exponentially. Depending on the species and growth conditions, when a nutrient or light starts to become limiting, the cell growth will become directly proportional to the nutrient or light still available, therefore the growth will look linear [163, 164]. Combination of limiting factors (either

more than one nutrient, light or other conditions) might slow down the growth even further until cells reach stationary phase and cell concentration will become roughly unchanged for some time (eg.: no net growth) until cell death becomes more noticeable (eg.: when conditions do not improve and cells cannot grow anymore). Visible cell death did not occur during the length of time used in the work with *Nannochloropsis oculata*.

The higher growth rate and biomass concentration achieved in the control from this study in comparison to the seed culture (threefold increase) (Figure A.6, Appendix A) is likely due to greater light availability for photosynthesis ($600 \mu\text{mol photons/m}^2\cdot\text{s}$ in the control vs. $100 \mu\text{mol photons/m}^2\cdot\text{s}$ in the seed culture). Typically, the greater the light availability, the greater is the nutrient uptake by the cells since they can use more energy to convert nutrients into more cell components and new cells. The higher light intensity used in this study also produced more heat, increasing the temperature of the microalgae suspension (from $23.5 \text{ }^\circ\text{C}$ control to $29 \text{ }^\circ\text{C}$ in the experiments). Enzymatic reactions in the cells are estimated to double with a $10 \text{ }^\circ\text{C}$ temperature rise (unless temperature is high enough to be harmful to the cells), also possibly meaning a double uptake of nutrients and double growth [165]. The light and temperature effects combined are a possible reason why the cells in the control of the experiments grew threefold greater in comparison with the seed culture.

5.1.2 SMF Experiments: Control and SMF Cultures

The SMF experiments were performed with the same light levels and the same conditions except for the different static magnetic field intensities.

5.1.2.1 Growth Curves and Final Biomass Yield (30-Day Runs)

From the literature [21, 17, 19, 20], SMF stimulation at 10 mT has shown positive growth stimulation, whether growth is represented in the form of specific growth rate (d^{-1}) or biomass concentration (g/L) (some authors measured only one of the parameters). For the work in this thesis, although the increase in biomass over time for each cell population (growth rate) was similar for all treatments until Day 14, overall, 10 mT had a longer and higher biomass accumulation over

time (Figure 4.1) showing the most positive stimulation. Since the differential among the experiments performed in this study was the overall biomass concentration during the 30-day experiment (instead of growth rate), biomass concentration was the chosen parameter for the indication of SMF effect on microalgae growth.

The overall growth at 10 mT was also confirmed by the final biomass concentration (Figure 4.2). The final biomass concentration of *N. oculata* had an increase of about 43% when treated at 10 mT (from 2.3 ± 0.1 g/L in the control to 3.3 ± 0.2 g/L for 10 mT). Comparing the results obtained at 10 mT with 5 mT and 15 mT, the final biomass concentration at 10 mT was 65% (from 2.0 ± 0.3 g/L in 5 mT to 3.3 ± 0.2 g/L for 10 mT) and 38% (from 2.4 ± 0.1 g/L in 15 mT to 3.3 ± 0.2 g/L for 10 mT) higher in comparison with 5 mT and 15 mT, respectively. In comparison with the control, the difference in final biomass concentration for 5 mT and 15 mT was statistically insignificant.

Numerous studies on the SMF effect on microalgae biomass concentration have been reported in the literature and include both fresh water (majority) and salt water microalgae (Table 5.1). Table 5.1 summarizes the findings regarding biomass concentration increase including our current study. For articles that tested different parameters in addition to SMF stimulation, the results for the optimal combination are shown.

The studies on Table 5.1 demonstrate that SMF affects microalgae and there is a SMF strength such that a beneficial effect exists on biomass concentration. Specifically, SMF intensities in the range 10 to 500 mT have been shown to increase biomass concentration on several microalgae species with enhancement from 10% to over 80%. In our current study, the biomass increase by *N. oculata* compares favourably and falls within the range of the biomass increase reported.

Table 5.1: Biomass increase due to SMF, in comparison with the control, reported in the literature, in addition to the increase obtained in this work. Values shown are the highest obtained in each study.

Species	Biomass increase in comparison with control	Optimal SMF Strength (mT)	Reference
<i>Chlorella pyrenoidosa</i>	12%	500	[14]
<i>Chlorella fusca</i>	21%	60, for 24 h/d	[15]
<i>Spirulina platensis</i>	47%	250	[18]
<i>Spirulina platensis</i>	10 %	10	[19]
<i>Dunaliella salina</i>	84%	10, with 1 mg/L Fe-EDTA	[20]
<i>Chlorella kessleri</i>	77%	10	[21]
<i>Nannochloropsis oculata</i>	43%	10	This work

Among the studies reported on Table 5.1, four studies including this one, reported an optimum SMF strength of 10 mT [19, 21, 20] for biomass concentration irrespective of the species and included both fresh water and salt water microalgae. More studies would be needed to determine how broadly applicable is the observed SMF strength of 10 mT on optimizing the enhancement of biomass. So far, results on microalgal biomass (or growth rate) increase have been varied depending on the SMF intensity studied and no clear pattern is observed.

5.1.2.2 Specific Growth Rates

Another approach to assess the SMF intensity effect is via changes in the specific growth rate of the cells. A couple of studies [19, 21] determined that the maximum growth rate coincided with the highest biomass concentration. A study on *Chlorella vulgaris* [17] determined that the growth rate was maximum at 10 mT (and approximately 72% higher than the control) although the corresponding effect on the biomass generated was not determined. In our study, little change in specific growth rate as a function of SMF exposure was observed although biomass concentration was significantly enhanced (43%) at 10 mT during a 30-day period. Similarly, from the growth curve provided by Li et al., 2007 [18], it could be observed that the highest biomass concentration (at 250 mT) did not coincide with the highest growth rate (at 550 mT) although all the growth rates and biomass concentration peaks were still higher than the control.

An inspection of the growth curve profiles (based on concentration vs. time or chlorophyll vs. time) of the articles covered in this discussion (Table 5.2) showed that at the optimal SMF strengths tested, there was a significant increase in growth rate since the beginning of SMF exposure [14, 16, 21, 18, 19] suggesting that although SMF stimulates increase in growth rate and/or microalgal biomass, the effect is not so prominent with *N. oculata* cells under SMF exposure (eg.: not an immediate increase in division but sustained division over time). Similarly, Deamici et al., 2016 [15] also found that at 60 mT, *Chlorella fusca* did not have a significant change in growth in comparison with the control until the Day 7 of cultivation.

5.1.2.3 Cell Size

Cell size can be a good indication of cell division rates although not always a relationship can be found. Small et al., 2012 [21] reported a microalgae reduction in size at 10 mT coupled with greater biomass yield and growth rate in comparison to the control and therefore, giving good indications that the smaller cells were due to faster division. Although there have been studies trying to relate cell size with growth rates [166], this relationship does not always exist and cell size alone does not determine growth [167]. For this work, *N. oculata* cells seemed to have the biggest size at 5 mT (Table 4.2 and 4.3) which theoretically could mean slower growth. However, all experimental conditions gave similar biomass concentration on Day 7 (when the cell size was measured) regardless of cell size (Figure 4.1), and therefore such a relationship regarding cell size and cell division could not be found.

Additionally, the relatively large standard deviation on Table 4.2 is most likely due to the non-uniformity in size of the cells, which would be caused either by a natural occurrence or due to the fact that the measurements were taken in 2-d. Since the cells are ellipsoid in shape, they might be oriented in space differently from each other at the time of the photos causing a non-uniform distribution in sizes. Also, experiments in flasks are not under optimal conditions, such as more uniform mixing and light exposure, and this can contribute to the non-uniformity of the cells size and morphology. A larger sample size may help to reduce the uncertainty in cell dimension measurements.

5.1.2.4 Media Composition and pH

Media composition can have a significant affect on the response of the algal cells to SMF stimulation. Yamaoka et al., 1992 [20] reported that when grown under SMF stimulation with Fe-EDTA concentration of 10 mg/L, the microalga *Dunaliella salina* suffered a negative impact on biomass concentration in comparison to when grown under SMF and a media with no added Fe-EDTA. In addition, when grown with SMF stimulation and a media containing 1 mg/L added Fe-EDTA, biomass concentration increased by approximately 84% compared to the control. The authors also observed that although the cells had positive results with SMF stimulation in comparison with the control, when no additional Fe-EDTA was added to the media, intensities above 10 mT were more beneficial for growth. For cells grown with added Fe-EDTA, the highest stimulation happened at 10 mT and decreased with stronger SMF intensities. These results suggest that components in the media might affect stimulation effects and consequently affect cell growth. For the microalga *N. oculata* studied in this thesis, the media had a Fe-EDTA concentration of approximately 11 mg/L. Based on the results obtained with the marine strain *Dunaliella salina* [20], it is possible that the biomass concentration of *N. oculata* could be further altered at 10 mT by adjusting the Fe-EDTA in the media.

The pH of the *Nannochloropsis oculata* media in this work was measured throughout the experiments. The decrease in pH from 8 to approximately 7 over the course of the study was observed for all runs. The decrease in pH was probably due to the initial consumption of components in the media by the cells followed by a decrease in CO₂ uptake by microalgae during the stationary phase (resulting in an increase in CO₂ levels in the media), therefore bringing the pH of the media closer to the neutral pH of 7. Although the pH strips used only provided a rather rough estimate of the pH, it was observed that regardless of the SMF intensity used, there were not significant changes in the media pH in comparison with the control. Deamici et al., 2016 [15] measured the pH in the beginning and at the end of the experiments for *Chlorella fusca* grown under control, 30 mT and 60 mT. The pH of all cases did not seem to vary significantly from each other. Li et al., 2007 [18] also investigated if SMF stimulation would affect the pH of the media when *Spirulina platensis* was grown using a SMF of 250 mT. Although the pH of the media changed over time (from 8.5 to around 11), the change was the same for both the control and 250

mT treatments. Therefore, SMF stimulation so far has not been shown to interfere significantly with the pH of microalgae media.

