THE EFFECT OF LIGHT INTENSITY AND MIXING ON THE EFFICACY OF A NOVEL PHOTOBIOREACTOR

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Declaration

I, William Henry Bedser, declare that this research report is my unaided work. It is being submitted to the Degree of Master of Science in Engineering to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.

(Signature of Candidate)

10 JULY 2021

Date

Abstract

Over the last two decades, there has been increased attempts to reduce reliance on dirty energy sources derived from fossil fuels, and more support for research into, and the development of, alternative energy sources derived from sustainable and reliable energy-rich oils. Photosynthetic algae cultivated in Photobioreactors (PBR) is a viable energy source, which when produced in large enough amounts can provide an alternative to fossil-derived fuels. Harnessing photosynthetic algae's inherent ability to convert light, carbon dioxide, and available nutrients into the water, sugars, oxygen, and energy-rich biomass combined with the treatment of municipal wastewater to an acceptable quality provides an alternative to fossil fuels. This research project aimed to; use a computer model to predict, and then conduct laboratory experiments to test, the extent to which a novel PBR efficiency is improved through enhancing the lighting conditions. For standard lighting conditions, both for the model and experiments, the only source of light to the PBR was overhead lights. The light conditions in the second PBR was enhanced through the use of reflective mirrors that were placed on either side of the growth compartments. Results from the model were compared to data obtained from three sets of laboratory experiments. The test culture used in laboratory studies was *Chlorella Vulgarus*, and each experimental run was carried over seven days with water samples being collected on days zero, one, three, five, and seven. All samples were assayed for pH, total nitrogen, ammonia, nitrate, phosphates, and chlorophyll-a. Total nitrogen, ammonia, nitrate, and phosphate were measured using commercial test kits as per standard methods: 108:14763 (total nitrogen), 54:14752 (ammonia), 139:09713 (nitrate), and 1.10428.0001 (phosphate). Chlorophyll-a was measured using a modified ESS Method 150.1 test. The pH was measured using calibrated pH-meter. On average algal growth, as measured by Chlorophyll-a production, in the novel PBR was substantially lower than the baseline PBR, which was used as a control. This set of results contradicted the prediction of the models. However, between experimental days, the light enhanced PBR's growth rates were significantly higher compared to the control PBR.

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List of Greek Symbols

Symbol		Description	Units
a	:	Fitting constant	
$\alpha_{x,\lambda}$	:	Specific light absorption coefficient	
b	:	Fitting constant	
C_n	:	biomass concentration	g-biomass / m^3
c	:	Fitting constant	
d	:	Fitting constant	
I	:	local irradiance	W/m^2
I_0	:	Incident irradiance	W/m^2
I_{av}	:	Average irradiance in the culture	$W\ m^{-2}$
I_e	:	Average irradiance at the energy compensation point	$\mu Em^{-2}\ s^{-1}$
I_h	:	Incident irradiance at hour h in a day	W/m^2
I_k	:	Microalga affinity for light	$\mu Em^{-2}\ s^{-1}$
K	:	Absorption coefficient	0.2 m^2 / g-biomass
K_i	:	Photoinhibition constant	W/m^2
$K_{i,j}$	:	Photoinhibition constant	$kJ\ cm^{-2}\ h^{-1}$
K_q	:	Dimensionless parameter to set the curve form, $K_q = K_c / (Q_{max} - Q_{min})$	
K_s	:	Saturation constant	W/m^2
l_{local}	:	local irradiance	W/m^2
l_{xy}	:	local irradiance at any location	
M_x	:	Molar mass of microalgae	
m	:	Number of fractions within the PBR	
P	:	Microbial Productivity	g-biomass / day
Q	:	Nutrient cell quota, Carbonn	$g\ g^{-1}$
Q_{max}		Maximum nutrient cell quota for algal existence, g g−1 Carbon	mol nutrient mol^{-1} Carbon
Q_{min}		Minimum nutrient cell quota for algal existence, g g−1 Carbon	mol nutrient mol^{-1} Carbon
S	:	Nutrient Concentration	$Mg\ L^{-1}$
V	:	Volume of the culture	m^3
x^*_e	:	Steady-state fraction of functional activated PSUs under continuous illumination	
α	:	Initial slope of the light response curve	
α'	:	Parameter	$E\ m^{-2}\ s^{-1}$
β	:	Sharpness coefficient	
μ	:	Specific Growth rate	h^{-1}
μ_{av}	:	Average growth rate	day^{-1}
μ_{m1}	:	Maximum value for μ,	day^{-1}
μ_{m2}	:	Specific growth rate at the absence of nutrient in the culture medium	day^{-1}
μ_{m3}	:	Specific growth rate at high nutrient concentration in the culture medium,	day^{-1}
μ_{max}	:	Maximum specific growth rate	h^{-1}

List of Abbreviations

Abbreviation		Description
PBR	:	Photobioreactor
PAR	:	Photosynthetic Active Radiation
TN	:	Total nitrogen
TP	:	Total phosphorus
GHG	:	Greenhouse gas
PE	:	Photosynthetic Efficiency
EG	:	Euglena Gracilis Medium
TM	:	Jaworski's Medium

Chapter 1: Introduction

1.1 Research Background

There is an increased demand for energy-rich oils, such as oils derived from sources other than sugarcane or corn, as potential alternatives to fossil fuels. These alternatives include fuels such as biodiesel, bio-alcohol, and refuse-derived fuels. Biofuels are derived from biological materials that are either living or freshly dead. Feedstock materials used in the production of biofuels include animal fats, vegetable oils, and microalgae. In recent years, microalgae have been identified as an important source of feedstock for biofuels (Slade and Bauen, 2013). Apart from their use as feedstock, algae are also useful as fertilizer, a source of nutrition and even as a replacement for chemical colouring agents in the textile and the food industry.

Photosynthetic algae are responsible for approximately 50% of all photosynthesis on our planet (Lenka, Bartosova and Gerulova', 2015). Algae's inherent ability to convert light, carbon dioxide, and surrounding nutrients into water, sugars and oxygen as they grow can be manipulated where algae are the base product for a variety of applications. The cultivation of algae feedstock can be done through two main processes: raceway pond systems and photobioreactors (Slade and Bauen, 2013). Algae can be cultivated in a photobioreactor where energy is provided through natural sunlight and the nutrients are provided by the municipal sewage. In both setups, algal growth is accompanied by the reduction in contaminant concentrations in the wastewater.

This study focuses on understanding the effects of light and mixing on microalgae photosynthesis in a novel batch photobioreactor.

1.2 Research Motivation

To ensure that future global energy demand is met, it is vital to look for viable and sustainable energy-rich alternatives to fossil fuels. Along with energy security, the protection of water resources through the effective and cost-efficient treatment of wastewater, globally, is paramount. PBRs can be used to both treat wastewater and thus contribute towards the protection of water resources as well as to provide feedstock for energy-rich oils. As a result, there is a need to research into and develop PBRs as a viable alternative solution that is resource neutral.

Large-scale use of PBRs for the treatment of municipal sewage is limited due to the capital and operational costs associated with PBRs. Several growth factors influence the cultivation of algae in a PBR. These factors include but are not limited to light intensity, duration of the light period, mixing, nutrient utilization rates, CO_2 transfer, and environmental conditions. Through the introduction of additional light to a PBR the algae's productivity would increase, this would lead to improved efficiency in the PBR design and ultimately reducing both capital and operational costs.

1.3 Hypothesis

In a photobioreactor, factors that will have the greatest impact on biomass growth are light intensity and nutrient availability, thus the increase of light would be coupled with an increase the biomass production due to nutrient utilization.

1.4 Problem Statement

Several factors affect the growth of microalgae in photobioreactors, and large-scale industrial systems are costly to construct and to operate, therefore uneconomical. Reducing the overall total costs of a photobioreactor by increasing biomass production might make PBR a viable alternative.

1.5 Objective

The objective of this research study was:

- To test the extent to which enhancing the lighting condition in a novel photobioreactor improves the bioreactor's efficiency.

The secondary objective of this research was:

- To compare algae growth rates achieved in a novel PBR to modelled growth rates.

1.6 Assumptions

The following assumptions were made:

- The radiation, used for modelling, in Johannesburg was assumed to similar to the data obtained from the De Aar weather station located in the Free State, South

Africa. The De Aar weather station is 1286 m above sea leave and approximately 635 km from Johannesburg (Appendix 1).

- Nutrient availability never limits growth within the photobioreactor.
- Dispersed radiation was negligible when compared to direct radiation.
- Suspended biomass was considerably larger than attached biomass.
- The model inputs for specific light absorption coefficient ($a_{x,\lambda}$), the molar mass of microalgae (M_x) and maximum specific growth rate (μ_{max}) were obtained from the literature.
- The growth segments were divided into half and only half the growth segment was modelled.
- Light intensity received by the growth segment was only from the overhead lighting and no other light sources were considered.
- Light refraction and reflection were not taken into account.

1.7 Outline of Report Structures

1.7.1 Literature Review

The literature review provides an overview of the urban water cycle, phase of algal growth, models to predict and track algal growth, use of photobioreactors for algal growth, and growth of algal species of *Chlorella Vulgarus.*

1.7.2 Model Development and Prediction

The model development and prediction chapter elaborates on the development of the model, which simulated microalgae growth. The parameters used in modelling the growth of *Chlorella vulgarus* were obtained from literature and assumed experimental parameters that will be used during the laboratory experiments. The model's predictions are presented and discussed.

1.7.3 Laboratory Studies: Methodology

This chapter elaborates on the equipment and methodology that were used to conduct the experiments.

1.7.4 Laboratory Results and Observation

The laboratory results and observation chapter documents the results and observations from laboratory studies.

1.7.5 Discussion and Conclusion

The discussion and conclusion chapter explores the data obtained during the experiments, provides insight and understanding of the differences or similarities and concludes the research.

1.7.6 Recommendations for Future Research

This chapter makes recommendations for possible future research within the study field.

Chapter 2: Literature Review

2.1 Introduction

South Africa's constitution informs that access to basic water and sanitation services are basic human rights (Constitution of the Republic Of South Africa, 1996). For many, this basic human right is realized through the urban water cycle. The urban water cycle is a representation of the average person's interaction with water and sanitation.

The urban water cycle comprises many interlinked components as shown in Figure 1 below. The first component is the abstraction of the raw water often from natural water resources, such as rivers and underground aquifers. The raw water is treated at water treatment facilities to meet prevailing water quality standards. In South Africa, potable water must meet the South African National Standards code SANS-241 Drinking Water Specification (SANS 241-1, 2011). The third component is the distribution of potable water from the treatment plant to places where it is needed. Water that has been used, and therefore contaminated to some extent, is collected by the sewer network and transmitted to wastewater treatment plants (fourth component). The fifth component is the treatment of wastewater at the wastewater treatment works. Effluent from wastewater treatment plants is usually discharged into environmental waters, hence closing the urban water cycle. In South Africa, the standard of effluent discharge is governed by the National Water Act, 1998.

In 2015, The World Health Organization estimated that only 2.9 Billion people had access to properly managed sanitation services while as recent as 2018, "2.3 Billion people still did not have access to basic sanitation facilities such as toilets or latrines" (WHO, 2018). The same WHO report concluded, "Poor sanitation continues to contribute towards the transmission of diseases such as cholera, dysentery, hepatitis A, typhoid, and polio". In South Africa, access to sanitation has improved over time with the 2016 census indicating that 60.6% of South Africans have access to flush toilets connected to a public sewerage system. This shows a sharp decrease in the number of South Africans who use bucket toilets or who have no access to sanitation services (Statistician-General, 2016). As the access to sanitation improves there needs to be an equal improvement in South Africa's ability to treat wastewater to discharge standards in order not to protect the environment from the effects of poorly treated wastewater.

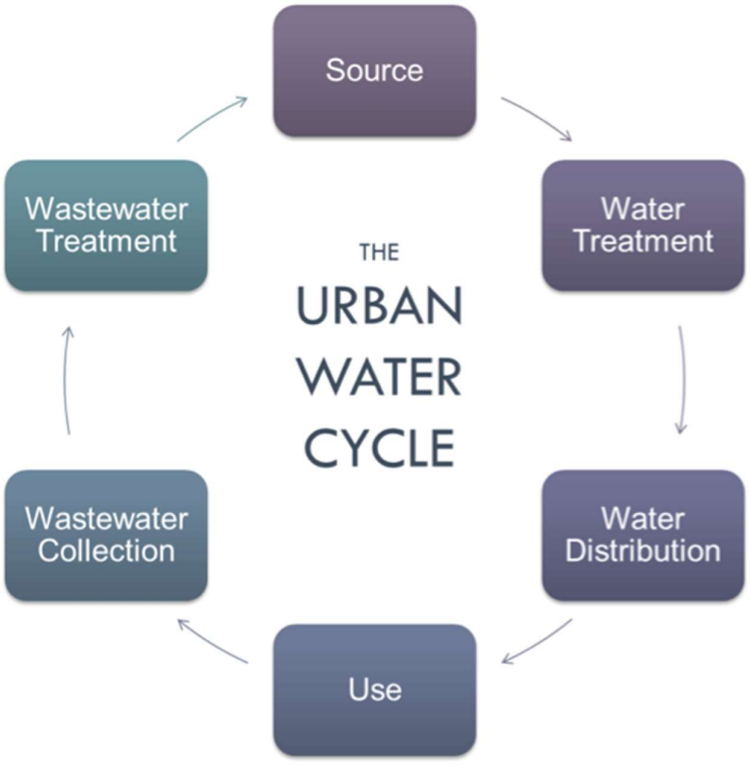


FIGURE 1: URBAN WATER CYCLE (SOWBY, 2014)

Since the early 1900's the collection and treatment of wastewater has been central to the protection of environmental waters. There was little consideration given to monetary resources, energy resources, and the biological constituents of the wastewater. The treatment of wastewater was seen as a burden and the costs were passed onto the producers of the wastewater. This caused the conventional wastewater treatment process to be resource-intensive. Due to the increased production of wastewater and resource limitations, there is a need to find alternative treatment processes that can mimic ecosystem processes and are less resource intense. Bio-fibre media reactors using suspended plant roots and photobioreactors that utilizes suspended algae growth are some of the possible alternatives (Engineering News, 2018).

Algae has been around for billions of years and is responsible for the majority of carbon dioxide conversion into oxygen and organic carbon. Algae has been cultivated for a variety of reasons, for example, food production, for many years. Algae is an ideal alternative fuel source to fossil fuel because it has a unique ability to be cultivated in difficult environmental conditions and its biomass can be converted into energy-rich oil.

2.2 Wastewater Treatment

Wastewater Treatment in South Africa dates back to the late 1800s with the first sewage purification plant in Wynberg, Western Cape, being designed in 1898. Between 1898 and January 1905, when this plant became operational, South Africa's first treatment plant was commissioned in Bloemfontein in November 1904 (Osborn, 1988). In Johannesburg, the first wastewater treatment works constructed was the Klipspruit Sewage Purification Works, which was commissioned on 1 November 1907. (Biggs, 1973).

According to the Department of Water and Human Settlements: Green Drop Certification of 2013, there were 824 wastewater treatment works in use within South Africa in 2013. The majority of these treatment works have a treatment capacity of less than 1 Ml/day (DWS, 2014). Oxidation ponds and trickling filters are the preferred treatment processes for small treatment works while the activated sludge process is the preferred treatment for larger treatment works.

The poor and inadequate treatment of municipal wastewater is considered a major threat to the water resources of South Africa. In 2013, 30.1% of all systems were considered to be in a critical risk category: indicating a score of between 0 to 30% for the Green Drop Certification (DWS, 2014). The target category for certification is to obtain a score of between 90 to 100 % that reflects an excellent score. It is difficult to ascertain if these statistics have improved since 2013 as the Department of Water and Sanitation has not commissioned the green drop assessments since 2014 (WISA, 2020). However, an independent study completed by Afriforum in 2020 concluded that from their sample of 118 wastewater treatment works, 90 works did not compile with national standards (Afriforum, 2020).

The year 2018 saw the commissioning of South Africa's first ecosystem-driven treatment plant (Engineering News, 2018). This system uses plants and bio-fibre media with suspended root systems submerged in the treatment basins. The process claims to reduce the sludge and energy requirements by approximately 30% when compared to the activated sludge treatment process (Engineering News, 2018).

In South Africa, the discharge of wastewater effluent into a water resource is governed by section 39 of the National Water Act, 1998 (Act No. 36 of 1998) and in particular section two of the Schedule to Government Notice No. 665 of 6 September 2014. Table 1 below, shows the water quality parameters that are regulated and their general limits. The relevance of these parameters to this study is indicated in the last column of the table.

TABLE 1: WASTEWATER LIMIT VALUES APPLICABLE TO DISCHARGE OF WASTEWATER INTO A WATER RESOURCE (NATIONAL WATER ACT 36 OF 1998, 2013)

Substance/Parameter	General Limit	Relevance to Study
Faecal Coliforms (per 100 ml)	1000	No
Chemical Oxygen Demand (mg/I)	75	No
pH	5.5 - 9.5	Yes
Ammonia (ionised and un-ionised) as Nitrogen (mg/I)	6	Yes
Nitrate/Nitrite as Nitrogen (mg/I)	15	Yes
Chlorine as Free Chlorine (mg/I)	0.25	No
Suspended Solids (mg/I)	25	No
Electrical Conductivity (mS/m)	70	No
Ortho-Phosphate as phosphorous (mg/I)	10	Yes
Fluoride (mg/I)	1	No
Soap, oil or grease (mg/I)	2.5	No
Dissolved Arsenic (mg/I)	0.2	No
Dissolved Cadmium (mg/I)	0.005	No
Dissolved Chromium (VI) (mg/I)	0.05	No
Dissolved Copper (mg/I)	0.01	No
Dissolved Cyanide (mg/I)	0.02	No
Dissolved Iron (mg/I)	0.3	No
Dissolved Lead (mg/I)	0.01	No
Dissolved Manganese (mg/I)	0.1	No
Mercury and its compounds (mg/I)	0.005	No
Dissolved Selenium (mg/I)	0.02	No
Dissolved Zinc (mg/I)	0.1	No
Boron (mg/I)	1	No

2.3 Algae

From giant seaweed, macro-algae, to small single-cell organisms, microalgae, algae has a wide range of sizes and forms. Algae is defined as a unicellular or multicellular, autotrophic, photosynthetic eukaryotes or prokaryotes (Abu-Orf et al., 2014). The majority of algae can be defined as photosynthetic, oxygen-producing aquatic bacteria, or protists (Graham, Wilcox and Graham, 2009, chap. 1). Cell multiplication in a unicellular organism is considered growth wherein a multicellular organism growth could be due to the increase in the size of the individual organisms.

Algae can be grouped into ten large divisions; namely cyanobacteria, glaucophytes, chlorarachniophytes, euglenoids, cryptomonads, haptophytes, dinoflagellates, photosynthetic stramenopiles, red algae and green algae (Graham, Wilcox and Graham, 2009, chap. 1). It is estimated that there are between 200,000 to 800,000 species of algae within these ten groups (*Algae - Types Of Algae - Various, Pigments, Divisions, and Chlorophylls - JRank Articles*, no date). The majority of industrial systems use only a small sample of species in their process and green algae are primarily uses instead of either two cyanobacteria or one red algal species (Randrianarison and Ashraf, 2017).

Tiwari, 2018 stated that there are four determining factors that should be considered when selecting an algal strain for the production of biofuels, these include oil content, production yield, downstream processing, and the microalgae's ability to adapt to environmental changes (Tiwari and Kiran, 2018). The cultivation of algae biomass from municipal wastewater must also consider the natural habitats in which the algal strain will be cultivated and thus freshwater algae is the only viable algae to be considered. Table 2 below illustrates the lipid content in g lipid per 100 g dry algae. Although *Chlorella* does not contain the highest liquid content, it can be cultivated in freshwater and thus suitable for wastewater treatment.

TABLE 2: LIPID CONTENT PRESENT IN MICROALGAE (DWIVEDI AND DWIVEDI, 2019)

Algal strain	**Habitat**	**Lipid content in g lipid per 100 g dry algae**
Chlorella sp.	Freshwater	28-32
Amphidimium carteri	Marine	>20
Tetraselmis	Marine	15-23
Dunaliella primolecta	Marine	23
Isochrysis sp.	Marine	25-33
!alassiosira pseudonana	Marine	>30
Nannochlorosis sp.	Marine	31-68
Porphyridium cruenturn	Marine	>40
Schizochytrium sp.	Marine	50-77
Monallanthus salina	-	>20
Phaeodactylum	-	20-30
Neochloris oleoabundans	-	35-54
Botryococcus braunii	-	25-75

2.3.1 Chlorella Vulgarus

Chlorella Vulgarus was used for all experimental work in this study. C*hlorella vulgarus* is a common fast-growing species of green alga that occurs as small spherical unicells and reproduces by autospores (Graham, Wilcox and Graham, 2009, chap. 18). It has been cultivated for both human and animal nutrition since 1970. *Chlorella vulgarus* can grow both autotrophically and heterotrophically (El-Sheekh and Fathy, 2009). In 2005 it was estimated that the annual production of *Chlorella* was 2000 Tonnes (Spolaore *et al.*, 2006). Table 3 below shows *Chlorella's* classification.

TABLE 3: CLASSIFICATION OF CHLORELLA VULGARIS (LENKA, BARTOSOVA AND GERULOVA', 2015)

Microalgae	Chlorella vulgaris Beyerink
Empire	Eukaryota
Kingdom	Plantae
Phylum	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	Chlorella
Type species	This is the type species (lectotype of the genus Chlorella
General Environment	This is a freshwater/terrestrial species

Chlorella Vulgaris has great potential as a resource for biodiesel production due to faster growth and easier cultivation (Lenka, Bartosova and Gerulova', 2015). Venkataraman established that *Chlorella vulgarus* has a constant overall rate of increase of between 2.5 and 2.9 when the temperature is 25 °C (Venkataraman, 1969, chap. 10).

The efficiency of *Chlorella Vulgaris* in reducing nutrients in wastewater is well documented with maximum removal rates recorded for total nitrogen, phosphorus, and COD of 84%, 95%, and 36% respectively (Choi and Lee, 2012). In addition to the nutrient removal abilities of *Chlorella*, *Chlorella* has an oil content of between 28 – 32 % dry wt. (Gouveia *et al.*, 2017). The ability of *Chlorella* to remove nutrients efficiently and its oil content makes it a good single culture alga for experimental research.

2.3.2 Algae Growth Phases

Algae has five growth phases, namely: lag or induction phase, exponential phase, declining relative growth phase, stationary phase, and the death phase as shown in Figure 2 below. During the lag phase, there is minimal net growth within the culture as the algae begin to adapt to the conditions within the culture (Lavens and Sorgeloos, 1996). The delay in growth can also be attributed to the non-viable cells being present and the viable cells' adjustment to the new environmental conditions (Lee, Jalalizadeh and Zhang, 2015).

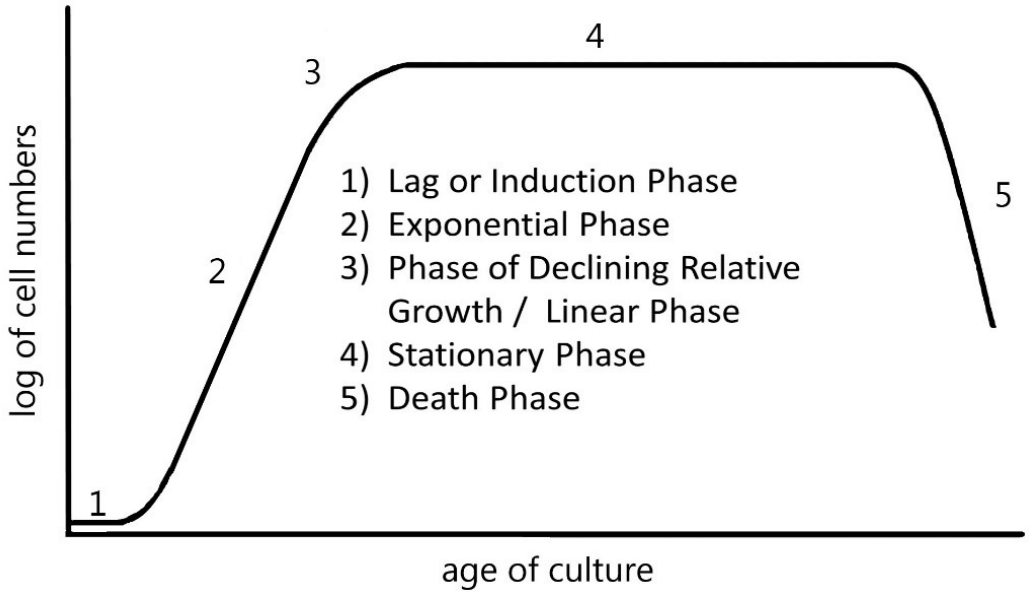


FIGURE 2: FIVE GROWTH PHASES OF MICROALGAE CULTURES (SVALDENIS, 2014, P. 9)

The Exponential phase is when the growth of the algae is according to a logarithmic function as shown below, where C is the concentration of cells, and m is the specific growth rate. Light intensity and temperature are key factors that influence the specific growth rate of algal species (Lavens and Sorgeloos, 1996, chap. 2). The definition of Generation Time is the time taken for a population to double in size. Given that for each time step, the population is doubling in size at a constant growth rate, the culture has entered into the exponential phase.

$$C_t = C_o e^{mt} \quad \ldots (1)$$

The Phase of Declining Relative Growth or Linear phase is when the growth is limited as the cell division slows down due to the limited nutrients, light, pH, carbon dioxide (Lavens and Sorgeloos, 1996, chap. 2). The concentration of algae within the culture has become dense causing the reduction of the light intensity available to individual cells.

The Stationary phase is when there is limited cell division due to a lack of growth sustaining nutrients and the cell numbers remain constant.

The final stage is the Death phase where the depletion of nutrients and a decline of water quality result in net cell death (Lavens and Sorgeloos, 1996, chap. 2). Factors that contribute to the death phase include overheating, pH disruption, the presents of impurities, or dwindling nutrients. (Lee, Jalalizadeh and Zhang, 2015).

2.3.3 Algae's Nutrient Requirements

Algae, like all living organisms, can only tolerate specific ranges of physical (e.g. temperature) and chemical (e.g. nutrient) environmental conditions. The principal nutrients needed for algal growth include carbon, phosphorus, nitrogen, sulphur, potassium, and magnesium (Venkataraman, 1969). Algae use nitrogen, in the form of nitrates, ammonia, or organic sources of nitrogen for general metabolism. The link between algae's digestion of nitrates and ammonia is related to the pH of the growth medium with some species, such as Chlorella, being able to drastically reduce the pH to levels as low as pH 3.0 (Venkataraman, 1969). Blair et al. (2014) suggest that there is a direct proportion between algae growth and the concentration of nitrogen in the medium. (Blair, Kokabian and Gude, 2014). Through the monitoring of pH and nitrogen during the experiments will provide insight into growth within the culture.

Phosphorus concentrations have a great effect on algae growth. The ratio of nitrogen to phosphorus needs to be approximately 30/1 to achieve optimal growth for most algal species (Venkataraman, 1969).

2.3.4 Utilization of Algae

Algal biomass has been cultivated for many uses. In the past, algal biomass has been used as food, food additives, animal feed, and pigments. As the global population increase with expanding economies, Slade and Bauen (2013) suggest that there is a great potential for microalgae to be a viable feedstock for biofuel production as they produce useful quantities of sugars and fats (Slade and Bauen, 2013). The added benefit associated with the cultivation of algae is the cleaning of the environment through the CO_2 conversions as it is estimated that 100,000 kg of dry algal biomass utilizes about 1,830,000 kg of CO_2 in its growth (Gouveia *et al.*, 2017).

Utilization of Algae for Food

The United Nations estimates that currently 800 million people are regularly undernourished and this number will increase to between two and three billion people by 2050. The main factor attributed to global diseases is the lack of access to nutritious food by large populations. This lack of nutritious food can lead to early death, mental retardation, and blindness. (Moomaw, Berzin and Tzachor, 2017). The Food and Agriculture Organization of the United Nations cites its greatest concern is the increased consumption of non-seasonal food, such as meat, fish, and dairy, by nations that are changing dietary habits. A sustainable means to produce proteins is required to meet the needs of current and future global populations. (Moomaw, Berzin and Tzachor, 2017). The provision of nutritious food to meet the demand for the current and future global population needs to be done through sustainable means.

Figure 3 below represents the estimated yield of Essential Amino Acids and their required water and land use. For example, to produce 1 kg of beef 148,007 litres of water and 80 ha of fertile land are required, where the same mass of Nannochloropsis, also known as Nanno, can be produced using only 20 litres of water on 6.171 ha of non-fertile land. (Moomaw, Berzin and Tzachor, 2017).

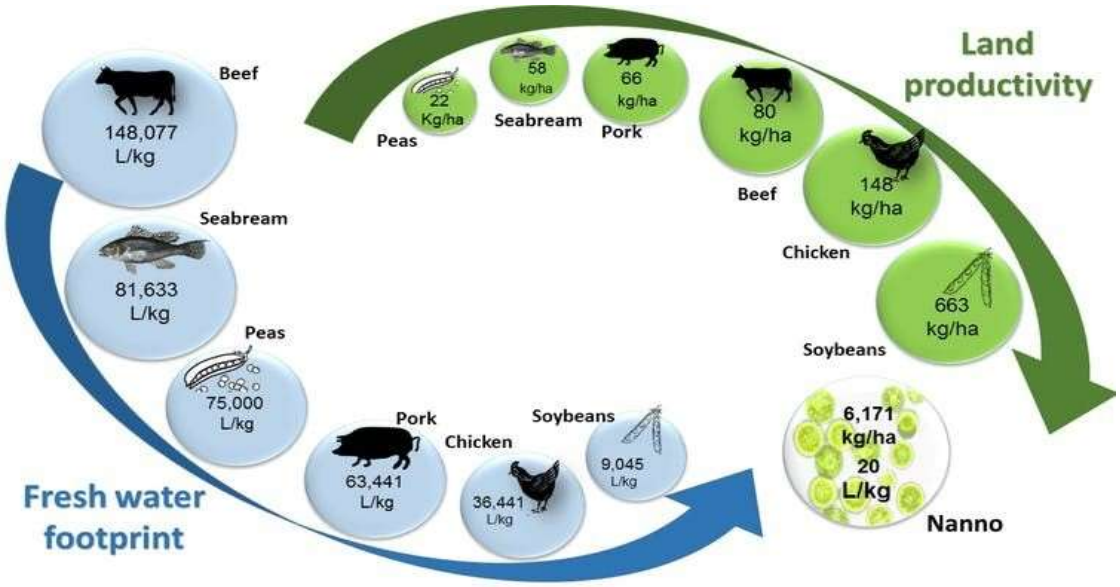


FIGURE 3: LAND AND WATER REQUIREMENTS FOR THE PRODUCTION OF ESSENTIAL AMINO ACIDS FROM VARIOUS SOURCES (MOOMAW, BERZIN AND TZACHOR, 2017)

Nannochloropsis is a marine unicellular microalgae species that have high photosynthetic efficiency (Ma *et al.*, 2016). However, Moomaw and colleagues state that for microalgae to become a viable alternative it is essential that cultivation, processing, education, and regulatory barriers are addressed (Moomaw, Berzin and Tzachor, 2017). Approximately between 5000 to 10000 tons of algae biomass is produced worldwide, mainly for high-value, low-volume food supplements, and nutraceuticals (Pienkos and Darzins, 2009).

