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LIMITS OF GROWTH OF SOME SIMPLE AQUATIC PLANTS

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Declaration

I declare that this thesis is my own unaided work. It is being submitted for the degree Doctor of Philosophy in Engineering to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.

.....
Michelle Low

..... day of ..... year .....

Abstract

The process of photosynthesis is of great importance as it is the reaction of carbon dioxide (CO_2) and water with the help of light, 'free' energy from the sun, to form useful carbohydrates and oxygen. Photosynthesis is therefore useful both in carbon dioxide mitigation and growing bio-feedstocks towards making biofuel.

This thesis aims to address two areas for analysing the photosynthesis process:

1. Looking at the physical limits of the growth; and
2. Improving the production rate of some aquatic plants, such as duckweed and microalgae.

To address the first aim, the fundamental concepts of thermodynamics were used to analyse the photosynthetic process. It was found that the theoretical minimum number of moles of photons (NP) required (9–17) is less than the values reported by other researchers, suggesting that the photosynthesis process is highly irreversible and inefficient (operating at 35% efficiency or less). This is because the number of moles of photons will increase with greater process irreversibility (when the entropy generated is greater than zero). If the photosynthesis process is indeed that irreversible then the removal of heat (the heat not used by other cellular processes) by the plant becomes a major problem. It is suggested that transpiration, and other cellular processes, are the processes by which that is done, and it is shown that the water needs of the plant for transpiration would dwarf those needed for photosynthesis. Knowing the fundamental limits to growth could also be of use because if an organism was growing at a rate close to this value there would be no advantage to try to do genetic modification to improve its rate.

Following the ideas presented above a spectrophotometer was used not only to obtain the absorption spectrum of algae, but it was also used to grow small samples at specific light wavelengths. The algae species researched was *Desmodesmus* spp., which, for example, is used to remediate waste water or as a

source of feedstock for biofuel production. It also tolerates high CO_2 concentrations. This simple experimental method demonstrated that a specific light wavelength (in particular the Secomam Prim spectrophotometer) 440 nm was preferred for the algae growth. It was recommended that this specific light wavelength would be best for growth. It might also be useful to know this fact particularly when designing photobioreactors, as this could reduce the amount of heat released into the surroundings and thus make the process more energy efficient. Interestingly, the wavelength for maximum growth corresponded to one of the peaks in the absorption spectra but there was no increase in growth rate corresponding to any of the other peaks.

To address the second aim, the author determined how well predictions on improving the growth of algae (*Desmodesmus* spp. for example), based on a theoretical model, would work when tested experimentally. What the researcher found was that the method improved algae production, using the same set of equipment. The production was improved by a factor of 1.28 and 1.26 (at product concentrations 1000 mg/L and 600 mg/L respectively) when retaining 40% of the algae suspension. The method may be particularly useful when large amounts of biomass are required as there is no extra cost of purchasing additional equipment. The same model was applied to a growth profile of duckweed (*Spirodela polyrhiza* 8483, which is convertible into biofuel or a source of food), and the author showed that the model could work if the duckweed was provided with an added carbon source. In order to find an economical and reliable alternative to bridge the scale gap between laboratory and industrial production, the author checked if duckweed species (*Spirodela polyrhiza* 8483, *Spirodela polyrhiza* 9509, *Lemna gibba* 8428, *Lemna minor* DWC 112, *Wolffia cylindracea* 7340 and *Wolffia globosa* 9527) could be cultivated in media less expensive than the basal laboratory medium (Schenk and Hildebrandt). The author found that duckweed can be cultivated more efficiently, and in a more cost-effective manner, in the alternative media types, while maintaining growth rates, RGR ≈ 0.09 day^{-1}, and starch contents, 5–17%(w/w), comparable with that obtained with the conventional laboratory media.

Thus, by looking at the photosynthesis process thermodynamically and experimentally, it is shown to be possible to improve the process by using concepts presented in this thesis.

To my loving and supportive parents
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To
David

To
Christine

To
Sushi-Sun

To
Lee Low Lok & Toy Ling and Chan Mhew-Ann & George Law

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"PhD is an apprenticeship, one cannot teach one how to do research."

- Prof D. Glasser

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Nomenclature

Abbreviations

-S	Without sucrose
Abs	Absolute value
AFZ	Association Française de Zootechnie
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BY	Biomass yield
CCS	Carbon capture and sequestration
CELSS	Controlled Ecological Life Support Systems
CIRAD	Centre de coopération Internationale en Recherche Agronomique pour le Développement
COP	Conference of the Parties
DW	Duckweed
exp	Exponential
FAME	Fatty acid methyl esters
FAO	Food and Agricultural Organization of the United Nations
HP	Hydroponic media
IG	Instrument Grade
INRA	Institut National de la Recherche Agronomique
IPCC	Intergovernmental Panel on Climate Change

LED	Light emitting diode
log	logarithm
MS	Murashige and Skoog
NADPH	reduced Nicotinamide Adenine Dinucleotide Phosphate
NDP	National development plan
NOAA	National Oceanic and Atmospheric Administration
NP	Number of photons (mol photons/mol substance) or moles of photons
NPK	Nitrogen, Phosphates, Potassium
NRF	National Research Foundation
OD	Optical density
PBR	Photobioreactor
PC	Previous culture
PEF	Partial Emptying and Filling
RDSC	Rutgers duckweed stock cooperative
RGR	Relative Growth Rate (also known as specific growth rate)
RSA	Republic of South Africa
SAB	Sodium Acetate Buffer
SAM	Swine artificial medium
SH	Schenk and Hildebrandt
TN	Total Kjedahl Nitrogen
USA	United States of America
WS	With sucrose

Greek Symbols

λ	Photon wavelength (nm)

ϕ or Φ	Retained fraction
$\triangle$	A difference between two points

Molecular Formulae

$(CHO_2O)_n$	Carbohydrate
$C_6H_{12}O_6$	Glucose
CO_2	Carbon dioxide
H_2O	Water
O_2	Oxygen

Subscripts and Superscripts

*	Intermediate
0	Basis/ Initial
A	Component A
f	Formation
g	Gas
gen	Generated
i	Component i
in	Flow of substance into a system
l	Liquid
max	Maximum value
min	Minimum value
out	Flow of substance out of a system
s	Solid

Mathematical Symbols

∞	Infinity

$E_{photons}(\lambda)$	Energy of the photon (kJ/mol)
$n_{photons}$	Number of moles of photons (mol photon/mol substance)
$\triangle H_{vap}$	Enthalpy of vaporization (kJ/mol)
n	Number of cycles
c	Speed of light (2.998 x 10^8 m/s)
h	Planck's constant (6.626 x 10^{-34} J.s)
k	Boltzmann constant (1.38 $\times$ 10 $^{-23}$ J/K)
C	Concentration
$C_{p,water}$	Specific heat of water (4.182 kJ/(L.K))
F	Frequency
G	Gibbs free energy (kJ/mol)
H	Enthalpy (kJ/mol)
m	Gradient of a line
N_A	Avogadro's number (6.022 x 10^{23} particles/mol)
P	Production rate (mass/time)
Q	Heat into or out of the system per mole of reaction (kJ/mol)
R	Universal gas constant (8.134472 J/(mol.K))
RF	Resonance frequency
RF_{max}	Maximum resonance frequency
RGR'	A light induced growth rate to be the value at a particular frequency minus the one without light
RGR'_{max}	A light induced growth rate to be the value at a particular frequency minus the one without light RGR at the maximum resonance frequency
S	Entropy (kJ/(mol.K))
T	Ambient temperature (25°C or 298.15 K)

t	Time
V	Volume
X	Mole fraction of a component
z	Distance

Chapter 1

Introduction

1.1 Background and motivation

1.1.1 Climate change

As of January 2013 the United States National Oceanic and Atmospheric Administration (NOAA) reported that the carbon dioxide (CO_2) level in the atmosphere was 389.69 ppm (McGee, 2010), but currently it is 407.57 ppm (McGee, 2016), or expressed in mole percent 0.040757 % (NOAA, 2016). However the upper safety limit in the atmosphere is reckoned to be 350 ppm CO_2 (McGee, 2010). The Intergovernmental Panel on Climate Change (IPCC) attributes the increase in carbon dioxide to the burning of fossil fuels, to the use of biomass as a fuel and to related industrial processes (Metz et al., 2005). Wilkinson and his co-workers (2007) add that greenhouse gases are a result of the socio-economic development, which results in urbanization, which in turn results in high energy demands.

Carbon dioxide is one of the main greenhouse gases which contribute to the increase in the temperature of the earth (Ziska et al., 2009), known as global warming. Britannica (2009) states that the IPCC reported that since the industrial revolution, the average global surface temperature has increased by 0.6°C, the sea level has risen by seventeen centimetres and in the northern hemisphere the average snow cover has decreased by four percent. The effects of global warming are therefore changing the behaviour of the weather, such as the melting of the ice caps and increasing sea levels (Ncube et al., 2013), which in turn could affect not only public health (Frumkin and McMichael, 2008), but animal life and populations living near to the coast and also plant

life (Ziska et al., 2009).

Climate change is also affecting energy policies, and authors such as Frumkin and McMichael (2008) and Ncube et al. (2013) make recommendations for adjustment in new or existing energy policies. Climate change is also a threat to the global population, whereby the poorer populations are more at risk from the effects of greenhouse gases (Campbell-Lendrum and Corvalán, 2007; St Louis and Hess, 2008). It is encouraged that better energy policies should be geared towards sustainability rather than short-term economic development in urbanization (McMichael et al., 2006; Campbell-Lendrum and Corvalán, 2007). Frumkin and McMichael (2008) agree that reducing greenhouse gases can also help reduce air pollution and that any result, negative or positive, can help guide energy policies. Ncube and his co-workers (2013) found that the South African local government infrastructure, such as water and electricity services, will be affected by climate change and one of their recommendations is that policies should be geared towards climate change mitigation and adaptation strategies.