5.2 Biochemical Content

5.2.1 30-Day Study

Major biochemical components of the *N. oculata* microalgal biomass at the end of the 30-day growth period were determined and reported (Figure 4.3). The biochemical components included proteins, carbohydrates, lipids, chlorophyll a and carotenoids. It can be seen that for the major components, there was not a significant difference in comparison with the control (except for an approximately 23% decrease in carbohydrate content at 15 mT). Pigment (chlorophyll a and carotenoids) content was significantly enhanced for the 10 mT treatment in comparison with the control (more than twofold). In the literature, not all authors have investigated biochemical composition and some authors only investigated biochemical composition partially. These results are summarized and compared to the results determined in the current study (Table 5.2).

For proteins, Li et al., 2007 [18] reported a small increase (less than 1%) in total amino acid content in *S. platensis* at a SMF of 250 mT. In other studies, protein content results were varied depending on SMF intensity and length of exposure [15, 21] The protein content in *Nannochloropsis oculata* remained fairly constant (around 25%) over the SMF strength of 5-15 mT (Figure 4.3).

Carbohydrate accumulation seems to have the largest variation in results (no change, decrease or increase) depending on the SMF intensity and length of exposure [15, 19, 21], therefore no particular trend has been observed in terms of SMF stimulation and it might be solely species-dependent. Hirano et al., 1998 [19] observed that the carbohydrate content in *Spirulina platensis* decreased 0.6-fold in comparison with the control when the cells were exposed to 70 mT SMF. The 0.6-fold decreased carbohydrate content at 70 mT along with lower pigment content overall (same chlorophyll a content but 20% lower phycocyanin pigment) and a 0.5-fold lower growth rate indicated lower photosynthetic ability of the cells.

For the species *Nannochloropsis oculata* investigated in this study, at the highest SMF intensity used (15 mT), the cells had a 23% decrease in carbohydrate content in comparison with the control. While chlorophyll a content ($0.30 \pm 0.04\%$) was at a similar level with the control ($0.37 \pm 0.09\%$), carotenoid content was 66.7% higher, therefore pigment synthesis was not affected negatively (Figure 4.3) and no change in growth rate in comparison with the control was observed (Figure 4.1). These results suggest that, unlike Hirano et al., 1998 [19], photosynthetic ability was not reduced at 15 mT. However, since application of SMF to biological systems has been associated with an increase or a change in the lifetime of free radicals [168], it is possible that the cells at 15 mT had to use additional energy to counteract any negative effects from increased oxidative stress, therefore causing a reduction in carbohydrate levels. Moreover, since the growth pattern and biomass concentration for the cells at 15 mT are similar to the control (Figures 4.1 and 4.2), this indicates that the effect of free radicals on the cells was not large enough to affect the cell growth negatively.

Lipid synthesis seems to be the least affected by SMF stimulation with most authors reporting no changes in lipid content (Table 5.2). Figure 4.5 shows that although there were some differences between the control and 5 mT in carbohydrates and pigments over time (despite similar growth, Figure 4.1), lipid synthesis was unaffected and accumulation over time was similar for both control and 5 mT, increasing equally for the same periods of time tested. The relative constancy of the lipid content under magnetic stimulation in the *N. oculata* in this work is significant as this microalga has a relative high lipid content and has been suggested as a promising candidate for biofuels production (Chapter 2, sections 2.2.1 and 2.2.3).

Table 5.2: Summary of SMF effect on biochemical content of species in the literature including this work.

In comparison with the control						
Species (SMF used)	Lipids	Carbohydrates	Proteins	Chlorophyll a	Carotenoids	Reference
<i>N. oculata</i> (0, 5, 10, 15 mT)	No change	Mostly no change (decreased 23% at 15 mT)	No change	Increased 1.9 to 2.0-fold (at 5 and 10 mT), no change at 15 mT	Increased 1.7 to 2.5-fold	This work
<i>C. pyrenoidosa</i> (0, 500 mT)	No change	-	-	Increased 10.5%	-	[14]
<i>C. fusca</i> (0, 30, 60 mT)	No change for 30 mT, decreased 18 to 23% for 60 mT	Mixed results depending on SMF strength and length of time	Mixed results depending on SMF strength and length of time	-	-	[15]
<i>S. obliquus</i> (0, 50, 100, 200, 300, 400, 500 mT)	-	-	-	Increased 27% at 100 mT (not significant at other SMF)	-	[16]
<i>C. kessleri</i> (0, 10 mT)	No change	Increased 8.5%	Increased 8.7%	Increased 15%	No change	[21]
<i>C. vulgaris</i> (0, 5, 10, 15, 25, 35, 45, 50 mT)	-	-	-	-	No change	[17]
<i>S. platensis</i> (0, 250 mT)	-	-	No change	Increased 31%	-	[18]
<i>S. platensis</i> (0, 5, 10, 20, 35, 40, 70 mT)	-	Mostly increased 6 to 20% (decreased 0.6-fold at 70 mT)	-	Not significant (but phycocyanin increased 16 to 54% in most cases)	-	[19]
<i>D. salina</i> (0, ~ 10, 23, 50, 130, 230 mT)	-	-	-	-	Mostly increase of β -carotene (3 to 45%)	[20]

While some authors reported no visible differences in pigment content (chlorophyll a or accessory pigments) when cells were grown under SMF stimulation (Table 5.2) and even a 20% decrease in phycocyanin content in *Spirulina platensis* at 70 mT with unchanged chlorophyll levels [19], pigment content seems to be the cell component most positively affected by SMF treatment with most authors reporting an increase in chlorophyll a (major pigment) or in accessory pigments or both. These results can be taken as an indication that one of the major effects of SMF is in stimulating chlorophyll (and/or other pigments) accumulation for increased light capture, a basic requirement for photosynthesis. The cells then have the potential to increase their energy capture and use it differently to produce cell components and grow depending on the species and other growth conditions (such as media composition).

For *Nannochloropsis oculata* reported in this study, carotenoid content increased 1.67 to 2.5-fold when the cells were grown under SMF stimulation. Accessory pigments, such as carotenoids, help harvest more light for the cells. More importantly, carotenoids possess antioxidant properties and help protect chlorophyll and the cells against oxidative stress [169, 40]. Since the only difference in the runs was the SMF stimulation, the cells were potentially stimulated to increase carotenoid production in response to free radical generation (Figure 4.3). Small et al., 2012 [21] noticed a 35% decrease in antioxidant activity with *Chlorella kessleri* cells when grown at 10 mT, in comparison with the control, and suggested that it was due to the increase in oxidative stress in the cells due to SMF stimulation. For this work, it is possible that the levels of free radicals were not large enough to cause a reduction in carotenoid content in the cells, except for 15 mT, which had a 29 to 33% lower carotenoid content than 5 and 10 mT, but still 67% higher than the control.

Based on the growth and biochemical composition of the final biomass in this study on *N. oculata*, it is likely that the increased growth at 10 mT was due to the higher chlorophyll a and carotenoid content in the cells. At a SMF of 10 mT, cells were able to utilize more light due to higher chlorophyll a concentration to harvest light until cell density was large enough to cause cell shielding [163]. Although some authors reasoned that SMF might increase the rate of the reactions inside the cells making the cells to accelerate cell growth [15, 19, 16, 17, 21], this did not seem to be applicable to *Nannochloropsis oculata* as SMF had little effect on its growth rate.

Among many possible sites of action, static magnetic fields have been shown to affect biological systems due to the action of free radicals or possible changes in biological membranes [168, 170, 171]. For this study, a more comprehensive analysis of the reactive oxygen species (ROS) and biochemical content would be beneficial in order to better understand the effects of each magnetic field intensity on the cells.

5.2.2 Control and 5 mT (10-Day vs. 30-Day)

Since the control and 5 mT had similar growth profiles (Figure 4.1), additional experiments were performed to determine biochemical composition changes at an intermediate time point (day 10) and compared to that at the end of the experimental run (day 30). As shown in Figure 4.5 it was observed that from day 10 to day 30, the control had an increase in carbohydrates and lipids while the pigments (chlorophyll a and carotenoids) decreased over time. When cells are exposed to high light intensities but do not need or cannot use the additional energy (eg.: due to nutrient limitation and reduced cell division), they reduce their pigment content in order to protect them against possible photodamage [172]. Under nitrogen limitation, the cells can also use chlorophyll as a nitrogen source to support the creation of enzymes or cell maintenance overall [173] leading to the observed decrease in the pigments over time.

Although a nutrient limitation could not be confirmed in this study, the behaviour of the control cells is typical of phosphorous or nitrogen-limited cultures [23]. It is well established that these conditions favour and in most cases, lead to an increase in lipid accumulation as being observed. However, there are exceptions as in the case of *Dunaliella salina* which experiences a lipid content decrease [174, 175].