Utilization of Algae in the production of Biofuel

Potential alternatives to fossil fuels derived from energy-rich oils, such as biodiesel, bio-alcohol and refuses-derived fuels, have recently seen an increase in demand. Biofuels are derived from biological materials, which can be either living or freshly dead. Feedstock materials used in the production of biofuels include animal fats, vegetable oils, and microalgae. In recent years, microalgae have been identified as an important source of feedstock for biofuels (Slade and Bauen, 2013).

Hannon et al. (2010) identified the following benefits of using microalgae as a feedstock for biofuels including (Hannon *et al*>, 2010);

- Algae grow in a wide range of aquatic environments.
- Algae is extremely efficient in its use of $C0_2$.

- All algae species can produce energy-rich oil from their biomass.
- Single-celled algae can duplicate through division, which provides a large advantage over terrestrial plants.
- Algae can be grown on non-fertile land that has no agricultural value and thus does not compete with food production; and
- Nutrient streams for algae production include municipal wastewater, flue gas of coal, or nutrient-rich fertilizer runoff from farming activities.

Although there are many advantages of using algae as a feedstock, the current challenges still plague its utilization. Several researchers have identified the following challenges (Ramírez-mérida, Zepka and Jacob-lopes, 2015), (Pienkos and Darzins, 2009):

- The commercial viability of a large-scale biofuel plant has not been tested as the technology is considered relatively new; and
- Further engineering research into the downstream cost-effective algae harvesting technology.

Table 4 below illustrates the area requirements to cultivate a variety of crops with the ability to produce 50% of the United States' biodiesel needs. Using corn as an example, 1540 M ha of land will be required to produce 50% of the U.S. fuel needs. In comparison, microalgae will require 2 to 4.5 M ha depending on the algae's oil content. Microalgae has a high oil-to-land ratio that makes them a realistic competitor as an alternative source of energy to fossil fuels. (Gouveia *et al.*, 2017).

TABLE 4: COMPARISON OF SOME SOURCES OF BIODIESEL (GOUVEIA *ET AL.*, 2017)

Crop	***Oil yield (L / ha)***	***Land Area needed (M ha)*** [a]	***Per cent of existing US cropping area*** [a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil Palm	5950	45	24
Microalgae (70% oil (by wt) in biomass)	136,900	2	1.1
Microalgae (30% oil (by wt) in biomass)	58,700	4.5	2.5

[a] *For meeting 50% of all transport fuel needs of the United States.*

2.4 Photobioreactors

The purpose of a bioreactor is to maintain and support adequate levels of bioactivity that mimics natural systems. A bioreactor is classified as a PBR when the biological reactor integrates a light source in its process (Us *et al.*, 2018). A photobioreactor is generally defined as a closed (or mostly closed) vessel for phototrophic production where energy is supplied via electric lights (Andersen, 2005). With greater photosynthetic efficiency and biomass concentrations, lower levels of water loss and contamination, and the ability to control environmental conditions, PBRs has some benefits over open-air systems (Huang *et al.*, 2017).

There are currently two major types of PBR's; open ponds and closed systems. Open ponds utilize shallow artificial lagoons or oxidation ponds similar to the conventional systems in wastewater treatment. Open ponds are usually not sterile as they are exposed to the atmosphere nor are they monoculture. Al-hadabi and Al-balushi (2012) proposed that almost all commercial producers use open ponds where the algae culture is mixed either by paddle wheels (raceway ponds) or by a centrally pivoted rotating arm (Al-hadabi and Al-balushi, 2012). The length to width ratio is an important parameter when designing an open pond (Gupta, Lee and Choi, 2015).

Close system PBRs are usually used for the cultivation of monoculture algae in sterile environments. The advantages that closed systems have over open pond systems include much higher productivity and a lower water loss due to evaporation (Posten, 2009). Close system PBRs can be configured in many geometric arrangements, for example, a bubble column, airlift, flat-plate, stirred tank, tubular, conical, torus and seaweed types. However, due to the capital and operational cost associated with these reactors, large-scale use of this treatment system is still to be proven as a viable option.

Open pond systems have some advantage over close systems, as they are easier to construct and operate. Table 5 below illustrates the advantages and limitations associated with different cultivation systems along with how mixing, temperature, and gas exchanges are controlled. Huang suggests that some of the advantages of a PBR include: higher photosynthetic efficiency, higher concentration and areal productive, low contamination, the prevention of water loss caused by evaporation and a precisely controlled environment (Huang *et al*>, 2017).

TABLE 5: THE MAJOR ADVANTAGES AND LIMITATIONS OF THE ALGAE CULTIVATION SYSTEM (GUPTA, LEE AND CHOI, 2015)

Cultivation Systems	Surface /Volume	Mixing	Temp. Control	Gas exchange	Advantages	Limitations
Open Ponds	High	Paddle Wheel	None	Poor, only achieved through surface aeration	• Cost-Effective • Simple and flexible design • Beneficial for mass cultivation	• Lower biomass productivity • Less control over culturing condition • Susceptible to contamination, occupy large land space and lower mass transfer Water and CO2 loss due to evaporation
Stirred Tank PBR	Low	Mechanical agitator	Heat Exchanger	Injection through sparger	• Good heat and mass transfer • Good light dispersion • Lower contamination issues • Simple design Moderate biomass productivity	• Low surface to volume ratio. • Heating issue due to agitation • Mechanical agitation requires extra energy, expensive, not scalable
Vertical column PBR	Low	Airlift/ bubble	-	Open gas exchange at headspace	• High mass transfer • No internal structure • Lack of moving parts • Good mixing with low shear stress • Lower photoinhibition	• Low surface area for illumination. • Expensive construction material • Limited scale up due to design constraints, shading effect issues
Horizontal tubular PBR	High	Re-circulation via pumps	Shading, over-lapping, water spraying	Injection into feed, dedicated degassing unit	• High surface to volume ratio • Low hydrodynamic stress • Suitable for outdoor cultivation, • Good biomass productivity • Cost-effective • The low mutual shading effect	• Dissolve Oxygen build up. Susceptible to photoinhibition • Fouling due to algal growth • Large space requirement • Poor temperature regulation
Flat-panel PBR	High	Airlift/ bubble from bottoms or side	Heat exchange coils	Open gas exchange at headspace	• High surface to volume ratio • Low space requirement • High photosynthetic efficiency, cheap and economic, low oxygen build-up	• Short light penetration depth. • Not scalable, requires many components, frequent fouling and clean up issues • Poor temperature regulation

Additional advantages of using algae for wastewater treatment include a reduction in the energy cost as the oxygen requirement of the bacteria is provided by the algae, a reduction in the quantities of hazardous solids sludge with possible heavy metals, a reduction in greenhouse gases, and the reduced cost and production of useful algae biomass (Gouveia *et al.*, 2016).

Flat-panel PBRs' have a high surface to volume ratio, which often results in high photosynthetic efficiencies. Generally, the flat panel PBR are either mixed using bubbling air or a pump, which keeps the algae cells in suspension. However, flat panel PBRs might be suitable for research but they have their limitation when it comes to scalability as highlighted in Table 5 (Gupta, Lee and Choi, 2015).

Some considerations need to be taken into account during the design of PBR's including. Light distribution, hydrodynamics, mass transfer, and growth kinetics, are closely interrelated to achieve high mass transfer rates and thus a high biomass growth (Huang *et al.*, 2017).

2.5 Factors that affect microalgae growth and effectiveness of photobioreactors

The rate of growth of microalgae cultivated in PBRs depends on several environmental conditions. These include light, mixing, CO_2 transfer, temperature, pH, and the availability of growth fuelling substrates and other nutrients.

2.5.1 Light

Light is one of the basic and most important requirements for photosynthesis-driven growth and this is no different for microalgae. Photosynthesis is a process where light energy, usually from the sun, is converted to chemical energy. Svaldenis stated the growth of algae that follows a pathway as follows (Svaldenis, 2014):

$$Water + carbon\ dioxide + light\ energy = glucose + oxygen + water \quad \ldots (2)$$

Sunlight is the main source of energy for plants and microalgae. (Carvalho *et al.*, 2011). The more microalgae are exposed to light, the faster they tend to grow, however, with overexposure to light the microalgae's photosynthesis activity can decline due to photoinhibition. The biological stress experienced by all photosynthetic organisms when they are exposed to excessive light is defined as Photoinhibition (Adir *et al.*, 2003). Oxygen evolving photosynthetic organisms is the process of generating oxygen through a chemical process in water and is the main process for generating oxygen in the earth's atmosphere.

Not only is the reproduction of algae in a PBR considered a green energy source, but it also can reduce the carbon dioxide level in the atmosphere. During photosynthesis, for every mole of carbon dioxide consumed a mole of oxygen is produced (Svaldenis, 2014). Algal biomass productivity can be calculated by dividing the total biomass obtained by the total volume of algae.

The cell density and the geometric design of the PBR influence light intensity and net growth rate. The denser the cell culture, the lesser the extent to which light can penetrate the culture resulting in reduced growth and productivity. This effect is referred to as the shading effect. The geometric design of PBR with shallower and vertically thinner cultures will minimize the effects of self-shading in dense cell cultures.

Chlorophyll is a photoreceptor with a very high extinction coefficient that specifically absorbs energy from sunlight. The green colour of chlorophyll is what gives algae its green appearance. Chlorophyll is made up of six different molecules (A, B, C, D, E and F) where Chlorophyll-a is found in each photosynthetic organisms and is the primary molecule responsible for photosynthesis (Fondriest, 2016).

The optimal light enhancement to the novel PBR is essential because there is a considerable drop in the transmission of light intensity through the culture because of chlorophyll's high absorption (Andersen, 2005, chap. 13). The geometric design of the PBR should take into consideration light- path lengths and ensure they remain short because as the concentration of the cell becomes dense, light absorption only takes place in the initial cell layers while the other cells remain in the dark.

The point at which light energy is required to result in net growth is called the compensation point (Andersen, 2005). The light saturation point can be defined as the point where the increase in light intensity does not increase the photosynthesis rate (Lopez, 2018). If excess light is provided to the culture, greater than the light saturation point, it will lead to photoinhibition.

The five growth phases of microalgae shown in Figure 2 correlate with the typical growth vs. light intensity curve for microalgae cultures shown in Figure 4 where I_s is light saturation and I_d is incident death. The limits of light exposure have been further established. The photo-synthetically active radiation received from solar irradiation should be within the range of 400 – 700 nm (Huang *et al.*, 2017). All these design parameters need to be considered to achieve high nutrient conversion and high-density growth.

It is misleading to believe that the more light exposure the algae receives, the more the algae will grow. As shown in Figure 4 below, there is a linear correlation between the algae's growth rate with increasing light intensity within the first two groups. When the growth reaches the light saturation point, I_s, there is no longer a linear relation and any additional light intensity will not result in additional growth. In mid-latitude regions, most algae species can adapt and grow with a small proportion of the full day's light (Posten, 2009).

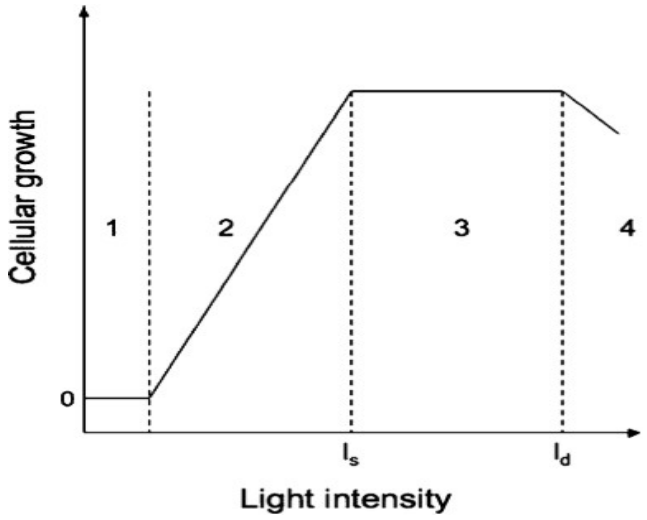


FIGURE 4: TYPICAL GROWTH VS. LIGHT INTENSITY CURVE FOR MICROALGA CULTURES (CARVALHO *ET AL.*, 2011)

It is not only the light intensity that is an important factor for the growth of microalgae but also the light/dark cycles that the cells are exposed to that will have a strong effect on algae growth. (Posten, 2009). The duration of the cycles has an impact on the growth rates of the biomass, as microalgae occur naturally it would be assumed that these dark periods should be similar to night but dark periods of several hours generally result in biomass loss (Carvalho *et al.*, 2011).

Flat plate PBRs are generally more efficient in sunlight utilization than tubular PBRs because they have a wider surface area (Gupta, Lee and Choi, 2015). This can be seen by the reduction in the diameter of tubular reactors from 10-30 cm in 1986 to tube diameters of <4 cm used in modern reactors. The smaller the diameter of the tubular reactor the shorter the path the light has to travel before it reacts with a cell and thus this increases the efficacy of the PBR. An example of this is the ability of flat panel bioreactor to produce 64 ton/ha of algae with a photosynthetic efficiency (PE) of 5% compared with the open pond method and tubular PBRs that can only produce 21 and 41 ton/ha with PE of 1.5 and 3 % respectively (Gupta, Lee and Choi, 2015). Photosynthetic efficiency is defined as the fraction of light energy (W/m^2) which, is converted into chemical energy, biomass ($\mu mol/m^2s$), during the photosynthesis in the algae. Photosynthetic processes occur over three distinct time frames namely primary photochemistry, electron shuttling and carbon metabolism (Carvalho *et al.*, 2011).

2.5.2 Photoinhibition

Photoinhibition in a PBR can cause great complications, algal death and is described as the decline in photosynthetic viability of oxygen-evolving photosynthetic organisms due to excessive illumination (Adir *et al.*, 2003). Figure 5 below reflects the typical photosynthesis vs irradiance curves for algal cells with three main areas shown in the graph where I_c is compensation light intensity and I_h is the point that denotes photoinhibitions. The first region is where the microalgae begin to grow; the second region is where the irradiance causes an increase in the photosynthesis process until it reaches its maximum photosynthetic rate (P_{max}). All excess light received will be dissipated either by heat or by fluorescence. The third region is after the photosynthesis process capacity reaches P_{max}, any additional light radiations received causes photoinhibition and photo-damage to the microalgae is the point of photoinhibition (Carvalho *et al.*, 2011).

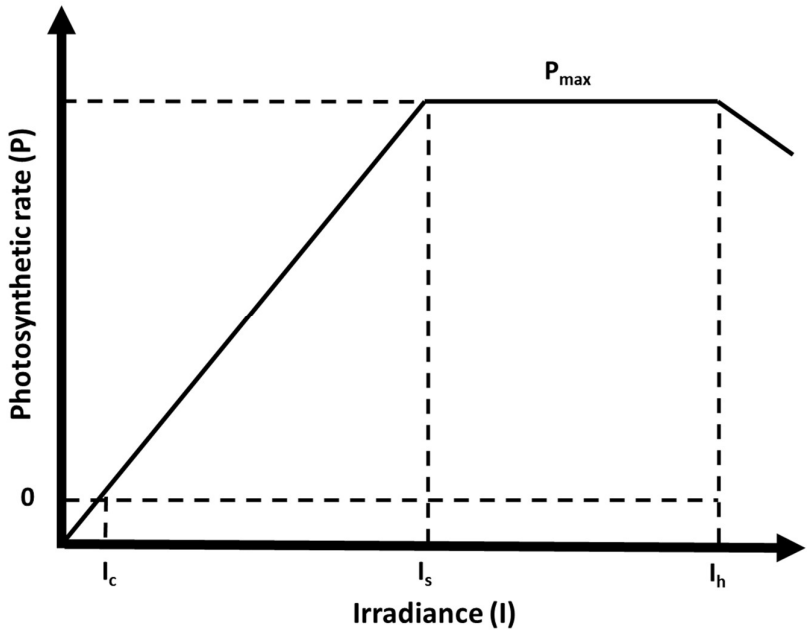


FIGURE 5: TYPICAL PHOTOSYNTHESIS (P) VS IRRADIANCE (I) CURVE FOR MICROALGAE CELLS (CARVALHO *ET AL.*, 2011)

2.5.3 Mixing

The inadequate mixing of an algae culture could lead to cells clumping together, attaching to the walls, and settling to the bottom of the reactor. Adequate mixing enables the equal distribution of nutrients and algae to aid mass transfer throughout the culture and ensures each cell is equally exposed to light (Huang *et al.*, 2017). The introduction of mixing will ensure that both the nutrients and the microalgae are in constant contact to optimize mass transfer. The force of the mixing is important as a too vigorous mixing of the tank could cause harm to the micro-algae.

Huang et al. (2017) argued that overmixing is also detrimental to the cell as it can cause damage, therefore it is recommended that for the mixing of a photobioreactor, the liquid velocities should be between 20–50 $cm \cdot s^{-1}$ (Huang *et al.*, 2017). Posten (2009) states that cells could potentially be damaged if the high velocities are high enough to cause micro eddies (Posten, 2009). The mixing intensity for the model should be included as design parameters to ensure that there are acceptable mass transfer and light/dark cycles.

2.6 Kinetic Modelling of Algae Growth

The algae growth can be predicted using various models, which are based either on a single substrate factor, light intensity factor or other factors. The accurate prediction of algal growth is key for both the upscaling of novel photobioreactors into full-scale process units able to be environmentally sustainable and commercially viable in the production of biomass for biofuel, food and pigment. The factors include models based on the light intensity, substrate depletions i.e. limiting substrate, or models that take into account multiple growth factors.

Table 6 below lists the models' sources, the algae species used and considerations made during the formulation of the models (Lee, Jalalizadeh and Zhang, 2015). Models initially took N, P and light as the factors that influence cell metabolism in natural environments, however, later models considered light factors and CO_2 as more important (Lee, Jalalizadeh and Zhang, 2015).

2.6.1 Models taking into account Light Intensity

The sufficient provision of light will affect the photosynthetic activity of photoautotrophic algae such as *Chlorella vulgarus*, under nutrient saturation. For algae to meet its maximum growth rate, it needs to reach its saturated light level. Light levels below or above the saturated light level can cause either light-limitation or photoinhibitions.

Models based on light intensity can be divided into three groups. The first light-limitation group of models consider assuming algae exist as individual cells. The second group considers light attenuation by cells and the third model group considers both light-limitations and photoinhibitions (Lee, Jalalizadeh and Zhang, 2015). The third group of models use two or three parameters to predict growth. Table 7 below is a list of models, which have been developed over time. What should be noted is the number of parameters, which each equation is based on. The greater the number of parameters included in a model, the greater the risk of overfitting the model into specific and sensitive experimental data.

TABLE 6: EXISTING MODELS USING MULTIPLE FACTORS (LEE, JALALIZADEH AND ZHANG, 2015)

Source	Species	Considered factors in the models					
		N	P	CO_2	Light	Temperature	Others
Kunikanleee and Kaneko, 1984	Scenedesmus dimorphus	✓	✓				
Zhang et al., 1999	Haematococcus pluvialis				✓		✓
Klausmeier et al.	Scenedusmus sp.	✓	✓				
Haario et al., 2009	Wild type algae (Chrysophycea)	✓	✓		✓	✓	
Bougaran et al., 2010	Selenastrum minutum	✓	✓				
	Isochrysis affinis galbana						
Bernard, 2011	Isochrysis galbana	✓			✓		
Filali et al., 2011	*Chlorella Vulgaris*			✓	✓		
Packer et al., 2011	Pseudochlorococcum sp.	✓			✓		
Spijkerman et al., 2011	Chlamydomonas acidophila		✓	✓			
Yang, 2011	Wild type algae	✓		✓	✓		
Franz et al., 2012	Chlamydomonas reinhardtii	✓		✓	✓	✓	
He et al., 2012	Chlorella sp.			✓	✓		
	Synechocystis PCC 6803 Tetraselmis suecica						
Pegallapati and Nirmalakhandan, 2012	Nannochloropsis salina			✓	✓	✓	
	Scenedesmus sp.						
Guest et al. 2013	Chlamydomonas reinhardtii	✓	✓		✓		
Ketheesan and Nirmalakhandan, 2013	Nannochloropsis salina Scenedesmus sp.	✓		✓	✓	✓	
Wu et al. 2013	Scenedesmus sp.	✓		✓	✓		

The growth kinetics equation (Lee, Jalalizadeh and Zhang, 2015), developed by Aiba (Aiba, 1982) and later expanded on by Chiu et al. (Chiu *et al.*, 2008) was used to determine the specific growth rate as it takes into account both light-limitations and photoinhibition as shown below (Zhang *et al.*, 2015).

$$\mu = \mu_{max} \frac{I_{av}}{K_s + I_{av} + \frac{I_{av}^2}{K_i}} - \mu_{min} \quad \dots (3)$$

Where μ is the specific growth rate (/ hour), μ_{max} is the maximum specific growth rate (/ day), I_{av} is the average irradiation in the reactor (W / m^2), K_s is photo-saturation constant (W / m^2), K_i is the Photoinhibition constant (W / m^2) and μ_{min} is the decay rate (/ day).

TABLE 7: MICROALGA GROWTH KINETIC MODELS FOR A FUNCTION OF LIGHT INTENSITY (LEE, JALALIZADEH AND ZHANG, 2015)

	Reference	***Equation***
Group 3	(Steele, 1962)	$\mu = \mu_{max} \frac{1}{I_{opt}} e^{(1-\frac{I}{I_{opt}})}$
	(Aiba, 1982)	$\mu = \mu_{max} \frac{I_{av}}{K_s + I_{av} + \frac{I_{av}^2}{K_i}}$
	(Lee, Erickson and Yang, 1987)	$\mu = \mu_{max} \frac{I_{av}}{K_s + K_{i,L} I_{av}^2}$ where $I_{av} = \frac{I_{In} + I_{out} + 2I_c}{2}$
	(Talbot *et al.*, 1991)	$\mu = 2\mu_{max}(1+\beta) \frac{\frac{I}{I_{opt}}}{\left(\frac{I}{I_{opt}}\right)^2 + 2\beta\left(\frac{I}{I_{opt}}\right) + 1}$
	(Bernard and Rémond, 2012)	$\mu = \mu_{max} \frac{I}{I + \frac{\mu_{max}}{\alpha}\left(\frac{I}{I_{opt}} - 1\right)^2}$
	(Molina Grima *et al.*, 1996)	$\mu = \mu_{max} \frac{I_{av}^{\left(b+\frac{c}{I}\right)}}{\left[I_K + \left(\frac{I}{K_{i,L}}\right)^a\right]^{\left(b+\frac{c}{I}\right)} + I_{av}^{\left(b+\frac{c}{I}\right)}}$
	(García-Malea *et al.*, 2006)	$\mu = \mu_{max} \frac{I_{av}^{(a+bI)}}{(c+dI)^{(a+bI)} + I_{av}^{(a+bI)}}$
	(Muller-Feuga, 1999)	$\mu = 2\mu_{max} \frac{(1-\frac{I_e}{I_{opt}})(\frac{I_{av}}{I_{opt}} - \frac{I_e}{I_{opt}})}{\left(1-\frac{I_e}{I_{opt}}\right)^2 + (\frac{I_{av}}{I_{opt}} - \frac{I_e}{I_{opt}})^2}$
	(Rubio Camacho *et al.*, 2003)	$\mu = \mu_{max} \frac{1}{\alpha'}(1 - x_e^*)$

The Beer-lambert Law (4 below), which considers only light intensity, is the simplest model as it only takes into consideration light absorption (Blanken *et al.*, 2016). The law states that there is an exponential decrease in light intensity as it penetrates a homogeneous section of culture (Politècnica De Catalunya, Thesis and Solimeno, 2017).

$$I_t = I_0 e^{-kcl_{xy}} \ldots (4)$$

Where I_t is the local irradiance (W^2 / m), I_0 is the Incident irradiance, k is the Absorption coefficient (m^2 / g-biomass), c is the biomass concentration (g-biomass / m^3) and l_{xy} is the light path at any location inside the reactor (m).

2.6.2 Models related to Substrate Depletion (Limiting Substrate)

The growth curve with the five phases, as illustrated below in Figure 6, will be obtained if the environmental conditions are conducive to the growth of the algal species at least at time zero (Lee, Jalalizadeh and Zhang, 2015). The algae enter into the death phase once the nutrients have been depleted. The measurement of nutrient concentrations is straight forward, which makes validating models based on substrate depletion relatively easy.

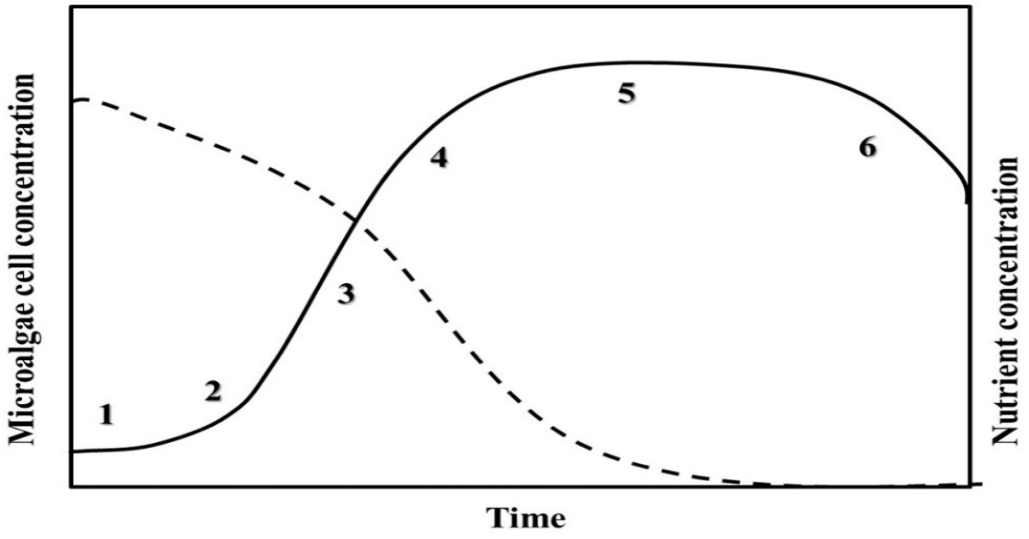


FIGURE 6: GROWTH PHASES IN MICROALGAE BATCH CULTURE (SOLID LINE) AND NUTRIENT CONCENTRATION (DASHED LINE); (1) LAG PHASE; (2) EXPONENTIAL PHASE; (3) LINEAR PHASE; (4) DECLINING GROWTH PHASE; (5) STATIONARY PHASE; AND (6) DEATH PHASE (UAN*G ET AL*, 2017)

Kinetic models that consider substrate depletion can be divided into two groups; the first group assumes that the growth rate is controlled by the external nutrient concentration. The second group assumes that the growth rate is depended on the internal nutrient concentration in the cell, measured by the cell quota, which is the amount of intercellular nutrient per cell (Lee, Jalalizadeh and Zhang, 2015). Verification of group two model's results is limited as the nutrient quota is difficult to determine.

The most common group one model is the Monod model, which describes the relationship between a single nutrient concentration and the growth of the microalgae. The Monod model is limited by its ability to predict growth inhibition due to high nutrient concentrations or very low nutrient concentrations. (Lee, Jalalizadeh and Zhang, 2015). An example of a group two model is the Droop model, which describes the growth of algae with nitrogen and phosphorus as the limiting nutrients in natural environments such as lakes and rivers (Droop, 1968).

Table 8 below is a list of the models, which have developed taking into account a single substrate.

TABLE 8: MICROALGA GROWTH KINETIC MODELS AS A FUNCTION OF A SINGLE SUBSTRATE (LEE, JALALIZADEH AND ZHANG, 2015)

	Reference	***Equation***
Group 1	(Monod, 2003)	$\mu = \mu_{max}\frac{S}{K_S + S}$
	(Andrews, 1968)	$\mu = \mu_{max}\frac{S}{K_S + S + \frac{S^2}{K_i}}$
	(Martínez Sancho, Jiménez Castillo and El Yousfi, 1997)	$\mu = \frac{\mu_{m1}S + \mu_{m2}K_s}{K_S + S}$
	(Martínez, Jiménez and El Yousfi, 1999)	$\mu = \frac{\mu_{m1}S + \mu_{m2}K_s + \mu_{m3}{S^2}/{K_i}}{K_S + S + {S^2}/{K_i}}$
Group 2	(Droop, 1968)	$\mu = \mu'_{max}(1 - \frac{Q_{min}}{Q})$
	(Caperon and Meyer, 1972)	$\mu = \mu^*_{max}\frac{Q - Q_{min}}{Q - Q_{min} + K_C}$
	(Flynn, 2002)	$\mu = \mu'_{max}\frac{(1 + K_q)(Q - Q_{min})}{(Q - Q_{min}) + K_q(Q_{max} - Q_{min})}$

2.6.3 Multiple growth factor kinetic models

Models which take into account multiple growth factors are often referred to as co-limitation models and the main assumption is that both multiple nutrient resources and light, and their interaction control overall microalgae growth (Lee, Jalalizadeh and Zhang, 2015). Threshold and Multiplicative models are the two different types of models. Threshold models are based on the idea that the overall growth rate is affected by the most limited resource among all resources required for cell growth as compared with multiplicative models assume that all resources equally contribute to microalgae growth (Lee, Jalalizadeh and Zhang, 2015).

2.7 Literature Review Summary and conclusion

The literature reviewed in this chapter shows the importance of the cultivation of algae coupled with the treatment of municipal wastewater. The cultivation and use of algae have been thoroughly researched as algae have been cultivated and used in food, food additives, and animal feed, and food and textile pigments for many years. The potential for algae to be a viable source of alternative fuel has increased research in methods of algae cultivation. The search and implementation of a proven sustainable alternative process for the treatment of municipal sewage would improve access to reliable sanitation services for large groups of people.

Combining the need for fuel with PBR and nutrients from municipal sewage is currently a potential solution, but its adoption into mainstream alternative is still hampered by the commercial viability testing of large-scale biofuel plants. A means to improve their viability is to increase the algae's productivity through a greater understanding of the environmental conditions that affect growth and how the manipulation of these environmental conditions can improve the efficiency of PBR.

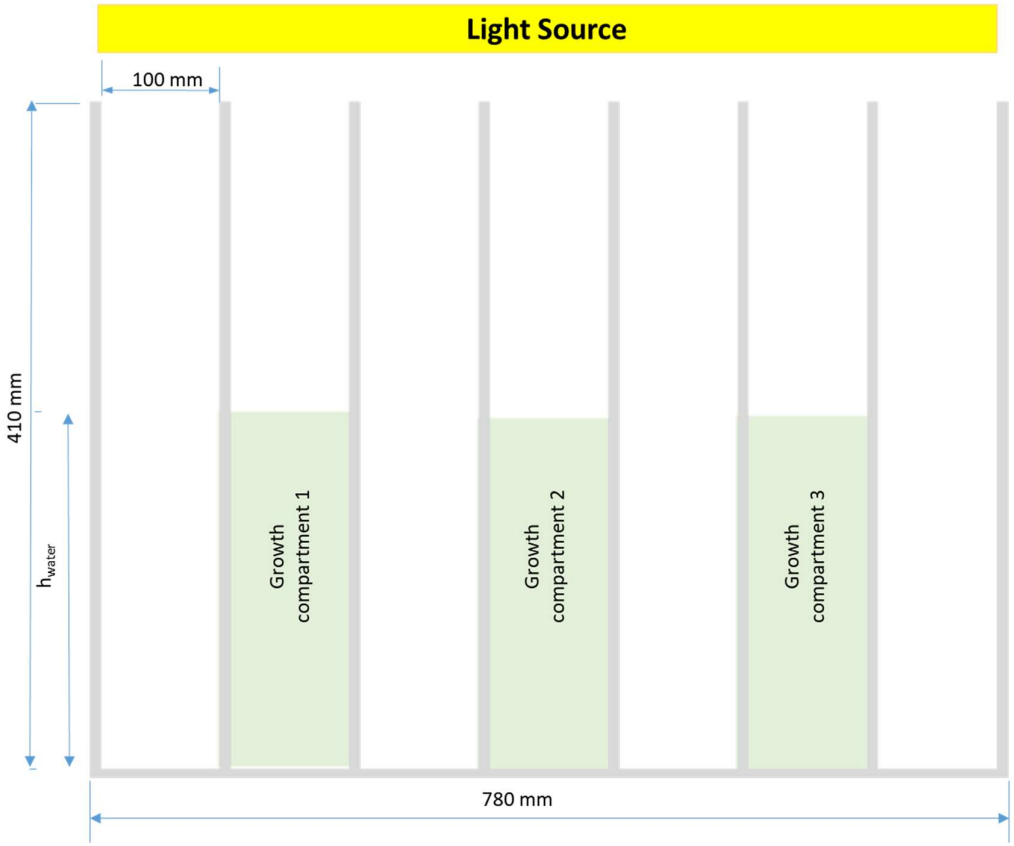
Previous research has shown that C*hlorella vulgarus* is a freshwater species that is fast growing and has been cultivated for both human and animal nutrition since the 1970s. *Chlorella vulgarus* also contain a high percentage of oil per dry-weight and is effective at nutrient removal.