Root et al. (2003) analysed the global warming effects on species including plants. In conclusion, Root and her colleagues (2003) identified a rapid temperature change which could lead to the possible extinction of some plants and animals. Thomas et al. (2004) used analytical methods to obtain estimates of the effect of the increase in the temperature of the earth on different taxa, possibly even extinction. They recommended that there should be an implementation of technologies not only to reduce greenhouse gases but also to reduce carbon dioxide via sequestration. McMichael et al. (2006) investigated the effects of what global warming can do, due to the change in climate to health in relation to thermal stress, floods and infectious diseases. The researchers recommended that the global research then being done should also be incorporated into global policies. Additionally, McMichael and his co-workers (2006) said that we are now living in an era where the greenhouse gases that are present, are exceeding the Earth's capacity to absorb them. Currently the carbon dioxide (CO_2) level in the atmosphere is 407.57 ppm (McGee, 2016), which is 0.040757 % mole percent (NOAA, 2016)

Therefore, from all of the above, carbon dioxide (a greenhouse gas), should be reduced as its increase is affecting not only the climate, but this has a

global influence on the health of humans, animals and plants, and it must ultimately be reflected in government policies.

1.1.2 Carbon dioxide mitigation

From the effect of carbon dioxide on the environment, the amount of carbon dioxide being emitted from industrial processes should be reduced. Metz et al. (2005) lists five possible ways in which this can be done:

1. Improve the energy efficiency of processes;
2. Reduce the dependence on carbon intensive fossil fuels;
3. Increase use of low carbon energy sources, such as nuclear energy or renewable energy sources such as solar, wind power, biomass and hydro;
4. Carbon dioxide sequestration through the enhancement of natural biological sinks; and
5. Carbon dioxide capture and storage.

The recommendations for non-biological forms of capturing carbon dioxide given by Metz et al. (2005) are graphically represented in Figure 1.1.1. However, biological sequestration of carbon dioxide from a flue-gas was not factored into the aforementioned recommendations, but was cited in Pires et al. (2011), referring to authors that suggest that biological systems such as trees or algae can capture carbon dioxide. The capture of carbon dioxide from industrial sources is not mitigation but rather carbon recycling (Packer, 2009).

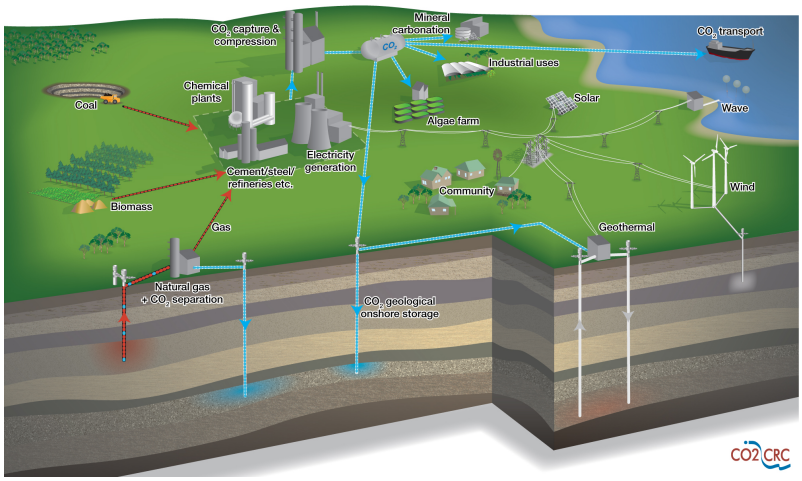

Figure 1.1.1: Schematic view of non-biological processes for capturing carbon dioxide from combustion (CO2CRC, 2016).

Carbon dioxide mitigation is of interest as carbon dioxide is still being produced as a by-product of many processes, and will probably continue to be so until fossil fuels run out. Stewart and Hessami (2005) reviewed papers on different techniques of sequestration of flue gases ranging from membrane adsorption technologies to geological and oceanic sequestration, none of which are economically feasible or sustainable. In concluding their review, Stewart and Hessami (2005) explained that the natural process of photosynthesis is sustainable, economical and viable to fix carbon dioxide in order to produce useful by-products. Stuart (2011) reviewed geological sequestration as well as biological recycling, and he concluded that in order for anything to have a significant impact, a combination of newly developed technology to reduce CO_2 emissions, such as carbon capture and sequestration (CCS) technology, and enhanced biological recycling (photosynthesis) to absorb the CO_2 from the atmosphere and plants should be utilized. This results in research opportunities in biomass cultivation as well as processing technologies (Stuart, 2011).

The research presented in this thesis will deal with natural biological systems which are capable of reducing carbon dioxide by a process called photosynthesis. The process of natural photosynthesis is of interest as it is the reaction of water and carbon dioxide with the help of light to form oxygen and carbohydrates. It is the most successful solar converter (Barber, 2007). Sunlight is freely available energy, is non-polluting (Barber, 2007) and is the largest available energy resource (Lewis and Nocera, 2006; McConnell et al., 2010).

Berg and his co-workers (2002) said that the mechanism of photosynthesis consists of two stages, namely light dependent and light independent. A simple reaction is represented by equation 1.1.1. Glucose is chosen as the representative of the carbohydrate as it can be considered to be a building block in many plant organisms, including various species such as algae and duckweed.

$$CO_2 + H_2O \rightarrow {}^1\!/_6 C_6H_{12}O_6 + O_2 \tag{1.1.1}$$

The product of photosynthesis is a renewable energy feedstock source, as opposed to the reducing supply of non-renewable energy from fossil based fuels. Bio-renewables are also environmentally safe, cleaner and emit fewer greenhouse gases than fossil fuels (Demirbas and Demirbas, 2011).

Barber (2007) said we should exploit all known technologies to produce energy

and at the same time mitigate carbon dioxide production. Whitesides and Crabtree (2007) highlighted fundamental research in carbon dioxide, where its uptake and its fixation within biological photosynthesis should be optimised. Tour and his colleagues (2010) argued that in the future carbon dioxide would be considered as a useful resource and not a waste product.

1.1.3 Types of aquatic plants

There are two types of photosynthetic aquatic plants:

1. Microphytes: Phytoplankton is an example and there are many species of microalgae, according to Barsanti and Gualtieri (2006) approximately 5000, and these move freely in the open water zone or in water columns (de Nie, 1987).

2. Macrophytes: These can be seen with the naked eye and are those that grow in or near water and are floating, submerged, or emergent (Hasan and Chakrabarti, 2009); examples of floating macrophytes are azolla, duckweeds, water hyacinths and filamentous algae.

1.1.4 Motivation for using an aquatic plant

Algae may play an important role in reducing carbon dioxide emissions from industrial plants, thereby alleviating the impact of CO_2 on the environment (Doucha et al., 2005). Furthermore, by the time of the Konur (2011) study, he found that research related to algae and bio-energy had increased exponentially, where the United States of America, China, Germany and England were the biggest contributors to algal research. Jeong et al. (2003) stated that since the beginning of the 1990's, research in biotechnology using microalgae as a CO_2 mitigator had been studied. Demirbas and Demirbas (2011) reported that microalgae was among the fastest growing species in the world. According to Packer (2009), research in the area of algal cultivation for CO_2 fixation and its potential conversion to biofuels was not a new idea; it had been suggested that it could be used in the earliest internal combustion engines before petroleum became the most economically feasible option. Additionally, Packer (2009) showed in his review paper a simple comparison (see Figure 1.1.2) the increasing growth rate potential of biomass with additional carbon dioxide.

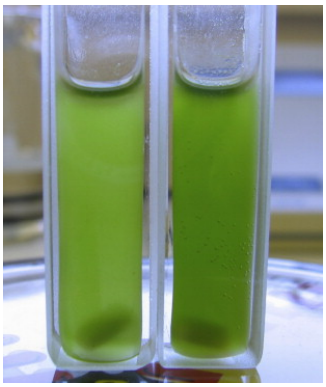

Figure 1.1.2: Comparison of algae (*chlamydomonas reinhardtii*) without CO_2 (on the left of the picture) and with CO_2 (on the right). This was to demonstrate the microalgae's potential to capture CO_2. Figure from Packer (2009).