Under 5 mT SMF exposure, the trend of increasing lipid accumulation from day 10 to day 30 are similar to that of the control. For carbohydrates, the cells accumulated higher amounts as compared to control and remained constant up to day 30. The higher carbohydrate content at an earlier time point can be taken as an indication of enhanced photosynthetic activity due to exposure to the SMF. The higher accumulation of carbohydrates at 5 mT in comparison with the control is consistent with the larger cells (Tables 4.2 and 4.3).

5.3 Productivity

Productivity was calculated based on the 30-day data. Although the content of the major components was not significantly affected by SMF treatment, since 10 mT had the highest increase in biomass (Figure 4.2), productivity for all components increased in comparison to other SMF intensities and to the control. Table 5.3 shows a summary of the productivity changes for 5, 10 and 15 mT in comparison with the control.

Table 5.3: Summary of productivity increase in comparison with the control (30-day cultivation).

SMF (mT)	Biomass Productivity (g/L/d)	Lipids Productivity (mg/L/d)	Carbohydrates Productivity (mg/L/d)	Proteins Productivity (mg/L/d)	Chlorophyll a Productivity (mg/L/d)	Carotenoids Productivity (mg/L/d)
5	No change	No change	No change	No change	No change	Increase (twofold)
10	Increase (45%)	Increase (57%)	Increase (38%)	Increase (38%)	Increase (threefold)	Increase (threefold)
15	No change	No change	No change	No change	No change	Increase (60%)

At 10 mT, the highest increase occurred for lipid productivity due to the 43% higher biomass concentration and since lipid content was 7.6% slightly larger at 10 mT (although not significant in comparison with the control).

The effect of magnetic stimulation using SMF on microalgae productivity of specific biochemical components is summarized in Table 5.4. There are some fairly large variations in the microalgae cultivation condition among the studies. For the articles where productivity was based on growth rate but sufficient data was available, product productivity was calculated as the product of biomass productivity and product content for better comparison with the way productivity was calculated for *Nannochloropsis oculata*. Since length of the experiments was not provided for *Dunaliella salina* [20], it was assumed that the cells were cultivated up to the same time point. For Li et al., 2007 [18], productivity of *S. platensis* was based on Day 7 biomass concentration results since further analysis for 250 mT and control experiments lasted 7 days. Nevertheless, some of the comparisons are quite instructive.

Table 5.4: Biochemical productivity increase in comparison with the control reported in the literature, in addition to the increase obtained in this work. Values shown are the highest obtained in each study.

Biochemical productivity increase in comparison with the control						
Species (Best SMF)	Lipids	Carbohydrates	Proteins	Chlorophyll a	Carotenoids	Reference
<i>N. oculata</i> (10 mT)	57%	38%	38%	Threefold	Threefold	This work
<i>C. pyrenoidosa</i> (500 mT)	10%	-	-	11%	-	[14]
<i>C. fusca</i> (30, 60 mT)	No change (at 30 mT, 1 h/d)	50% (at 60 mT, 24 h/d)	9% (at 30 mT, 1h/d)	-	-	[15]
<i>C. kessleri</i> (10 mT)	47%	92%	93%	Twofold	Twofold	[21]
<i>S. platensis</i> (250 mT)	-	-	No change	31%	-	[18]
<i>S. platensis</i> (10 mT)	-	31%	-	10%	-	[19]
<i>D. salina</i> (10 mT)	-	-	-	-	Threefold (at 1 mg/L Fe-EDTA)	[20]

The major biochemical components of interest are the lipids and the carotenoids. Lipids are of interest as they are essential for biodiesel and biojet fuels production. Carotenoids are some of the most potent natural antioxidants with high biomedical values.

Of the sparse data in the literature summarized in Table 5.4, it can be seen that the SMF has a significant effect in enhancing both lipids and carotenoids production in microalgae. From the four species tested for lipid productivity including the species used in this study, 10 mT SMF stimulation has been shown the most beneficial, and the enhancement is the highest for *Nannochloropsis oculata* among all the results reported suggesting that it is a promising microalga for biodiesel applications under SMF stimulation. For carotenoids production, *N. oculata* again has the highest enhancement among all the results reported irrespective of the magnetic field intensity and the algal species.

For the other biochemical products including carbohydrates, proteins and chlorophyll a, there is also a general increase in their productivities. These products, although less valuable, are still useful as animal feed (carbohydrate and protein) and colorants (green chlorophyll), which could

help further improve the economics associated with microalgal biomass based biodiesel production. In the case of *Chlorella kessleri*, the almost doubling in carbohydrates productivity makes it an attractive candidate for alcohol production in addition to biodiesel production. However, *N. oculata* can still be regarded as more attractive as it can be grown in brackish and seawater. *Chlorella kessleri* is a freshwater species and would require the use of potable water for cultivation.

CHAPTER 6 – Conclusions and Future Work

6.1 Conclusions

To enhance the attractiveness of using microalgae for biofuels production, the use of static magnetic fields (SMFs) to stimulate algal biomass, lipids and high value co-products production was investigated. The microalga *Nannochloropsis oculata* was the chosen algal species since it grows well and can accumulate high lipid content.

The effect of static magnetic field intensities (control, 5, 10, 15 mT) on *Nannochloropsis oculata* growth was studied to determine the optimal conditions for maximum biomass, lipid and high value co-products productivities.

Over the range of SMF intensity (0–15 mT) studied, it was determined that a SMF intensity of 10 mT gave the highest biomass yield. High value co-products including chlorophyll a and carotenoids productivities were also the highest when *N. oculata* cells were exposed to 10 mT SMF. Additionally, *N. oculata* had the highest lipid productivity (57% increase in comparison to the control) among all algal species that have been investigated for lipid production when cultivated under SMF stimulation.

Based on the results reported in this thesis, it can be concluded that SMF stimulation is an effective alternative to enhance microalgal biomass yield. When taking into account the high lipid contents and increased yield of the high value co-products of chlorophyll a and carotenoids, the economics of the use of *N. oculata* for biofuels production is significantly improved. The SMF used in this study is also well below the recommended limit for exposure [144] and therefore, no known major safety concerns are associated with its use to enhance microalgae growth.

6.2 Future Work

This study was performed on a laboratory scale, additional experiments in different photobioreactor designs and larger scale systems would be required to confirm the observed SMF effects in scaled up experiments.

Since SMF stimulation is an external stressor, it can possibly be combined with other growth stimulation methods such as nitrogen limitation or CO₂ addition for potential further increase in lipid productivity or even with wastewater or seawater, if needed. Additionally, although the effect of SMF stimulation on growth is considered reversible once the source is removed, the effect stays with the cells for a few days [21]. Tu et al., 2015 [16] has reported that a two-hour stimulation of the microalga *Scenedesmus obliquus* was enough to increase growth significantly in comparison with the control. Although more research is needed for different species and test conditions, these results suggest that there is a possibility to improve growth using SMF stimulation without utilizing power for 24 h/d, which could save on electricity costs. Alternatively, permanent magnets can be used once a SMF strength has been selected.

It would also be beneficial to test both 10 mT and 15 mT on Day 10, or track changes in biochemical composition with time for all, in addition to a more comprehensive metal analysis for all runs since it was observed in this study that there was a difference in biochemical composition at day 10 between the control and 5 mT. An investigation of consumption of nutrients (phosphates and nitrates), oxidative species generation and membrane lipid structure could give more indications if SMF influences different arrangements and composition of lipid membranes. For cell measurements and imaging, it would be advised to use a larger population and to try and find better buffers and techniques to obtain the best quality images for comparison.

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- [175] The British Columbia Innovation Council, "Microalgae Technologies & Processes for Biofuels/Bioenergy Production in British Columbia: Current Technology, Suitability & Barriers to Implementation," *Seed Science*, 2009.

Appendix A: Supplementary Results and Information

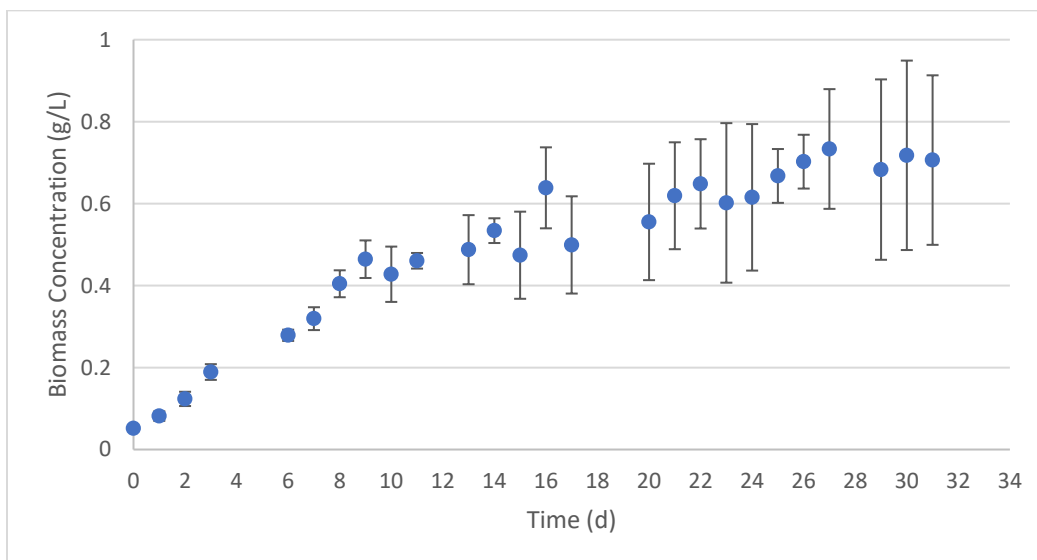


Figure A.1: Growth curve of seed culture (duplicates)