There have been many models developed to predict algae growth, some of these methods consider a single factor, light or nutrient consumption, while others consider many factors, nutrient concentration, light, carbon dioxide concentration, temperature, etc. From previous studies, it has been shown that models, with a large number of parameter, run the risk of overfitting the results into specific and sensitive experimental data. This study will therefore focus on a single parameter modelling.

In conclusion, this review has outlined the factors that affect the growth of algae in a PBR and how they can be controlled. In this study, the water quality parameters that were analysed to track algal growth were pH, nitrogen species (TN, ammonia, nitrates), phosphates and chlorophyll-a. The model development and predictions, Chapter 3, is based on this review and the review assisted with the construction of the models.

Chapter 3: Model Development and Predictions

Two models were developed to track the effects of the impact of modifying the light regime, light intensity and duration, on the algal growth and biomass productivity. The first model was used as the baseline model that was exposed to standard growth condition. The second model was exposed to the same standard growth conditions, however, the lighting conditions were improved through the introduction of mirrors on either side of the growth compartments. Models 1 and 2 were developed using the dimensions of a laboratory PBR that has been previously used in research (Chirwa and Nkgoeng, 2016). The PBR is a rectangular tank constructed out of 10 mm thick Perspex with an overall dimension of 780 mm (width) x 410 mm (height) and 520 mm (depth). Each tank is divided into seven 100 mm wide compartments. Figure 7 is an illustration of the PBR tanks showing their dimensions, light source, and growth compartment for models 1 and 2. Model 1 only considered a light source directly above while model 2 took into consideration the additional light intensity received from the light enhancement provided by the reflection due to the mirrors, the mirrors extended the entire depth of the tank.



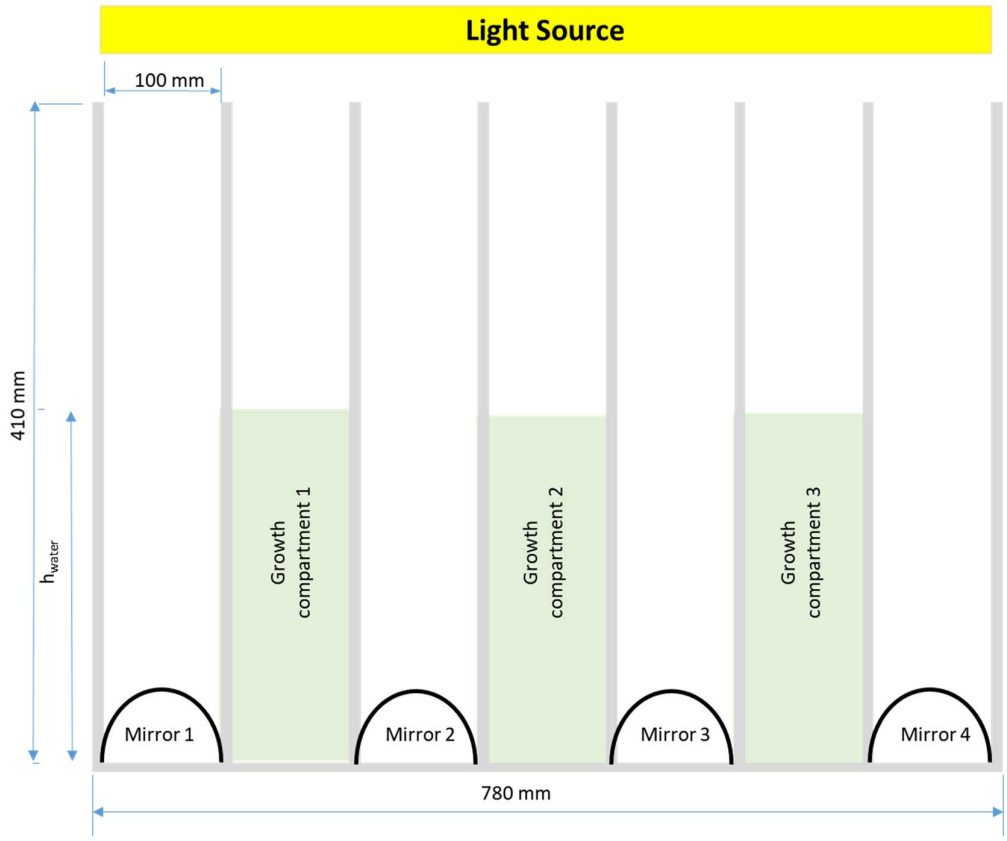


FIGURE 7: PBR TANK DIMENSIONS FOR MODEL 1 (TOP) AND MODEL 2 (BOTTOM)

3.1 Procedure for models development based on Light Intensity

The sequential modelling procedure, elaborated on below, was followed for each of the two models. A Cartesian coordinate system was used for position validation in the models. The Cartesian coordinate system is important in establishing the position of individual growth fraction's position in space within the PBR and the fraction's relative distance from the light source. The light path used for the calculation of local irradiation can only be done if the relative positions of the fractions to the surface is known. Model 1 and 2 coordinate origin (0, 0) was based on the PBR tank illustrated in Figure 8 below. It is assumed that each growth compartment and fractions are exposed to the same environmental and nutrient conditions and therefore should produce a similar photosynthetic efficiency.

3.1.1 Model Procedure

The modelling procedure is outlined below:

Step 1:

The algae's exposure to light radiation is a function of the location relative to the open surface and therefore the light path must be determined. The growth segment was divided into individual compartments of 1 mm in thickness, the 1 mm thick layer was divided into 1 mm depth increments as shown in Figure 8 below. Each of these individual 1 mm by 1 mm parts is known as the fraction of the culture.

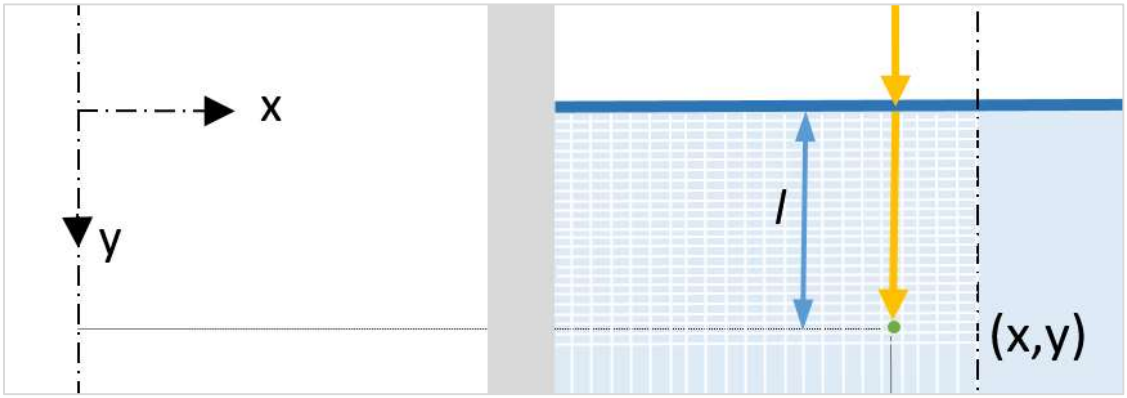


FIGURE 8: CULTURE SEGMENTS USED FOR EACH MODEL

Step 2:

The light path for each of the fractions is calculated relative to the surface parallel to the overhead light source for model 1 and both the overhead and reflected light source for model 2. The local irradiance for each fraction per hour is calculated using the Beer-Lambert Law (5).

$$I_{xy} = I_h e^{-kC_n l_{xy}} \quad \ldots (5)$$

Where I_{xy} is the local irradiance at the location (x,y) (W^2 / m), I_h is the Incident irradiance corresponding to the hour of the day (W/m^2), k is the Absorption coefficient (m^2 / g-biomass), c_n is the biomass concentration (g-biomass / m^3) and l_{xy} is the local irradiance at any location inside the growth compartment at position x,y (m).

Step 3:

Each of the local radiance calculated in step 3, are added together, and then divided by the number of fractions to calculate the average Local radiance for a given hour.

$$I_{av} = \frac{\sum_0^{xy} I_0 e^{-kC_n l_{xy}}}{m} \ldots (6)$$

Where *m* is the number of fractions within the PBR as shown in Figure 9.

Step 4:

The specific growth rate per hour is calculated, using the hourly average local radiance calculated in the step above, using equation 7 below

$$\mu_h = \mu_{max} \frac{I_{av}}{K_s + I_{av} + \frac{I_{av}^2}{K_i}} - \mu_{min} \ldots (7)$$

Where μ_h is specific growth rate (/hour), μ_{max} is the maximum specific growth rate (/day), I_{av} is the average irradiation in the reactor (W/m^2), K_s is photo-saturation constant (W/m^2), K_i is the Photoinhibition constant (W /m^2) and μ_{min} is the decay rate (/day).

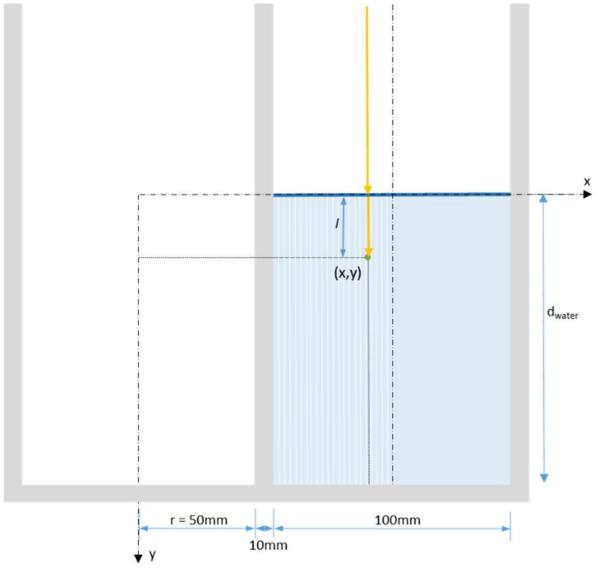


FIGURE 9: GENERAL LAYOUT OF MODEL 1

Step 5:

The average growth rate for each day is calculated using equation (8) below:

$$\mu_{av} = \frac{\sum_{n=1}^{24}\left(\mu_{max}\frac{I_{av(n)}}{K_s + I_{av(n)} + \frac{I_{av(n)}^2}{K_i}} - \mu_{min}\right)}{24} \ldots (8)$$

Where μ_{av} is the average growth rate per day and n is the hour of the day.

Step 6:

Microbial productivity is calculated for the algal culture using equation (9) below:

$$P = \mu_{av} C V \ldots (9)$$

Where P is the microbial productivity (g-biomass / day), V is the volume of the culture (m^3) and C is the biomass concentration (g-biomass / m^3).

The same steps were followed in the assembly of model 2. Model 2 took into account the additional light intensity provided by the introduction of the mirror. This was done by including additional local irradiation to equation (5).

3.1.2 Model Parameters

For each of the models' parameters the parameters shown in Table 9 were used:

TABLE 9: MODEL PARAMETER VALUES

Parameter	**Symbol**	**Initial Value**	**Units**
Initial Biomass Concentration	C_0	1000	g-biomass / m^3
Absorption Co-efficient	k	0.2	m^2/g-biomass
The maximum specific growth rate	μ_{max}	4	4/d
Saturation constant	K_s	5	W/m^2
Photoinhibition constant	K_i	200	W/m^2
Decay rate	μ_{min}	0.1	Day^{-1}
Volume of culture	V	0.0078	m^3
Incident Irradiance corresponding to the hour of the day during either summer or winter solstice.	I_h	Refer to Appendix 1	W/m^2

3.2 Model Prediction

The two models were developed using the principle depicted above in an excel spreadsheet. Each model culture was divided into 1 mm x 1 mm fractions for model 1 and 2 with each excel cell representing the specific culture's fraction. The calculation for both winter and summer for both models can be found in Appendix 5.

3.2.1 Local Irradiation

For each of the culture fractions within the PBR growth compartment, the local irradiation was calculated using the incident radiation, light path length (depth) and the biomass concentration at the beginning of each time interval (1 hour). The incident radiation used in the model was for the De Aar weather station over the winter and summer solstice.

The local irradiation is a function of the many factors, biomass concentration, incident irradiance and light path length, and a coefficient, absorption coefficient. The incident irradiance that the cultures are exposed to for the duration of the modelling period is assumed to be at either the summer or winter solstice (constant), and the model's volume is assumed constant over the seven days. The reactors are considered batch reactors as there is no inflow nor outflow for the duration of the experiment, also, the loss of volume due to evaporation was considered negligent.

The only variation that affects the local irradiation was the change in the biomass concentration; as the concentration of biomass increased the shading effect occurred. Figure 10 illustrates the summer model's midday irradiation within the culture. For model 1 day one, local irradiation drops from approximately 1000 W/m^2 on the surface to 22 W/m^2 within the 10 mm. On day three, the reduction is more rapid as the reduction surface irradiation from 1000 W/m^2 drop to approximately zero within the first 1 mm. On day seven, this loss of irradiation is experienced within the 1 mm. In contrast, for model 2 day one, the local irradiation drops from approximately 1885 W/m^2 on the surface to 752 W/m^2 within the 10 mm. On day three, the reduction is more rapid with the surface irradiation decreasing from 1150 W/m^2 to 3.15 W/m^2 at 2 mm. On day seven, this loss of irradiation is experienced within the first 1 mm.

The distinct reduction in the local irradiation over a short distance could be attributed to the high absorption ability of Chlorophyll-a and with the increase in the biomass concentration, there is an increase in the self-shading effect reducing the light's ability to penetrate the culture.

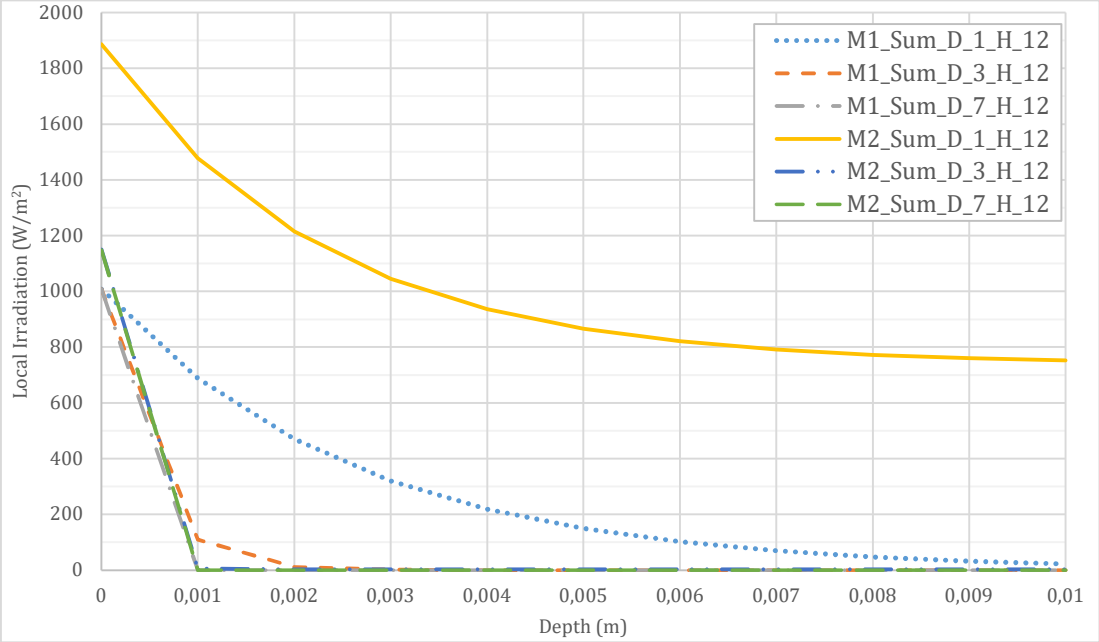


FIGURE 10: MODEL 1 AND 2 ON DAY ONE, THREE AND SEVEN MIDDAY LOCAL IRRADIATION COMPARISON (MIDDAY LOCAL IRRADIATION)

The seven-day average local irradiation for both the summer and winter solstice was calculated and the results are shown in Figure 11. The figure shows that an increase in the summer maximum hourly local irradiation over the 24 hours is just below 27.02 W/m^2 for model 2 where model 1 maximum is relatively constant at around 10.3 W/m^2 during the day. A similar trend can be seen for the winter models, where model 2 maximum hourly local irradiation is 25 W/m^2 compared to model 1's maximum local irradiation of 10.68 W/m^2.

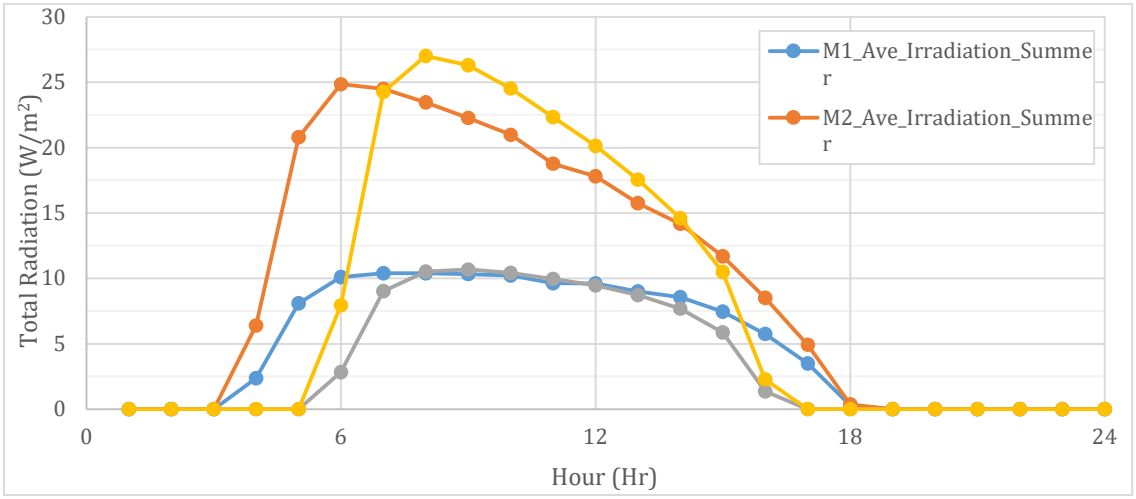


FIGURE 11: MODEL 1 AND 2 AVERAGE LOCAL IRRADIATION FOR SUMMER AND WINTER SOLSTICE

3.2.2 Biomass Concentration Predictions

Summer Solstice

Using the hourly average irradiation in the culture segment the specific growth rate was calculated for each hour, and following that, the specific growth rate per hour was calculated. The hourly biomass concentration was calculated using the previous hour's biomass concentration and the specific growth rate for the current hour. The summer model's results are plotted in Figure 12 below. The model predicts that model 2 biomass concentration should be 15.16% greater biomass concentration than model 1 over seven days.

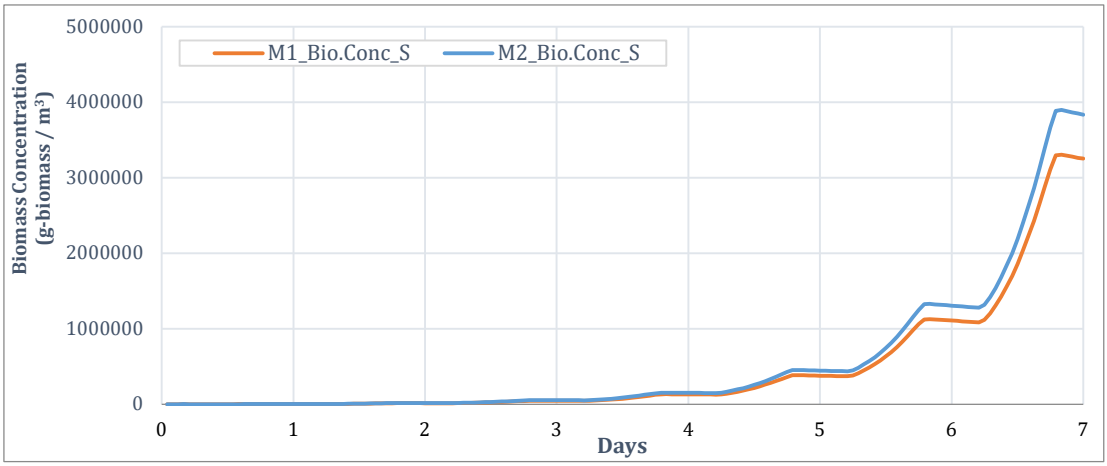


FIGURE 12: BIOMASS CONCENTRATION OVER A SUMMER SEVEN DAY PERIOD FOR MODEL 1 AND 2

Winter Solstice

Following the same modelling procedure, as outlined above for the summer solstice, the winter model results are plotted in Figure 13 below. Similar to the summer predictions, the winter solstice models predict that model 2 biomass concentration should be 19.68% greater than model 1 biomass concentration at the end of seven days.

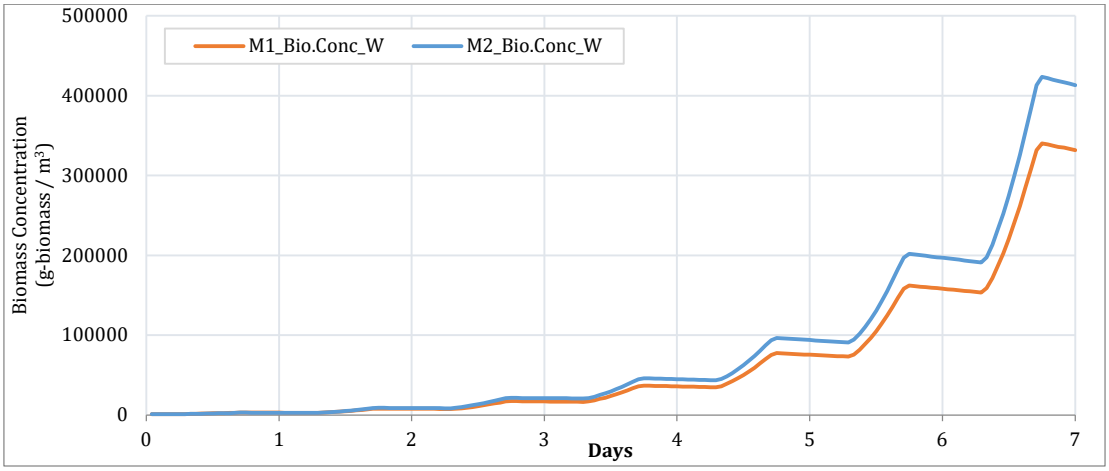


FIGURE 13: BIOMASS CONCENTRATION OVER A WINTER SEVEN DAY PERIOD FOR MODEL 1 AND 2

3.2.3 Model Productivity

Each model's microbial productivity was calculated using step 6 outlined above. The summer and winter productivity for each model is shown in Figure 14 and Figure 15 below. For the summer model sets, model 1 initial productivity is marginally greater than model 2. Model 2 productive improves daily and is greater than model 1. By day seven, model 2 productivity is 15.16% greater than model 1.

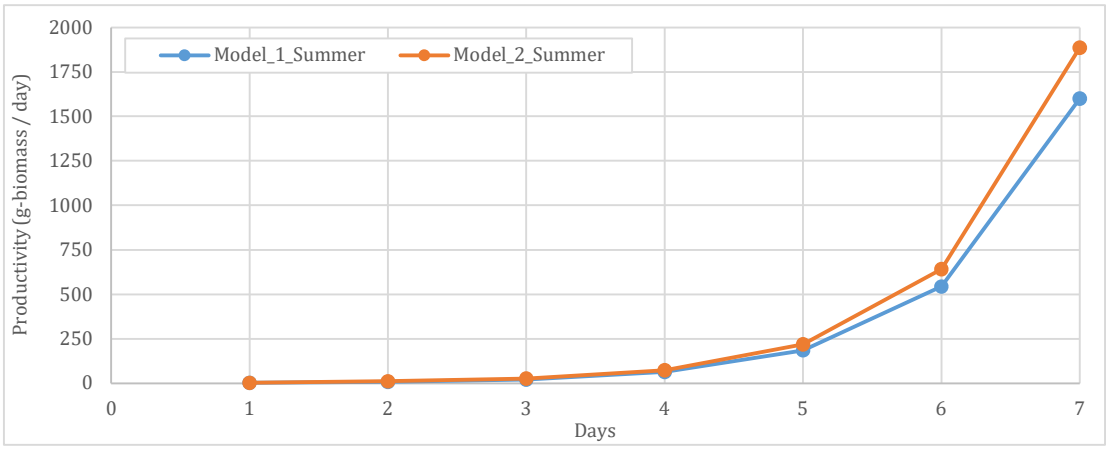


FIGURE 14: PREDICTED PRODUCTIVITY FOR MODEL 1 AND 2 DURING SUMMER

For the winter models, a similar trend is predicted for both models. Model 1 productivity is marginally greater on day one. However, by day seven model 2 productivity is 19.68% greater than model 1.

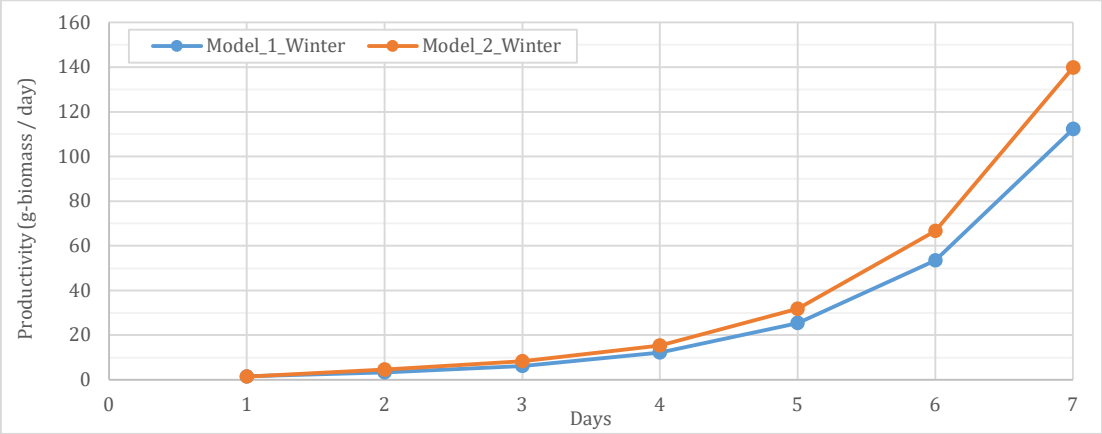


FIGURE 15: PREDICTED PRODUCTIVITY FOR MODEL 1 AND 2 DURING WINTER

3.2.4 Model Prediction Conclusion

Through the introduction of reflective mirrors, the light intensity in the PBR is increased and the model predicts a positive impact on productivity. This increase in productivity would have a significate effect on the mass of biomass produced and hence yielding a greater volume of energy-rich oils per unit volume within the PBR. The increase in productivity could reduce the construction costs associated with PBR as a PBR with a design based on model 2 could potentially reduce its dimensions by 15 to 20 % and it will still have the capability of producing the equivalent biomass of larger PBR.

Chapter 4: Laboratory Studies: Methodology

This chapter describes the laboratory experiments carried out in this study.

4.1 Laboratory Equipment

The tanks used in this study, shown in Figure 16 below, were constructed for a previous project. The tanks are made of 10 mm thick Perspex with overall dimensions of 780 mm (length) x 410 mm (height) and 520 mm (breath). The internal dimensions of the growth segments were 100 mm (length) x 500 (height) mm and 400 mm (breath). Figure 16 is a photograph of tank 1's set up for experiment two before the introduction of the culture as described in Chapter 3, figure 7. Before the start of each experimental run, the tanks were thoroughly washed with water and soap and dried with paper towels.

FIGURE 16: SETUP OF TANK 1 EXPERIMENT 2

Once the experiments were set up, the culture in each growth segment was mixed using a course bubble air mixing system, which introduced air to the bottom of the growth segment. The mixing system was made up of a laboratory compressor, a network of 5 mm diameter silicon tubing with several holes made on them, plastic valves, 90°, and T-piece bends. Supplying the air into the different growth compartments using this system ensured that each growth segment was continuously aerated and mixture throughout the entire experimental period.

4.2 Preparation of Algae Stock

The algal culture used in the laboratory experiments, *Chlorella vulgarus* (CCAP 211/11B), was obtained from SAMS Limited, Scotland. The culture was grown in a combination of Euglena Gracilis medium (EG) and Jaworski's Medium (JM) as per the culture bank's instructions. The protocol followed to prepare the EG:JM medium is detailed in Appendix 3. The steps followed to increase the volume of the initial algae culture of 45 ml to 4 litres is describe below and show in Figure 17.

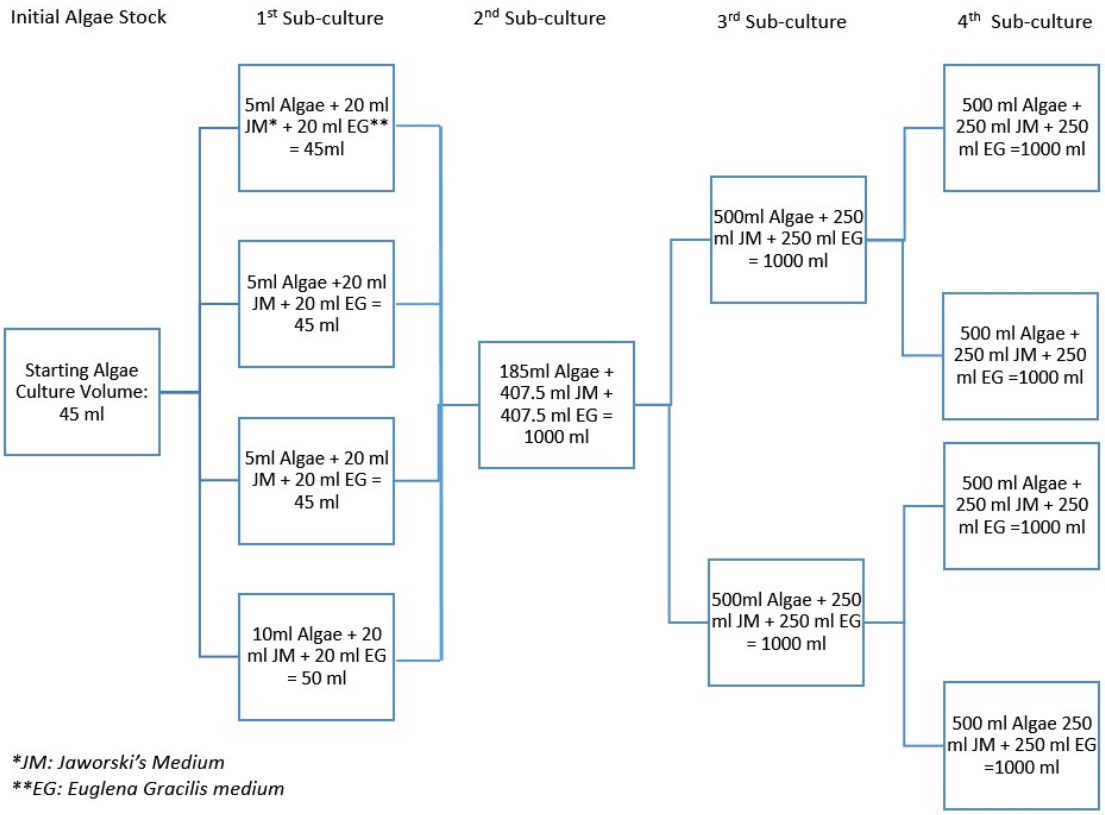


FIGURE 17: STAGES OF THE SUB-CULTURING OF THE ALGAE STOCK

The initial cultivation of the algae was done in four sterilized 50 ml Schott bottles. The bottles were each filled with 20 ml of each growth medium. One of these Schott bottles was inoculated with 10 ml of the stock culture while the remaining three Schott bottles were each inoculated with 5 ml of the stock culture.