Not only can algae be used to reduce the amount of CO_2 being released into the atmosphere but it can be converted to renewable energy (such as hydrogen or biodiesel), food products or pharmaceutical goods (Jeong et al., 2003; Demirbas and Demirbas, 2011; Kumar et al., 2011; Skjånes et al., 2013). Sorokin and Krauss (1958) highlighted the genetic make-up of different species having an influence on growth, as well as their response to external conditions. Depending on the type of species, they can produce various types of products because of differences in their protein, carbohydrates or oil content (Demirbas and Demirbas, 2011). However, Wijffels and his co-workers (2010) suggested that all of the microalgae components should be used for the process to be economical. Green algae is an important source of food for zooplankton (organisms living in fresh water or the sea) (NHM, 2013a). It is a potential biofuel contributor in that certain parts contain oils (Demirbas and Demirbas, 2011 gave a range of 2% to 40%). However, the current technology for efficient conversion into viable products is not thought to be economically feasible and needs to be researched further (NHM, 2013b). Microalgae are known as one of the feedstocks for third generation biofuels, which does not raise the issues with the first and second generation biofuels, such as the competition between food and fuel and debates about the use of arable land (Singh et al., 2011). This thesis would aim to propagate the microalgae, and investigate what would define the limits of its production. A more detailed outline is found in Section 1.2

Chisti (2007) suggested that microalgae is the only source of renewable biodiesel (a form of solar energy according to Demirbas and Demirbas, 2011), because microalgae will be able to meet global fuel requirements. Pimentel

(2008) said that there is a need for laboratory and field research for the production of the algae and oil. Hossain et al. (2008) reported that algae is one of the best sources for biodiesel and it is a versatile feedstock for several sources of biofuel, such as methane in the anaerobic digestion of algae, biodiesel from the algal oil and biohydrogen. Pokoo-Aikins and her co-workers (2010) list advantages of using algae for biodiesel production, namely the high oil yield and its ability to sequestrate carbon dioxide. Demirbas and Demirbas (2011) argued that algae can grow on land not suitable for food crops and on any type of water such as wastewater. It can also take up nutrients from the wastewater (Wijffels et al., 2010; Singh et al., 2014). The review by Zhao and Su (2014), concluded that it is important that after carbon dioxide fixation, microalgae should be used as a biofuel because of the global energy crisis.

Wilkinson and his co-workers (2007) suggested that shifting towards renewable energy would help industry towards cleaner energy. In an article by Yang et al. (2008), they mention that despite 50 years of research, commercial viability had not yet been achieved. Taylor (2010) reported a commercial interest in the growth of algae for the use of biofuels. For example, a company in Australia had a pilot plant, later decommissioned, that demonstrated the ability to grow algae from coal-fired flue gas. However, they were only researching improved oil extraction (Taylor, 2010). Although, commercial companies such as Algenol Biofuels were reported to be investigating the possibility of growing algae and extracting the lipid economically (Algenol, 2013). Packer (2009) suggested the need for basic research of the algal technology, and that within the right economic environment it would be able to provide a more stable carbon recycling technology. Fischer et al. (2011) discussed a fundamental need for continuous research on many topics such as strain selection and cultivation of microalgae.

There are two ways to cultivate algae, in open systems such as ponds, or closed systems which include photobioreactors (PBR). The latter is more expensive for producing microalgae than the former because of the associated costs for example construction material (Demirbas and Demirbas, 2011; Singh et al., 2014). Furthermore, issues such as contaminants (from wastewater) can decrease the efficiency of PBRs (Zhang et al., 2014b). However, Posten (2009) suggested that higher algae area production and reduction of water loss (from evaporation) gives PBRs an advantage over open-ponds, and the reactors can

provide a controlled environment for microalgae growth (Zhang et al., 2014b).

PBRs are pieces of equipment where one can introduce flue gas into the system for the purpose of mitigating CO_2. A review by Kumar et al. (2011), referred to other researchers in the field focussing mainly on the ability of microalgae to sequestrate CO_2 from flue gases (at high temperatures) and the factors affecting it in the context of PBRs. Kumar and his colleagues (2011) list factors which affect carbon dioxide sequestration, namely, light, pH, O_2 removal, suitable design of the PBR, culture density, and the proper mixing within the reactor. In the report by Rawat et al. (2013), of the companies he surveyed who want to commercialise the production of algae, 52% will opt for closed systems, 26% proposed open systems and 22% will use settings which are natural for the cultivation of the algae.

Singh and his co-workers (2014) reported that it is cost intensive to cultivate microalgae. The Demirbas and Demirbas (2011) review included a section on the economic feasibility of producing biofuel from microalgae, which was not yet economically feasible by comparison with other agricultural biomass. The economic feasibility studies focussed their calculations on photosynthetic efficiency, scale-up assumptions, and long-term cultivation issues (Demirbas and Demirbas, 2011). Potential biomass yield and the costs associated with production of that biomass and its value added products were added to the discussions (Demirbas and Demirbas, 2011). These authors added that an enormous supply of cheap biomass is needed for biodiesel to become the choice as an alternative fuel. They noted that innovative technology to cultivate algae could reduce the price and compete with (or replace) fossil-based fuels. Wijffels et al. (2010) concluded that the propagation of microalgae was immature, and needed a multidisciplinary approach, for example, PBR operation, design and scale-up, in order to produce microalgae in an economical manner.

Rawat et al. (2013) found that moving from small to large scale cultivation of microalgae (for biodiesel production) needs careful planning. This includes a selection of affordable media since artificial media (basal salt media) are not suitable for pilot or even large-scale processes (Rawat et al., 2013). On the other hand, the researchers reported that although the use of wastewater would reduce costs, its use for large-scale purposes had not been well established. Singh et al. (2014) found quoting from other researchers in the field that

in all stages of the production of biodiesel, including cultivation and harvesting of the microalgae, energy needs to be minimised. At that stage, researchers in the field acknowledge that producing biofuel from microalgae, was not a sustainable process because of the amounts of water, nutrients and energy that were needed (Gautam et al., 2014).

Chisti (2007) gave a list of seven options on molecular engineering scale for enhancing algal biology for the manufacture of biofuels. One of these, which is of interest, is to enhance the biomass growth rate. Issues of biofuel production from microalgae such as microalgae harvesting and oil extraction from the algal species will not be the focus of this thesis. These open the doors for additional research which is beyond the scope of this thesis, which is limited to analysing the growth limitations of the feedstock for biofuel. However, Eriksen (2008) argued that future improvements for microalgae culturing depends on ongoing research and development such as will be done in this thesis.

Algae is a viable aquatic plant source that can be used to reduce carbon dioxide and to clean water, and it has a range of potential products, therefore its production has the potential to be turned into a sustainable process. With this in mind the natural photosynthetic process will be studied to evaluate more fundamental issues regarding the growth of the chosen microphyte microalgae, *Desmodesmus* spp. These algae are readily and locally available and will be studied in order to understand the limits to its growth. This will be done in order to make recommendations for improving its production.

1.2 Research aims and objectives

The research set out in this thesis aimed to meet the following major objectives. The focus would be on what defines the limits of production of the aquatic plant. This would be done in order to help with design considerations when growing an aquatic plant such as algae or duckweed, in photobioreactors. The intention was to use these results to help reduce South Africa's green house gas production (NPC, 2013). Furthermore, the end purpose of this research was to investigate means of producing a feedstock that will supply the next generation of biofuels, and to gain insight into the photosynthesis process. This would be done with two approaches:

1. Evaluating the physical limits of growth by:

(a) using the first and second law of thermodynamics; and

(b) exploring whether a specific light wavelength is more suitable for growth using a piece of analytical equipment as both the reactor and the measuring device.

2. Determining production improvements by:

(a) improving a batch reactor's performance by applying a theoretical model using algae as the case study;

(b) checking if the model be applied to another aquatic plant source from the macrophytes such as duckweed; and

(c) propagating other species to find cheaper media than the very expensive artificial media.

1.3 Layout of the thesis

The thesis is organized into seven chapters, including the Introduction. In the introductory chapter the background and motivation are presented as well as the research objectives of the thesis. Each chapter has its own reference list. Chapters one and seven are written as normal thesis chapters.

The reader will see that each of chapters two to six has a review of scientific literature related to the body of work of that particular chapter. The reason for this format is that the research for each chapter is presented as a paper for publication. Chapters two to six can be read as independent research reports. The reader can refer to Appendix A for the individual Chapter's plagiarism report. The reader can also refer to Appendix B for the copyright clearance of figures not drawn by the author. Therefore, for instance, there will be overlaps between chapters three and four as well as chapters five and six. Each chapter will, however, provide an input towards the overall objective of the thesis. What follows is a short description of the content of each chapter.

Chapter 2: A black-box thermodynamic analysis of the photosynthesis process: determining operational limits.

The thermodynamic limits of the photosynthesis reaction are explored by applying mass, energy and entropy balances of the process.

Chapter 3: A black-box experimental analysis on the growth of microalgae *Desmodesmus* spp. at specific light wavelengths.

Experiments with algae at different specific light wavelengths are done in a spectrophotometer. Some of the findings are related to the results from Chapter 2.

Chapter 4: The application of batch partial emptying and filling to improve the production rate of *Desmodesmus* spp. algae on a laboratory scale.

The opportunity for improving productivity by applying theoretical concepts put forward by other authors is both investigated theoretically and checked experimentally.

Chapter 5: Comparison between different media used for duckweed propagation: steps toward optimized scale-up production of duckweed biomass.

Duckweed, an aquatic plant which has a faster growth rate than algae was investigated. It is also a compelling candidate as a source of biofuel. This research work was done at Rutgers University while on study leave. The intention was to bridge the gap between lab and large scale production by finding affordable media in which to cultivate duckweed (other than wastewater).

Chapter 6: Determining the partial emptying and filling (PEF) potential of *Spirodela polyrhiza* 8483.

This is an extension of the method used in Chapter 4, but applied to duckweed (with or without a carbon source) with the intention to determine theoretically whether its production rate can be improved by changing the operational method. This work was also done at Rutgers University.

Chapter 7 Conclusions.

This chapter is a general discussion linking Chapters 2 to 6 and is based on the results obtained in relation to the objectives set out for the thesis. Recommendations are also included for future directions for the work.

References

Algenol, 2013. Algenol Biofuels. [Online]. Available at: http://www.algenolbiofuels.com/, [accessed 18 March 2013].