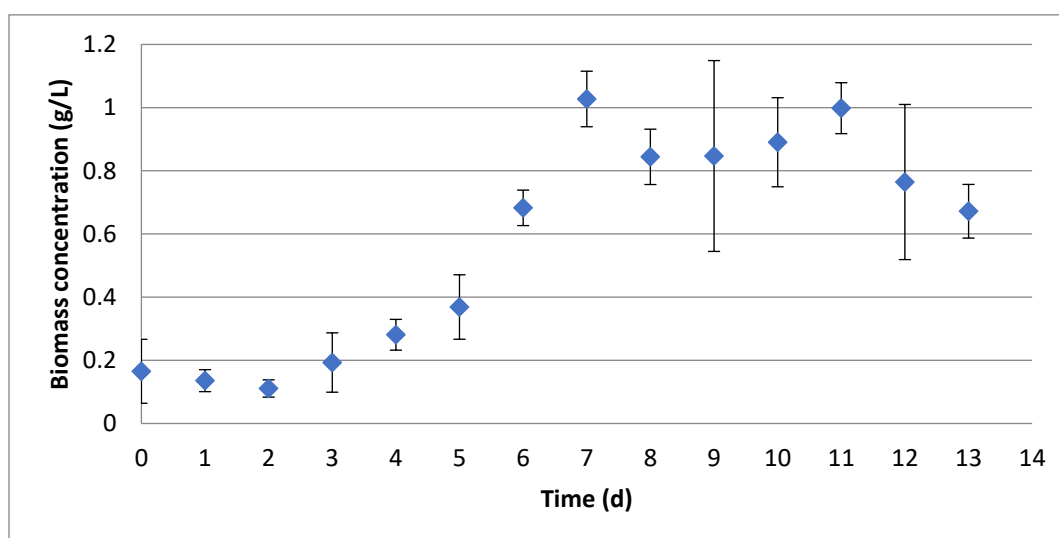


Figure A.2: Biomass concentration versus time for the control, in triplicates (inconsistent measurements due to not keeping the volume constant by daily water addition)

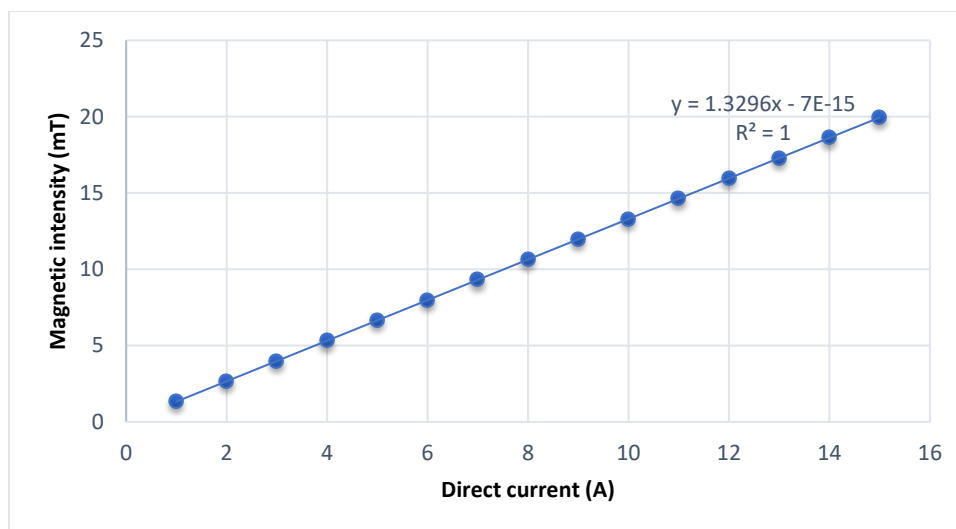


Figure A.3: Solenoid calibration curve – relationship between current (A) and static magnetic field intensity (mT).

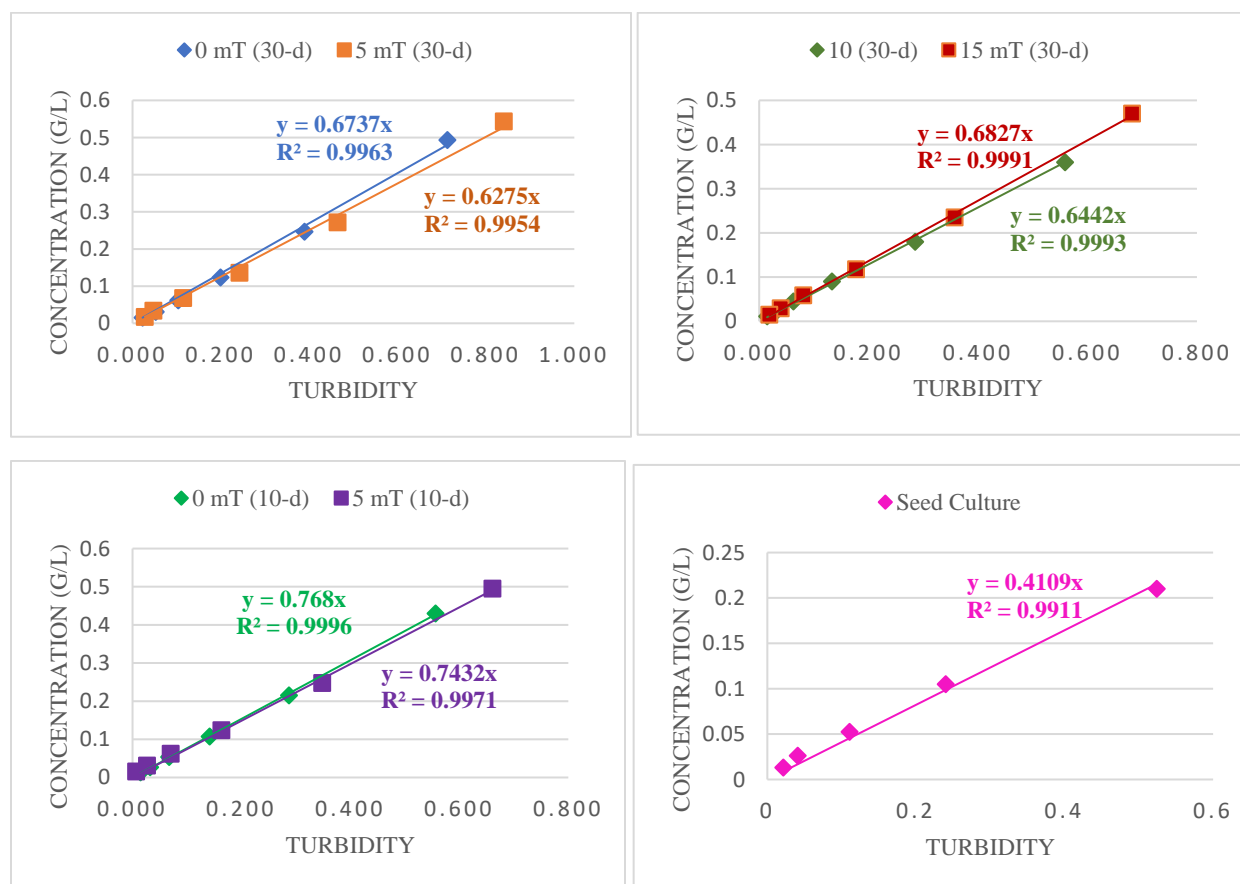


Figure A.4: Calibration curves of the Growth Curves for the 30-d and 10-d Experiments and the Seed Culture.

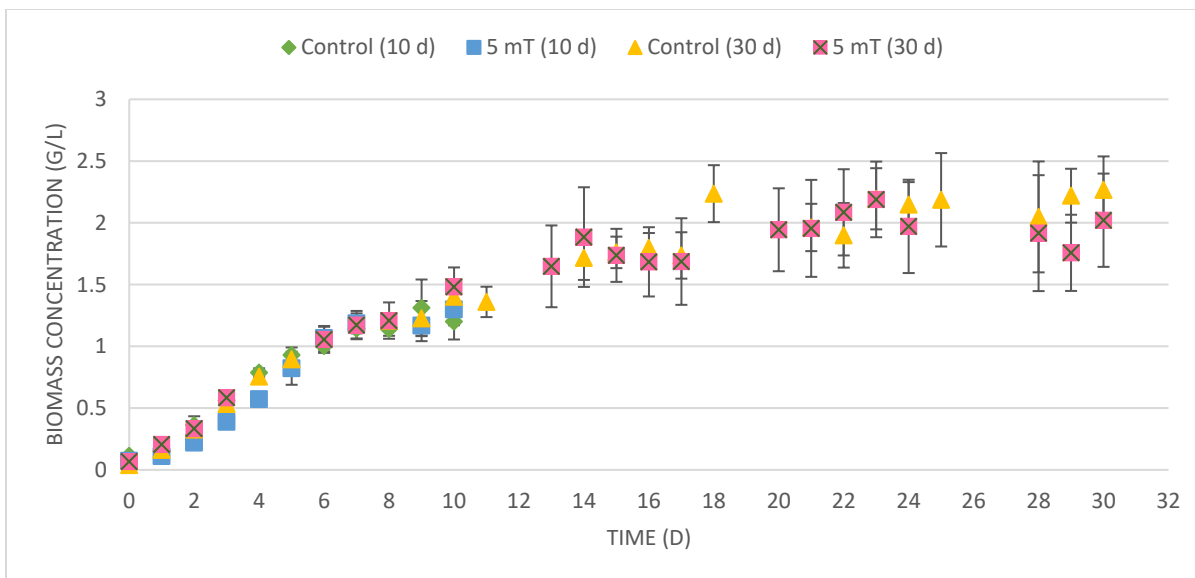


Figure A.5: Biomass concentration versus time curve for the control (0 mT) and 5 mT at different time points. Mean \pm standard deviation is shown (n=3).