For all the rounds of sub-culturing, the intensity of the green pigmentation of the culture was used as a proxy for algal density. This is because the colouration of the culture mixes comes from chlorophyll-a, a pigment that photosynthetic algae produce during their growth. Figure 18, Figure 19, and Figure 20 below show the density of the algal culture preparations over five days. It was observed that there was a slight increase in the density of the culture from day zero to day three and again from day three to five.

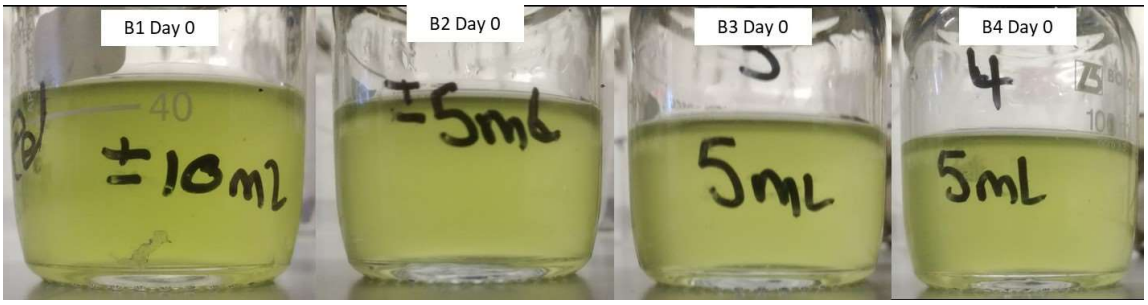


FIGURE 18: DAY ZERO OF ALGAE INCUBATION MEDIUM

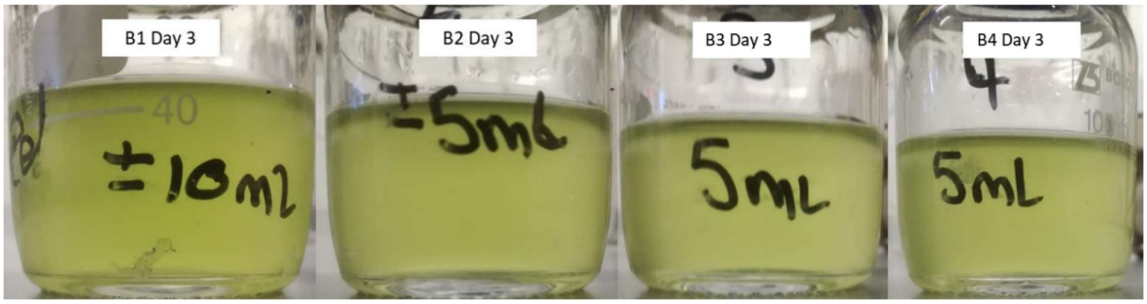


FIGURE 19: DAY THREE OF ALGAE INCUBATION MEDIUM

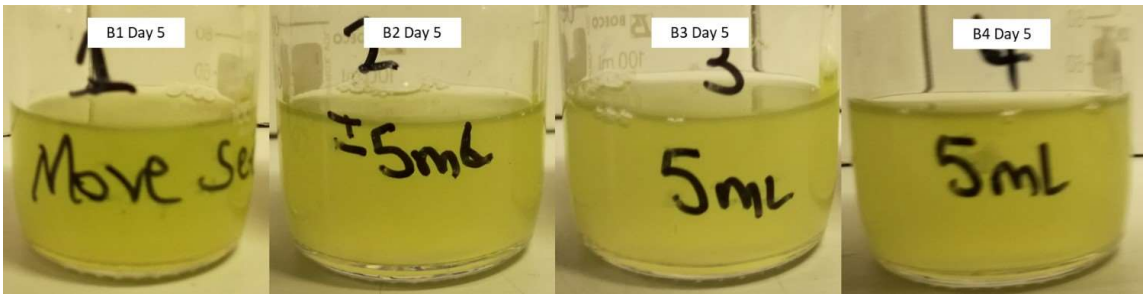


FIGURE 20: DAY FIVE OF THE ALGAE INCUBATION MEDIUM

Following the first round of sub-culturing, which lasted for seven days, the culture mixes from all four Schott bottles were combined into a large Schott bottle and mixed. The total volume of the culture mix was 185 ml at this stage. This up-scaled stock culture was then brought to a volume of 1000 ml through the addition of EG:JM medium. The 1000 ml algal mix was incubated at room temperature for two days. Figure 21 and Figure 22 show the differences in the density of the culture mix at days zero and two. There was a visible decrease in the intensity of colour after two days of incubation. It is important to note that the culture mix was more turbid after incubation.

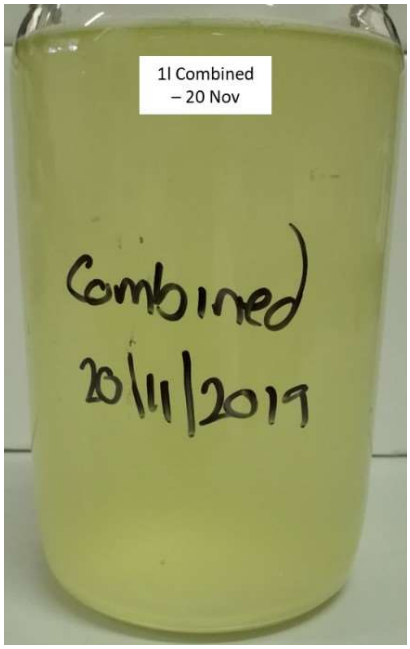


FIGURE 21: COMBINED ALGAE MEDIUM (DAY ZERO)

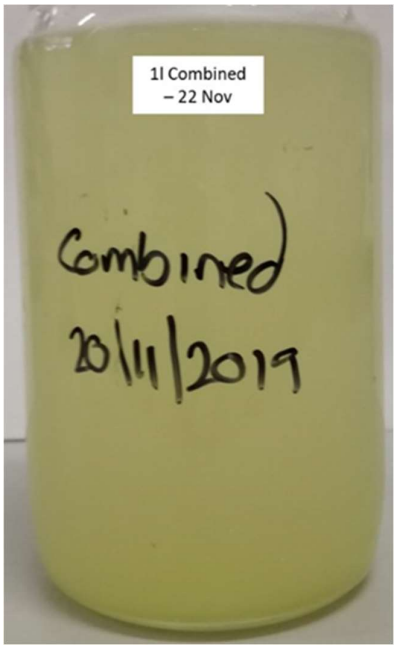


FIGURE 22: COMBINED ALGAE MEDIUM (DAY TWO)

The 48 hours "old", 1000 ml, culture mix was doubled through the addition of the EG:JM medium. The 2-litre mix was then split into two 1-litre Schott bottles and incubated at room temperature. Figure 23 below shows the differences in algal density between day zero and day three of incubation. There was a visible loss/reduction of the green colour with the culture mix becoming more cloudy with time.

FIGURE 23: 2 LITRE COMBINED DAY ZERO (LEFT) AND DAY THREE (RIGHT)

The culture mix from the two 1-litre Schott bottles was mixed into one container and up-scaled to 4-litre though the addition of EG:JM medium. After upscaling the culture mixture, it was divided into four 1-litre Schott bottles and further incubated at room temperature for seven days as shown in Figure 24 below. After seven days, the culture mixes in the four Schott bottles were mixed, and 3-litre of the stock culture used to inoculate the PBR for the first round of experiments while the remaining 1-litre was used to replenish the stock back up to 4-litre, following the procedure described above.

FIGURE 24: 4 LITRE COMBINED DAY ZERO

4.3 Photobioreactor's Laboratory Experiments

Two PBRs were used for laboratory studies, with the first tank serving as the control tank and the second tank serving as the test tank. The two PBRs were treated the same way in terms of how they were sanitized before each experimental run, volumes of cultural inoculum added to each PBR per experimental run, volumes of water added to each PBR per experimental run, nutrient supply, and mixing conditions. The difference between the two PBRs was the mirrors placed between growth segments at the bottom of the tanks.

The hypothesis was that providing additional light to one of the PBR would result in an improvement to algal growth and nutrient depletion. For the first experimental run each PBR was inoculated with 1.5 litres of the of stock culture, 16.5 litres of chlorine-free potable water, and 18 mL each of Nutriplex Gro, Nutriplex Micro and Nutriplex Bloom (all Nutriplex stock solutions were purchased from Hydroponics, Fourways), see Table 10 below for their composition. The Butriplex solution provided nutrients at levels similar to those seen in municipal wastewater.

The first experiment run was a preliminary experimental run, primarily aimed at determining optimal nutrient requirements and lighting conditions for the study. Data from this run was used to streamline growth conditions for experimental runs 2, 3, and 4. The laboratory overhead fluorescent lighting provided the external light for the tanks during the first experiment.

For the second, third, and fourth experimental runs everything was set up as detailed above with one exception: only 10 ml, each, of Nutriplex Gro, Nutriplex Micro and Nutriplex Bloom was added to each PBR. The laboratory overhead lighting did not provide sufficient control over the light intensity and duration; therefore, a dedicated overhead lighting source was used for the second, third and fourth experimental runs, shown in Figure 16. The on and off durations of the new lighting source was controlled using a timer that provided 12 hours of light and 12 hours of dark.

The enrichment of water with Nutriplex nutrient solutions was done to achieve nutrient levels similar to those found in municipal wastewater. The quantities used for each of the Nutriplex nutrient solutions was in line with previous experiments conducted under similar condition (Chirwa and Nkgoeng, 2016). Each experimental run was carried over seven days under the same conditions. As the light intensity was provided by the overhead lights, the only variation to light intensity was that the light was either on or off and not the varied light intensity seen during a natural day. The mixing conditions did not change during the experiments.

The water quality parameters that were tracked during the experiments were chosen because they are among parameters regulated for municipal WWTP effluent disposal except for the measurement of Chlorophyll-a, which was used to monitor algae growth. In addition, algae use nitrogen and phosphorus for growth. These parameters were measured on days zero, one, two, three, five, and seven.

The parameters measured were:

- pH level,
- Total Nitrogen,
- Ammonia,
- Nitrate,
- Phosphates, and
- Chlorophyll-a

For the first experimental run, samples from each of the growth segments were withdrawn and tested individually, however for the remaining three experiment runs; a composite sample was prepared by taking aliquots of equal volume from each growth segment and mixing them. Along with the water quality parameters listed above, any visible changes in the water in the PBRs were recorded. The PBRs were also photographed each day that the samples for water quality testing were collected.

All water quality testing was conducted at the School of Civil and Environmental Engineering. All chemical parameters except for pH were measured with a Spectroquant Pharo 300 spectrophotometer, following the standard testing methods (Uv and Spectrophotometer, no date) listed below:

- Chlorophyll- Modified ESS Method 150.1
- Total Nitrogen – Method 108: 14763
- Ammonia – Method 54: 14752
- Nitrate – Method 139: 09713
- Phosphates – Method 1.10428.0001

pH was measured using a calibrated pH-meter.

TABLE 10: COMPOSITION OF NUTRIPLEX GRO, MICRO, AND BLOOM

Name	Chemical Compound	Composition
Nutriplex Gro (*Nutriplex Gro 1L - Greenthumb Hydroponics*, 2018)	Nitrogen (N)	2%
	Ammonia Nitrogen (NH_3N)	1.5%
	Nitrate Nitrogen (NO_3)	0.5%
	Phosphate (P_2O_5)	1%
	Potassium (K_2O)	6%
	Magnesium EDTA (Mg)	0.8%
Nutriplex Micro (*Nutriplex Micro 1L - Greenthumb Hydroponics*, 2018)	Nitrogen (N)	5.0 %,
	Ammonia Cal Nitrogen (NH_3-N)	1.0%
	Nitrate Nitrogen (NO_3)	4.0%
	Soluble Potash (K_2O)	1.2%
	Calcium (Ca)	3.8%
	Cobalt (Co)	0.0007%
	Iron DTPA (Fe)	0.2%
	Manganese EDTA (Mn)	0.1%
	Molybdenum EDTA (Mo)	0.006%
	Zink EDTA (Zn)	0.025%
	Copper EDTA (Cu)	0.02%
	Boor (B)	0.016%
	Amino Acids	
Nutriplex Bloom (*Nutriplex Bloom 1L - Greenthumb Hydroponics*, 2018).	Phosphate (P_2O_5)	5%
	Potassium (K_2O) 4%,	4%
	Magnesium (Mg) 3%,	3%
	Sulphur (S) 5%	5%
	Amino Acids	

The ESS Method 150.1 used to measure chlorophyll-a was modified as detailed below:

1. A 20 ml sample was removed from each growth segment for a total sample volume of 60 ml was collected per tank and placed in a dark box.
2. The 60 ml sample was filtered through a 5 μm filter paper.
3. The filter paper was folded and placed in a 50 ml centrifuge tube that was topped-up with 15 ml of acetone aqueous solution. Each sample was placed back into the dark box until each sample was filtered and topped-up.
4. All samples were sonified in a sonicator for 5 min at a frequency of 40 kHz.
5. Once the samples were sonicated, they were placed in a dark container. The dark box was placed in a fridge for a minimum of 18 hrs at a temperature of 5°C.
6. The samples were transferred into 2 ml centrifuge tubes and then they were clarified in a centrifuge for 20 minutes at a speed of 2750 rpm.
7. The clear extract was then transferred into the 10 mm cell. The spectrophotometer's multi-wavelength function was used to determine the absorbance at 750, 665, 663, 645 and 630 nm wavelength. The spectrophotometer was zeroed using a 10 mm cell, which consisted of the acetone aqueous solution.

The final chlorophyll-a concentration was determined using the absorbance results and the equation below.

The absorbance recorded at 750 nm was subtracted from the absorbance that was recorded at 630, 645, and 663 to ensure that the turbidity obtained is correct. Final Chlorophyll was calculated using equation 10 below.

$$\text{Chlorophyll a } \left(\frac{\mu g}{L}\right) = \frac{[11.64(Abs663) - 2.16(Abs645) + 0.1(Abs630)]E(F)}{V(L)} \quad \ldots (10)$$

Where: F= Dilution Factor
E = the volume of acetone used for extraction (mL)
V = the volume of water filtered (L)
L = the cell path length (cm)

Chapter 5: Laboratory Results and Observations

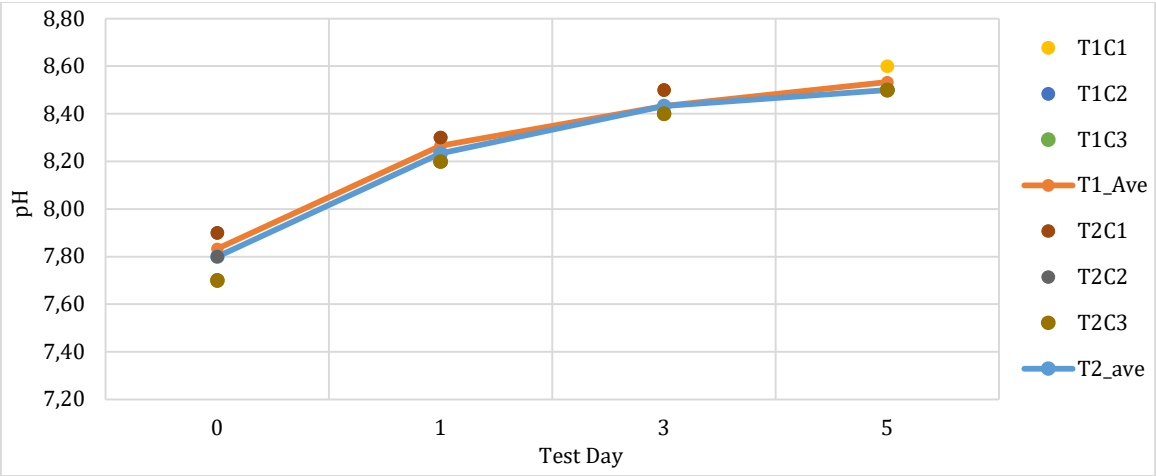
This chapter details the results and observations from the laboratory experiments conducted in this study.

5.1 Experiment Run 1

The first experimental run was carried out from 2 December 2019 (day zero) to 7 December 2019 (day five). For each PBR, test samples were taken from each growth segment. A set of selected water quality parameters were measured on days zero, one, three, and five, and the results were recorded per growth segment and an average reading was calculated. A full set of results can be found in Appendix 4. The results from experiment run 1, presented below, are not included in the Discussion and Conclusion section as this run was primarily carried out to calibrate the testing methods.

5.1.1 pH

Figure 25 below illustrates the pH recorded on test day zero, one, three, and five. On day zero, the minimum, maximum, and average pH reading in tank 1 were 7.70, 7.90, and 7.83 respectively. On the same day, the minimum, maximum, and average pH readings in tank 2 were 7.70, 7.90, and 7.80 respectively. The pH in both tank clustered around their respective averages and increased over the test period. On day five, the minimum, maximum, and average pH readings in tank 1 were 8.50, 8.60, and 8.53 respectively. The minimum, maximum, and average pH readings on day five in tank 2 were all 8.50.



T# represents Tank Number
C# represents Growth Segment Number

FIGURE 25: EXPERIMENT RUN 1 - PH RESULTS

5.1.2 Total Nitrogen

Figure 26 below illustrates the TN concentrations recorded on test day zero, one, three, and five. On day zero, the minimum, maximum, and average TN concentration in tank 1 were 89.00, 104.00, and 94.00 mg/L respectively. The minimum, maximum, and average TN concentration in tank 2 were 104.00, 109.00, and 105.67 mg/L respectively. An error was discovered for the day one results and these reading were disregarded. On day five, the minimum, maximum, and average TN concentrations in tank 1 were 44.00, 91.00, and 64.33 mg/L respectively. The minimum, maximum, and average TN concentrations in tank 2 were 46.00, 90.00, and 61.67 mg/L respectively. There was an overall decrease in both tanks over the experimental run.

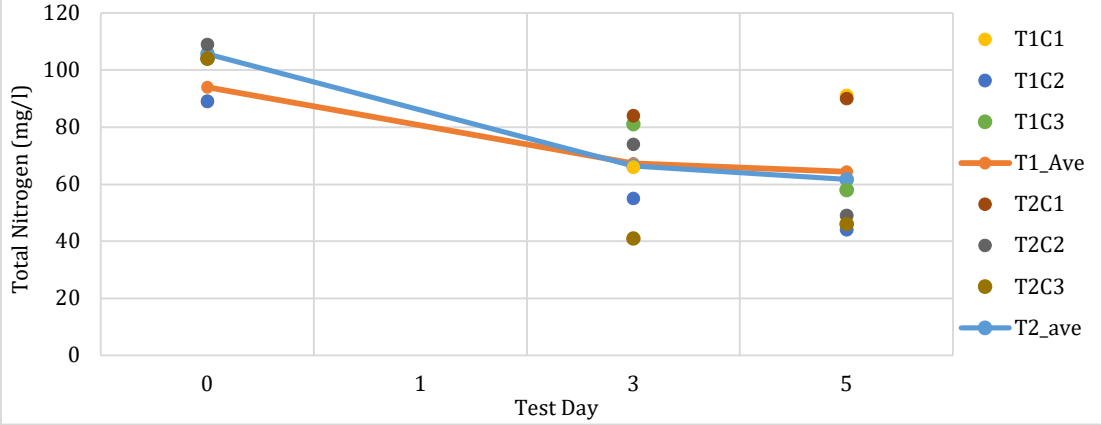


FIGURE 26: EXPERIMENT RUN 1 - TOTAL NITROGEN

5.1.3 Ammonia

Figure 27 below illustrates the ammonia concentrations recorded on test day zero, one, three, and five. On day zero, the minimum and maximum ammonia concentration in tank 1 were 0.77, 0.96, and 0.89 mg/L respectively. The minimum, maximum, and average ammonia concentration in tank 2 was 0.85, 1.06, and 0.94 mg/L respectively. The ammonia increased over the test period and the results cluster around their respective tank averages. On day five, the minimum, maximum, and average ammonia concentration in tank 1 were 4.27, 5.00, and 4.51 mg/L respectively. The minimum, maximum, and average ammonia concentration in tank 2 was 2.10, 5.29, and 3.79 mg/L respectively.

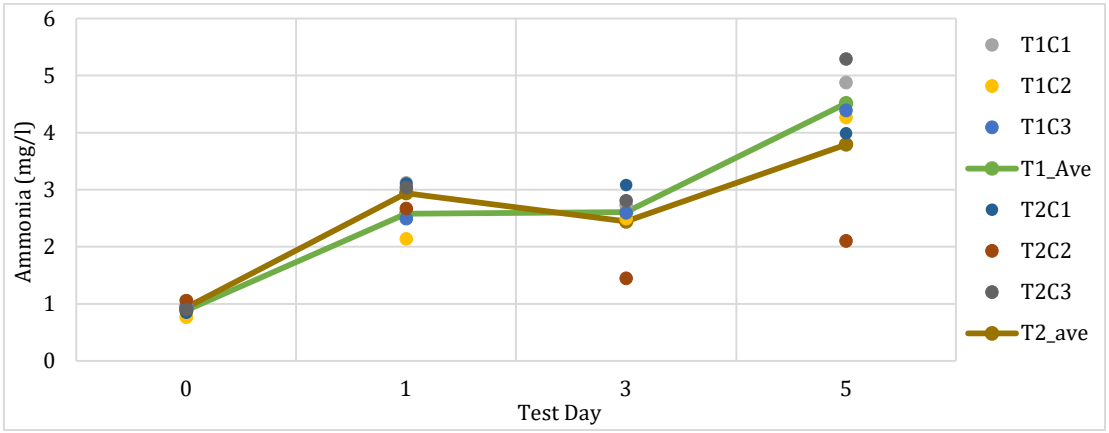


FIGURE 27: EXPERIMENT RUN 1 - AMMONIA

5.1.4 Nitrate

Figure 28 below illustrates the nitrate concentrations recorded on test day zero, one, three, and five. On day zero, the minimum and maximum nitrate concentration in tank 1 were 4.91, 12.50, and 9.97 mg/L respectively. All three-growth compartments nitrate concentration in tank 2 were above 12.50 mg/L. The nitrate decreased over the first three days of testing and then the nitrate concentration increase to high concentrations on day five. On day five, all growth segments' nitrate concentration in both tanks were measured greater than 25.00 mg/L, which is the detection limit for the testing kit used in this study. Therefore, the minimum, maximum, and average nitrate concentration for day five could not be determined.

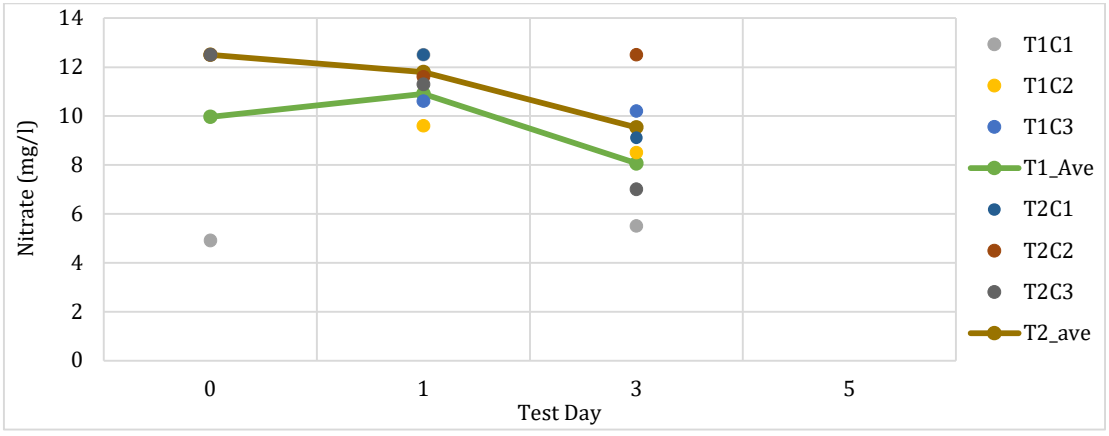


FIGURE 28: EXPERIMENT RUN 1 – NITRATE

5.1.5 Phosphates

Phosphate was measured with a colour stick indicator test with the measuring ranges, as shown in Figure 29 below. The test is carried out as prescribed and the stick colour is compared with the colour chart. This form of testing is an indicative test and does not yield accurate measurement, therefore many of the results overlap.

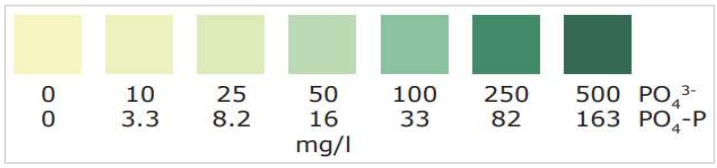


FIGURE 29: MEASUREMENT RANGE OF THE PHOSPHATE TEST

The phosphate concentration recorded on test day zero, one, three, and five is illustrated in Figure 30 below. On day zero, for all three-growth segments' phosphate concentrations for both tanks were all 33 mg/L. Tank 1 had a phosphate concentration of 33 mg/L until day five. Tank 2 phosphate concentration dropped to 16 mg/L on day one in all three-growth segments and it maintained that concentration for the duration of the experimental run.

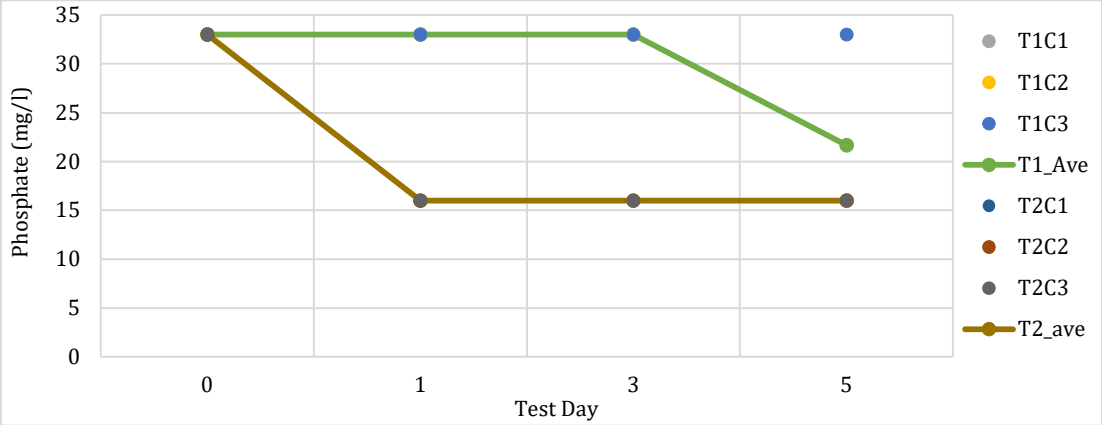


FIGURE 30: EXPERIMENT RUN 1 – PHOSPHATES

5.1.6 Chlorophyll-a

Figure 31 below illustrates the chlorophyll-a concentrations recorded from day zero until day five. The chlorophyll-a measurements for day zero and one are not shown as the results were incorrect due to an experimental error and were excluded from the result set. On day three, the minimum, maximum, and average chlorophyll-a concentration in tank 1 was 16.39, 27.94, and 23.74 µg/l respectively. The minimum, maximum, and average chlorophyll-a concentration in tank 2 were 14.90, 37.40, and 25.02 µg/l respectively.

The chlorophyll-a concentration increased on day five. The minimum, maximum, and average concentration tank 1 were 28.70, 46.23 and 39.14 µg/l respectively. The minimum, maximum, and average chlorophyll-a concentration in tank 2 was 28.77, 41.49, and 34.35 µg/l respectively.

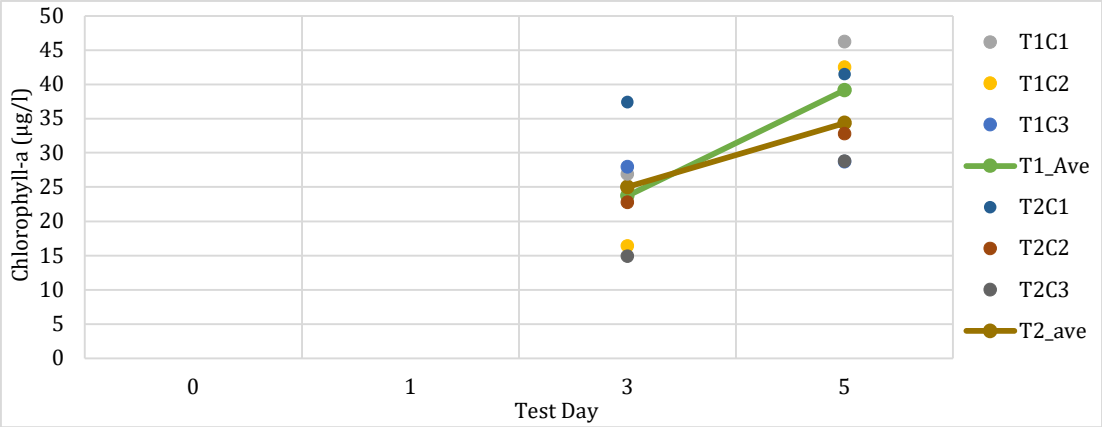


FIGURE 31: EXPERIMENT RUN 1 - CHLOROPHYLL-A

5.1.7 Photographic images were taken during Experiment 1

For each experimental run, both PBRs were photographed one each test day and all photographs were taken before any sampling. Figure 32 and Figure 33 show images of the two PBRs throughout the first experimental run. The images show a similar change in colour from a light green colour to a brownish-green colour over the initial four days of the experiment. The biofilm growth is visible in the photographs from day three and five. By day five, there was a substantial amount of fine "sediment" accumulated at the bottom of each of the growth segments and some in floating in suspension.