Barber, J., 2007. Biological solar energy. Philosophical Transactions of

the Royal Society A: Mathematical, Physical and Engineering Sciences 365 (1853), 1007–1023.

Barsanti, L., Gualtieri, P., 2006. Algae: anatomy, biochemistry, and biotechnology, 1st Edition. CRC (Taylor and Francis), Florida, USA.

Berg, J. M., Tymoczko, J. L., Stryer, L., 2002. The light reactions of photosynthesis. In: Biochemistry, 5th Edition. WH Freeman, New York, USA, Ch. 19.

Britannica, 2009. Global Warming. [Online]. Available at: http://www.britannica.com/EBchecked/topic/235402/global-warming, [accessed 26 July 2010].

Campbell-Lendrum, D., Corvalán, C., 2007. Climate change and developing-country cities: implications for environmental health and equity. Journal of Urban Health 84 (1), 109–117.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnology Advances 25 (3), 294–306.

CO2CRC, 2016. CO2CRC. [Online]. Available at: http://www.co2crc.com.au/imagelibrary3/general.php, [accessed 19 January 2016].

de Nie, H. W., 1987. The decrease in aquatic vegetation in Europe and its consequences for fish populations. EIFAC Occasional Paper (19), 1–52.

Demirbas, A., Demirbas, M. F., 2011. Importance of algae oil as a source of biodiesel. Energy Conversion and Management 52 (1), 163–170.

Doucha, J., Straka, F., Lívanskỳ, K., 2005. Utilization of flue gas for cultivation of microalgae *Chlorella* sp. in an outdoor open thin-layer photobioreactor. Journal of Applied Phycology 17 (5), 403–412.

Eriksen, N. T., 2008. The technology of microalgal culturing. Biotechnology Letters 30 (9), 1525–1536.

Fischer, B., Richardson, J., Outlaw, J., Allison, M., 2011. Economic feasibility of commercial algae oil production in the United States. [Online]. Available at: http://purl.umn.edu/98834, [accessed 27 February 2011].

Frumkin, H., McMichael, A. J., 2008. Climate change and public health: thinking, communicating, acting. American Journal of Preventive Medicine 35 (5), 403–410.

Gautam, K., Pareek, A., Sharma, D., 2014. Exploiting microalgae and macroalgae for production of biofuels and biosequestration of carbon dioxide- A review. International Journal of Green Energy (just-accepted).

Hasan, M., Chakrabarti, R., 2009. Use of algae and aquatic macrophytes as

feed in small-scale aquaculture. A review. Fisheries and Aquaculture Technical Paper (FAO) (531), 1–123.

Hossain, A., Salleh, A., Boyce, A., Chowdhury, P., Naqiuddin, M., 2008. Biodiesel fuel production from algae as renewable energy. American Journal of Biochemistry and Biotechnology 4 (3), 250–254.

Jeong, M., Gillis, J., Hwang, J., 2003. Carbon dioxide mitigation by microalgal photosynthesis. Bulletin-Korean Chemical Society 24 (12), 1763–1766.

Konur, O., 2011. The scientometric evaluation of the research on the algae and bio-energy. Applied Energy 88 (10), 3532–3540.

Kumar, K., Dasgupta, C., Nayak, B., Lindblad, P., Das, D., 2011. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresource Technology 102 (8), 4945–4953.

Lewis, N. S., Nocera, D. G., 2006. Powering the planet: Chemical challenges in solar energy utilization. Proceedings of the National Academy of Sciences 103 (43), 15729–15735.

McConnell, I., Li, G., Brudvig, G. W., 2010. Energy conversion in natural and artificial photosynthesis. Chemistry and Biology 17 (5), 434–447.

McGee, M., 2010. CO_2 Now. [Online]. Available at: http://co2now.org/, [accessed 26 July 2010].

McGee, M., 2016. CO_2 Now. [Online]. Available at: http://co2now.org/, [accessed 31 May 2016].

McMichael, A. J., Woodruff, R. E., Hales, S., 2006. Climate change and human health: present and future risks. The Lancet 367 (9513), 859–869.

Metz, B., Davidson, O., De Coninck, H., Loos, M., Meyer, L., 2005. IPCC, 2005: IPCC special report on carbon dioxide capture and storage. Prepared by Working Group III of the Intergovernmental Panel on Climate Change.

Ncube, M., Zikhali, P., Musango, J. K., 2013. The impact of climate variability on water and energy demand: the case of South African local governments. Water and Environment Journal 27 (1), 29–41.

NHM, 2013a. *Desmodesmus* subspicatus (pond scum, green weed). [Online]. Available at: http://www.nhm.ac.uk/nature-online/species-of-the-day/scientific-advances/industry/desmodesmus-subspicatus/index.html, [accessed 19 March 2013].

NHM, 2013b. Uses for green algae. [Online]. Available at: http://www.nhm.ac.uk/nature-online/species-of-the-day/scientific-advances/

industry/desmodesmus-subspicatus/green-algae/index.html, [accessed 19 March 2013].

NOAA, 2016. Earth System Research Laboratory: Trends in Atmospheric Carbon Dioxide. [Online]. Available at: http://www.esrl.noaa.gov/gmd/ccgg/trends/, [accessed 31 May 2016].

NPC, 2013. National Development Plan Vision 2030. [Online]. Available at: http://policyresearch.limpopo.gov.za/handle/123456789/941, [accessed 31 May 2015].

Packer, M., 2009. Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. Energy Policy 37 (9), 3428–3437.

Pimentel, D., 2008. A brief discussion on algae for oil production: Energy issues. In: Pimentel, D. (Ed.), Biofuels, Solar and Wind as Renewable Energy Systems. Springer Netherlands, pp. 499–500.

Pires, J., Martins, F., Alvim-Ferraz, M., Simões, M., 2011. Recent developments on carbon capture and storage: An overview. Chemical Engineering Research and Design 89 (9), 1446–1460.

Pokoo-Aikins, G., Nadim, A., El-Halwagi, M., Mahalec, V., 2010. Design and analysis of biodiesel production from algae grown through carbon sequestration. Clean Technologies and Environmental Policy 12 (3), 239–254.

Posten, C., 2009. Design principles of photo-bioreactors for cultivation of microalgae. Engineering in Life Sciences 9 (3), 165–177.

Rawat, I., Kumar, R. R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Applied Energy 103 (0), 444–467.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., Pounds, J. A., 2003. Fingerprints of global warming on wild animals and plants. Nature 421 (6918), 57–60.

Singh, A., Olsen, S. I., Nigam, P. S., 2011. A viable technology to generate third-generation biofuel. Journal of Chemical Technology and Biotechnology 86 (11), 1349–1353.

Singh, B., Guldhe, A., Rawat, I., Bux, F., 2014. Towards a sustainable approach for development of biodiesel from plant and microalgae. Renewable and Sustainable Energy Reviews 29 (0), 216–245.

Skjånes, K., Rebours, C., Lindblad, P., 2013. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Critical Reviews in Biotechnology 33 (2), 172–215.

Sorokin, C., Krauss, R. W., 1958. The effects of light intensity on the growth rates of green algae. Plant Physiology 33 (2), 109–113.

St Louis, M. E., Hess, J. J., 2008. Climate change: impacts on and implications for global health. American Journal of Preventive Medicine 35 (5), 527–538.

Stewart, C., Hessami, M.-A., 2005. A study of methods of carbon dioxide capture and sequestration-the sustainability of a photosynthetic bioreactor approach. Energy Conversion and Management 46 (3), 403–420.

Stuart, B. J., 2011. Addressing the grand challenge of atmospheric carbon dioxide: geologic sequestration vs. biological recycling. Journal of Biological Engineering 5 (1), 1–8.

Taylor, R., 2010. Efficient algae–the next biofuel? [Online]. Available at: http://www.ecosmagazine.com/paper/EC10041.htm, [accessed 27 February 2011].

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F., De Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A., Midgley, G., Miles, L., Ortega-Huerta, M., Peterson, A., Phillips, O., Williams, S., 2004. Extinction risk from climate change. Nature 427 (6970), 145–148.

Tour, J. M., Kittrell, C., Colvin, V. L., 2010. Green carbon as a bridge to renewable energy. Nature Materials 9 (11), 871–874.

Whitesides, G. M., Crabtree, G. W., 2007. Don't forget long-term fundamental research in energy. Science 315 (5813), 796–798.

Wijffels, R. H., Barbosa, M. J., Eppink, M. H., 2010. Microalgae for the production of bulk chemicals and biofuels. Biofuels, Bioproducts and Biorefining 4 (3), 287–295.

Wilkinson, P., Smith, K. R., Beevers, S., Tonne, C., Oreszczyn, T., 2007. Energy, energy efficiency, and the built environment. The Lancet 370 (9593), 1175–1187.

Yang, H., Xu, Z., Fan, M., Gupta, R., Slimane, R. B., Bland, A. E., Wright, I., 2008. Progress in carbon dioxide separation and capture: A review. Journal of Environmental Sciences 20 (1), 14–27.

Zhang, S., Liu, H., Fan, J., Yu, H., 2014b. Cultivation of *Scenedesmus dimorphus* with domestic secondary effluent and energy evaluation for biodiesel production. Environmental Technology 36 (7), 929–936.

Zhao, B., Su, Y., 2014. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renewable and Sustainable Energy Reviews 31 (0), 121–132.

Ziska, L. H., Epstein, P. R., Schlesinger, W. H., 2009. Rising CO_2, climate change, and public health: exploring the links to plant biology. Environmental Health Perspectives 117 (2), 155–158.