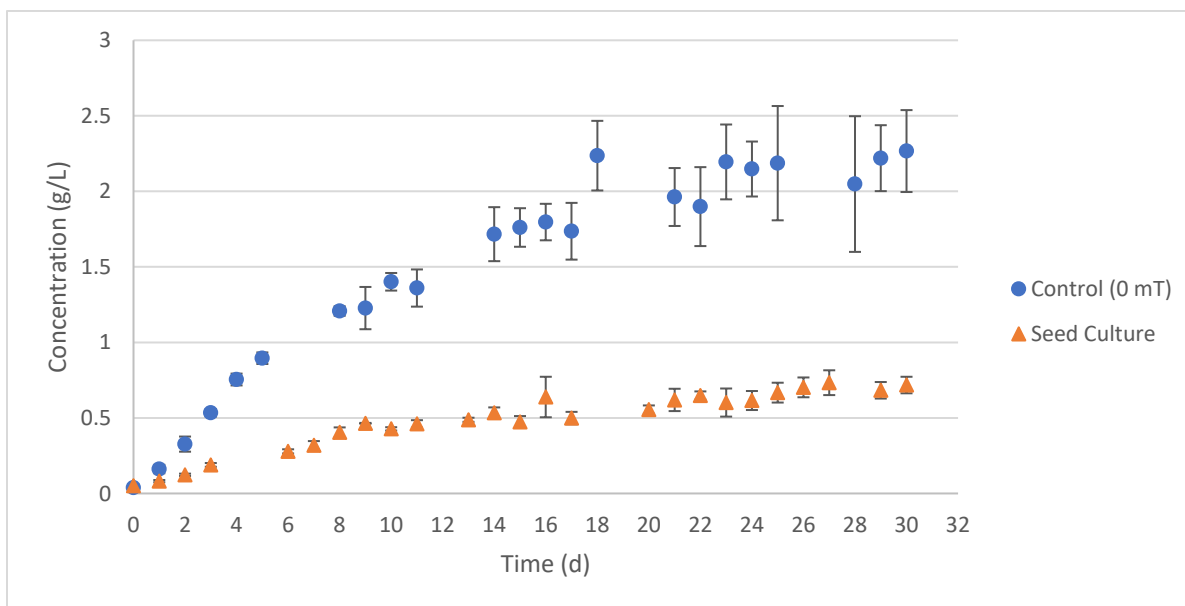


Figure A.6: Biomass concentration versus time for the control (0 mT) and the seed culture together

Appendix B: Growth Curve Fitting

B.1 Monod

B.1.1 Monod Codes

Function Monod

```
function [mu] = monod_original( p, s )
%p(1)= umax; p(2)= Ks

for i=1:length(s)
    mu(i) = p(1).*(s(i))./(p(2)+s(i));

    if mu(i)<0
        mu(i)=0;
    end
end

mu=mu';
end
```

Script for monod_original

```
s0=[0.035817139    %s is substrate vector calculated from biomass density and
0.03372888        % "0" part denotes SMF intensity
0.032215293
0.025470272
0.025296194
0.023960137
0.022894347
0.021780462
0.022272901
0.01463246
0.014104106
0.013679716
0.013544684
0.010161025
0.00882607
0.003424777
0.00626594
0.006166543
0.004556613
0.003345384
0.001323002
0.001316126];

s5=[0.038365196    % for 5 mT
```

```
0.027164997
0.025783298
0.025060803
0.024008706
0.020369163
0.017122515
0.017122515
0.013366842
0.010580335
0.010756514
0.010432796
0.011997257
0.012441449
0.012487212
0.006321655
0.005717479
0.003752575
0.002283115
0.000295455
0
0];
```

```
s10=[0.047030598    % for 10 mT
0.04457159
0.041795396
0.038286931
0.034927506
0.031801138
0.029697866
0.027833108
0.026102292
0.024000426
0.021621581
0.019219073
0.01672912
0.015123476
0.012921094
0.011139647
0.009030754
0.007266097
0.005626039
0.003909704
0.002908824
0.001752076];
```

```
s15=[0.041343099    % for 15 mT
0.038701957
0.032095001
0.026717638
0.02563638
0.023100355
0.021328292
0.019786407
0.018186675
0.016436922
0.015231279
```

```
0.012786581
0.010720998
0.010391647
0.010062295
0.010062295
0.008435232
0.008435232
0.006249176
0.003963147
0.003473421
0.003178572];
```

```
mu0=[0.3027737 % calculated growth rate, averaged and smoothed
0.125133534 % for 0 mT
0.081661576
0.29965466
0.006831064
0.049492384
0.03791676
0.038451599
-0.016574792
0.23198233
0.014215898
0.011328412
0.003699538
0.085481072
0.031243286
0.118473698
-0.130112742
-0.017233634
0.044858415
0.030092191
0.044194395
-0.004623883];
```

```
mu5=[0.240513256 % for 5 mT
0.589177352
0.053721503
0.028885328
0.038316543
0.120932173
0.096876991
0
0.096294165
0.068276489
-0.001680879
0.008167201
-0.037144389
-0.01244232
-0.002122773
0.14638203
0.011712668
0.023098428
0.040128699
-0.005807327
```

```
-0.000810087
-0.057695359];

mu10=[0.453756007      % for 10 mT
0.430575058
0.326476128
0.311081583
0.227599607
0.174333785
0.103189826
0.082617108
0.070614225
0.078986653
0.082517755
0.076947645
0.074220807
0.045084088
0.05893257
0.045049559
0.05079431
0.040553704
0.036371221
0.036650914
0.020751958
0.023463094];

mu15=[0.572776004      % for 15 mT
0.215149485
0.5274093
0.282309386
0.048377727
0.101848908
0.060389702
0.044571417
0.056497159
0.046008746
0.038591276
0.06541334
0.05662057
-0.009619591
-0.009713027
0
0.075184289
0
0.057180882
0.095733463
0.00323214
0.021705789];

upb = [4, 0.15]; %upper bound guesses for umax and ks
lbp = [0,0]; %lower bound guesses for umax and ks
pg = [0.2,0.03]; %intial guesses for umax and ks

options = optimset('lsqcurvefit')      % creates options structure used by
lsqcurvefit function
```

```

options = optimset(options, 'TolX', 1e-9, 'TolFun', 1e-
9, 'MaxFunEvals', 4e20, 'MaxIter', 4e20, 'FinDiffType', 'Central',
'FinDiffRelStep', 1e-16 );

p0 = lsqcurvefit(@monod_original, pg, s0, mu0, lbp, upb, options) %gives
values of constants(coefficients) umax and ks for 0mT
p5 = lsqcurvefit(@monod_original, pg, s5, mu5, lbp, upb, options) %for 5mT
p10 = lsqcurvefit(@monod_original, pg, s10, mu10, lbp, upb, options) %for
10mT
p15 = lsqcurvefit(@monod_original, pg, s15, mu15, lbp, upb, options) %for
15mT

mumod0 = monod_original (p0, s0) % recalculates the specific growth rate
based on the constants obtained from the model for 0 mT
mumod5 = monod_original (p5, s5) % for 5 mT
mumod10 = monod_original (p10, s10) % for 10 mT
mumod15 = monod_original (p15, s15) % for 15 mT

%this section calculates a vector of squared error for each
%element which could be used as error bars on the graph (residual)
rse0=( (mumod0-mu0).^2).^0.5./length(mumod0).^0.5
rse5=( (mumod5-mu5).^2).^0.5./length(mumod5).^0.5
rse10=( (mumod10-mu10).^2).^0.5./length(mumod10).^0.5
rse15=( (mumod15-mu15).^2).^0.5./length(mumod15).^0.5

%this section plots the data, with residual root squared error as error
%bars

hold on
errorbar(s0,mumod0,rse0,'b-')
errorbar(s5,mumod5,rse5,'r-')
errorbar(s10,mumod10,rse10,'g-')
errorbar(s15,mumod15,rse15,'m-')

legend('0 mT', '5 mT', '10 mT', '15 mT')
xlabel('PO4 (g/L)');
ylabel('mu, 1/d');

```

B.1.2 Constants Obtained from the Model (μ_{\max} and K_s)

Table B.1: Predicted constants obtained from Monod

SMF (mT)	μ_{\max}	K_s
0	0.3245	0.0546
5	0.3084	0.0395
10	1.0979	0.1373
15	1.0511	0.1161

B.1.3 Specific Growth Rate vs. Substrate Concentration Curves

The predicted constants μ_{\max} and K_s by the model were used to recalculate the specific growth rate data. Figure B.1 shows the curve of the specific growth rate vs. phosphate concentration predicted by the model and the error in comparison with the experimental specific growth rate values.