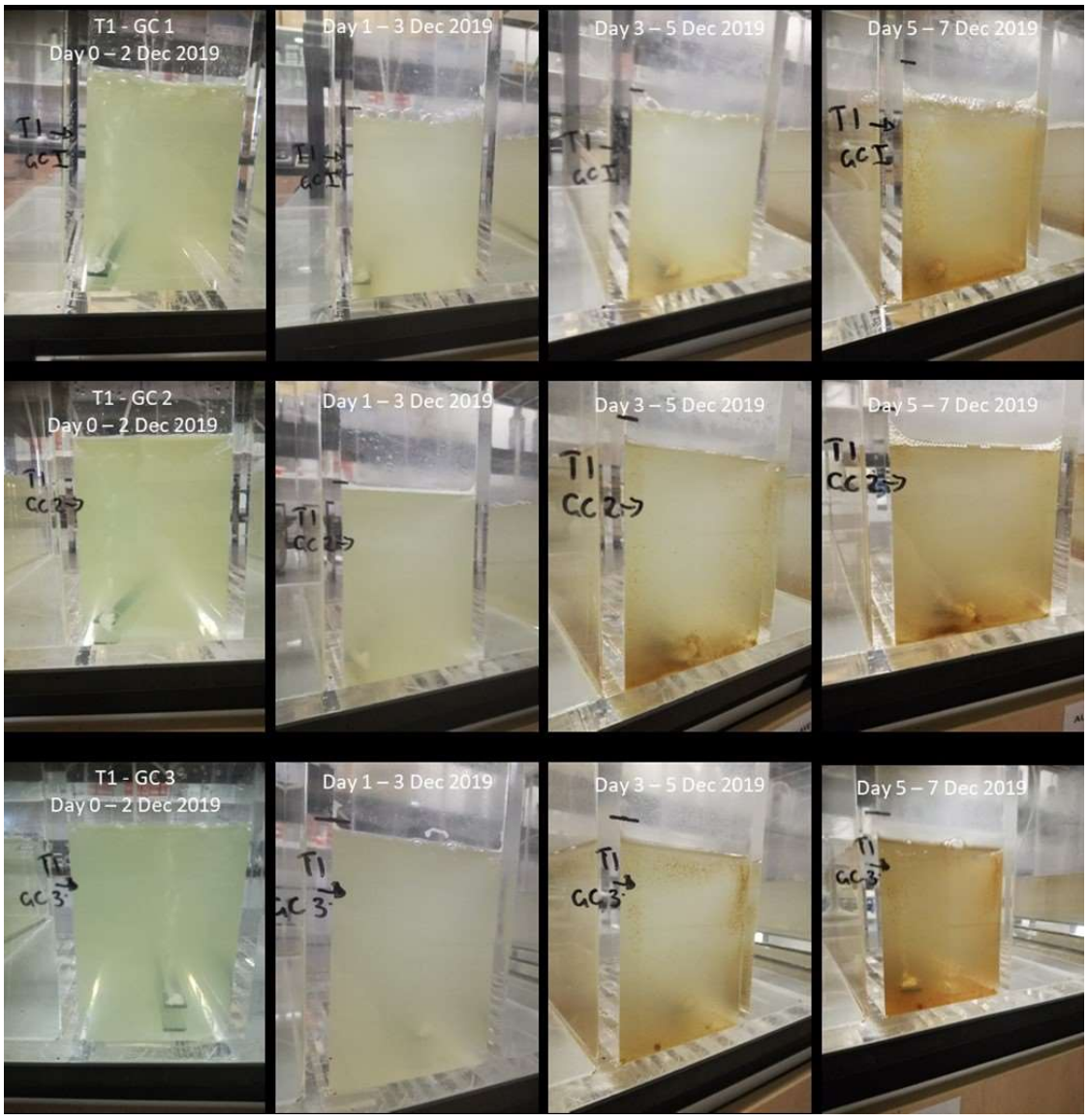


FIGURE 32: EXPERIMENT RUN 1 - TANK 1 PHOTOGRAPHS FROM DAY ZERO TO FIVE

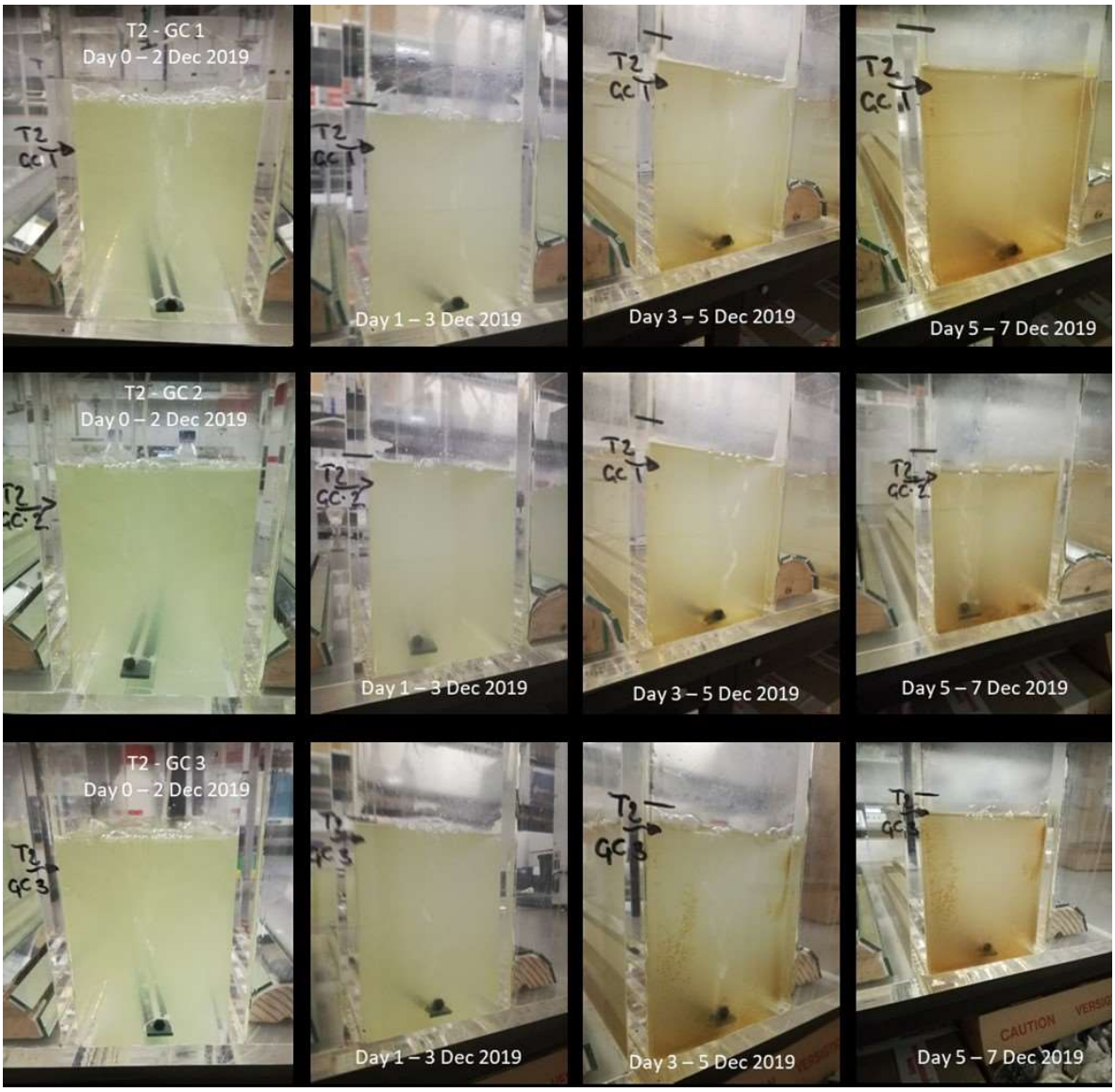


FIGURE 33: EXPERIMENT RUN 1 - TANK 2 PHOTOGRAPHS FROM DAY ZERO TO FIVE

5.2 Experiment Run 2, 3 and 4

This section details the results from experimental runs 2, 3, and 4. Following each experimental run, the two PBR tanks were cleaned and then prepared as detailed in Chapter 4: Laboratory Studies: Methodology. The algae culture was prepared and introduced into each growth cell along with the nutrient sources. The second, third and fourth experimental runs were carried out from 10 December 2019 (day zero) to 17 December 2019 (day seven), 6 January 2020 (day zero) to 13 January 2020 (day seven) and the 18 January 2020 (day zero) to 25 January 2020 (day seven) respectively.

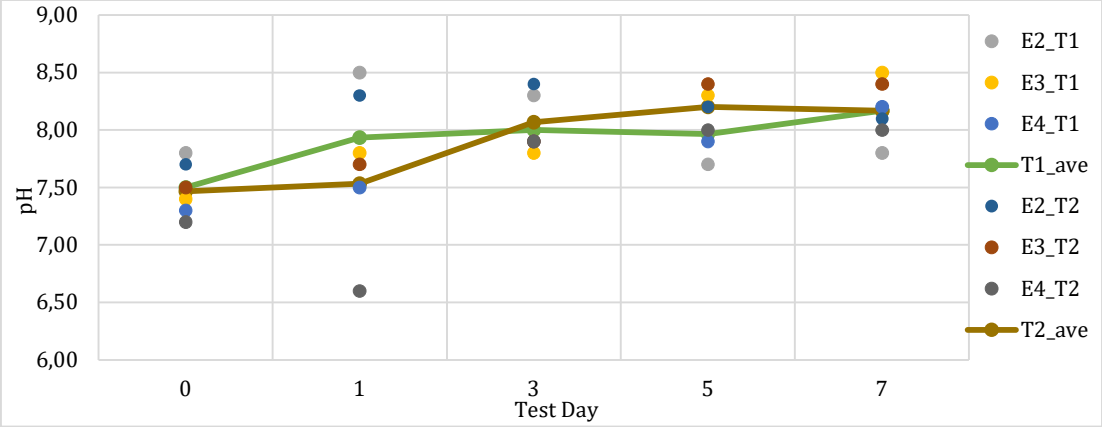
Three adjustments were made to these three experimental runs from experimental run 1. The first adjustment was that a combined composite sample was tested instead of each growth segment. The second adjustment was that thee nutrient concentration was reduced by lowering the volume of each nutrient source to 10 ml from 18 ml. This was done to reduce the initial nutrient concentration to prevent the measured parameters to be within the test kits range of measurement. The third adjustment was the light source for each tank was provided for by a timer controlled overhead lighting systems that provided both tanks with the same light intensity for the same duration each day. The laboratories fluorescent light provided an inconsistent amount of light and the duration varied.

A set of selected water quality parameters were measured on day zero, one, three, five and seven as described in Chapter 4. The full set of results can be found in Appendix 4 and the summary of results are shown below.

5.2.1 pH

Figure 34 below illustrates the pH readings recorded on test day zero, one, three, five, and seven. On day zero, the minimum and maximum pH reading in tank 1 were 7.3 and 7.8 and for tank 2, the minimum and maximum pH reading were 7.2 and 7.7. The average pH reading for day zero for tank 1 and 2 were 7.5 and 7.47 respectively. On day one, the minimum and maximum pH reading in tank 1 were 7.5 and 8.5. For tank 2, the minimum and maximum pH reading on the day were 6.6 and 8.3. The average pH reading for day one for tank 1 and 2 were 7.93 and 7.53 respectively.

The minimum, maximum, and average pH readings gradually increased in both tanks over the test period. On day seven, the minimum and maximum pH reading in tank 1 were 7.8 and 8.5. For tank 2, the minimum and maximum pH reading on day seven were 8.0 and 8.4. The average pH reading for day seven for tank 1 and 2 were both 8.17. The average percentage increase in pH for tank 1 and tank 2 was 8.89% and 9.38% respectively over the experiment's period.



E# references the Experiment Number.
T# References the tank number.

FIGURE 34: EXPERIMENT RUN 2, 3, AND 4 - PH RESULTS

5.2.2 Total Nitrogen

Figure 35 below illustrates the TN concentrations recorded on test day zero, one, three, five, and seven. The measurement generally was clustered around the averages for each tank except for a few outliers.

On day zero, the minimum, maximum, and average TN concentration in tank 1 were 55.00, 80.00, and 67.33 mg/L respectively. The minimum, maximum, and average TN concentration in tank 2 were 67.00, 75.00, and 69.67 mg/L respectively. The minimum, maximum, and average TN concentration remained constant between day one and three with only minor reduction variation recorded. In each of the experiment run, the TN concentration decreased from day zero until day five.

On day seven, the minimum, maximum, and average TN concentration in tank 1 were 62.00, 78.00, and 69.67 mg/L respectively. The minimum, maximum, and average TN concentration in tank 1 are 72.00, 97.00, and 81.67 mg/L respectively. The concentration profiles of TN were similar for all three tests as seen in Figure 35 below. It can be seen that for all three experimental runs and the average concentration for each tank there is an apparent linear decrease in the TN concentration from day zero until day five.

The average TN decrease over the initial five days was 21.29% and 32.06% for tank 1 and 2 respectively. However, there was an increase in the TN concentration on day seven. This resulted in an overall increase in TN concentration of 3.47% and 17.22% for tank 1 and 2 respectfully.

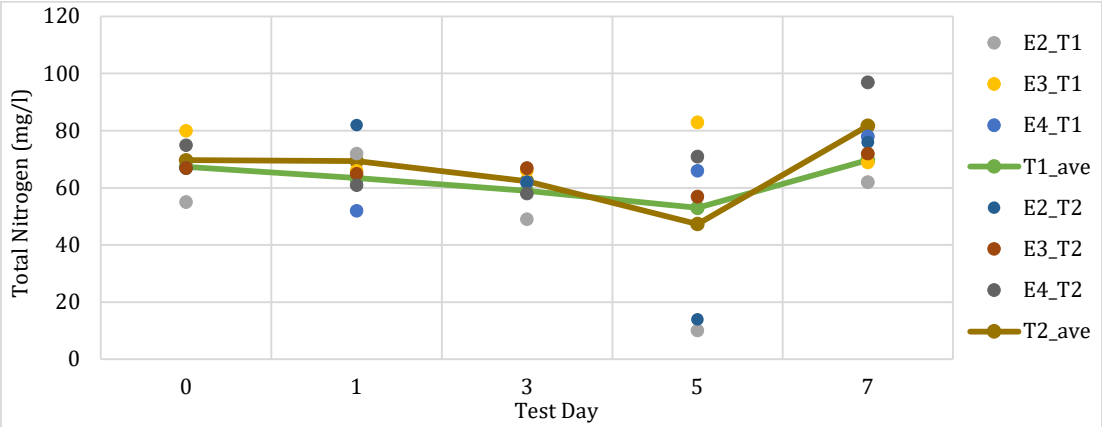


FIGURE 35: EXPERIMENT RUN 2, 3, AND 4 - TOTAL NITROGEN

5.2.3 Ammonia

Figure 36 below illustrates the ammonia concentrations recorded on test day zero, one, three, five, and seven. On day zero, the minimum, maximum, and average ammonia concentration in tank 1 were 1.70, 4.60, and 3.07 mg/L respectively. The minimum, maximum, and average ammonia concentration in tank 2 was 1.70, 4.50, and 3.07 mg/L respectively. The minimum, maximum, and average ammonia concentration increased from day zero until day five. On day five, the minimum, maximum, and average ammonia concentration in tank 1 were 6.9, 8.6, and 7.73 mg/L respectively. The minimum, maximum, and average ammonia concentration in tank 2 was 6.80, 8.20, and 7.73 mg/L respectively.

The ammonia concentration decreased from day five to seven where tank 1 minimum, maximum and average ammonia concentration was 3.40, 7.80, and 5.90 mg/L respectively, which represents an average decrease of 31.07% between test days. Tank 2 minimum, maximum, and average ammonia concentration were 4.10, 7.10 and 6.07 mg/L respectively, which represented a 27.47% decrease between test days.

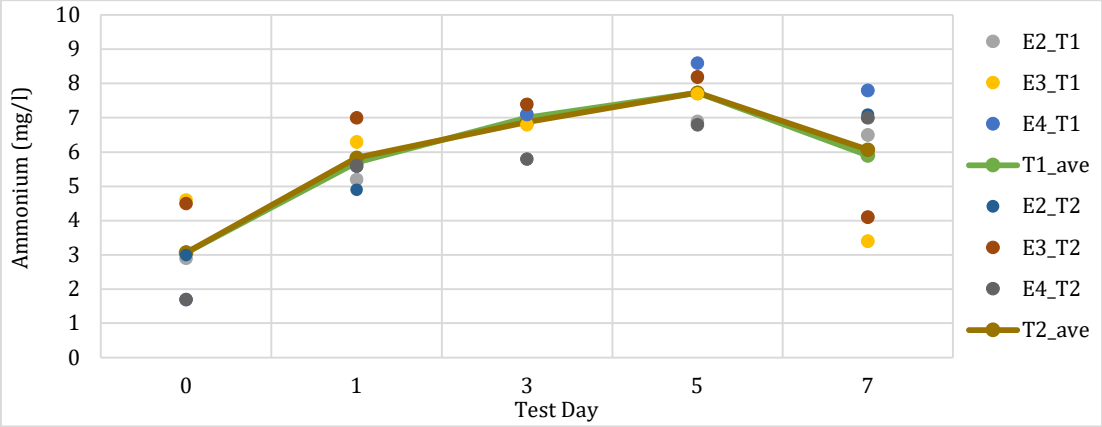


FIGURE 36: EXPERIMENT RUN 2, 3, AND 4 - AMMONIA

5.2.4 Nitrate

Figure 37 below illustrates the nitrate concentrations recorded on test day zero, one, three, five, and seven. On day zero, the minimum, maximum, and average nitrate concentration in tank 1 were 12.50, 17.40, and 15.43 mg/L respectively. The minimum, maximum, and average nitrate concentration in tank 2 were 12.50, 17.70, and 15.83 mg/L respectively. On day one, there was a decrease in the nitrate concentration in tank 2 for all three experimental runs; however, this only occurred on experimental run two in tank 1. For the experimental run three and four, tank 1 experienced an increase in nitrate concentration. On day one, the minimum, maximum, and average nitrate concentration in tank 1 were 7.20, 21.00, and 15.17 mg/L respectively. The minimum, maximum, and average nitrate concentration in tank 2 were 10.60, 17.70, and 13.63 mg/L respectively.

The minimum, maximum, and average nitrate concentration increased throughout the experiments. On day seven, the minimum, maximum, and average ammonia concentration in tank 1 were 15.7, 19.40, and 17.70 mg/L respectively. The minimum, maximum, and average ammonia concentration in tank 2 was 19.70, 21.30, and 19.47 mg/L respectively. The measured nitrate concentrations were not clustered closely around their calculated averages as seen in Figure 37 below. Tank 2 experienced a nitrate concentration reduction of 16.14% from day zero to day one, but then the concentration increased over the remaining period for each experimental run. This resulted in an overall average increase in the nitrate concentration of 22.95% for tank 2.

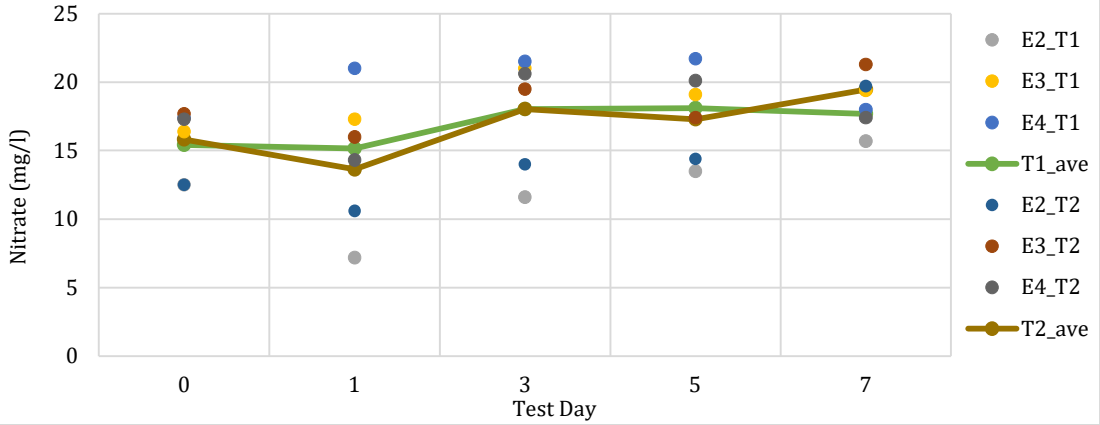


FIGURE 37: EXPERIMENT RUN 2, 3, AND 4 – NITRATE

5.2.5 Phosphates

The phosphate measurements were determined using a colour stick indicator test. The clustering of the measurements does not represent any significant insight into the accuracy of the phosphate testing due to the subjective nature of the strip test used. Figure 38 below illustrates the phosphate concentrations recorded on test day zero, one, three, five, and seven. On day zero, the minimum, maximum, and average phosphate concentration in tanks 1 and 2 were 16, 33, and 21.67 mg/L respectively. On day one, phosphate concentration in tanks 1 and 2 was measured at 16 mg/L for all three experimental runs. There was a gradual decrease in the phosphate concentration until day seven where all three experimental runs and both tank's phosphate concentration was 8 mg/L.

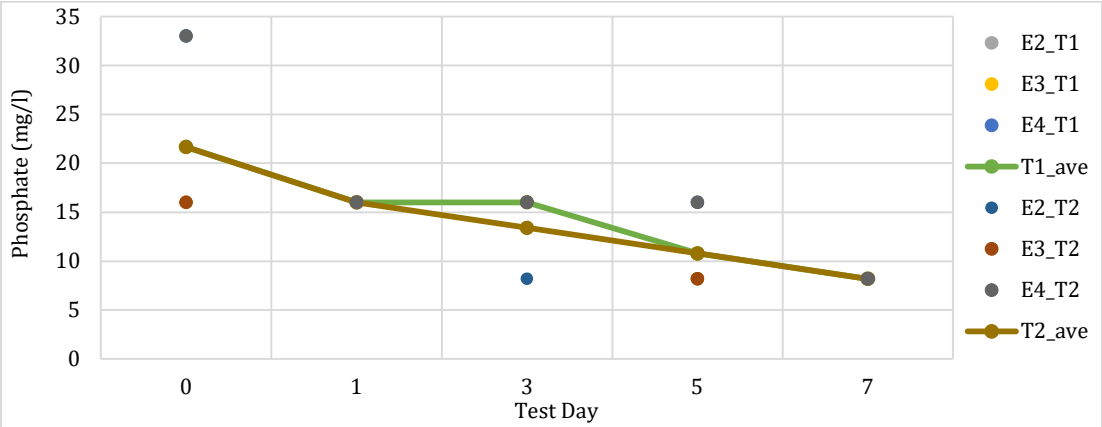


FIGURE 38: EXPERIMENTAL RUN 2, 3, AND 4 - PHOSPHATE CONCENTRATION

5.2.6 Chlorophyll-a

Figure 39 below illustrates the chlorophyll-a concentrations recorded on test day zero, one, three, five, and seven. On day zero, the minimum, maximum, and average chlorophyll-a concentration in tank 1 was 10.04, 18.40, and 14.51 µg/L respectively. The minimum, maximum, and average chlorophyll-a concentration in tank 2 were 8.32, 11.03, and 9.85 µg/L respectively.

It is also noticeable that for tank 1, these increases and decreases between test days were mostly single digits with the largest increase in the concentration of 9.02% on day seven and the largest decrease of 14.38% on day one. Overall, the increase and decrease in concentration cancelled each other out, as there was only a marginal increase in the concentration on day seven when compared to day zero. Tank 2 experienced greater shifts in chlorophyll-a concentration from test day to test day with the largest increase recorded was 30.41% on day five and the largest decrease in concentration was 88.04% on day seven.

The results for tank 1 are not clustered around the average, as there is some measurement that is either considerably higher or lower than the average. However, the clustering of the results for tank 2 is closer to the average concentration. On day seven, the minimum, maximum, and average Chlorophyll-a concentration in tank 1 was 8.33, 20.40, and 14.57 µg/l respectively. The minimum, maximum, and average Chlorophyll-a concentration in tank 2 were 4.66, 9.86, and 7.80 µg/l respectively.

However, using the average concentration, Tank 1 concentration did not vary as dramatically as tank 2 and over the seven-day test period tank 1 concentration increase by 0.43%. In comparison, tank 2 overall performance was dismal as the tank produced a large concentration decrease of 20.83% over the same period. On a test day to test day comparison, in the instances where tank 1 increases its concentration, tank 2 recorded a decrease. This is also true for test days when tank 2 increased its concentration and tank 1 concentration would decrease.

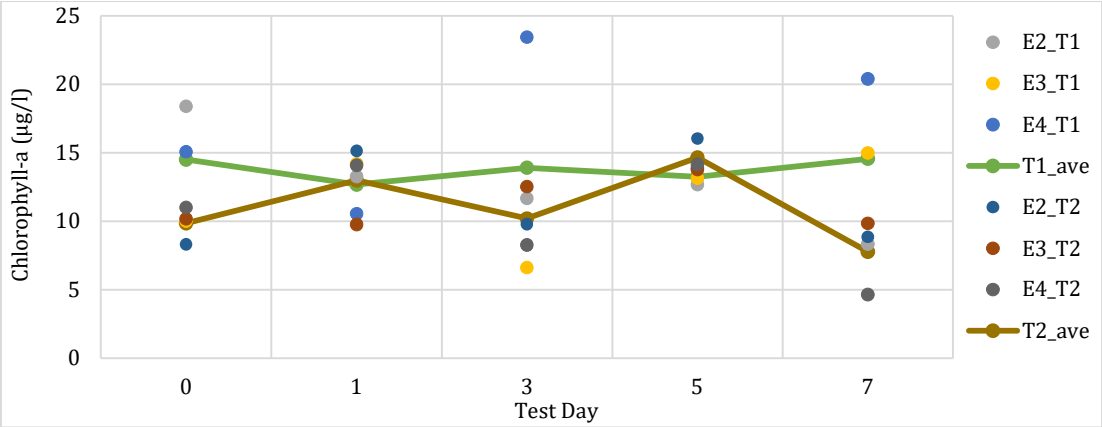


FIGURE 39: EXPERIMENT RUN 2, 3, AND 4 - CHLOROPHYLL-A

5.2.7 Photographic Images take during Experiment 2, 3 and 4

Photographs of each growth segments of each tank were taken at approximately the same time in the day and before any of the samples being taken. The photographs were also taken at a similar position. Figure 40 and Figure 41 below are the photographs for each tank from day zero to seven for experimental run 2. For experimental run 3, Figure 42 and Figure 43 below are the photographs for tank 1 and tank 2 from day zero until day seven. For experimental run 4, Figure 44 and Figure 45 below are the photographs for tank 1 and tank 2 from day zero to seven.

For experimental run 2, both sets of photographs show that there was a sudden accumulation of biofilm growth from day one until day three and a relative unchanged image from day three through until day seven. There also seems to be an accumulation of matter on the floor of each growth segment. These observations hold for both tank 1 and tank 2. The liquid colour however does not change throughout the test cycle and remains a murky greenish colour.

It was observed during experimental run 3, that both tanks experienced an increase in biofilm accumulation between day zero and day one where the colour had a slight brown colour. On day three, the colour changed to brown with matter accumulation on the bottom of each tank. The images showed very little change in the culture's colour from day three through until day seven.

It was observed in experimental run 4, that both tanks experienced an increase in biofilm growth from day one to day three and a relatively stationary growth from day three through until day seven. The tanks colour changes from a murky green colour to a brown colour from day one to day three and then the colour did not change for the remainder of the experimental run. Similar to experimental runs 2 and 3 there was an accumulation of matter at the bottom of each growth segment.

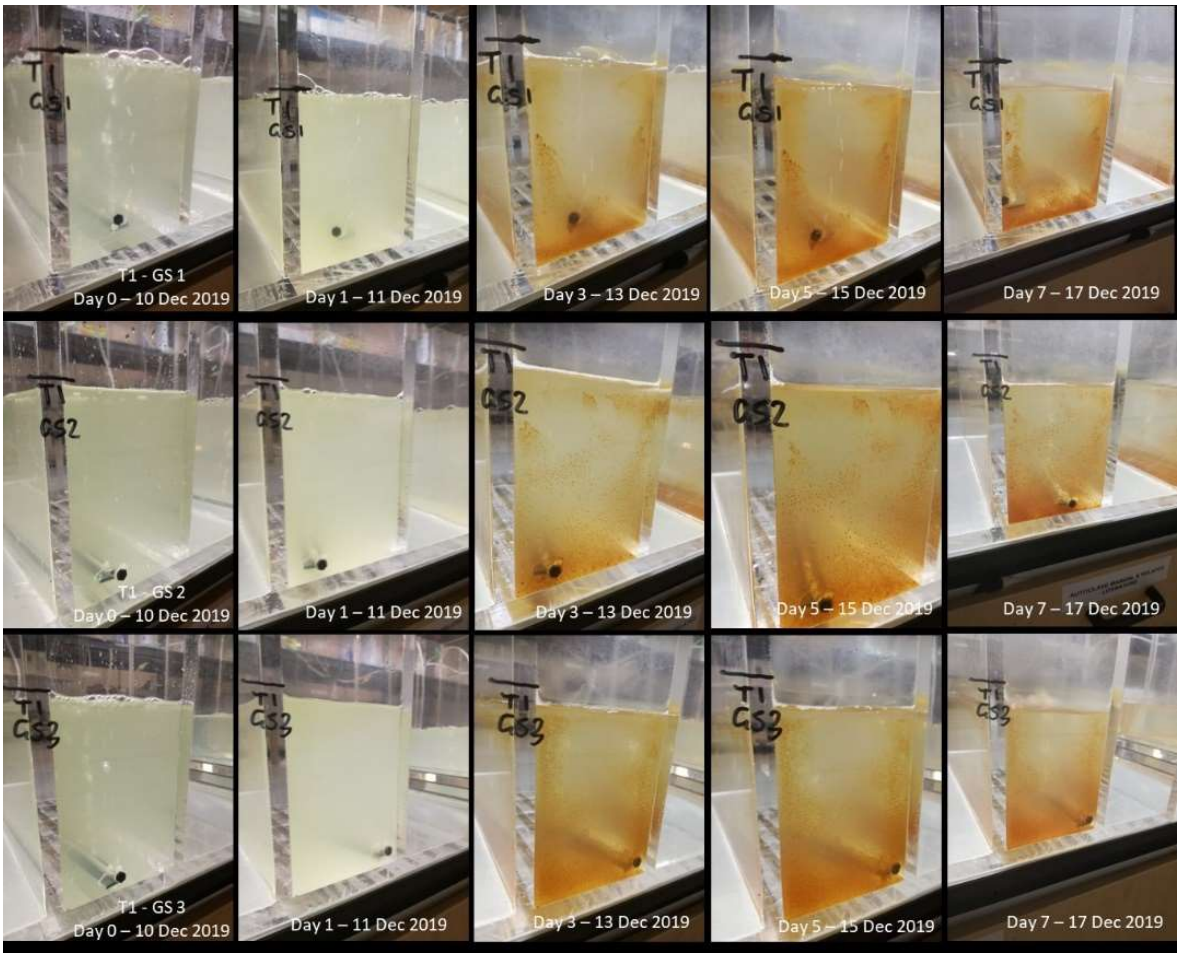


FIGURE 40: EXPERIMENT RUN 2 - TANK 1 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