Chapter 2

A black-box thermodynamic analysis of the photosynthesis process: determining the operational limits

- This chapter presents a model on the number of photons required for photosynthesis. The research does not use any experimental data, but rather presents a simple ('black-box') thermodynamic analysis of the photosynthesis reaction. This will help to provide insights into the thermodynamic limits of photosynthesis when growing a plant species, such as algae. The thinking behind the development of the minimum number of photons model was done with Kok H,W.[†], and he submitted his findings in his MSc Dissertation (Kok, 2011). Any further analysis presented in this chapter is that of the PhD candidate.

- This chapter will be submitted for peer review. An abbreviated form of this chapter was also presented orally at the 2010 ISCRE 21 in Philadelphia, USA as well as the 2010 AIChE Spring Meeting & 6th Global Congress on Process Safety, USA.

- The contribution of the other co-authors (Hildebrandt, D.[*];Glasser, D.[†*]; Matambo, T.[*]) towards the work is primarily that of supervision or collaboration, and the work and write-up was conducted by the author of this thesis.

Abstract

The process of photosynthesis is of interest as it is the reaction of carbon dioxide and water with the help of light and 'free' energy from the sun, to form useful carbohydrates and oxygen. Photosynthesis is therefore useful in carbon dioxide mitigation and in growing bio-feedstocks towards making biofuels. The aim of this chapter was to implement a theoretical analysis using thermodynamics to analyse the photosynthetic process, in the visible light spectrum, in order to better understand the process. This was performed by quantifying the minimum light energy requirements (number of photons or quantum requirement) at specific light wavelengths (λ) needed for the photosynthetic process. The theoretical number of moles of photons (NP), that is the minimum number of photons obtained in this chapter (9–17), are less than the values reported by other researchers. The literature values, if correct, would suggest that the rejection of heat is a big issue in photosynthesis, and consequently the management of heat would be a very important issue in plant physiology. Furthermore it suggests that the photosynthetic process is very irreversible and inefficient (operating at 35% efficiency or less) at utilizing photon energy as it would need large amounts of water to satisfy the heat being released, the amount of heat which is not used by other cellular processes. This is because the number of moles of photons will increase with greater process irreversibility. If the photosynthesis is indeed that irreversible then the removal of heat by the plant becomes a major problem. It is suggested that transpiration is the process by which that is done, and it is shown that the water needs of the plant for transpiration would dwarf those needed for photosynthesis.

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2.1 Introduction

2.1.1 Photosynthesis process

Carbon dioxide (CO_2) has been in the limelight for quite some time because of its gradual concentration increase in the atmosphere and its potential contribution to the greenhouse gas effect. This is because it is a contributing

factor to undesirable climate change. Sequestration is of importance as carbon dioxide is still being produced from fossil fuels, and will probably be so for many years to come. An effective method of carbon dioxide reduction can be achieved by the photosynthesis process used by plants. Not only do biological systems have the ability to sequestrate carbon dioxide, but the products have the potential to be used as an alternate renewable fuel (Gouveia, 2011).

The main aim of this chapter is to theoretically analyse the thermodynamics of the photosynthesis process in order not only to understand the minimum limits of operation of the process better, but to understand the role of light and the wavelengths of light (λ) that are needed. It is also the purpose of the author to further understand what the issues around it are. These theoretical constraints of the reaction or process can then potentially help when applying the models for design considerations to photosynthesis systems such as algal ponds and photobioreactors.

Berg and his co-workers (2002) said that the mechanism of photosynthesis consists of two stages, namely:

1. Light dependent: where the chlorophyll absorbs the photons, transfers that energy to two reaction centres or special photosystems (Moroney and Ynalvez, 2001) and turns that into useful chemical energy: such as ATP (adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate. ATP is a molecule which stores energy, energy that can be used for cellular reactions or processes (OpenStax-College, 2013); and

2. Light independent: using the ATP and NADPH from the light dependant stage, the CO_2 is converted to useful sugars, such as glucose, via the Calvin cycle, using an enzyme called RuBisCO–ribulose-1,5-bisphosphate carboxylase/oxygenase.

However, the generalized photosynthesis process or mass balance is shown below as equation 2.1.1. This involves the inputs of carbon dioxide, water (H_2O), and photons (the light energy from the sun which is the driving force for the reaction) to form oxygen (O_2) and carbohydrates $(CH_2O)_n$.

$$nCO_2 + nH_2O + \text{light} \rightarrow (CH_2O)_n + nO_2 \tag{2.1.1}$$

Glucose ($C_6H_{12}O_6$) is one of the simplest organic 'building blocks' in nature, and for the purposes of this chapter it is chosen to be the carbohydrate of choice as it has relevancy to many plant forms, including algae (which is a source of biofuel and takes up CO_2). It is also a basic unit of carbohydrate (Hill and Govindjee, 2014) and contributes to the formation of other products. The advantage of glucose as the chosen product for the synthesis analysis is revealed in the ready availability of both its enthalpy and Gibbs free energy of formation data, compared to that of starch or cellulose. Most journal articles, such as Bisio and Bisio (1998); Andriesse and Hollestelle (2001); Albarrán-Zavala and Angulo-Brown (2007); Petela (2008) and Feng et al. (2010) have used the standard enthalpy of formation of glucose to represent the carbohydrate in photosynthesis. The revised process is shown here:

$$6CO_{2(g)} + 6H_2O_{(l)} + \text{light} \rightarrow C_6H_{12}O_{6(s)} + 6O_{2(g)} \tag{2.1.2}$$

For autotrophic plants, such as microalgae, light is the main source of energy during the photosynthesis process to produce organic compounds (Carvalho et al., 2011). Hall and Rao (1987) mentioned that the main chlorophyll types found in most plant forms, including algae, are chlorophyll a and chlorophyll b. For chlorophyll a, the absorption peaks are 420 nm and 660 nm and for chlorophyll b the peaks occur at 435 nm and 643 nm (Hall and Rao, 1987). The chlorophyll species are most receptive to photons at these wavelengths, and thus are more readily photo-excited. However, chlorophyll a and chlorophyll b have different absorption peaks in different solvents, but in general both have maximas in the 400–500 nm and the 600–700 nm ranges (McDonald, 2003).

There is however, a debate as to how many moles, or the number of moles photons (NP), are required for photosynthesis (Osborne and Geider, 1987; Govindjee, 1999). The debate on the number of photons (also known as quantum requirement in other reports) has not yet led to a conclusive number. However, Lam et al. (1986) report from other researchers in the field that the values range from 4 to 13 NP/O_2 although the minimum number is thought to be greater than 8 NP/O_2 and this is the accepted value. Furthermore, Hill and Govindjee (2014) and Höxtermann (2007) gave an abbreviated account of the controversy as to how many photons were found experimentally by researchers in the field, a controversy of either three and four or 8 to 12 or even 16 to 20 per mole of oxygen formed. Therefore, the literature gives between 24 and 90 photons that will be required to produce 1 mole of glucose (Raven and

Johnston, 1991; Govindjee, 1999; Raven and Geider, 2004; Albarrán-Zavala and Angulo-Brown, 2007).

Additionally, it has to be taken into consideration that different methods and assumptions were used to measure the NP, such as manometry and calorimetry (Magee et al., 1939; Govindjee, 1999). Hoch and Kok (1963) suggested that in a manometer system, one cannot tell what gases (concentration) are produced by using pressure differences alone, especially in photosynthesis, where the same gas is being produced and consumed simultaneously. Researchers have measured photosynthesis by manometric techniques. Millan-Almaraz et al. (2009) reported that this involves measuring the variation of the O_2 pressure directly by keeping CO_2 constant via the addition of a chemical buffer (such as bicarbonate) to absorb the CO_2 gas. However, this technique had limitations because variables such as temperature and the composition of the air can influence the pressure readings (Millan-Almaraz et al., 2009).

Despite the debate on the number of photons, it is accepted that photosynthesis has two light reactions as it has two photosynthesis reactions (Bolton and Hall, 1991; Hill and Govindjee, 2014), and the Z-scheme accounts for a minimum of eight to ten quanta (or NP) per mole of oxygen formed (Hill and Govindjee, 2014). Respiration is the reverse reaction of photosynthesis, and Magee et al. (1939) found that the respiration rate is not proportional to the rate of photosynthesis and oxygen concentration. Light is an important factor in the growth of algae. Jeon et al. (2005), referred to Evers (1991), who suggested that it is important to understand and quantify the light dependence of the activity of the microalgae.

2.1.2 Thermodynamics

Since this chapter's work is based on the limits of the photosynthesis reaction using the first and second law of thermodynamics, this section reviews authors using thermodynamics on the photosynthesis process.

2.1.2.1 First and Second Laws

A thermodynamic approach which included the energy, entropy and exergy analysis of photosynthesis was examined by Petela (2008). Energy is defined

as the maximum useful work which is destroyed by irreversibility, and expresses the real ability to do work (Petela, 2008). The researcher recommended that several detailed exergy evaluations should be carried out on photosynthesis, namely: temperature, light intensity and carbon dioxide concentration.