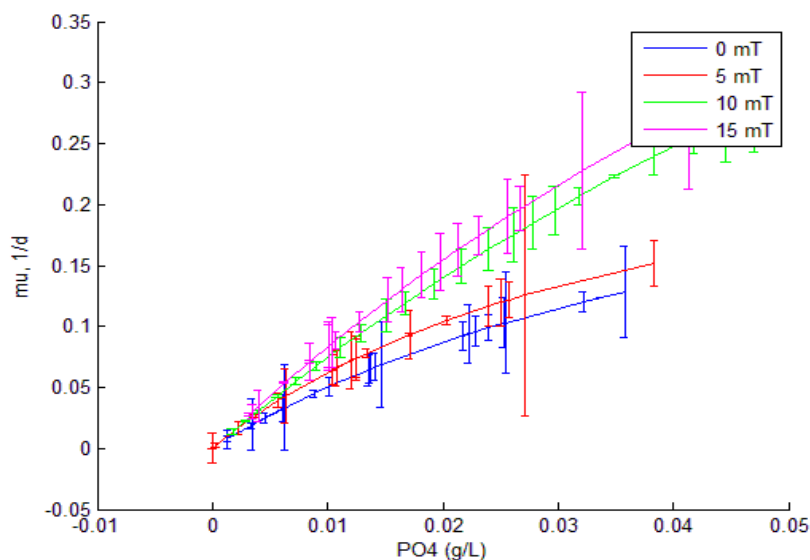


Figure B.1: Monod predicted specific growth rate vs. substrate concentration (phosphate) with error bars showing deviation from experimental data

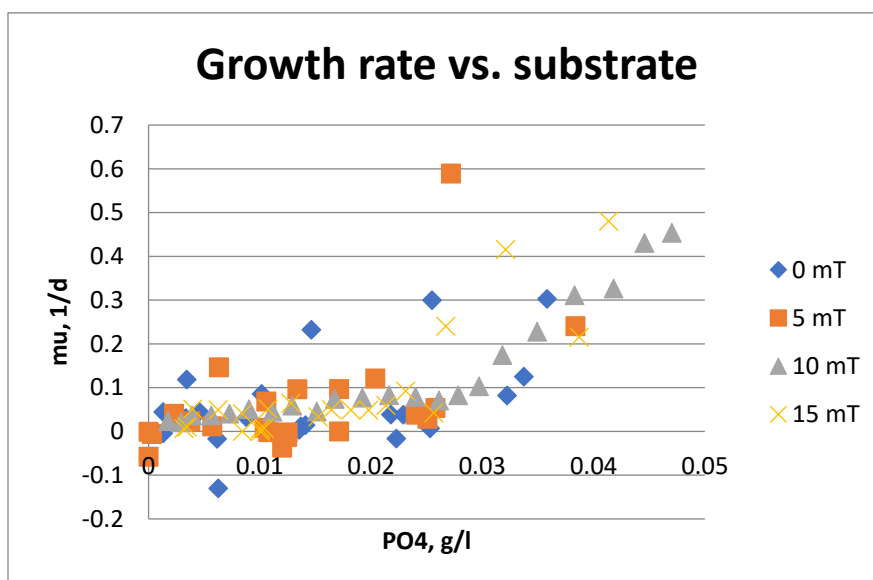


Figure B.2: Specific growth rate vs. substrate concentration (phosphate) from experimental data

B.2 Modified Monod

B.2.1 Modified Monod Codes

Modified Monod Function

```
function [ mu ] = monad( p,s )
% p is parameters and s is substrate vector
%p(1)=mumax p(2)=ks p(3)=so
for i=1:length(s) % do it element by element so we can check if s>so

    if s(i)<p(3)
        mu(i)=0; % if s<so then set mu to zero, otherwise we d get negative
growth rate
    else
        mu(i)=p(1).*(s(i)-p(3))./(p(2)+s(i)-p(3));
    end

end

mu=mu'; % mu must be a column vector for lsqcurvefit, so transpose it
```

Modified Monod Script

```
% Same S0, S5, S10, S15, mu0, mu5, mu10, mu15 vectors as the "Script for
monod_original" file plus the rest of the code below

ubp=[4,0.15,0.03]; % upper bounds on mu, ks, and so
lbp=[0,0,0]; % lower bounds on same
pg=[0.2,0.03,0.01]; % initial guess on the same

options = optimset('lsqcurvefit') % creates options structure used by
lsqcurvefit function
options = optimset(options, 'TolX',1e-9, 'TolFun',1e-
9, 'MaxFunEvals',4e20, 'MaxIter',4e20, 'FinDiffType', 'Central',
'FinDiffRelStep',1e-16 );
p0=lsqcurvefit(@monad,pg,s0,mu0,lbp,ubp,options) %lsqcurvefit gives the
coefficients of the equation
p5=lsqcurvefit(@monad,pg,s5,mu5,lbp,ubp,options)
p10=lsqcurvefit(@monad,pg,s10,mu10,lbp,ubp,options)
p15=lsqcurvefit(@monad,pg,s15,mu15,lbp,ubp,options)

mumod0=monad(p0,s0)
mumod5=monad(p5,s5)
mumod10=monad(p10,s10)
mumod15=monad(p15,s15)

%this section calculates a vector of squared error for each
%element which could be used as error bars on the graph (residual)
```

```

rse0=( (mumod0-mu0).^2).^0.5./length(mumod0).^0.5
rse5=( (mumod5-mu5).^2).^0.5./length(mumod5).^0.5
rse10=( (mumod10-mu10).^2).^0.5./length(mumod10).^0.5
rse15=( (mumod15-mu15).^2).^0.5./length(mumod15).^0.5

%this section plots the data, with residual root squared error as error
%bars

hold on
errorbar(s0,mumod0,rse0,'b-')
errorbar(s5,mumod5,rse5,'r-')
errorbar(s10,mumod10,rse10,'g-')
errorbar(s15,mumod15,rse15,'m-')

legend('0 mT', '5 mT', '10 mT', '15 mT')
xlabel('PO4 (g/L)');
ylabel('mu, 1/d');

```

B.2.2 Constants Obtained from the Model (μ_{\max} and K_s)

Table B.2: Predicted constants obtained from Modified Monod

SMF (mT)	μ_{\max}	K_s	S0
0	0.3966	0.0503	0.0063
5	0.3904	0.0167	0.0124
10	1.0447	0.036	0.0235
15	1.0445	0.0266	0.0196

B.2.3 Specific Growth Rate vs. Substrate Concentration Curve

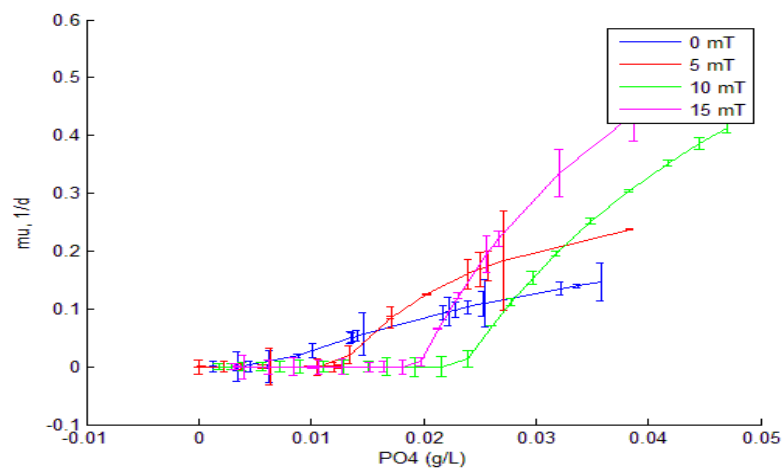
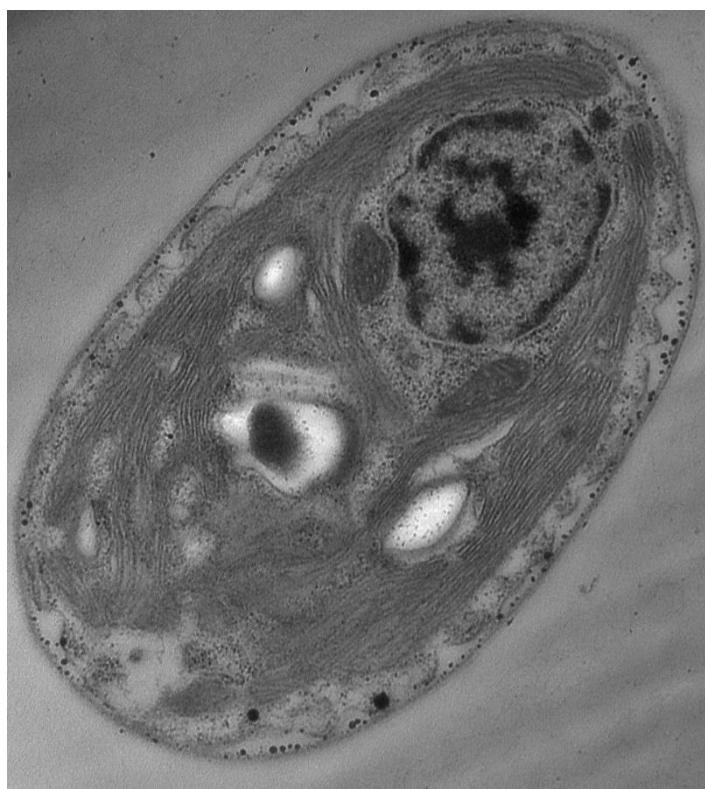
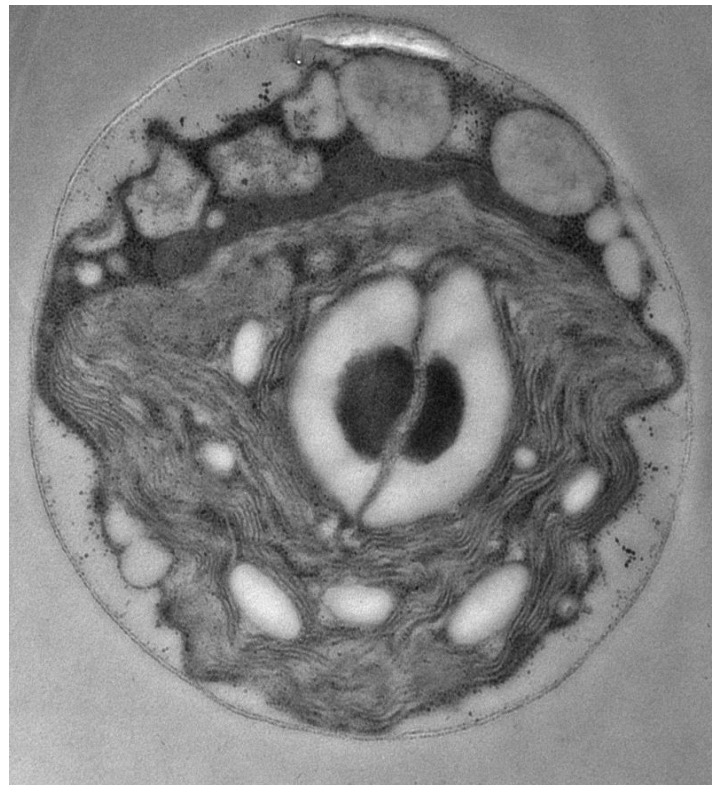
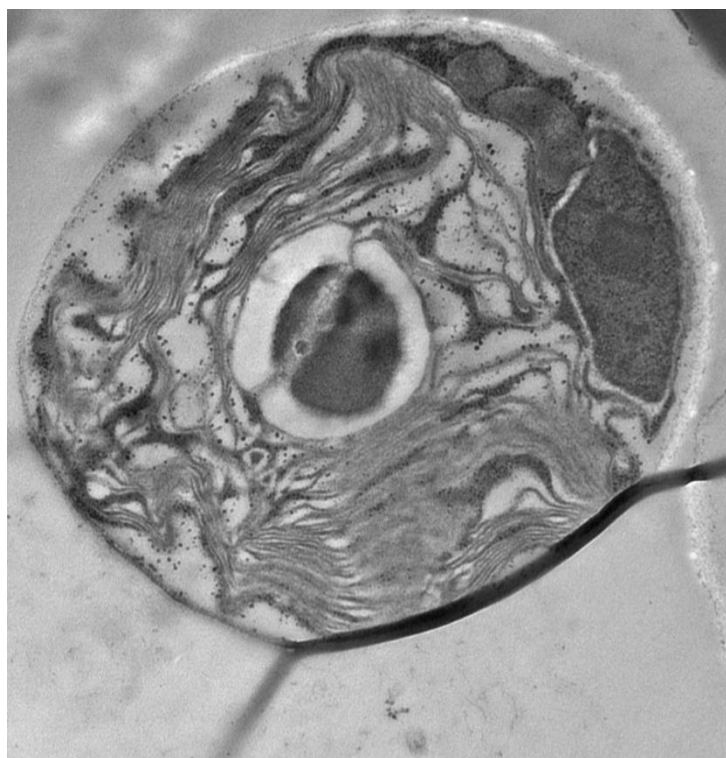