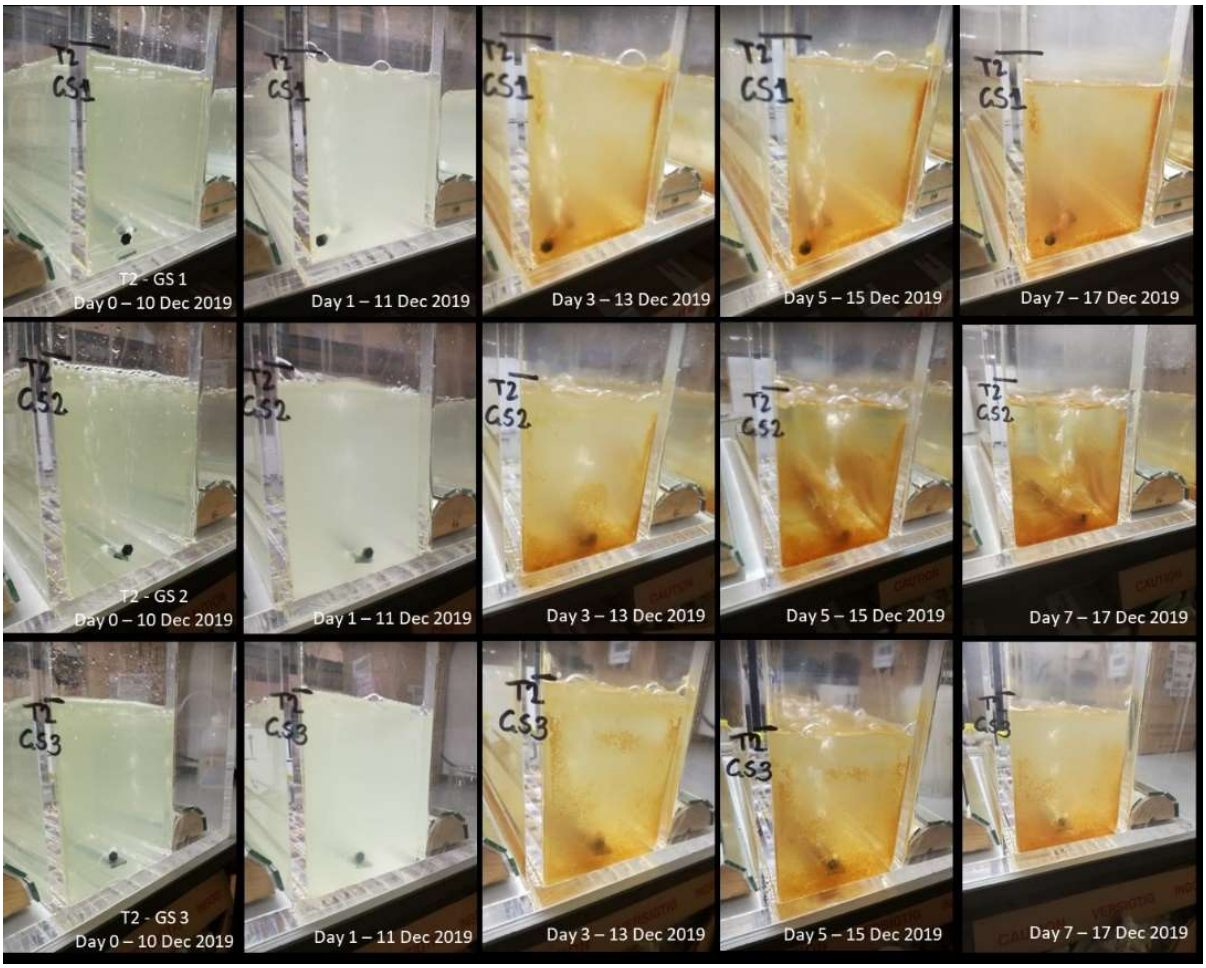


FIGURE 41: EXPERIMENT RUN 2 - TANK 2 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

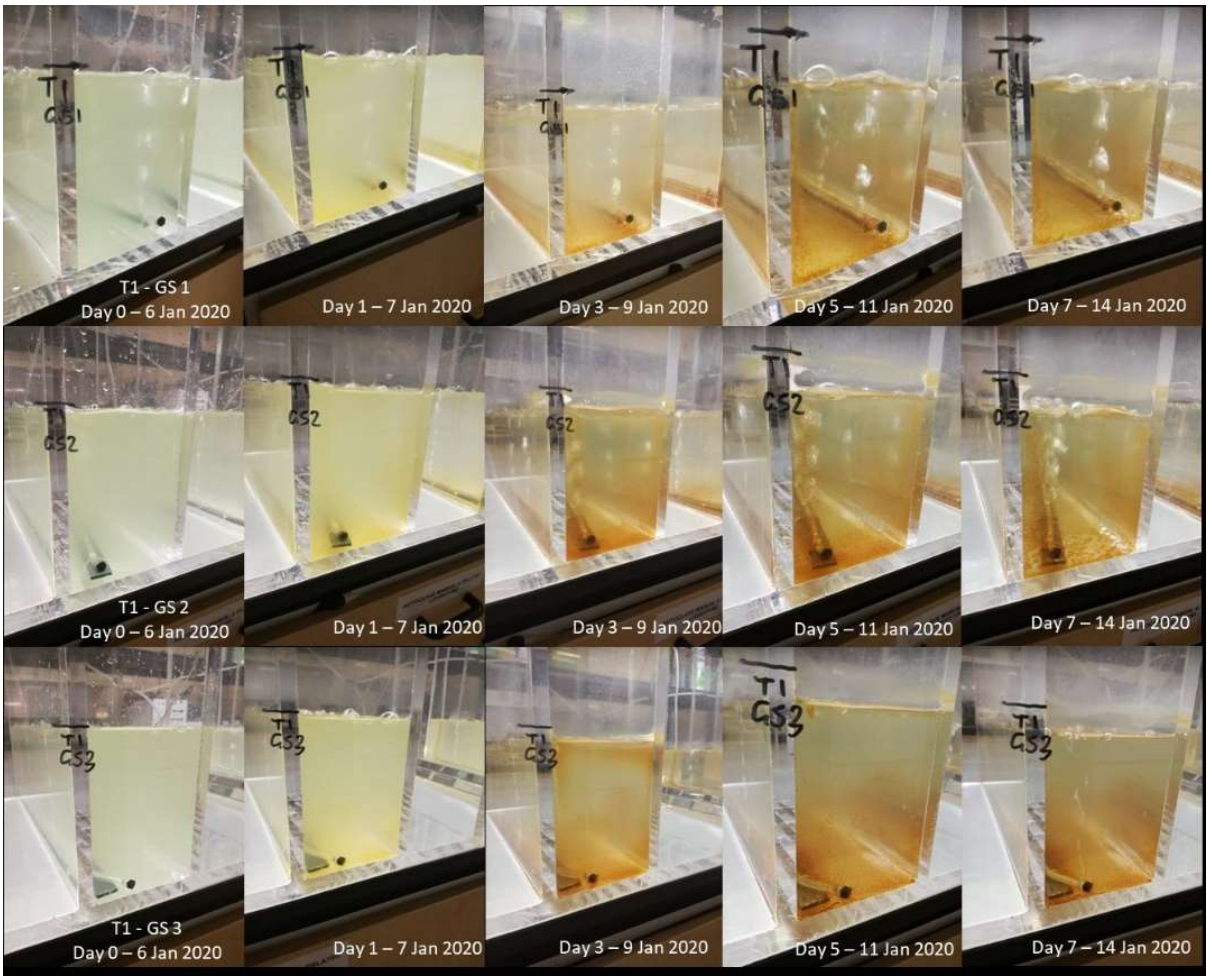


FIGURE 42: EXPERIMENT RUN 3 - TANK 1 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

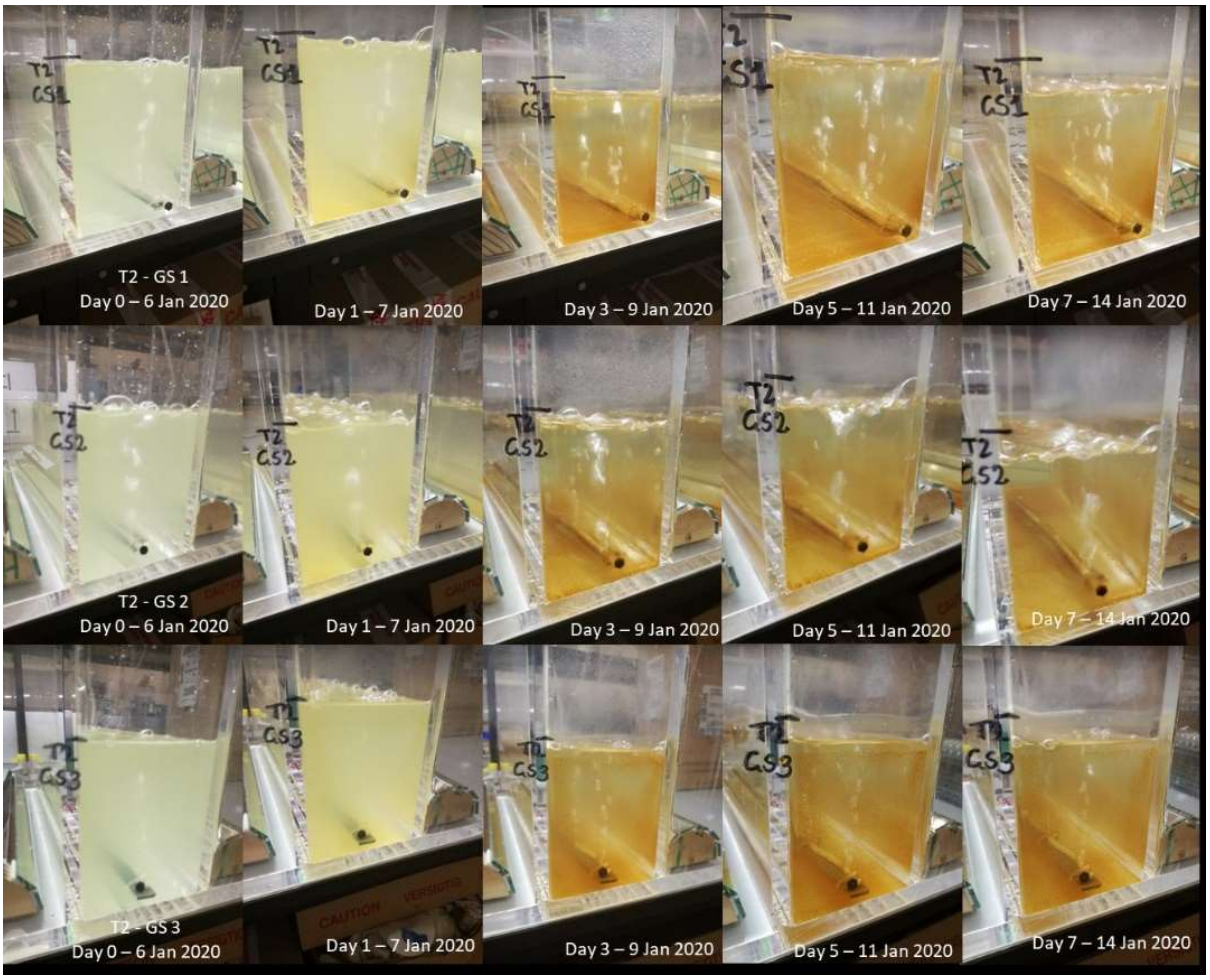


FIGURE 43: EXPERIMENT RUN 3 - TANK 2 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

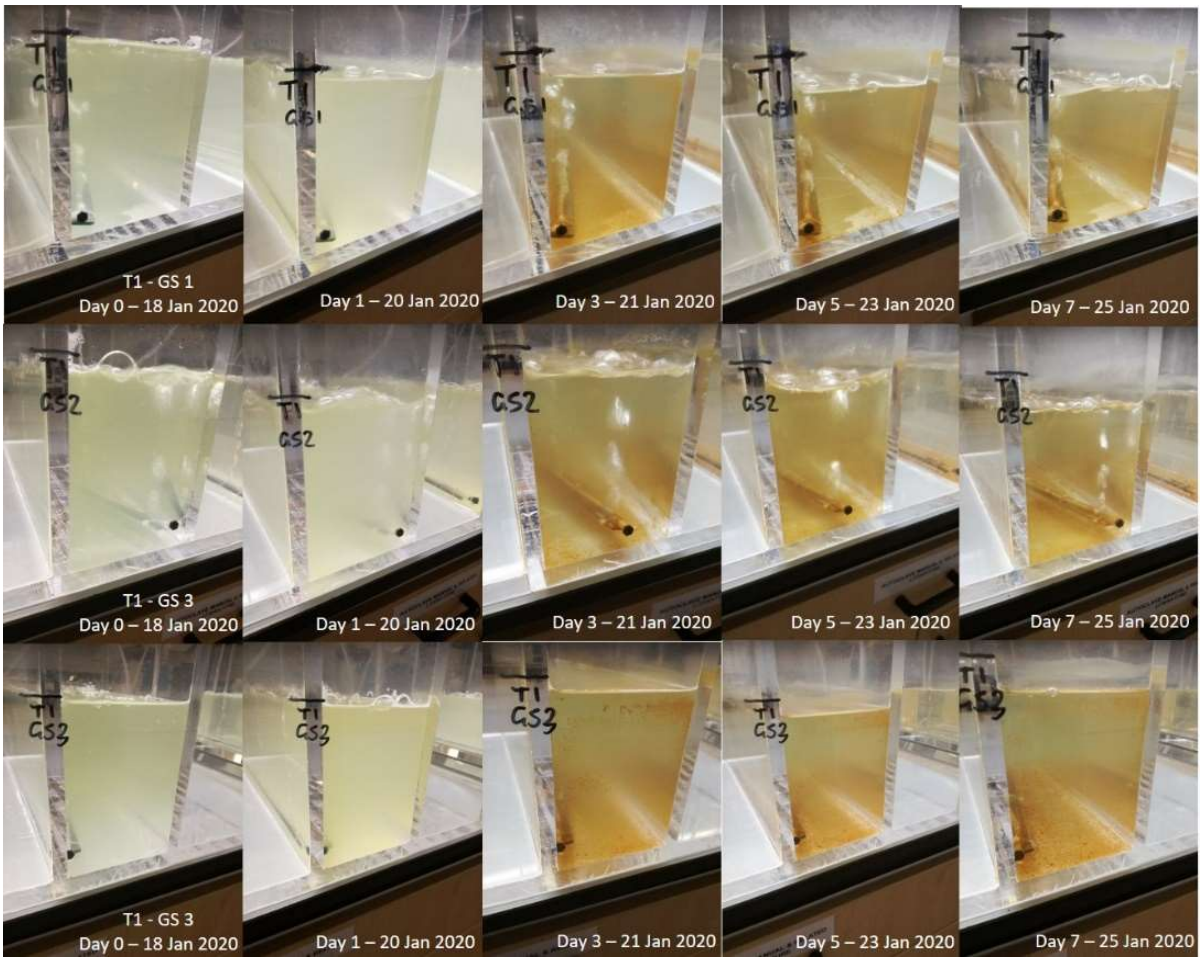


FIGURE 44: EXPERIMENT RUN 4 - TANK 1 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

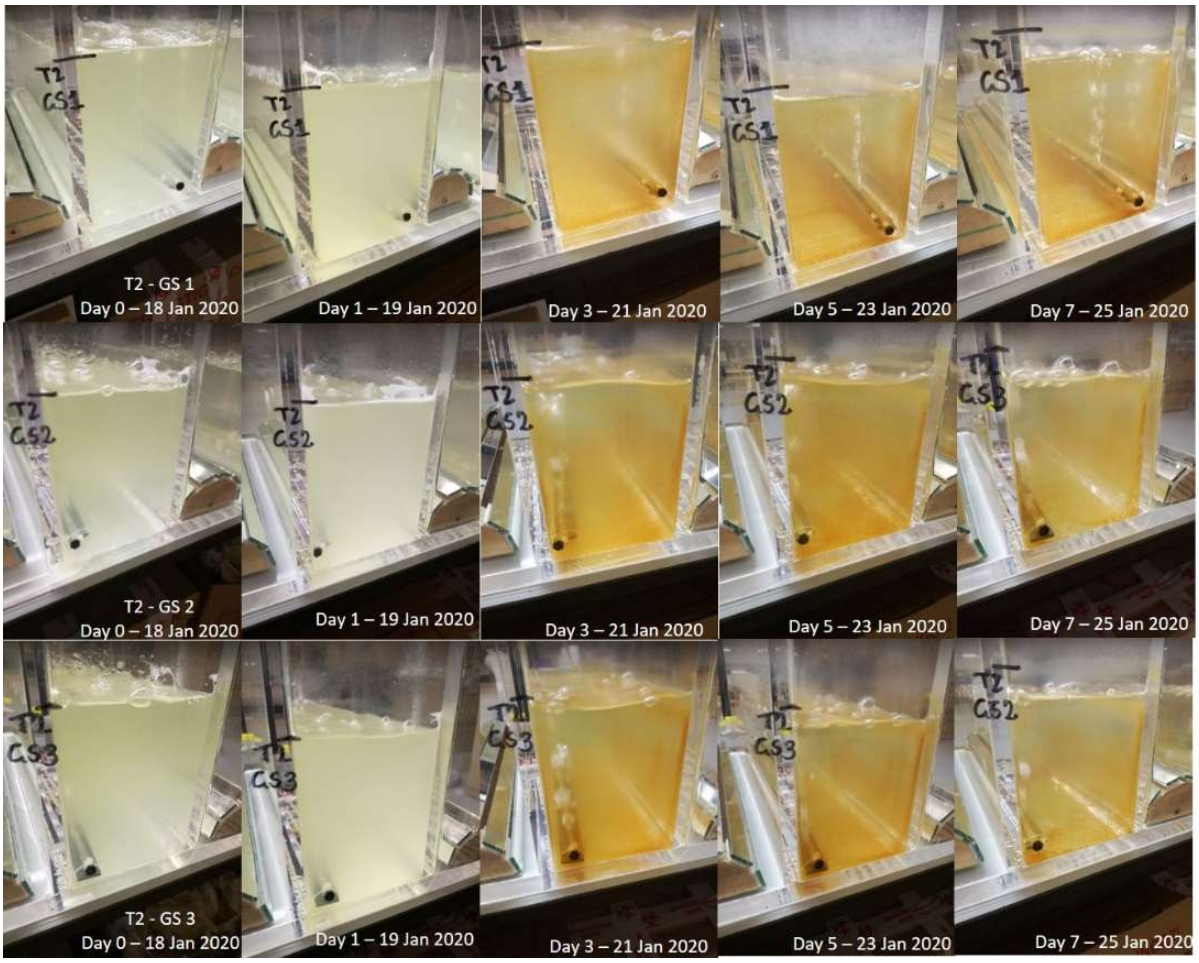


FIGURE 45: EXPERIMENT RUN 4 - TANK 2 PHOTOGRAPHS FROM DAY ZERO TO SEVEN

Chapter 6: Discussion and Conclusion

This chapter discusses the results obtained from laboratory studies and concludes the research. The discussion only considers results from experimental runs two, three, and four. The results from experimental run one are not discussed in this chapter as it was preliminary work, whose main purpose was to generate data to assist with modifying experimental design where needed.

6.1 pH

The pH was measured at the beginning of each of the experimental test days and it was observed that the pH increased from almost neutral to more basic as the experiment proceeded. It is suggested by Koca et al. (2007) that there is a linear relationship between an increase in pH and degradation of chlorophyll-a concentration (Koca, Karadeniz and Selen Burdurlu, 2007). The increase in pH over the test period and the observed colour change and matter accumulation on the bottom of each growth segment correlate with the decrease in chlorophyll-a concentration over the test period and the corresponding increase in the nutrient levels at the end of each experiment, is in line with the relationship found by Koca et al. (2007)

6.2 Nitrogen

6.2.1 Total Nitrogen

There is an apparent linear decrease in the TN concentration from day zero until day five. Day five results were the lowest TN concentration in both tanks. On the final day of testing, the TN concentration would increase to above the initial concentration readings. The average TN reduction over the initial five days was 21.29% and 32.06% for tank 1 and 2 respectively, however with the increase recorded on day seven. This resulted in an overall increase in TN concentration of 3.47% and 17.22% for tank 1 and 2 respectively.

TN reduction rates between 21.29% and 32.06% are considerably lower than the results recorded by Singh et al. (2017) who recorded TN reduction rates between 78.3 % and 87,9% for *Chlorella* (Singh, Birru and Sibi, 2017). It is important to note the differences in the experimental setup between the work done by Singh et al. (2017) and the work done in this study. For example, Singh et al. (2017) used municipal wastewater diluted with distilled water as a substrate for algae and not the nutrient mix as was the case with this study. The second difference is that their experiment was conducted over a duration of ten days compared with this experiment that was conducted over a duration of seven days.

The removal rate found by Wang et al. (2010) was also significantly greater than what was records in this study. This study used wastewater collected from a local wastewater treatment plant and at four process locations. The locations were before the primary settling tank, after the primary settling tank, after activated sludge tank and centrate from sludge centrifuge. Wang et al. (2010) reported removal rates of 68.4%, 68.5%, 50.8 % and 82.8% compared to the maximum removal rate of 32% from this study for tank 2 on day five (Wang *et al.*, 2010). A possible reason for the large discrepancies between results by Wang et al. (2010) and this study is the wastewater used by Wang was collected at four different locations within the wastewater treatment works, which all contained different levels of inorganics and organics. Through the incorporation of an organic substrate, the *Chlorella* growth is accelerated (Wang *et al.*, 2010). In comparison, this research used an enriched nutrient solution with a limited number of nutrients.

6.2.2 Ammonia

The Ammonia concentration followed a similar pattern for both tanks with a low concentration reading on day zero and a steady increase in concentration until day five. On day seven, there is a decrease in the concentration readings but the overall increase in ammonia concentration was 92.39% and 97.83% for tank 1 and 2 respectively. Choi and Lee (2013) reported that *Chlorella Vulgaris* effectively removed ammonia from wastewater when the initial ammonia concentrations were between 5.22-25.24 mg/L (Choi and Lee, 2013). During these experimental runs, the ammonia was not removed from *Chlorella Vulgaris* but the ammonia concentrations increased over the test period.

This could be attributed to the limited growth of algae as was seen with the decrease of the chlorophyll-a concentrations.

6.2.3 Nitrate

The measured nitrate concentrations were not clustered closely around their calculated tank averages; however, both tanks average measurements followed a similar trend. Tank 2 experienced a nitrate reduction of 16.14% between day zero to day one, but then the concentration increased over the remaining period for each experimental run. This resulted in an overall average increase in the nitrate concentration of 22.95% for tank 2.

Tank 1 followed a similar trend to tank 2, except there was a small reduction of 1.76% in initial nitrate concentration from day zero until day one and the overall average increase in nitrate concentration was 14.69% over the entire experimental run. It was expected that nitrate concentration would decrease over the experimental run as it is removed by *Chlorella Vulgaris*.

For tank 2, the correlation between the relative opposite relationship between nutrient concentration and algae growth can be seen. The change from day zero to day one saw a decrease in the nitrate concentration and an increase in the Chlorophyll-a concentration of 24.26% between these days. Similar on day seven, the Chlorophyll-a concentration experiences a decrease and there was a corresponding increase in the nitrates concentration.

There is an established correlation between the different nitrogen species concentrations and chlorophyll-a growth. Research by Bbalali et. Al (2013) showed that when the concentration of dissolved nitrogen species, nitrate, nitrite and ammonia, was at their lowest, the chlorophyll-a concentration was at its highest (Bbalali *et al.*, 2013). This correlation was best presented on day one in tank 2 where the Chlorophyll-a concentration was high and the nitrate concentration was at its lowest measured concentration. Then on day seven, the nitrate concentration was at its peak while the Chlorophyll-a concentration was at the lowest reading.

A range of 6.8 – 10 is considered the optimal organic N/P ratio for the growth of freshwater algae (Wang *et al.*, 2010). The average N/P ratio for tank 1 and tank 2 on day one is 0.71 and 0.72 respectively, which indicates there is a high nitrogen limitation as the ratios are much lower than the optimal ratio. This ratio improves throughout the experiment with tank 1 and tank 2 ratios on day seven being 2.16 and 2.37 respectively, however, these ratios are still significantly below the optimum range.

6.3 Phosphate

The phosphate measurements were determined using a colour stick indicator test that had a large measurement range between the different colour indicators that produced a linear relationship. This method of measurement may lead to subjective results as a person doing the test might not be able to determine where the colour on the test stick correlated to the colours within the measurement range, especially when the phosphate concentration is below 10mg/L.

The clustering of the measurement around their respective tank averages does not represent any significant insight into the accuracy of the phosphate testing due to the limited measurement ranges and to the subjective nature of the strip-test used.

However, both tanks exhibited a similar linear decrease in phosphate concentration over the eight days. Bbalali et al. (2013) reported that there is no significate correlation between chlorophyll-a and phosphorus depletion (Bbalali *et al.*, 2013) and Havens (2004) found that there was no support for the view that coloured lakes produce less Chlorophyll-a per unit of TP than clear lakes (Havens, 2004).

Phosphate was the only nutrient whose concentrations decreased over the entire test period as both tanks average phosphate concentration reduced by 62.15% for all three experimental runs. Delgadillo- Mirquez et al. (2016) found that a combination of algae and bacteria culture was able to remove between 72 to 83%. (Delgadillo-Mirquez *et al.*, 2016).

6.4 Chlorophyll-a

For each experimental run, the same procedure was followed however, there was a considerable difference in the initial measured concentration in each tank on day zero for experimental run two and four. The differences in the initial chlorophyll concentration make it difficult to link changes in chlorophyll concentration to the growth of algal biomass during the experiments. In setting up different experimental runs, 500 ml of algal stock from each of three one-litre Schott algae stock bottles were mixed with water and nutrient sources. It is important to reiterate that the 500 ml were not all taken from the same up-scaled culture although they were all from the original culture stock.

Examining the average Chlorophyll-a concentration over the seven-day test period, tank 1 experienced a relatively stable concentration with an overall marginal increase of 0.43%. In comparison, the overall Chlorophyll-a concentration in tank 2 was disappointing as the tank produced a large concentration decrease of 20.83% over the seven-day test period. On a test day to test day comparison, in the instances where tank 1 increased its concentration, tank 2 recorded a decrease. This is also true for test days when tank 2 increased its concentration and tank 1 concentration would decrease.

It is important to note that for tank 1, the fluctuations between test days were mostly single digits with the largest increase in the concentration of 9.02% on day seven and the largest decrease of 14.38% on day one. Tank 2 experienced greater shifts in chlorophyll-a concentrations from test day to test day with the largest increase recorded being 30.41% on day five and the largest drop being 88.04% on day seven. When this decrease is scrutinised with the increase in the concentration of the substrate towards the end of each experimental run, the accumulation of visual matter observed in the photographic evidence and the decrease in the chlorophyll-a concentration could be due to algal death.

The light and dark cycles that were imposed on each of the photobioreactor tanks was through overhead lighting set to illuminate the reactor for 12 hours a day and then it was switched off for 12 hours. This was constant for all three of the experimental runs and tank 2 had reflective mirrors, which increased the incident light transfer by reflecting light into the sides of the column for each of the growth segment. Each growth segment was mixed thoroughly by air being pumped into the bottom of each segment. "Beside light transfer, the most important task of photo-bioreactors is to feed the algal cells with carbon dioxide for photosynthesis and to remove the produced oxygen from the medium" (Posten, 2009).

From the experiments completed during this research, it can be seen that the modification to the light regime does not always yield increased algal growth and biomass production. A possible explanation is that the level of enhancement achieved may have been within the range that trigger photoinhibition for *Chlorella Vulgarus*. In the natural environment, the light-dark cycles usually do not include 12-hour bouts of light bombardment.

6.5 Conclusion

This report aimed to find out the extent to which the increasing light intensity within a novel PBR, would increase biomass production. A mathematic model predicted that this objective is achievable with a large increase in biomass productivity predicted. The predictions obtained from the models informed that through the introduction of mirrors the algae productivity would be increased by between 15 – 20 %. In this study, four experimental runs were carried out from 2 December 2019 to 25 January 2020. In contrast, the data obtained from the experiments showed a decrease in phosphate concentration while the remaining nutrient concentrations increased over the experimental period. The Chlorophyll-a concentration in tanks with the light enhancement saw two significate daily increases. The overall Chlorophyll-a concentration for the tank, with the light enhancement, saw decreases greater than 20% at the end of the experiments. The average Chlorophyll-a concentration in the tank without any light enhancement saw a small increase of 0.43% over the duration of the experiment.

The unexpected difference between the model predictions and the experimental results could be attributed to several possible reasons. The light intensity used in the model was provided from the De-Aar weather station and represented either the summer or winter solstice. The light intensity provided during the experimental runs was supplied by an overhead fluoresce light, which provided 12 continuous hours of light and 12 continuous hours of darkness. It is difficult to make comparisons between the model prediction and the experimental results as the light intensity was not varied to mimic a natural day's light intensity, and it was not measured. Another factor is the long light/dark cycles that provided excessive illumination to both tanks could have resulted in photoinhibition and this was not incorporated into the mathematical modelling.

In conclusion, a tank with a small compartment and enhanced light distribution, using mirrors, did not yield any improvements to algae growth when compared with a tank with small compartments and no light enhancement mirrors as predicted in the modelling of these tanks.

Chapter 7: Recommendations for Future Research

The cost-effective treatment of municipal wastewater and the protection of water resources are of global interest. The search for a viable and sustainable energy-rich alternative to fossil fuels is of global interest. Therefore, the provision of energy-rich oil through the treatment of municipal sewage could provide a unique solution to these two problems.

The models, PBR design and growth parameters utilised for this research are based on available literature. However, the results from the experiments were not in line with what was expected. Several explanations for the departure from what was expected are provided in previous chapters. Further testing of the efficiency of the novel photobioreactor design should be carried out with several alternative parameters. For example, experiments could be carried out using different algal species and mixed-cultures could also be tried out instead of only using pure cultures. Water quality parameter testing can also be measured on a more frequent basis so that changes can be monitored more closely.

In addition, the light intensity provided in a PBR is one of the primary factors that affect growth. Further research should incorporate calibrated light intensity, which mimics the natural environment.

Finally, the use of physical municipal wastewater collected at various process stages should be considered for future research. This would provide insight into whether PBRs are suitable for use in either primary, secondary, or tertiary treatment processes. The nutrient concentration and their ratios have a direct impact on the growth productivity within PBR.

References

Abu-Orf, M., Bowden, G., Pfrang, W., Tchobanoglous, G.. Stensel, H., Tsuchiashi, R. Burton, F. (2014) *Wastewater Engineering Treatment and Resource Recovery*. 5th Editio. McGraw-Hill Education.

Adir, N. *et al.* (2003) *Photoinhibition-a historical perspective **, *Photosynthesis Research*. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.326.8143&rep=rep1&type=pdf (Accessed: 7 December 2018).

Afriforum (2020) *AfriForum announces its blue and green drop report - AfriForum*. Available at: https://afriforum.co.za/en/afriforum-announces-its-blue-and-green-drop-report/ (Accessed: 21 December 2020).

Aiba, S. (1982) 'Growth kinetics of photosynthetic microorganism', *Microbial reactions*, (Springer, Berlin, Heidelberg), pp. 85–156.

Al-hadabi, H. and Al-balushi, T. (2012) 'A Critical Review of Wastewater Treatment in Photobioreactors for Improving Microalgae Growth', *Proceedings of the World Congress on Engineering 2012 Vol III*, III, pp. 4–6.

Algae - Types Of Algae - Various, Pigments, Divisions, and Chlorophylls - JRank Articles (no date). Available at: https://science.jrank.org/pages/200/Algae-Types-algae.html (Accessed: 28 December 2020).

Andersen, R. A. (2005) *Algal Culturing Techniques*. 1st Editio. Elsevier Inc.

Andrews, J. F. (1968) 'A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates', *Biotechnology and Bioengineering*, 10(6), pp. 707–723. doi: 10.1002/bit.260100602.

Bbalali, S. *et al.* (2013) 'Relationships between nutrients and chlorophyll-a concentration in the international Alma Gol Wetland, Iran', *Journal of Aquaculture Research and Development*, 4(3). doi: 10.4172/2155-9546.1000173.

Bernard, O. and Rémond, B. (2012) 'Validation of a simple model accounting for light and temperature effect on microalgal growth', *Bioresource Technology*, 123, pp. 520–527. doi: 10.1016/j.biortech.2012.07.022.

Biggs, D. C. (1973) 'Klipspruit Sewage Purification Works', *The Civil Engineer in South Africa*, pp. 225–227.