There have been authors that examined photosynthesis microscopically such as closely looking at the photosystems. Jennings et al. (2005) examined the photosystems of the plant with regard to the photon absorption and primary photochemistry. They concluded that there was a negative entropy production in photosynthesis. However, Lavergne (2006) argued that photosynthesis does not decrease the entropy on earth. Jennings et al. (2006) clarified that the second law of thermodynamics for primary photochemistry can be violated at high thermodynamic efficiencies. Conversely, Knox and Parson (2007a) ascertained that the conclusion by Jennings et al. (2006) was incorrect as they left out a variable at the initial photo-excitation stage which contributes to the entropy production and therefore allows the second law to be true. The final reply from Jennings et al. (2007) argued that there was negative entropy production in photosynthesis because of the high efficiency that occurred from the photon absorption stage to the primary charge separation event. Knox and Parson (2007b) criticized the paper by Jennings et al. (2007) and asserted their grounds and understanding why Jennings et al. were incorrect in their assumptions. Nonetheless, authors such as Yourgrau and Van der Merwe (1968) and von Stockar and Liu (1999) have also encountered a negative entropy value in their thermodynamic calculations for microorganisms. Furthermore, the references to the second law of thermodynamics (Jennings et al., 2006) might indicate that non-equilibrium thermodynamics might be at play within the biological processes.

Brittin and Gamow (1961) derived an approximation for the maximum entropy change per molecule formed by considering that the photosynthetic reaction, equation 2.1.2, was exposed to monochromatic radiation from the sun. They came to the conclusion that the photosynthetic reaction is consistent with the second law of thermodynamics if the entropy conversion has at least an efficiency of 10 percent. Yourgrau and Van der Merwe (1968) evaluated the entropy balance for photosynthesis and improved on the Brittin and Gamow (1961) work. They included the amount of energy being capable of being absorbed by the leaf and expressed that as an efficiency. Furthermore the flux

parameters, specific radiation intensity of the frequency and the specific intensity of entropy radiation, were included. Their final derived equation was in terms of the amount of diluted radiation that the leaf will absorb and release. Their conclusion was if the efficiency was less than 88%, the entropy of the monochromatic radiation would be greater than that of the radiation for the reacting molecules.

Albarrán-Zavala and Angulo-Brown (2007) did a thermodynamic analysis on photosynthesis, by using the Brittin and Gamow (1961) approximation and a Carnot cycle evaluation of the photosynthesis pathway. They evaluated several photosynthetic pathways, at specific wavelengths, including the pathway that is followed by Cyanobacteria, known as blue-green algae, which is shown by equation 2.1.2. They derived the entropy of the universe equation describing the overall system, which included the entropy of the sun, the earth and the photosynthetic organism undergoing photosynthesis, assuming 60 NP were participating. They further derived another formula highlighting that they needed to calculate the Sun's radiation correctly to get the entropy change to the earth. This was done by using the assumptions that the light is a photon gas i.e. black body radiation and that there is a dilution factor as the Sun's radiation enters the earth. Kirwan (2004) found that the photon entropy is independent of wavelength which is contrary to the assumptions made by Albarrán-Zavala and Angulo-Brown (2007).

2.1.2.2 Energy balance: heat release from photosynthesis

A possible term from the process of photosynthesis is released heat (Q) into the environment. Milo (2009) highlights the finding that photons in the band gap energy (about 700 nm) will absorb more energy but the rest will result in heat lost. Von Stockar et al. (2011) used calorimetry to confirm that photosynthesis is indeed endothermic, however, because of the excess light, there was a lot of heat generation. The researchers also suggest the intrinsic thermodynamic force of the irreversible entropy generation is what drives the growth, but speculate that -500 kJ/Carbon-mol is the expected order of magnitude.

Maskow et al. (2010) report that Magee et al. (1939) were successful in photocalorimetric studies on the study of photosynthesis using algae, *Chlorella vulgaris*, for the intention of determining the number of photons required per mole of glucose. Although in the data by Magee et al. (1939) it was shown that the

amount of heat evolved within their calorimeter ranged between 0.013–0.015 Watts (depending on the light intensity, a result obtained when subtracting the effects of respiration away from photosynthesis). According to a review by Wadsö (1995) and more recently by Maskow et al. (2010) not much photo-calorimetric studies have been carried out. Photo-calorimetric studies could be useful for the determination of heat released from the microbial processes. Maskow et al. (2010) attributes the lack of reports to complications experienced, such as heat from the light sources, heat dissipation and maintaining an isothermal photobioreactor. They go on to warn future photo-calorimetrists, that most of the sun's energy is dissipated as heat, because only a fraction of the heat is absorbed and stored by the plant. However, they also note that changes in metabolism is correlated to heat production in batch and continuous cultivations.

To help with the optimization of photo-autotrophic bio-processors, there are scientists, such as Janssen et al. (2007) and Mukhanov and Kemp (2009), who are working on improving their photo-calorimeters. Johansson and Wadsö (1997) used micro-calorimetric techniques (with different concentrations of carbon dioxide) and found that the Gibbs Free Energy of formation of spinach leaves had a similar value to that of glucose. Patino et al. (2007) managed to also measure the heat flux with time from the growth of *Chlorella vulgaris*, when taking away the effects of the light and stirring. Their results revealed that fed-batch mixotrophic (autotrophic with heterotrophic—glucose as a carbon source) growth released more energy than a batch process. Additionally, they found that while autotrophic growth was endothermic, heterotrophic growth released more energy than mixotrophic growth, as the latter favoured photosynthesis more when the light intensity increased (see more in Patino et al., 2007). On the other hand, Lamprecht et al. (1991) used a calorimeter to measure the temperature rise within a thermogenic plant (a lily which releases heat during inflorescence) and found it was 7^{o}C above ambient temperature, a result from a high heat production during flowering.

Losing large amounts of heat is considered a reduction in efficiency. Janssen et al. (2005) found for their experiment using bio-calorimetry (using *Chlorella vulgaris*) that the photosynthetic efficiency improved from 7.1% to 10.5% by increasing the light intensity and light storage within the species. This is an important factor for optimization to increase productivity. A review by Wil-

helm and Jakob (2011) cited researchers who found for a group of algae (a diatom), that 70% of heat was lost via florescence, while in a green algae culture, only 35% of heat was lost. They went on to add that other researchers found for different compositions of algae (lipid, protein or carbohydrate content) that the NP was different. Zhu et al. (2008) reported that in C3 and C4 plants the solar energy to biomass conversion is 4.6–6%, and list possible areas where the energy losses are occurring:

- that not all energy is transferred to photosynthesis when non-photosynthetic pigments absorb the energy from the wavelengths;
- energy loss with the species, particularly in the chlorophyll, reaction centre and carbohydrate synthesis; and
- via photorespiration and respiration.

The limiting factor of the photosynthesis process is not often light energy but the efficiency of the photosynthetic system (Milo, 2009). Photosynthetic efficiency is 35% assuming the use of the accepted number of 8 photons at 680 nm (Mauzerall, 2013). The review by Carvalho et al. (2011) suggested that photosynthetic efficiency ranged between 6.5–40%. They included a summary of approaches to optimise the efficiency via light or genetic engineering. In the report by Emerson and Lewis (1943), they confirmed that the quantum yield is independent of light intensity over green, blue and red light. However, at low intensities, for wavelengths beyond 685 nm, the efficiency of photosynthesis decreases but can be enhanced by supplementation of wavelengths between 644 and 680 nm. That research led them to the two photon-systems which supported Einstein's law of photochemistry. The law states that the number of absorbed quanta should be in proportion to the primary photochemical action (Emerson and Lewis, 1943), which in turn supports the postulate for 8–10 photons to oxidize water to O_2 (Hill and Govindjee, 2014).

Perlman (2014) reported that plants release moisture through transpiration which contributes to approximately 10% of the moisture found in the atmosphere. Additionally, as the temperature goes up so does transpiration (Perlman, 2014). On the other hand, Jasechko et al. (2013) had shown in their study that transpiration represents 80–90% of the terrestrial evapotranspiration. It has been suggested that 90% of the water that is taken up by plants is used for transpiration. This is defined as Transpiration $= \frac{\text{Mass } H_2O \text{ transpired}}{\text{Mass dry matter produced}}$ (Biocyclopedia, 2012). Factors that affect transpiration depend on its type,

size, light intensity, temperature, relative humidity, and wind speed (Biocyclopedia, 2012; Perlman, 2014). Additionally, transpiration helps to cool the plant (Covert, 2015).

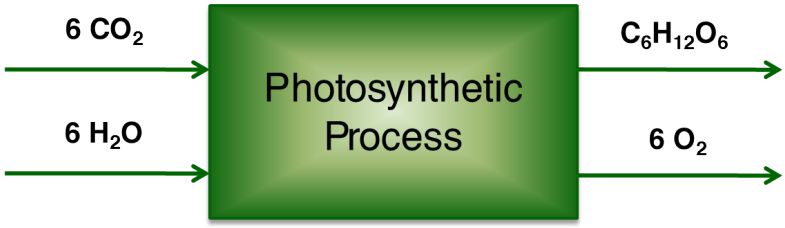
2.1.3 Options toward optimization in photosynthesis

To improve productivity of algae, Simionato et al. (2013) suggest this can be done via genetic engineering (such as suppressing pathways in the biological system or producing an enzyme) and optimization of the operational parameters in the photobioreactor. This includes better control for improving production in the system. Some authors review different genetic engineering methods that could be used to improve crops to be efficient carbon dioxide absorbers in order to turn them into food (Mann, 1999) or using algae as efficient biofuel contributors (Radakovits et al., 2010; Schuhmann et al., 2012). Zeng et al. (2011) mention papers that try to genetically engineer the photosynthetic activity by trying to improve the selectivity of one of the components within the photosynthetic mechanism. Ruan et al. (2012) review trans-genetic engineering applications towards improving the biological methods of absorbing carbon dioxide in plants. However, if the biological system has already reached its thermodynamic limit it may not be able to make more of that material regardless of its manipulation. In the study by Mussgnug et al. (2007), to protect itself against photo-damage under high light, the absorbed photons are wasted as heat and fluorescence, they used RNAi technology (a form of genetic engineering) on green algae under high light intensities to reduce fluorescence and heat losses.