Figure B.3: Modified Monod predicted specific growth rate vs. substrate concentration (phosphate) with error bars showing deviation from experimental data

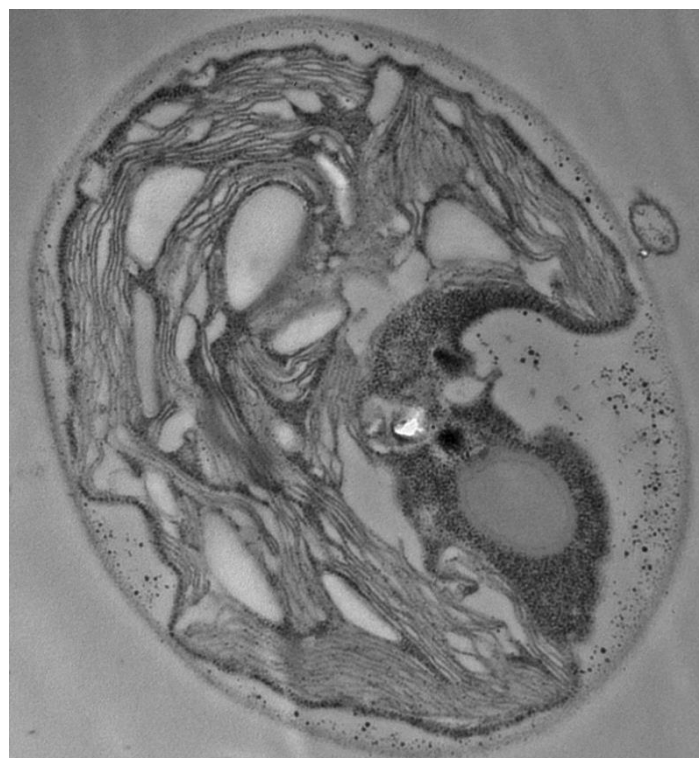
Appendix C: Additional TEM and SEM Images and Results