Blair, M. F., Kokabian, B. and Gude, V. G. (2014) 'Light and growth medium effect on Chlorella vulgaris biomass production', *Journal of Environmental Chemical Engineering*, 2(1), pp. 665–674. doi: 10.1016/j.jece.2013.11.005.

Blanken, W. *et al.* (2016) 'Predicting microalgae growth', *Algal Research*, 14, pp. 28–38. doi: 10.1016/j.algal.2015.12.020.

Caperon, J. and Meyer, J. (1972) 'Nitrogen-limited growth of marine phytoplankton-I. changes in population characteristics with the steady-state growth rate', *Deep-Sea Research and Oceanographic Abstracts*, 19(9), pp. 601–618. doi: 10.1016/0011-7471(72)90089-7.

Carvalho, A. P. *et al.* (2011) 'Light requirements in microalgal photobioreactors: An overview of biophotonic aspects', *Applied Microbiology and Biotechnology*, 89(5), pp. 1275–1288. doi: 10.1007/s00253-010-3047-8.

Chirwa, T. and Nkgoeng, M. (2016) *Testing of a Novel Photobioreactor*. doi: 10.1115/1.802915.ch1.

Chiu, C. *et al.* (2008) 'Evaluating the Efficiency of a novel Photobioreactor Design', pp. 0–18.

Choi, H. J. and Lee, S. M. (2012) 'Effects of microalgae on the removal of nutrients from wastewater: Various concentrations of Chlorella vulgaris', *Environmental Engineering Research*, 17(S1), pp. 3–8. doi: 10.4491/eer.2012.17.S1.S3.

Choi, H. J. and Lee, S. M. (2013) 'Performance of Chlorella vulgaris for the removal of ammonia-nitrogen from wastewater', *Environmental Engineering Research*, 18(4), pp. 235–239. doi: 10.4491/eer.2013.18.4.235.

Constitution of the Republic Of South Africa (1996) *Constitution of the Republic Of South Africa*. South Africa.

Delgadillo-Mirquez, L. *et al.* (2016) 'Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture', *Biotechnology Reports*, 11, pp. 18–26. doi: 10.1016/j.btre.2016.04.003.

Droop, M. R. (1968) 'Vitamin B12 and Marine Ecology. IV. The Kinetics of Uptake, Growth and Inhibition in Monochrysis Lutheri', *Journal of the Marine Biological Association of the United Kingdom*, 48(3), pp. 689–733. doi: 10.1017/S0025315400019238.

Dwivedi, N. and Dwivedi, S. (2019) 'Bio-Oil Production from Algal Feedstock', *Liquid Biofuel Production*, (December), pp. 351–371. doi: 10.1002/9781119459866.ch11.

DWS (2014) 'Executive Summary of the Green Drop Report', 152, pp. 1–7. Available at: https://www.dwa.gov.za/Documents/RSP.aspx.

El-Sheekh, M. M. and Fathy, A. A. (2009) 'Variation of Some Nutritional Constituents and Fatty Acid Profiles of Chlorella vulgaris Beijerinck Grown under Auto and Heterotrophic Conditions', *International Journal of Botany*, 5(2), pp. 153–159. doi: 10.3923/ijb.2009.153.159.

Engineering News (2018) 'M&R Water, partner launch innovative wastewater treatment demo plant', pp. 1–13. Available at: http://www.engineeringnews.co.za/article/mr-water-partner-launch-innovative-wastewater-treatment-demo-plant-2018-04-25 (Accessed: 22 August 2018).

Flynn, K. J. (2002) 'How Critical is the Critical N:P Ratio?', *Journal of Phycology*, 38(5), pp. 961–970. doi: 10.1046/j.1529-8817.2002.t01-1-01235.x.

Fondriest (2016) *Algae, Phytoplankton and Chlorophyll - Environmental Measurement Systems*, *Fondriest Environmental Inc.* Available at: https://www.fondriest.com/environmental-measurements/parameters/water-quality/algae-phytoplankton-chlorophyll/ (Accessed: 3 May 2019).

García-Malea, M. C. *et al.* (2006) 'Continuous production of green cells of Haematococcus pluvialis: Modeling of the irradiance effect', *Enzyme and Microbial Technology*, 38(7), pp. 981–989. doi: 10.1016/j.enzmictec.2005.08.031.

Gouveia, L. *et al.* (2016) 'Microalgae biomass production using wastewater: Treatment and costs. Scale-up considerations.', *Algal Research*, 16, pp. 167–176. doi: 10.1016/j.algal.2016.03.010.

Gouveia, L. *et al.* (2017) 'Biodiesel from microalgae', in *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products*, pp. 235–258. doi: 10.1016/B978-0-08-101023-5.00010-8.

Graham, L., Wilcox, L. and Graham, J. (2009) *Algae (2nd Edition)*. Benjamin Cummings; 2 edition (November 9, 2008).

Gupta, P., Lee, S. M. and Choi, H. J. (2015) 'A mini review: photobioreactors for large scale algal cultivation', *World Journal of Microbiology and Biotechnology*, 31(9), pp. 1409–1417. doi: 10.1007/s11274-015-1892-4.

Hannon, M. *et al.* (2010) 'Biofuels from algae: challenges and potential.', *Biofuels*, 1(5), pp. 763–784. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21833344 (Accessed: 8 March 2019).

Havens, K. E. (2004) 'The Phosphorus-Chlorophyll Relationship in Lakes : Potential Influences of Color and Mixing Regime', (August 2014). doi: 10.1080/07438140409354243.

Huang, Q. *et al.* (2017) 'Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms', *Engineering*, 3(3), pp. 318–329. doi: 10.1016/J.ENG.2017.03.020.

Koca, N., Karadeniz, F. and Selen Burdurlu, H. (2007) *Effect of pH on chlorophyll degradation and colour loss in blanched green peas*.

Lavens, P. and Sorgeloos, P. (1996) *Manual on the Production and Use of Live Food for Aquaculture: 2.3. Algal production*, *University of Ghent*. Rome. doi: M-44.

Lee, E., Jalalizadeh, M. and Zhang, Q. (2015) 'Growth kinetic models for microalgae cultivation: A review', *Algal Research*, 12, pp. 497–512. doi: 10.1016/j.algal.2015.10.004.

Lee, H. Y., Erickson, L. E. and Yang, S. S. (1987) 'Kinetics and bioenergetics of light-limited photoautotrophic growth of Spirulina platensis', *Biotechnology and Bioengineering*, 29(7), pp. 832–843. doi: 10.1002/bit.260290705.

Lenka, B., Bartosova, A. and Gerulova', K. (2015) 'Cultivation of Microalgae (Chlorella Vulgaris) for Biodiesel Production', *Materials Science and Technology*, 23(26), pp. 81–86.

Lopez, J. . (2018) *Influence of Light on Crop Growth*, *Premier Horticulture Ltd.* Available at: https://www.pthorticulture.com/en/training-center/influence-of-light-on-crop-growth/ (Accessed: 18 December 2020).

Ma, X. N. *et al.* (2016) 'Lipid production from Nannochloropsis', *Marine Drugs*, 14(4). doi: 10.3390/md14040061.

Martínez, M. E., Jiménez, J. M. and El Yousfi, F. (1999) 'Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga Scenedesmus obliquus', *Bioresource Technology*, 67(3), pp. 233–240. doi: 10.1016/S0960-8524(98)00120-5.

Martínez Sancho, M., Jiménez Castillo, J. M. and El Yousfi, F. (1997) 'Influence of phosphorus concentration on the growth kinetics and stoichiometry of the microalga Scenedesmus obliquus', *Process Biochemistry*, 32(8), pp. 657–664. doi: 10.1016/S0032-9592(97)00017-4.

Molina Grima, E. *et al.* (1996) 'A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances', *Journal of Biotechnology*, 45(1), pp. 59–69. doi: 10.1016/0168-1656(95)00144-1.

Monod, J. (2003) *The Growth of Bacterial Cultures*, *Annual Review of Microbiology*. doi: 10.1146/annurev.mi.03.100149.002103.

Moomaw, W., Berzin, I. and Tzachor, A. (2017) 'Cutting Out the Middle Fish: Marine Microalgae as the Next Sustainable Omega-3 Fatty Acids and Protein Source', *Industrial*

Biotechnology, 13(5), pp. 234–243. doi: 10.1089/ind.2017.29102.wmo.

Muller-Feuga, A. (1999) 'Growth as a function of rationing: A model applicable to fish and microalgae', *Journal of Experimental Marine Biology and Ecology*, 236(1), pp. 1–13. doi: 10.1016/S0022-0981(98)00194-4.

National Water Act 36 of 1998 (2013) *Revision of General Authorisations in Terms of Section 39 of the National Water Act, 1998 (Act No. 36 Of 1998) (The Act)*, *Government Gazette No. 19182*.

Nutriplex Bloom 1L - Greenthumb Hydroponics (2018). Available at: https://gthydro.co.za/products/193-nutriplex-bloom-1l-4522015.html (Accessed: 6 September 2018).

Nutriplex Gro 1L - Greenthumb Hydroponics (2018). Available at: https://gthydro.co.za/products/196-nutriplex-gro-1l.html (Accessed: 6 September 2018).

Nutriplex Micro 1L - Greenthumb Hydroponics (2018). Available at: https://gthydro.co.za/products/198-nutriplex-micro-1l.html (Accessed: 6 September 2018).

Osborn, D. W. (1988) 'Sewage purification in South Africa - past and present', 14(3), p. 139. Available at: http://www.wrc.org.za/Lists/Knowledge Hub Items/Attachments/4141/509 abstract.pdf (Accessed: 12 September 2018).

Pienkos, P. T. and Darzins, A. (2009) 'The promise and challenges of microalgal-derived biofuels', *Biofuels, Bioproducts and Biorefining*, 3(4), pp. 431–440. doi: 10.1002/bbb.159.

Politècnica De Catalunya, U., Thesis, P. and Solimeno, A. (2017) *Numerical Modelling Of Microalgae Systems for Wastewater Treatment*. Available at: https://upcommons.upc.edu/bitstream/handle/2117/109353/TAS1de1.pdf (Accessed: 1 March 2019).

Posten, C. (2009) 'Design principles of photo-bioreactors for cultivation of microalgae', *Engineering in Life Sciences*, 9(3), pp. 165–177. doi: 10.1002/elsc.200900003.

Ramírez-mérida, L. G., Zepka, L. Q. and Jacob-lopes, E. (2015) 'Current Status, Future Developments and Recent Patents on Photobioreactor Technology', *Recent Patents on Engineering,* 9(vi), pp. 80–90. doi: 10.2174/1872212109666150206235316.

Randrianarison, G. and Ashraf, M. A. (2017) 'Microalgae: a potential plant for energy production', *Geology, Ecology, and Landscapes*, 1(2), pp. 104–120. doi: 10.1080/24749508.2017.1332853.

Rubio Camacho, F. *et al.* (2003) 'A mechanistic model of photosynthesis in microalgae', *Biotechnology and Bioengineering*, 81(4), pp. 459–473. doi: 10.1002/bit.10492.

SANS 241-1 (2011) 'SANS 241-1 : 2011 SOUTH AFRICAN NATIONAL STANDARD Drinking water Part 1 : Microbiological, physical, aesthetic'.

Singh, R., Birru, R. and Sibi, G. (2017) 'Nutrient Removal Efficiencies of *Chlorella vulgaris* from Urban Wastewater for Reduced Eutrophication', *Journal of Environmental Protection*, 08(01), pp. 1–11. doi: 10.4236/jep.2017.81001.

Slade, R. and Bauen, A. (2013) 'Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects', *Biomass and Bioenergy*, 53(July), pp. 29–38. doi: 10.1016/j.biombioe.2012.12.019.

Sowby, R. B. (2014) 'The Urban Water Cycle: Sustaining Our Modern Cities – National Geographic Blog', *Changing Planet - National Geography Blog*. Available at: https://blog.nationalgeographic.org/2014/03/19/the-urban-water-cycle-sustaining-our-modern-cities/ (Accessed: 16 April 2020).

Spolaore, P. *et al.* (2006) 'Commercial applications of microalgae', *Journal of Bioscience and Bioengineering*, 101(2), pp. 87–96. doi: 10.1263/jbb.101.87.

Statistics South Africa (2016) *The state of basic service delivery in South Africa: In-depth analysis of the Community Survey 2016 data*. Available at: www.statssa.gov.za (Accessed: 22 August 2018).

Steele, J. H. (1962) 'Environmental control of photosynthesis in the sea', *Limnology and Oceanography*, 7(2), pp. 137–150. doi: 10.4319/lo.1962.7.2.0137.

Svaldenis, A. (2014) *Cultivating Algae in a Photobioreactor*.

Talbot, P. *et al.* (1991) 'A comparative study and mathematical modelling of temperature, light and growth of three microalgae potentially useful for wastewater treatment', *Water Research*, 25(4), pp. 465–472. doi: 10.1016/0043-1354(91)90083-3.

Tiwari, A. and Kiran, T. (2018) 'Biofuels from Microalgae', i(tourism), p. 13. Available at: http://dx.doi.org/10.5772/intechopen.73012.

Uang, H. Q. *et al.* (2017) 'Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms', *Engineering*, 3(3), pp. 318–329. doi: 10.1016/J.ENG.2017.03.020.

Us, A. *et al.* (2018) *Photobioreactor - Definition, Glossary, Details - Oilgae*. Available at: http://www.oilgae.com/ref/glos/photobioreactor.html (Accessed: 16 August 2018).

Uv, S. and Spectrophotometer, V. I. S. (no date) 'Pharo 300', pp. 0–20.

Venkataraman, G. S. (1969) *The Cultivation of Algae*. New Delhi: Indian Council of Agricultural Research.

Wang, L. *et al.* (2010) 'Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant', *Applied Biochemistry and Biotechnology*, 162(4), pp. 1174–1186. doi: 10.1007/s12010-009-8866-7.

WHO (2018) *Key facts*, *World Health Organization*. Available at: http://www.who.int/en/news-room/fact-sheets/detail/physical-activity (Accessed: 21 August 2018).

WISA (2020) *WISA calls for reinstatement of the Green drop, Blue drop and No drop certification programmes for the safe supply of water and disposal of wastewater - Water Institute of Southern Africa*, *www.wisa.org.za*. Available at: https://wisa.org.za/2020/09/23/wisa-calls-for-reinstatement-of-the-green-drop-blue-drop-and-no-drop-certification-programmes-for-the-safe-supply-of-water-and-disposal-of-wastewater/ (Accessed: 21 December 2020).

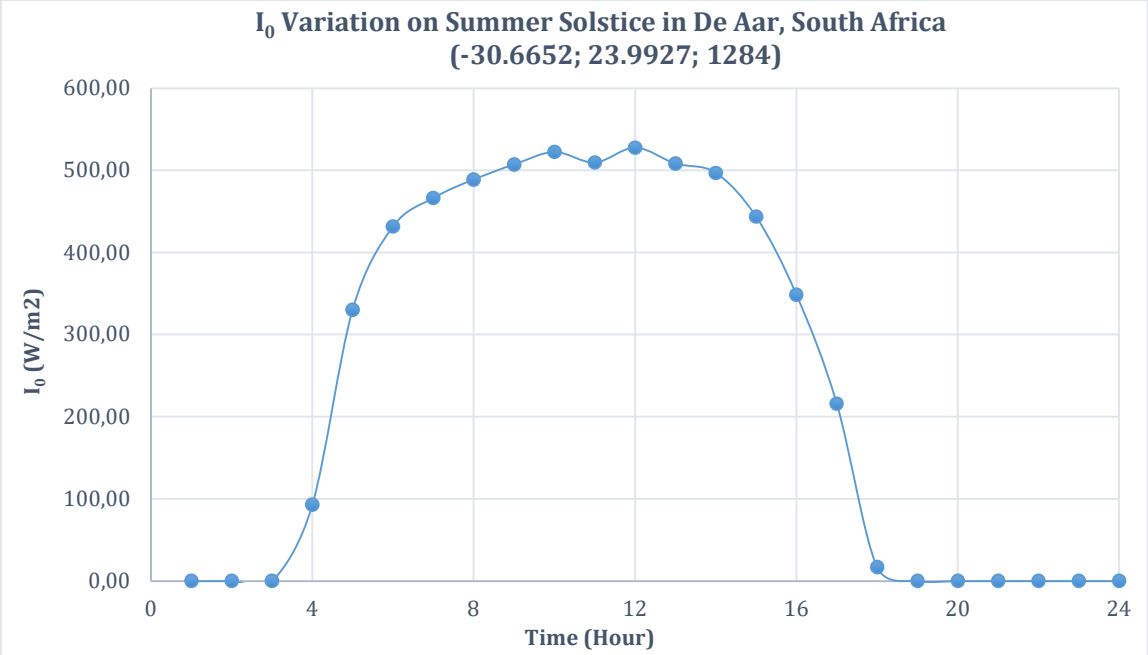
Zhang, D. *et al.* (2015) 'Modelling of light and temperature influences on cyanobacterial growth and biohydrogen production', *ALGAL*, 9, pp. 263–274. doi: 10.1016/j.algal.2015.03.015.

APPENDIX

Appendix 1: Solar Radiation for summer and winter solstice

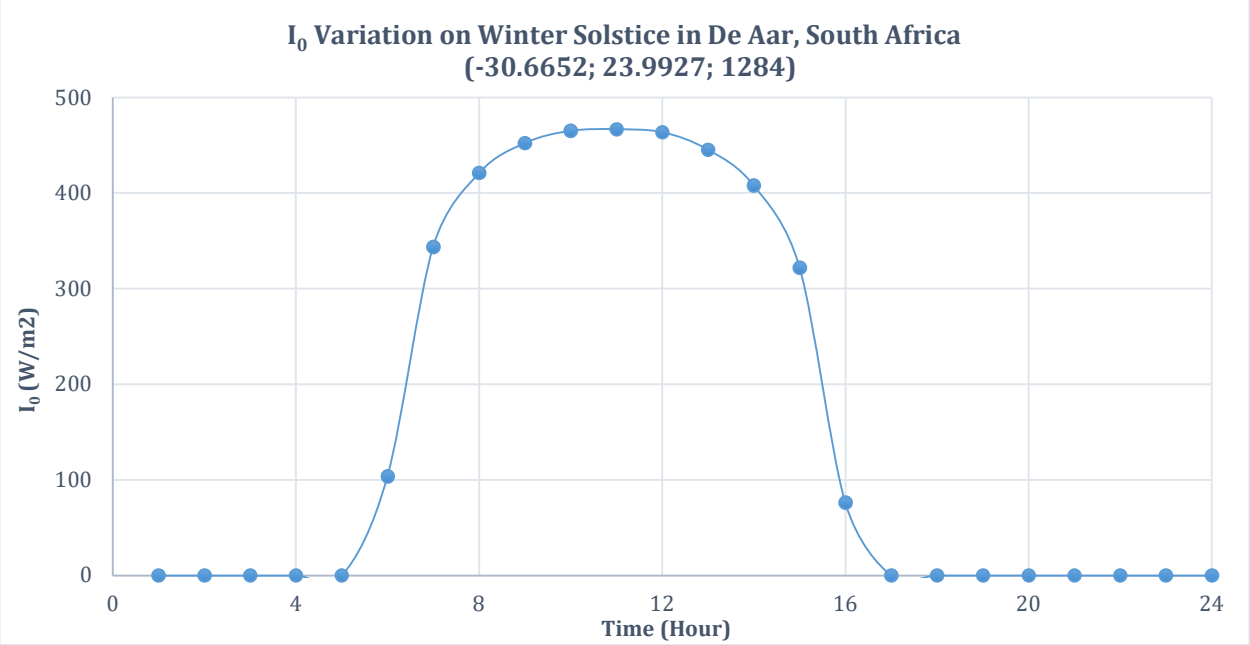
Summer

Hour	Direct Radiation (w/m²)	Diffuse Radiation (W/m²)	Total Radiation (W/m²)	Total Rad (PAR)
1	0	0	0	0.00
2	0	0	0	0.00
3	0	0	0	0.00
4	11	190	202	92.70
5	43	675	718	330.08
6	58	880	938	431.57
7	67	946	1014	466.30
8	108	954	1063	488.78
9	125	978	1102	507.13
10	116	1020	1135	522.28
11	102	1006	1108	509.63
12	111	1036	1147	527.70
13	124	981	1105	508.20
14	133	947	1080	496.96
15	90	874	964	443.56
16	71	687	758	348.63
17	41	429	469	215.92
18	3	33	37	16.80
19	0	0	0	0.00
20	0	0	0	0.00
21	0	0	0	0.00
22	0	0	0	0.00
23	0	0	0	0.00
24	0	0	0	0.00



Winter

Hour	Direct Radiation (w/m²)	Diffuse Radiation (W/m²)	Total Radiation (W/m²)	Total Rad (PAR)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	8	218	226	104
7	29	718	748	344
8	38	877	915	421
9	43	940	983	452
10	46	965	1011	465
11	46	969	1015	467
12	44	964	1008	464
13	41	927	968	445
14	36	851	887	408
15	25	675	700	322
16	4	161	166	76
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0



Appendix 2: Apparatus

The following apparatus will be used for the experiments:

- *Bath Sonicator*
- *Filter paper of 5.0 µm pore size*
- *Spectrophotometer*
- *2 ml micro-centrifuge tubes*
- *Dark box (for this experiment an old test kit box was used)*
- *Pipettes, lab beakers and pipette tips*
- *An aqueous solution of 90 parts acetone and 10 parts distilled water*
- *0.1 N Hydrochloric acid*
- *Magnesium carbonate*
- *Autoclave*

Appendix 3: EG-JM Growth Medium

culturecollection of algae and protozoa

media recipes

EG:JM

Medium

1:1 mixture

See separate recipes. Mix then autoclave at 15 psi for 15 minutes.

EG (Euglena gracilis Medium)

Freshwater algae and protozoa

Stock	**per litre**
(1) $CaCl_2$ stock solution:	
$CaCl_2$	1.0 g

Medium	**per litre**
Sodium acetate trihydrate	1.0 g
"Lab-Lemco" powder (Oxoid L29) *	1.0 g
Tryptone (Oxoid L42) *	2.0 g
Yeast extract (Oxoid L21)*	2.0 g
$CaCl_2$ stock solution (1)	10.0 ml

Add constituents above and make up to 1 litre with deionized water. For agar add 15 g per litre Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply
* Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

CCAP (Culture Collection of Algae and Protozoa), Dunstaffnage Marine Laboratory,
Oban, Argyll, PA37 1QA, UK
Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk

culturecollection of algae and protozoa

media recipes

JM (Jaworski's Medium)

Freshwater algae

Stocks		**per 200 ml**
(1)	$Ca(NO_3)_2.4H_2O$	4.0 g
(2)	KH_2PO_4	2.48 g
(3)	$MgSO_4.7H_2O$	10.0 g
(4)	$NaHCO_3$	3.18 g
(5)	EDTAFeNa	0.45 g
	$EDTANa_2$	0.45 g
(6)	H_3BO_3	0.496 g
	$MnCl_2.4H_2O$	0.278 g
	$(NH_4)6Mo_7O_{24}.4H_2O$	0.20 g
(7)	Cyanocobalamin	0.008 g
	Thiamine HCl	0.008 g
	Biotin	0.008 g
(8)	$NaNO_3$	16.0 g
(9)	$Na_2HPO_4.12H_2O$	7.2 g

Medium	**per litre**
Stock solutions 1 - 9	1 ml each

Make up to 1 litre with deionized water. For agar, add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply
* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

CCAP (Culture Collection of Algae and Protozoa), Dunstaffnage Marine Laboratory,
Oban, Argyll, PA37 1QA, UK
Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk

Appendix 4: Laboratory Data

Experimental Run 1 Laboratory Data

Test Day				02 Dec 19	03 Dec 19	05 Dec 19	07 Dec 19	09 Dec 19
Tank	**Growth Segment**	**Test**	**Units**	**D0**	**D1**	**D3**	**D5**	**D7**
Tank 1	Growth Cell 1	pH		7.9	8.3	8.5	8.6	
		Ammonia	mg/L	0.96	3.12	2.73	4.88	
		Total Nitrogen	mg/L	89		66	91	
		Phosphates	mg/L	33	33	33	16	
		Nitrates	mg/L	4.91	12.5	5.5	25	
		Chlorophyll	mg/L			26.892	46.2276	
	Growth Cell 2	pH		7.9	8.3	8.4	8.5	
		Ammonia	mg/L	0.77	2.14	2.49	4.27	
		Total Nitrogen	mg/L	89		55	44	
		Phosphates	mg/L	33	33	33	16	
		Nitrates	mg/L	12.5	9.6	8.5	25	
		Chlorophyll	mg/L			16.3896	42.4944	
	Growth Cell 3	pH		7.7	8.2	8.4	8.5	
		Ammonia	mg/L	0.94	2.49	2.6	4.39	
		Total Nitrogen	mg/L	104		81	58	
		Phosphates	mg/L	33	33	33	33	
		Nitrates	mg/L	12.5	10.6	10.2	25	
		Chlorophyll	mg/L			27.936	28.698	
Tank 2	Growth Cell 1	pH		7.9	8.3	8.5	8.5	
		Ammonia	mg/L	0.85	3.11	3.08	3.99	
		Total Nitrogen	mg/L	104		84	90	
		Phosphates	mg/L	33	16	16	16	
		Nitrates	mg/L	12.5	12.5	9.1	25	
		Chlorophyll	mg/L			37.398	41.4888	
	Growth Cell 2	pH		7.8	8.2	8.4	8.5	
		Ammonia	mg/L	1.06	2.67	1.45	2.1	
		Total Nitrogen	mg/L	109		74	49	
		Phosphates	mg/L	33	16	16	16	
		Nitrates	mg/L	12.5	11.6	12.5	25	
		Chlorophyll	mg/L			22.7748	32.7912	
	Growth Cell 3	pH		7.7	8.2	8.4	8.5	
		Ammonia	mg/L	0.9	3.04	2.81	5.29	
		Total Nitrogen	mg/L	104		41	46	
		Phosphates	mg/L	33	16	16	16	
		Nitrates	mg/L	12.5	11.3	7	25	
		Chlorophyll	mg/L			14.8992	28.7676	

		Day	0	1	3	5	7
T1_GC1	750	nm			0.048	0.038	
	665	nm			0.048	0.096	
	663	nm	-0.081		0.047	0.081	
	645	nm	-0.083		0.048	0.083	
	630	nm	-0.083		0.048	0.069	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	30.8744		26.892	46.2276	
T1_GC2	750	nm			0.029	0.041	
	665	nm			0.028	0.078	
	663	nm	-0.111		0.028	0.068	
	645	nm	-0.115		0.026	0.058	
	630	nm	-0.115		0.034	0.42	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	42.2056		16.3896	42.4944	
T1_GC3	750	nm			-0.035	0.039	
	665	nm			-0.048	0.068	
	663	nm	-0.131		-0.049	0.05	
	645	nm	-0.134		-0.051	0.05	
	630	nm	-1.29		-0.054	0.043	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	54.576		27.936	28.698	

		Day	0	1	3	5	7
T2_GC1	750	nm			0.065	0.046	
	665	nm			0.065	0.086	
	663	nm	-0.127		0.065	0.072	
	645	nm	-0.134		0.065	0.07	
	630	nm	-0.133		0.071	0.046	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	48.0856		37.398	41.4888	
T2_GC2	750	nm			0.041	0.033	
	665	nm			0.04	0.063	
	663	nm	-0.114		0.04	0.057	
	645	nm	-0.108		0.042	0.056	
	630	nm	-0.114		0.047	0.04	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	44.2032		22.7748	32.7912	
2_GC3	750	nm			0.03	0.027	
	665	nm			0.028	0.056	
	663	nm	-0.106		0.027	0.05	
	645	nm	-0.1		0.031	0.049	
	630	nm	-0.106		-0.01	0.033	
	F		1		1	1	
	E	ml	10		15	15	
	V	l	0.05		0.05	0.05	
	L	cm	5		5	5	
	Chl	ug/l	41.1376		14.8992	28.7676	

Experimental Run 2, 3 and 4 Laboratory Data

pH					
Day	0	1	3	5	7
E2_T1	7.80	8.50	8.30	7.70	7.80
E3_T1	7.40	7.80	7.80	8.30	8.50
E4_T1	7.30	7.50	7.90	7.90	8.20
Ave. T1	7.50	7.93	8.00	7.97	8.17
E2_T2	7.70	8.30	8.40	8.20	8.10
E3_T2	7.50	7.70	7.90	8.40	8.40
E4_T2	7.20	6.60	7.90	8.00	8.00
Ave. T2	7.47	7.53	8.07	8.20	8.17
Tank 1	*day diff.*	*-0.60*	*1.30*	*0.10*	*0.00*
	day %	*5.46%*	*0.83%*	*-0.42%*	*2.45%*
Tank 2	*day diff.*	*0.07*	*0.53*	*0.13*	*-0.03*
	day %	*0.88%*	*6.61%*	*1.63%*	*-0.41%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*0.80*	*8.89%*
		Tank 2		*0.70*	*9.38%*

Ammonia (mg/L)					
Day	0	1	3	5	7
E2_T1	2.90	5.20	7.10	6.90	6.50
E3_T1	4.60	6.30	6.80	7.70	3.40
E4_T1	1.70	5.60	7.10	8.60	7.80
Ave. T1	3.07	5.70	7.00	7.73	5.90
E2_T2	3.00	4.90	7.40	8.20	7.10
E3_T2	4.50	7.00	7.40	8.20	4.10
E4_T2	1.70	5.60	5.80	6.80	7.00
Ave. T2	3.07	5.83	6.87	7.73	6.07
Tank 1	*day diff.*	*3.90*	*0.20*	*1.00*	*0.20*
	day %	*46.20%*	*18.57%*	*9.48%*	*-31.07%*
Tank 2	*day diff.*	*2.77*	*1.03*	*0.87*	*-1.67*
	day %	*47.43%*	*15.05%*	*11.21%*	*-27.47%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*5.30*	*92.39%*
		Tank 2		*3.00*	*97.83%*

Total Nitrogen (mg/L)					
Day	0	1	3	5	7
E2_T1	55.00	72.00	49.00	10.00	62.00
E3_T1	80.00	66.00	66.00	83.00	69.00
E4_T1	67.00	52.00	62.00	66.00	78.00
Ave. T1	67.33	63.33	59.00	53.00	69.67
E2_T2	67.00	82.00	62.00	14.00	76.00
E3_T2	67.00	65.00	67.00	57.00	72.00
E4_T2	75.00	61.00	58.00	71.00	97.00
Ave. T2	69.67	69.33	62.33	47.33	81.67
Tank 1	*day diff.*	*-14.00*	*-3.00*	*13.00*	*26.00*
	day %	*-6.32%*	*-7.34%*	*-11.32%*	*23.92%*
Tank 2	*day diff.*	*-0.33*	*-7.00*	*-15.00*	*34.33*
	day %	*-0.48%*	*-11.23%*	*-31.69%*	*42.04%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*22.00*	*3.47%*
		Tank 2		*12.00*	*17.22%*

Phosphate (mg/L)					
Day	0	1	3	5	7
E2_T1	16.00	16.00	16.00	8.20	8.20
E3_T1	16.00	16.00	16.00	8.20	8.20
E4_T1	33.00	16.00	16.00	16.00	8.20
Ave. T1	21.67	16.00	16.00	10.80	8.20
E2_T2	16.00	16.00	8.20	8.20	8.20
E3_T2	16.00	16.00	16.00	8.20	8.20
E4_T2	33.00	16.00	16.00	16.00	8.20
Ave. T2	21.67	16.00	13.40	10.80	8.20
Tank 1	*day diff.*	*-17.00*	*0.00*	*0.00*	*-7.80*
	day %	*-35.42%*	*0.00%*	*-48.15%*	*-31.71%*
Tank 2	*day diff.*	*-5.67*	*-2.60*	*-2.60*	*-2.60*
	day %	*-35.42%*	*-19.40%*	*-24.07%*	*-31.71%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*-24.80*	*-62.15%*
		Tank 2		*-13.47*	*-62.15%*