2.2 Approach

Many experimentalists have found that different numbers of photons are required for photosynthesis and this has contributed to the controversy on what is actually required. In this chapter, the focus will be to try to find the thermodynamic limits of the minimum number of moles of photons required. To do this we will define the system as a 'black-box' that contains the photosynthesis process reaction. This involves the overall process reaction whereby the main focus of the analysis are the thermodynamic properties of the input and output streams (Patel et al., 2005). Figure 2.2.1 illustrates the method on the photosynthetic process mass balance assuming the products of photosynthesis are glucose and oxygen only. Thus the analysis it is not concerned with what

is happening inside, but with only the inputs and outputs of the system. This will help to determine the theoretical limits of operation, and as said by Hill and Govindjee (2014), one can determine what can and cannot be done once the limits are known.



$$\text{Products} = C_6H_{12}O_6 + 6O_2$$

$$\text{Reactants} = 6CO_2 + 6H_2O$$

Figure 2.2.1: Black-box overview of the photosynthesis process (making only glucose and oxygen) taking into consideration only the inputs and outputs of the biological system. The inner workings of the photosynthetic process are not shown, nor are they needed for this approach.

In the paper by Petela (2008), the first law of thermodynamics is mentioned to help provide a better understanding of the process and draws attention to areas of it that can be optimized. Petela (2008) also emphasised that the entropy balance allows one to identify the causes of entropy generation and therefore minimize it. In this chapter, in addition to the first law, the second law of thermodynamics is included to analyse the photosynthesis process in order to determine the NP that are required. The basis is set so that the enthalpy and Gibbs free energy of all elements at 25°C and 1 bar are equal to zero. The formation data used in this chapter are given in Table 2.2.1. Kok (2011) reported that the theoretical calculations would not be impacted by the choice of phase (gas or liquid) because the enthalpy of formation and Gibbs free energy differences between both phases are small.

Table 2.2.1: Formation data of the products and reactants for the photosynthesis process retrieved from Wagman et al. (1982). Gas is represented by (g), liquid by (l) and solid by (s).

kJ/mol	CO_2**(g)**	H_2O**(l)**	$C_6H_{12}O_6$**(s)***	O_2**(g)**
$\triangle H$	-393.51	-285.83	-1262.19	0
$\triangle G_f^\circ$	-394.36	-237.19	-915.9	0

*Glucose data from Alberty and Goldberg (1992)

2.2.1 Energy balance

The approach begins by considering an energy balance:

$$H_{in} + \frac{1}{2}\triangle u^2 + g\triangle z + E_{photons}(\lambda) = H_{out} + Q + w_s \tag{2.2.1}$$

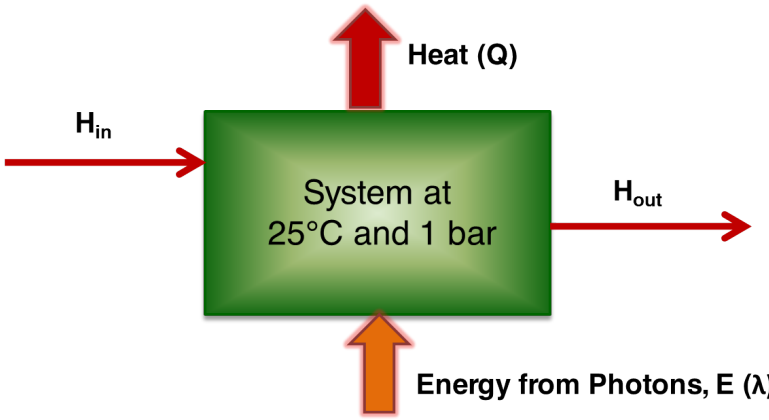


Figure 2.2.2: Input and output energy balance flows shown for the photosynthetic process. H_{in} and H_{out} correspond to the enthalpies of the inputs and outputs, Q represents heat exchange with the surroundings and $E_{photons}(\lambda)$ is the energy supplied from the photons, in the form of light.

The process shown in Figure 2.2.2 takes into account the energy of the photons $E_{photons}(\lambda)$. Additionally when there is no kinetic $\left(\frac{1}{2}\triangle u^2\right)$ or potential energy $(g\triangle z)$ as well as shaft work (w_s), then the energy balance reduces to:

$$H_{in} + E_{photons}(\lambda) = H_{out} + Q \tag{2.2.2}$$

where

H_{in} and H_{out}	= Enthalpies of the inputs and outputs respectively (kJ/mol);
$E_{photons}(\lambda)$	= Energy supplied from the photons, in the form of light, which depends on wavelength(λ) (kJ/mol); and
Q	= Heat exchanged with the surroundings (kJ/mol).

Equation 2.2.2 reduces to:

$$\triangle H_{process} = H_{out} - H_{in} = E_{photons}(\lambda) - Q \tag{2.2.3}$$

The $H_{process}$, which is the enthalpy of the process, is determined from the mass balance, shown by equation 2.1.2 to produce 1 mol of glucose. It can be calculated from formation data shown earlier in Table 2.2.1 where the stoichiometric constants from the products and reactants are x and m:

$$\triangle H_{process} = \sum \triangle x H^0_{f,products} - \sum \triangle m H^0_{f,reactants} \tag{2.2.4}$$

which then becomes:

$$\triangle H_{process} = \triangle H_{reaction} = 2814\,kJ/mol \tag{2.2.5}$$

The value is positive, which means that the process of photosynthesis is endothermic and needs energy to drive it. This energy is supplied from the photons in the form of light. It should be noted that in this chapter's system of equations the heat (or energy transfer between the system and surroundings, Q) is the energy rejected to the surroundings from the system. If the value becomes negative, because of our derivation, it would imply heat will be required for the system. Thus, if it is a positive number some energy will be lost to the surroundings due to the biological process.

In this chapter, a collection of photons are used as opposed to a photon gas. (Leff, 2002, gave a detailed general background review as to what a photon gas comprises.) The energy from the light is taken as energy from a photon which is given by the Plank relation multiplied by $n_{photons}$, which represents the number of moles of photon (designated as NP with units of moles). This relation depends on h (Plank's constant), N_A (Avogadro number), c (speed of light) and λ (photon wavelength). This results in equation 2.2.6. Teixeira and

Wadsö (1994) also used equation 2.2.6 for analysis within a photo-calorimeter.

$$\mathrm{E_{photons}} = \mathrm{n_{photons}} \frac{hc\mathrm{N_A}}{\lambda} \tag{2.2.6}$$

Therefore, the energy balance (equation 2.2.3) becomes:

$$\triangle \mathrm{H_{process}} = \mathrm{n_{photons}} \frac{\lambda}{hc\mathrm{N}_A} - \mathrm{Q} \tag{2.2.7}$$

This yields a number of moles of photons (NP) equation with respect to Q and $\triangle \mathrm{H_{process}}$:

$$\mathrm{n_{photons}} = (\triangle \mathrm{H_{process}} + \mathrm{Q}) \frac{\lambda}{hc\mathrm{N_A}} \tag{2.2.8}$$

If it were an adiabatic process where $\mathrm{Q} = 0\,\mathrm{kJ/mol}$ equation 2.2.3 and equation 2.2.8 are then reduced to equation 2.2.9. This would correspond to the minimum number photons that can be used by the system in order to produce 1 mol of glucose:

$$\mathrm{n_{photons,min}} = \triangle \mathrm{H_{process}} \frac{\lambda}{hc\mathrm{N_A}} \tag{2.2.9}$$

Therefore, at different wavelengths, the number of moles of photons ($\mathrm{n_{photons,min}}$) required per mole of product formed (or reactant consumed) can now be determined, and is shown in Figure 2.3.1, which will be discussed later.

2.2.2 Entropy balance

Figure 2.2.3 illustrates the chemical transformations that should occur around the system. The second law of thermodynamics will be used for the entropy balance.

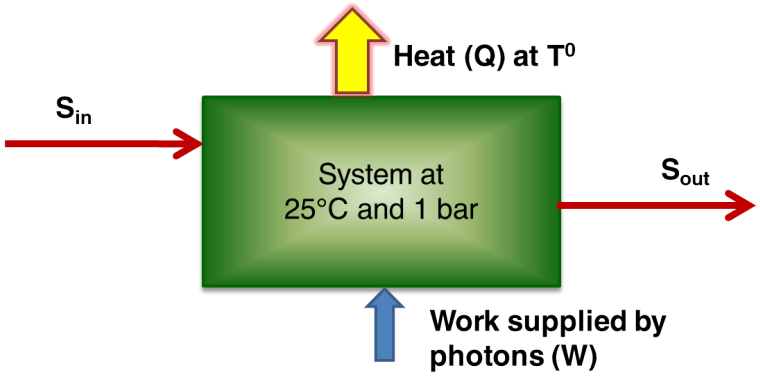


Figure 2.2.3: The work flows in and out of the system when considering the work limited approach. Inner workings are not shown. Heat is released to the surroundings at 298.15 K and work for the process is supplied by the photons.

For the photosynthetic process, the entropy balance can be considered as:

$$S_{in} + S_{gen} + S_{photons} = S_{out} + \frac{Q}{T} \tag{2.2.10}$$

where

S_{in} and S_{out}	= Entropy of the inputs and outputs respectively ($kJ/mol \cdot K$);
$S_{photons}$	= Entropy of the photons ($kJ/mol \cdot K$);
S_{gen}	= Entropy generated by the system ($kJ/mol \cdot K$); and
Q	= Heat released to the surroundings at basis temperature T=298.15 K ($kJ/mol \cdot K$).