0 mT Images

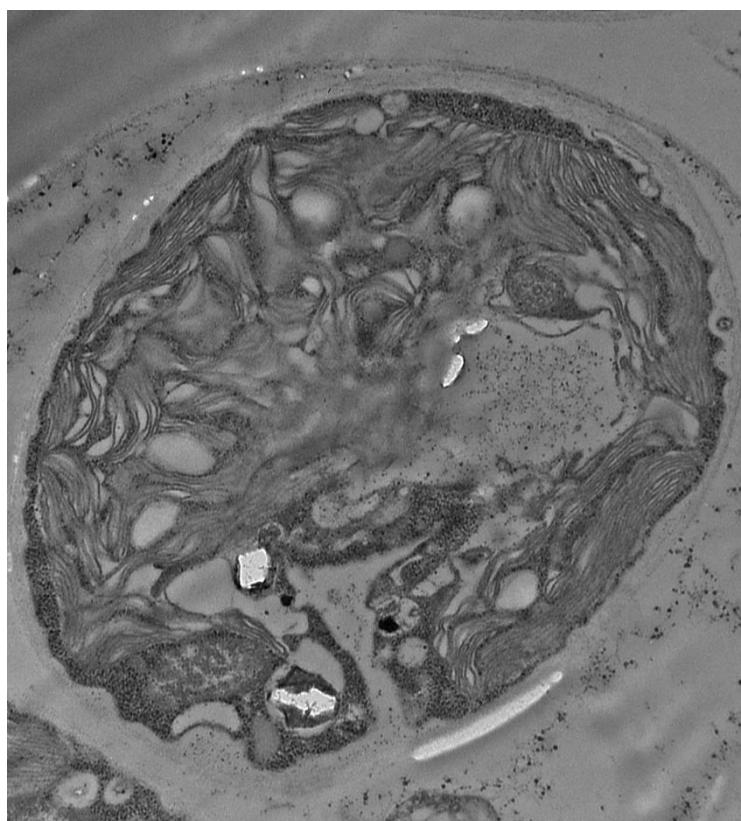


10 mT Images

500 nm



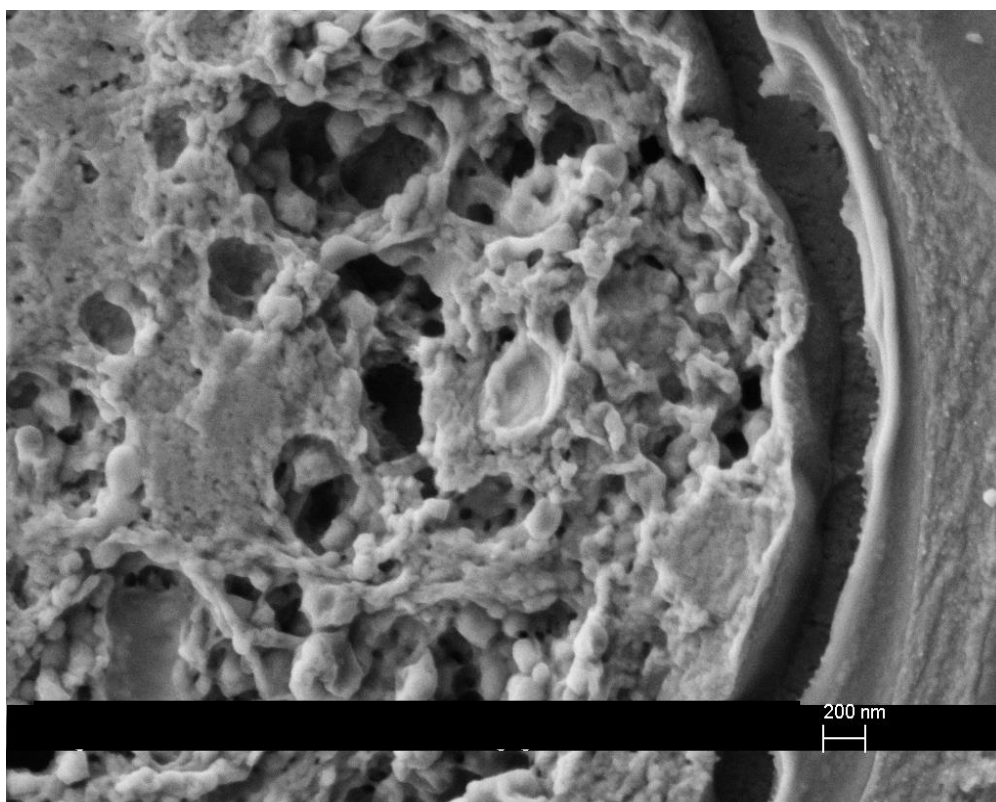
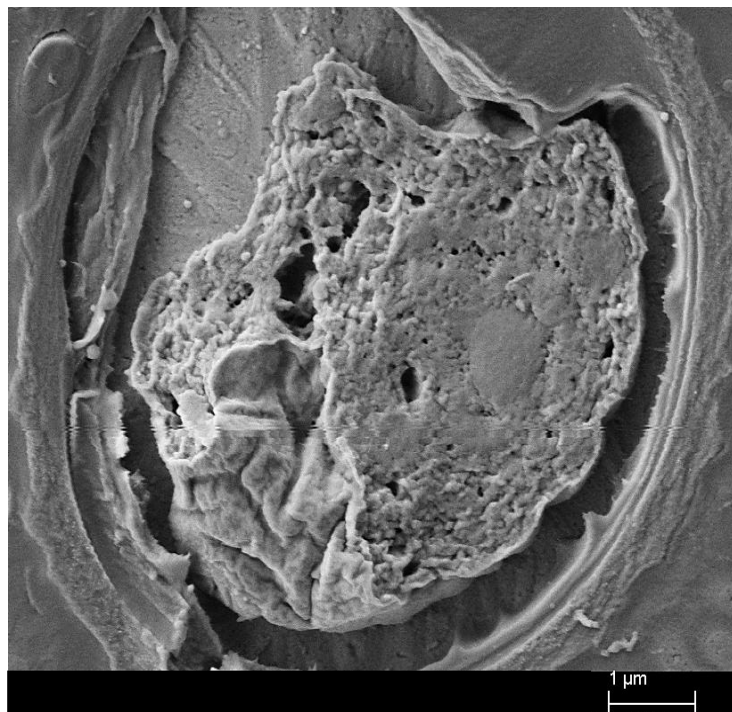
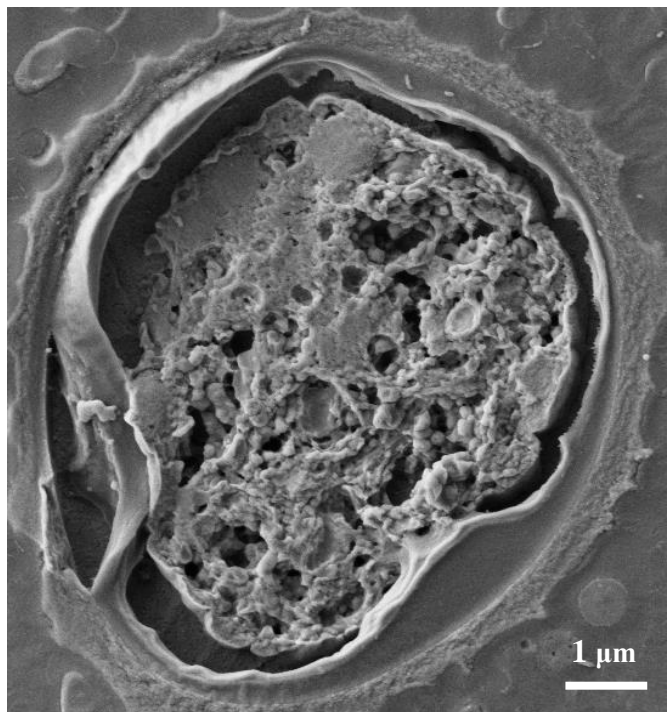
500 nm



500 nm



500 nm

SEM Pictures (10 mT)

SEM EDX Sample Result

Project 1

Spectrum processing :
No peaks omitted

Processing option : All elements analyzed (Normalised)
Number of iterations = 2

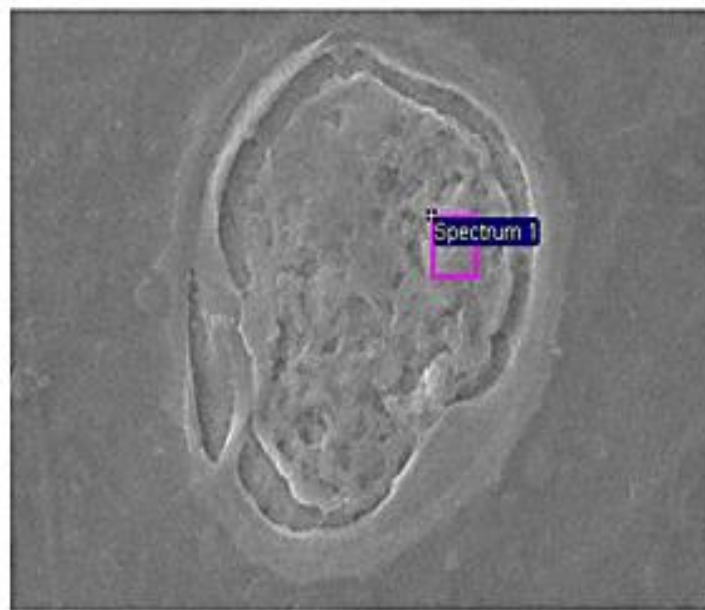
Standard:

C CaCO₃ 1-Jun-1999 12:00 AM

O SiO₂ 1-Jun-1999 12:00 AM

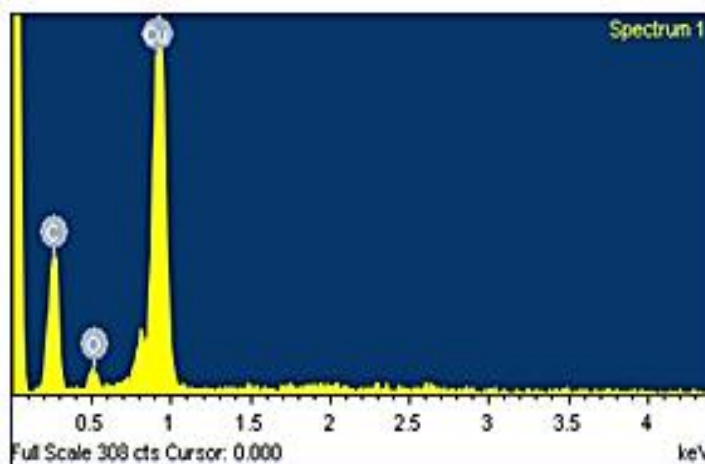
Cu Cu 1-Jun-1999 12:00 AM

Element	Weight %	Atomic %
CK	33.11	70.15
OK	2.57	4.09
Cu L	64.32	25.76
Totals	100.00	



Electron Image 1

7 μ m



INCA

Comment:

Curriculum Vitae

Manuella Oliveira

UNIVERSITY EDUCATIONAL BACKGROUND

- 2014 – 2017 **Master of Engineering Science, M.E.Sc**
 Chemical and Biochemical Engineering
 The University of Western Ontario, London, ON, Canada
- 2010 – 2014 **Bachelor of Engineering Science, B.E.Sc.**
 Chemical Engineering
 The University of Western Ontario, London, ON, Canada

SCHOLARSHIPS, HONOURS AND AWARDS

- Jul. 2016 First Place Presentation Award in Research Competition
 (Research Bridges 2016: Biochemical Group)
- Mar. 2016 Waldemar Bebris Ontario Graduate Scholarship
- Sep. 2015 The Ivan Malek Scholarship in Biochemical Engineering
- Jun. 2015 Graduate Student Teaching Award in Biochemical Engineering
- Jun. 2015 Ontario Graduate Scholarship (OGS)
- Mar. 2014 First Prize Award for Undergraduate Research Project Presentation
- 2013, 2014 NSERC Undergraduate Student Research Award (USRA)
- 2011 - 2013 Dean's Honor List
- Nov. 2012 Tom Ng Engineering Award

CONFERENCE PROCEEDINGS

*Presenter

Oliveira, M.*, Mika, A., Wan, W. (2016). Magnetic Stimulation on the Growth of the Microalga *Nannochloropsis oculata*. Oral presentation at the 2016 Research Bridges Symposium. Sarnia, ON, Canada.

Oliveira, M.*, Mika, A., Wan, W. (2016). Magnetic Stimulation on the Growth of the Microalga *Nannochloropsis oculata*. Poster presentation at the 18th Annual CScE Ontario-Quebec Biotechnology Meeting. Waterloo, ON, Canada.

Mika, A.*, Small, D.P., Oliveira, M.B., Wan, W.K. (2011). Production and characterization of polyhydroxybutyrate (PHB) from cyanobacterium *Spirulina platensis*. Poster presentation at the 61st Canadian Chemical Engineering Conference. London, ON, Canada.

RELATED WORK EXPERIENCE

2015, 2016
(Winter term) Graduate Teaching Assistant
CBE 4403: Biochemical Engineering Processes
Department of Chemical and Biochemical Engineering
The University of Western Ontario, London, ON, Canada

2014, 2015
(Fall term) Graduate Teaching Assistant
CBE 4493: Polymer Engineering
Department of Chemical and Biochemical Engineering
The University of Western Ontario, London, ON, Canada

RELATED EXTRACURRICULAR ACTIVITIES

2015-2016 Director of Communications
Chemical & Biochemical Engineering Graduate Society (CBEGS)
The University of Western Ontario, London, ON, Canada

Oct. 2011 Front Desk Volunteer
61st Canadian Chemical Engineering Conference (CSCChE 2011)
London, ON, Canada

PROFESSIONAL MEMBERSHIPS

2016 Professional Engineers Ontario (PEO)
Engineer in Training (EIT)