Nitrate (mg/L)					
Day	0	1	3	5	7
E2_T1	12.50	7.20	11.60	13.50	15.70
E3_T1	16.40	17.30	21.00	19.10	19.40
E4_T1	17.40	21.00	21.50	21.70	18.00
Ave. T1	15.43	15.17	18.03	18.10	17.70
E2_T2	12.50	10.60	14.00	14.40	19.70
E3_T2	17.70	16.00	19.50	17.40	21.30
E4_T2	17.30	14.30	20.60	20.10	17.40
Ave. T2	15.83	13.63	18.03	17.30	19.47
	day				
Tank 1	*diff.*	*-3.00*	*6.30*	*-0.50*	*-2.70*
	day %	*-1.76%*	*15.90%*	*0.37%*	*-2.26%*
	day				
Tank 2	*diff.*	*-2.20*	*4.40*	*-0.73*	*2.17*
	day %	*-16.14%*	*24.40%*	*-4.24%*	*11.13%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*0.10*	*14.69%*
		Tank 2		*3.63*	*22.95%*

Chlorophyll-a					
Day	0	1	3	5	7
E2_T1	18.40	13.29	11.69	12.70	8.33
E3_T1	10.04	14.19	6.64	13.15	14.99
E4_T1	15.09	10.57	23.45	13.92	20.40
Ave. T1	14.51	12.69	13.93	13.26	14.57
E2_T2	8.32	15.16	9.79	16.04	8.87
E3_T2	10.19	9.77	12.55	13.80	9.86
E4_T2	11.03	14.09	8.28	14.15	4.66
Ave. T2	9.85	13.00	10.20	14.66	7.80
	day				
Tank 1	*diff.*	*3.06*	*-5.81*	*5.88*	*-9.50*
	day %	*-14.38%*	*8.91%*	*-5.05%*	*9.02%*
	day				
Tank 2	*diff.*	*3.16*	*-2.80*	*4.46*	*-6.87*
	day %	*24.26%*	*-27.44%*	*30.41%*	*-88.04%*
		day 0 to 7		*diff.*	*%*
		Tank 1		*-6.38*	*0.43%*
		Tank 2		*-2.05*	*-20.83%*

Appendix 5: Model 1 and 2 Calculations

Model 1 – Summer Growth Model

Input Data			
Initial Biomass Concentration	c_0	1000	g-biomass/m^3
Absorption Co-efficient	k	0.2	0.2 m^2/g-biomass
Maximum specific growth rate	μ_{max}	4	4/d
Saturation constant	K_s	5	W/m^2
Photoinhibition constant	K_i	200	W/m^2
Decay rate	*b*	0.1	0.1/d

Tank Dimensions			
Width	w	0.7	m
Breath	b	0.1	m
Depth	d	0.15	m
Volume	V	0.0105	m^3

Day	Hours	M1_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
1	1	1000	0.00	-0.004		
	2	996	0.000	-0.004		
	3	992	0.000	-0.004		
	4	988	7.446	0.093		
	5	1080	24.467	0.121		
	6	1211	28.887	0.122		
	7	1359	28.207	0.122		
	8	1525	26.766	0.122		
	9	1711	25.190	0.122		
	10	1920	23.585	0.121		
	11	2152	20.977	0.120		
	12	2410	19.863	0.119		
	13	2697	17.547	0.117		
	14	3014	15.804	0.115		
	15	3361	13.047	0.111		
	16	3734	9.540	0.102		
	17	4114	5.543	0.082		
	18	4452	0.410	0.008		
	19	4490	0.000	-0.004		
	20	4471	0.000	-0.004		
	21	4453	0.000	-0.004		
	22	4434	0.000	-0.004		
	23	4416	0.000	-0.004		
	24	4397	0.000	-0.004	0.065	3.005

Day	Hours	M1_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
2	1	4379	0.000	-0.004		
	2	4361	0.000	-0.004		
	3	4343	0.000	-0.004		
	4	4325	2.305	0.048		
	5	4307	8.230	0.097		
	6	4514	10.450	0.105		
	7	4952	10.680	0.105		
	8	5470	10.579	0.105		
	9	6047	10.406	0.105		
	10	6682	10.199	0.104		
	11	7381	9.510	0.102		
	12	8148	9.449	0.102		
	13	8978	8.773	0.099		
	14	9890	8.303	0.097		
	15	10870	7.205	0.092		
	16	11927	5.528	0.082		
	17	13026	3.357	0.062		
	18	14095	0.257	0.004		
	19	14974	0.000	-0.004		
	20	15033	0.000	-0.004		
	21	14971	0.000	-0.004		
	22	14908	0.000	-0.004		
	23	14846	0.000	-0.004		
	24	14785	0.000	-0.004	0.053	8.225
3	1	14723	0.000	-0.004		
	2	14662	0.000	-0.004		
	3	14600	0.000	-0.004		
	4	14540	1.412	0.032		
	5	14479	5.030	0.078		
	6	14949	6.542	0.089		
	7	16121	6.991	0.091		
	8	17549	7.254	0.092		
	9	19147	7.463	0.093		
	10	20917	7.636	0.094		
	11	22871	7.413	0.093		
	12	25028	7.648	0.094		
	13	27360	7.347	0.093		
	14	29942	7.173	0.092		
	15	32723	6.395	0.088		
	16	35733	5.023	0.078		
	17	38868	3.110	0.059		
	18	41912	0.242	0.004		
	19	44400	0.000	-0.004		
	20	44557	0.000	-0.004		
	21	44371	0.000	-0.004		
	22	44186	0.000	-0.004		
	23	44002	0.000	-0.004		
	24	43819	0.000	-0.004	0.047	21.750

Day	Hours	M1_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_Sum
d	Hr	c	Iav	μ	μavg	P
day	hours	g - biomass / m3	W/m2	per day	per day	g-biomass / day
4	1	43636	0.000	-0.004		
	2	43455	0.000	-0.004		
	3	43273	0.000	-0.004		
	4	43093	1.335	0.031		
	5	42914	4.753	0.076		
	6	44240	6.214	0.087		
	7	47608	6.714	0.090		
	8	51732	7.037	0.091		
	9	56364	7.301	0.093		
	10	61511	7.519	0.094		
	11	67210	7.337	0.093		
	12	73510	7.597	0.094		
	13	80334	7.316	0.093		
	14	87893	7.155	0.092		
	15	96044	6.386	0.088		
	16	104872	5.019	0.078		
	17	114065	3.109	0.059		
	18	122995	0.242	0.004		
	19	130295	0.000	-0.004		
	20	130754	0.000	-0.004		
	21	130209	0.000	-0.004		
	22	129667	0.000	-0.004		
	23	129127	0.000	-0.004		
	24	128589	0.000	-0.004	0.047	63.223
5	1	128053	0.000	-0.004		
	2	127519	0.000	-0.004		
	3	126988	0.000	-0.004		
	4	126459	1.335	0.031		
	5	125932	4.752	0.076		
	6	129823	6.213	0.087		
	7	139705	6.713	0.090		
	8	151806	7.037	0.091		
	9	165401	7.301	0.093		
	10	180503	7.519	0.094		
	11	197228	7.337	0.093		
	12	215713	7.597	0.094		
	13	235739	7.316	0.093		
	14	257921	7.155	0.092		
	15	281839	6.386	0.088		
	16	307744	5.019	0.078		
	17	334723	3.109	0.059		
	18	360928	0.242	0.004		
	19	382349	0.000	-0.004		
	20	383696	0.000	-0.004		
	21	382097	0.000	-0.004		
	22	380505	0.000	-0.004		
	23	378920	0.000	-0.004		
	24	377342	0.000	-0.004	0.047	185.523

Day	Hours	M1_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_Sum
d	Hr	c	Iav	μ	μavg	P
day	hours	g - biomass / m3	W/m2	per day	per day	g-biomass / day
6	1	375769	0.000	-0.004		
	2	374204	0.000	-0.004		
	3	372645	0.000	-0.004		
	4	371092	1.335	0.031		
	5	369546	4.752	0.076		
	6	380964	6.213	0.087		
	7	409963	6.713	0.090		
	8	445474	7.037	0.091		
	9	485367	7.301	0.093		
	10	529683	7.519	0.094		
	11	578762	7.337	0.093		
	12	633006	7.597	0.094		
	13	691772	7.316	0.093		
	14	756865	7.155	0.092		
	15	827052	6.386	0.088		
	16	903071	5.019	0.078		
	17	982239	3.109	0.059		
	18	1059138	0.242	0.004		
	19	1121997	0.000	-0.004		
	20	1125951	0.000	-0.004		
	21	1121259	0.000	-0.004		
	22	1116587	0.000	-0.004		
	23	1111937	0.000	-0.004		
	24	1107303	0.000	-0.004	0.047	544.413
7	1	1102690	0.000	-0.004		
	2	1098095	0.000	-0.004		
	3	1093520	0.000	-0.004		
	4	1088963	1.335	0.031		
	5	1084426	4.752	0.076		
	6	1117933	6.213	0.087		
	7	1203029	6.713	0.090		
	8	1307235	7.037	0.091		
	9	1424302	7.301	0.093		
	10	1554347	7.519	0.094		
	11	1698368	7.519	0.094		
	12	1857547	7.597	0.094		
	13	2031645	7.316	0.093		
	14	2222815	7.155	0.092		
	15	2428946	6.386	0.088		
	16	2652204	5.019	0.078		
	17	2884709	3.109	0.059		
	18	3110552	0.242	0.004		
	19	3295161	0.000	-0.004		
	20	3306771	0.000	-0.004		
	21	3292993	0.000	-0.004		
	22	3279272	0.000	-0.004		
	23	3265614	0.000	-0.004		
	24	3252007	0.000	-0.004	0.047	1600.135

Model 2 – Summer Growth Model

Input Data			
Initial Biomass Concentration	c_0	1000	g-biomass/m^3
Absorption Co-efficient	k	0.2	0.2 m^2/g-biomass
Maximum specific growth rate	μ_{max}	4	4/d
Saturation constant	K_s	5	W/m^2
Photoinhibition constant	K_i	200	W/m^2
Decay rate	*b*	0.1	0.1/d

Tank Dimensions			
Width	w	0.1	m
Breath	b	0.15	m
Depth	d	0.52	m
Volume	V	0.0078	m^3

Day	Hours	M2_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per hour	per day	g-biomass / day
1	1	1000	0.00	-0.004		
	2	996	0.000	-0.004		
	3	992	0.000	-0.004		
	4	988	32.068	0.122		
	5	1108	100.467	0.103		
	6	1223	117.666	0.098		
	7	1343	114.369	0.099		
	8	1476	107.627	0.101		
	9	1625	99.936	0.103		
	10	1793	91.775	0.106		
	11	1983	79.543	0.110		
	12	2201	62.832	0.115		
	13	2455	61.454	0.116		
	14	2739	52.616	0.119		
	15	3064	40.953	0.121		
	16	3436	27.957	0.122		
	17	3856	15.019	0.114		
	18	4296	1.023	0.024		
	19	4400	0.000	-0.004		
	20	4381	0.000	-0.004		
	21	4363	0.000	-0.004		
	22	4345	0.000	-0.004		
	23	4327	0.000	-0.004		
	24	4309	0.000	-0.004	0.064	2.153

Day	Hours	M2_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per hour	per day	g-biomass / day
2	1	4291	0.000	-0.004		
	2	4273	0.000	-0.004		
	3	4255	0.000	-0.004		
	4	4238	5.740	0.084		
	5	4220	20.543	0.120		
	6	4573	24.338	0.121		
	7	5120	22.912	0.121		
	8	5741	20.931	0.120		
	9	6435	18.997	0.119		
	10	7207	17.207	0.117		
	11	8061	14.869	0.114		
	12	9003	13.640	0.112		
	13	10030	11.944	0.109		
	14	11153	10.645	0.105		
	15	12364	8.762	0.099		
	16	13665	6.427	0.088		
	17	15019	3.762	0.067		
	18	16340	0.280	0.005		
	19	17431	0.000	-0.004		
	20	17513	0.000	-0.004		
	21	17440	0.000	-0.004		
	22	17367	0.000	-0.004		
	23	17295	0.000	-0.004		
	24	17223	0.000	-0.004	0.061	8.181
3	1	17151	0.000	-0.004		
	2	17080	0.000	-0.004		
	3	17008	0.000	-0.004		
	4	16938	1.522	0.035		
	5	16867	5.431	0.081		
	6	17452	6.999	0.091		
	7	18872	7.347	0.093		
	8	20592	7.505	0.094		
	9	22504	7.631	0.094		
	10	24612	7.741	0.095		
	11	26932	7.473	0.094		
	12	29484	7.681	0.094		
	13	32241	7.363	0.093		
	14	35287	7.179	0.092		
	15	38568	6.397	0.088		
	16	42118	5.024	0.078		
	17	45813	3.110	0.059		
	18	49401	0.242	0.004		
	19	52334	0.000	-0.004		
	20	52518	0.000	-0.004		
	21	52300	0.000	-0.004		
	22	52082	0.000	-0.004		
	23	51865	0.000	-0.004		
	24	51649	0.000	-0.004	0.048	19.256

Day	Hours	M2_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per hour	per day	g-biomass / day
4	1	51433	0.000	-0.004		
	2	51219	0.000	-0.004		
	3	51006	0.000	-0.004		
	4	50793	1.335	0.031		
	5	50581	4.753	0.076		
	6	52145	6.214	0.087		
	7	56114	6.714	0.090		
	8	60975	7.037	0.091		
	9	66436	7.301	0.093		
	10	72501	7.519	0.094		
	11	79219	7.337	0.093		
	12	86644	7.597	0.094		
	13	94688	7.316	0.093		
	14	103598	7.155	0.092		
	15	113205	6.386	0.088		
	16	123610	5.019	0.078		
	17	134446	3.109	0.059		
	18	144972	0.242	0.004		
	19	153576	0.000	-0.004		
	20	154117	0.000	-0.004		
	21	153475	0.000	-0.004		
	22	152835	0.000	-0.004		
	23	152199	0.000	-0.004		
	24	151565	0.000	-0.004	0.047	55.357
5	1	150933	0.000	-0.004		
	2	150304	0.000	-0.004		
	3	149678	0.000	-0.004		
	4	149054	1.335	0.031		
	5	148433	4.752	0.076		
	6	153019	6.213	0.087		
	7	164667	6.713	0.090		
	8	178931	7.037	0.091		
	9	194954	7.301	0.093		
	10	212755	7.519	0.094		
	11	232468	7.337	0.093		
	12	254256	7.597	0.094		
	13	277860	7.316	0.093		
	14	304006	7.155	0.092		
	15	332197	6.386	0.088		
	16	362731	5.019	0.078		
	17	394530	3.109	0.059		
	18	425418	0.242	0.004		
	19	450666	0.000	-0.004		
	20	452254	0.000	-0.004		
	21	450369	0.000	-0.004		
	22	448493	0.000	-0.004		
	23	446625	0.000	-0.004		
	24	444764	0.000	-0.004	0.047	162.441

Day	Hours	M2_Bio.Conc_S	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_Sum
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per hour	per day	g-biomass / day
6	1	442911	0.000	-0.004		
	2	441065	0.000	-0.004		
	3	439227	0.000	-0.004		
	4	437397	1.335	0.031		
	5	435575	4.752	0.076		
	6	449033	6.213	0.087		
	7	483213	6.713	0.090		
	8	525069	7.037	0.091		
	9	572091	7.301	0.093		
	10	624325	7.519	0.094		
	11	682173	7.337	0.093		
	12	746110	7.597	0.094		
	13	815376	7.316	0.093		
	14	892100	7.155	0.092		
	15	974828	6.386	0.088		
	16	1064429	5.019	0.078		
	17	1157743	3.109	0.059		
	18	1248382	0.242	0.004		
	19	1322472	0.000	-0.004		
	20	1327132	0.000	-0.004		
	21	1321602	0.000	-0.004		
	22	1316096	0.000	-0.004		
	23	1310614	0.000	-0.004		
	24	1305153	0.000	-0.004	0.047	476.682
7	1	1299715	0.000	-0.004		
	2	1294299	0.000	-0.004		
	3	1288906	0.000	-0.004		
	4	1283536	1.335	0.031		
	5	1278188	4.752	0.076		
	6	1317682	6.213	0.087		
	7	1417982	6.713	0.090		
	8	1540808	7.037	0.091		
	9	1678791	7.301	0.093		
	10	1832073	7.519	0.094		
	11	2001827	7.519	0.094		
	12	2189448	7.597	0.094		
	13	2394653	7.316	0.093		
	14	2619981	7.155	0.092		
	15	2862942	6.386	0.088		
	16	3126091	5.019	0.078		
	17	3400140	3.109	0.059		
	18	3666336	0.242	0.004		
	19	3883930	0.000	-0.004		
	20	3897615	0.000	-0.004		
	21	3881375	0.000	-0.004		
	22	3865202	0.000	-0.004		
	23	3849103	0.000	-0.004		
	24	3833065	0.000	-0.004	0.047	1401.060

Model 1 – Winter Growth Model

Input Data			
Initial Biomass Concentration	c_0	1000	g-biomass/m^3
Absorption Co-efficient	k	0.2	0.2 m^2/g-biomass
Maximum specific growth rate	μ_{max}	4	4/d
Saturation constant	K_s	5	W/m^2
Photoinhibition constant	K_i	200	W/m^2
Decay rate	*b*	0.1	0.1/d

Tank Dimensions			
Width	w	0.1	m
Breath	b	0.15	m
Depth	d	0.52	m
Volume	V	0.0078	m^3

Day	Hours	M1_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
1	1	1000	0.00	-0.004		
	2	996	0.000	-0.004		
	3	992	0.000	-0.004		
	4	988	0.000	-0.004		
	5	983	0.000	-0.004		
	6	979	8.400	0.098		
	7	1075	25.594	0.122		
	8	1206	28.284	0.122		
	9	1353	27.461	0.122		
	10	1519	25.565	0.122		
	11	1704	23.275	0.121		
	12	1910	21.028	0.120		
	13	2139	18.428	0.118		
	14	2391	15.448	0.115		
	15	2666	11.220	0.107		
	16	2951	2.460	0.051		
	17	3100	0.000	-0.004		
	18	3087	0.000	-0.004		
	19	3074	0.000	-0.004		
	20	3061	0.000	-0.004		
	21	3048	0.000	-0.004		
	22	3036	0.000	-0.004		
	23	3023	0.000	-0.004		
	24	3011	0.000	-0.004	0.048	1.138

Day	Hours	M1_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
2	1	2998	0.000	-0.004		
	2	2985	0.000	-0.004		
	3	2973	0.000	-0.004		
	4	2961	0.000	-0.004		
	5	2948	0.000	-0.004		
	6	2936	3.364	0.062		
	7	2924	11.182	0.107		
	8	3106	13.098	0.111		
	9	3438	13.097	0.111		
	10	3819	12.536	0.110		
	11	4243	11.749	0.108		
	12	4710	10.942	0.106		
	13	5219	9.900	0.103		
	14	5772	8.576	0.098		
	15	6367	6.439	0.088		
	16	6993	1.456	0.033		
	17	7609	0.000	-0.004		
	18	7862	0.000	-0.004		
	19	7830	0.000	-0.004		
	20	7797	0.000	-0.004		
	21	7765	0.000	-0.004		
	22	7732	0.000	-0.004		
	23	7700	0.000	-0.004		
	24	7668	0.000	-0.004	0.041	2.452
3	1	7636	0.000	-0.004		
	2	7604	0.000	-0.004		
	3	7572	0.000	-0.004		
	4	7541	0.000	-0.004		
	5	7509	0.000	-0.004		
	6	7478	1.926	0.042		
	7	7447	6.393	0.088		
	8	7760	7.690	0.095		
	9	8441	7.988	0.096		
	10	9239	7.949	0.096		
	11	10125	7.742	0.095		
	12	11094	7.490	0.094		
	13	12145	7.033	0.091		
	14	13282	6.316	0.087		
	15	14494	4.907	0.077		
	16	15758	1.145	0.027		
	17	16978	0.000	-0.004		
	18	17434	0.000	-0.004		
	19	17362	0.000	-0.004		
	20	17289	0.000	-0.004		
	21	17217	0.000	-0.004		
	22	17145	0.000	-0.004		
	23	17074	0.000	-0.004		
	24	17003	0.000	-0.004	0.035	4.602

Day	Hours	M1_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
4	1	16932	0.000	-0.004		
	2	16862	0.000	-0.004		
	3	16791	0.000	-0.004		
	4	16721	0.000	-0.004		
	5	16652	0.000	-0.004		
	6	16582	1.550	0.035		
	7	16513	5.140	0.079		
	8	17095	6.266	0.087		
	9	18449	6.678	0.089		
	10	20053	6.820	0.090		
	11	21845	6.807	0.090		
	12	23814	6.733	0.090		
	13	25959	6.450	0.088		
	14	28287	5.893	0.085		
	15	30777	4.647	0.075		
	16	33380	1.098	0.026		
	17	35891	0.000	-0.004		
	18	36818	0.000	-0.004		
	19	36664	0.000	-0.004		
	20	36512	0.000	-0.004		
	21	36359	0.000	-0.004		
	22	36208	0.000	-0.004		
	23	36057	0.000	-0.004		
	24	35907	0.000	-0.004	0.033	9.104
5	1	35757	0.000	-0.004		
	2	35608	0.000	-0.004		
	3	35460	0.000	-0.004		
	4	35312	0.000	-0.004		
	5	35165	0.000	-0.004		
	6	35018	1.496	0.034		
	7	34873	4.956	0.078		
	8	36063	6.066	0.086		
	9	38868	6.514	0.088		
	10	42199	6.698	0.089		
	11	45930	6.721	0.090		
	12	50040	6.676	0.089		
	13	54523	6.414	0.088		
	14	59394	5.873	0.084		
	15	64611	4.637	0.075		
	16	70067	1.096	0.026		
	17	75332	0.000	-0.004		
	18	77274	0.000	-0.004		
	19	76952	0.000	-0.004		
	20	76632	0.000	-0.004		
	21	76312	0.000	-0.004		
	22	75994	0.000	-0.004		
	23	75678	0.000	-0.004		
	24	75363	0.000	-0.004	0.032	18.945

Day	Hours	M1_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M1_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
6	1	75048	0.000	-0.004		
	2	74736	0.000	-0.004		
	3	74424	0.000	-0.004		
	4	74114	0.000	-0.004		
	5	73805	0.000	-0.004		
	6	73498	1.494	0.034		
	7	73192	4.951	0.078		
	8	75689	6.061	0.086		
	9	81573	6.512	0.088		
	10	88562	6.696	0.089		
	11	96391	6.720	0.090		
	12	105014	6.676	0.089		
	13	114422	6.414	0.088		
	14	124645	5.873	0.084		
	15	135592	4.637	0.075		
	16	147043	1.096	0.026		
	17	158092	0.000	-0.004		
	18	162167	0.000	-0.004		
	19	161492	0.000	-0.004		
	20	160819	0.000	-0.004		
	21	160149	0.000	-0.004		
	22	159482	0.000	-0.004		
	23	158817	0.000	-0.004		
	24	158156	0.000	-0.004	0.032	39.751
7	1	157497	0.000	-0.004		
	2	156840	0.000	-0.004		
	3	156187	0.000	-0.004		
	4	155536	0.000	-0.004		
	5	154888	0.000	-0.004		
	6	154243	1.494	0.034		
	7	153600	4.951	0.078		
	8	158840	6.061	0.086		
	9	171190	6.512	0.088		
	10	185855	6.696	0.089		
	11	202286	6.696	0.089		
	12	220382	6.676	0.089		
	13	240096	6.414	0.088		
	14	261547	5.873	0.084		
	15	284519	4.637	0.075		
	16	308546	1.096	0.026		
	17	331731	0.000	-0.004		
	18	340282	0.000	-0.004		
	19	338864	0.000	-0.004		
	20	337453	0.000	-0.004		
	21	336047	0.000	-0.004		
	22	334647	0.000	-0.004		
	23	333252	0.000	-0.004		
	24	331864	0.000	-0.004	0.032	83.397

Model 2 – Winter Growth Model

Input Data			
Initial Biomass Concentration	c_0	1000	g-biomass/m^3
Absorption Co-efficient	k	0.2	0.2 m^2/g-biomass
Maximum specific growth rate	μ_{max}	4	4/d
Saturation constant	K_s	5	W/m^2
Photoinhibition constant	K_i	200	W/m^2
Decay rate	*b*	0.1	0.1/d

Tank Dimensions			
Width	w	0.1	m
Breath	b	0.15	m
Depth	d	0.52	m
Volume	V	0.0078	m^3

Day	Hours	M2_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
1	1	1000	0.00	-0.004		
	2	996	0.000	-0.004		
	3	992	0.000	-0.004		
	4	988	0.000	-0.004		
	5	983	0.000	-0.004		
	6	979	36.235	0.122		
	7	1099	105.682	0.102		
	8	1211	116.091	0.098		
	9	1330	112.156	0.100		
	10	1462	103.486	0.102		
	11	1612	92.833	0.106		
	12	1782	82.041	0.109		
	13	1977	69.780	0.113		
	14	2200	56.248	0.117		
	15	2459	38.866	0.122		
	16	2758	7.994	0.096		
	17	3023	0.000	-0.004		
	18	3010	0.000	-0.004		
	19	2998	0.000	-0.004		
	20	2985	0.000	-0.004		
	21	2973	0.000	-0.004		
	22	2961	0.000	-0.004		
	23	2948	0.000	-0.004		
	24	2936	0.000	-0.004	0.047	1.081

Day	Hours	M2_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
2	1	2924	0.000	-0.004		
	2	2912	0.000	-0.004		
	3	2899	0.000	-0.004		
	4	2887	0.000	-0.004		
	5	2875	0.000	-0.004		
	6	2863	10.410	0.105		
	7	2851	34.670	0.122		
	8	3150	37.574	0.122		
	9	3535	35.021	0.122		
	10	3966	31.246	0.122		
	11	4451	27.206	0.122		
	12	4996	23.467	0.121		
	13	5607	19.624	0.119		
	14	6286	15.703	0.115		
	15	7034	10.903	0.106		
	16	7844	2.288	0.048		
	17	8675	0.000	-0.004		
	18	9091	0.000	-0.004		
	19	9053	0.000	-0.004		
	20	9015	0.000	-0.004		
	21	8978	0.000	-0.004		
	22	8940	0.000	-0.004		
	23	8903	0.000	-0.004		
	24	8866	0.000	-0.004	0.049	3.374
3	1	8829	0.000	-0.004		
	2	8792	0.000	-0.004		
	3	8756	0.000	-0.004		
	4	8719	0.000	-0.004		
	5	8683	0.000	-0.004		
	6	8647	2.816	0.056		
	7	8611	9.370	0.101		
	8	9089	10.865	0.106		
	9	10010	10.649	0.105		
	10	11070	10.025	0.103		
	11	12235	9.294	0.101		
	12	13501	8.615	0.098		
	13	14864	7.806	0.095		
	14	16329	6.810	0.090		
	15	17881	5.174	0.079		
	16	19492	1.188	0.028		
	17	21041	0.000	-0.004		
	18	21626	0.000	-0.004		
	19	21536	0.000	-0.004		
	20	21446	0.000	-0.004		
	21	21357	0.000	-0.004		
	22	21268	0.000	-0.004		
	23	21179	0.000	-0.004		
	24	21091	0.000	-0.004	0.038	6.232

Day	Hours	M2_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
4	1	21003	0.000	-0.004		
	2	20915	0.000	-0.004		
	3	20828	0.000	-0.004		
	4	20741	0.000	-0.004		
	5	20655	0.000	-0.004		
	6	20569	1.594	0.036		
	7	20483	5.288	0.080		
	8	21222	6.416	0.088		
	9	22927	6.781	0.090		
	10	24941	6.881	0.090		
	11	27184	6.839	0.090		
	12	29643	6.747	0.090		
	13	32318	6.454	0.088		
	14	35218	5.893	0.085		
	15	38320	4.646	0.075		
	16	41561	1.097	0.026		
	17	44687	0.000	-0.004		
	18	45840	0.000	-0.004		
	19	45649	0.000	-0.004		
	20	45459	0.000	-0.004		
	21	45270	0.000	-0.004		
	22	45081	0.000	-0.004		
	23	44893	0.000	-0.004		
	24	44706	0.000	-0.004	0.033	11.393
5	1	44520	0.000	-0.004		
	2	44335	0.000	-0.004		
	3	44150	0.000	-0.004		
	4	43966	0.000	-0.004		
	5	43783	0.000	-0.004		
	6	43600	1.495	0.034		
	7	43419	4.955	0.078		
	8	44901	6.064	0.086		
	9	48393	6.513	0.088		
	10	52540	6.697	0.089		
	11	57185	6.721	0.090		
	12	62301	6.676	0.089		
	13	67882	6.414	0.088		
	14	73947	5.873	0.084		
	15	80442	4.637	0.075		
	16	87235	1.096	0.026		
	17	93790	0.000	-0.004		
	18	96208	0.000	-0.004		
	19	95807	0.000	-0.004		
	20	95408	0.000	-0.004		
	21	95010	0.000	-0.004		
	22	94615	0.000	-0.004		
	23	94220	0.000	-0.004		
	24	93828	0.000	-0.004	0.032	23.585

Day	Hours	M2_Bio.Conc_W	Average irradiation	Specific Growth Rate	Avg. Specific Growth	M2_Prod_W
d	Hr	c	I_{av}	μ	μ_{avg}	P
day	hours	g - biomass / m^3	W/m^2	per day	per day	g-biomass / day
6	1	93437	0.000	-0.004		
	2	93047	0.000	-0.004		
	3	92660	0.000	-0.004		
	4	92274	0.000	-0.004		
	5	91889	0.000	-0.004		
	6	91506	1.494	0.034		
	7	91125	4.951	0.078		
	8	94234	6.061	0.086		
	9	101560	6.512	0.088		
	10	110261	6.696	0.089		
	11	120008	6.720	0.090		
	12	130744	6.676	0.089		
	13	142458	6.414	0.088		
	14	155185	5.873	0.084		
	15	168815	4.637	0.075		
	16	183071	1.096	0.026		
	17	196828	0.000	-0.004		
	18	201901	0.000	-0.004		
	19	201060	0.000	-0.004		
	20	200223	0.000	-0.004		
	21	199389	0.000	-0.004		
	22	198558	0.000	-0.004		
	23	197731	0.000	-0.004		
	24	196907	0.000	-0.004	0.032	49.491
7	1	196086	0.000	-0.004		
	2	195269	0.000	-0.004		
	3	194456	0.000	-0.004		
	4	193645	0.000	-0.004		
	5	192838	0.000	-0.004		
	6	192035	1.494	0.034		
	7	191235	4.951	0.078		
	8	197759	6.061	0.086		
	9	213134	6.512	0.088		
	10	231393	6.696	0.089		
	11	251849	6.696	0.089		
	12	274379	6.676	0.089		
	13	298925	6.414	0.088		
	14	325631	5.873	0.084		
	15	354231	4.637	0.075		
	16	384146	1.096	0.026		
	17	413011	0.000	-0.004		
	18	423657	0.000	-0.004		
	19	421892	0.000	-0.004		
	20	420135	0.000	-0.004		
	21	418385	0.000	-0.004		
	22	416641	0.000	-0.004		
	23	414905	0.000	-0.004		
	24	413177	0.000	-0.004	0.032	103.831