The assumptions that will be taken forward in the analysis will be as follows:

- For a reversible process the internal entropy generation term is zero, i.e. $S_{gen} = 0\,kJ/(mol \cdot K)$. In nature this process is naturally irreversible, but for the purposes of this analysis this is the limit of performance. Therefore the number of photons obtained from this study, under this assumption, will be the minimum number of photons that will be required. If S_{gen} increases, the number of photons will increase, that is, this value would increase with increasing irreversibility, and this will be discussed further in Section 2.3.3.2. Furthermore, even though it is not the focus of this chapter, inner processes may need to be modelled using non-equilibrium thermodynamics to obtain a better approximation of the

real numbers. A recent techno-economic study on algal biofuels by Louw et al. (2016) reveals that the bioreactor design influences energy and water consumption balance. Thus, if one were in a detailed design stage of a bio-reactor, one should expand this study using non-equilibrium thermodynamics in order to obtain the correct values for water and energy consumption within the reactor.

- $S_{photons}$ refers to intrinsic entropy of the photons. Here it is assumed that all of the photons are coming in as work. Additionally, Kirwan (2004) suggested that the entropy of a photon gas is dependent on photon wavelength, as well as the pressure, volume and temperature of the system. However, as mentioned earlier, a collection of photons rather than a photon gas, is what is being assumed for this analysis. Gudkov (1998) suggested that light carries no thermodynamic entropy since it is a form of high grade energy, and therefore we will take $S_{photons} = 0\,kJ/(mol \cdot K)$. In the dissertation by Kok (2011), they found that $S_{photons}$ had a small effect on the NP, which further supports the assumption made here.

As a result, equation 2.2.10 reduces to equation 2.2.11:

$$S_{out} = S_{in} - \frac{Q}{T} \tag{2.2.11}$$

$$\therefore \triangle S_{process} = -\frac{Q}{T} \tag{2.2.12}$$

It can be seen that if Q were to be positive, $\triangle S_{process}$ will be negative. Thus the process would not occur spontaneously.

2.2.3 Relating the energy and entropy balance

Rather than using the entropy in our analysis we have found it more convenient to use Gibbs free energy. This is because it is related directly to work, which we find an easier concept to understand and explain than the entropy. Thus inputs (G_{in}) and outputs (G_{out}) will be used to relate the entropy and the energy balance to the work of the system, and to determine the NP for the chemical transformation of the photosynthesis process:

$$G = H - TS \tag{2.2.13}$$

Substituting the inputs and outputs of the system into equation 2.2.13, the following equations are derived:

$$G_{in} = H_{in} - TS_{in} \tag{2.2.14}$$

$$G_{out} = H_{out} - TS_{out} \tag{2.2.15}$$

$$G_{out} - G_{in} = H_{out} - TS_{out} - (H_{in} - TS_{in}) \tag{2.2.16}$$

Rewriting the above equations gives

$$\triangle G_{process} = \triangle H_{process} - T\triangle S_{process} \tag{2.2.17}$$

And substituting in equation 2.2.3 and 2.2.12

$$\triangle G_{process} = (E_{photons}(\lambda) - Q) - T\left(-\frac{Q}{T}\right) \tag{2.2.18}$$

Results in the following for a reversible process:

$$\triangle G_{process} = E_{photons}(\lambda) \tag{2.2.19}$$

Similarly, as done for the enthalpy of the process, we can calculate the $G_{process}$ from the formation data:

$$\triangle G_{process} = \sum \triangle x G^0_{f,products} - \sum \triangle m G^0_{f,reactants} \tag{2.2.20}$$

We obtain the following result, which represents the Gibbs free energy of the process, per 1 mol of glucose formed:

$$\triangle G_{process} = \triangle G_{reaction} = 2873\,kJ/mol \tag{2.2.21}$$

We note a positive Gibbs free energy change implies that work is required for the process (Patel et al., 2005):

$$\triangle G_{process} = E_{photons}(\lambda) = \text{work} \tag{2.2.22}$$

This gives the following equation for NP when S_{gen} and S_{photon} are zero, and the results are shown in Figure 2.3.1:

$$n_{photons} = (\triangle G_{process})\frac{\lambda}{hcN_A} \tag{2.2.23}$$

Equation 2.2.23 corresponds to the minimum number of moles photons required to be supplied to a reversible process which makes 1 mol of glucose. Knowing both the Gibbs free energy and the enthalpy for the reversible process, we can calculate the heat exchange resulting from the photosynthesis process. Thus if we substitute equation 2.2.19 into 2.2.3, we obtain the amount of energy produced by the system irrespective of the wavelength of the light:

$$\mathrm{Q} = \triangle \mathrm{G}_{\mathrm{process}} - \triangle \mathrm{H}_{\mathrm{process}} = 59.95\,\mathrm{kJ/mol} \tag{2.2.24}$$

Now, the entropy of the process at the basis can be determined from the enthalpy and Gibbs free energy of formation of the components ($C_6H_{12}O_6$, O_2, CO_2 and H_2O). By rearranging equation 2.2.13 we obtain:

$$\triangle \mathrm{S}^0_{\mathrm{f,component}} = \frac{\triangle \mathrm{H}^0_{\mathrm{f,component}} - \triangle \mathrm{G}^0_{\mathrm{f,component}}}{\mathrm{T}} \tag{2.2.25}$$

Then applying the difference between the inputs and outputs we obtain:

$$\triangle \mathrm{S}_{\mathrm{process}} = \sum \triangle \mathrm{xS}^0_{\mathrm{f,products}} - \sum \triangle \mathrm{mS}^0_{\mathrm{f,reactants}} \tag{2.2.26}$$

Using the values in Table 2.2.1, $\triangle \mathrm{S}^0_{\mathrm{f,component}}$ can be calculated using equation 2.2.25, to give the values in Table 2.2.2.

Table 2.2.2: Calculated formation data for entropy of formation using equation 2.2.13 and data from Table 2.2.1.

$\mathbf{J/(mol \cdot K)}$	CO_2**(g)**	H_2O **(l)**	$C_6H_{12}O_6$**(s)**	O_2**(g)**
$\triangle \mathrm{S}^\circ_{\mathrm{f}}$	0.002851	-0.1631	-1.161	0

This will result in a negative entropy value when using equation 2.2.26 and Table 2.2.2:

$$\triangle \mathrm{S}_{\mathrm{process}} = \triangle \mathrm{S}_{\mathrm{reaction}} = -0.1997\,\mathrm{J/\,(mol \cdot K)}$$

The $\triangle \mathrm{S}_{\mathrm{surrounding}}$ should be equal to or greater than the absolute value of $\triangle \mathrm{S}_{\mathrm{process}}$ to yield a positive $\triangle \mathrm{S}_{\mathrm{universe}}$. As discussed in the previous section, the entropy of photosynthesis is well known to have a negative value, that is, it cannot occur spontaneously but needs work input, in this case via the photons.

2.3 Results and Discussion

2.3.1 Reversible and adiabatic

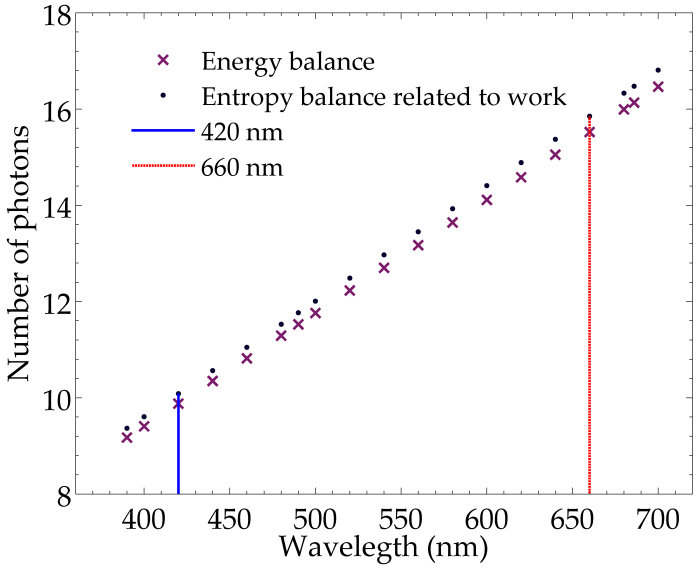


Figure 2.3.1: Minimum required number of moles of photons for the production of 1 mol of glucose for a reversible process. It results in a straight line from the origin for either the enthalpy or the entropy approaches. However, the entropy process governs the limit as more photons will be required, as a consequence the overall heat produced from the process is Q=59.95 kJ/mol.

From Figure 2.3.1 we see that there appears to be two possible numbers of photons for the photosynthesis process to occur. The two are related to two different assumptions concerning the process. The first was that the process was adiabatic, the second that it was reversible. If we take the reversible assumption then it turns out that the Gibbs free energy for the process would be positive. From the second law we know this is a process that cannot occur without extra work being supplied, and so this is the limiting assumption. That is the upper graph is the one we will use in the rest of the chapter.

The NP ($n_{photons}$) required per mole of reactant consumed or product formed can then be determined at different wavelengths and this is represented by the upper straight line as seen in Figure 2.3.1. If one takes 420 nm and 660 nm as the wavelengths that are being fed into the system, then the minimum amount NP to produce 1 mol of glucose at 420 nm is just below 10, and at 660 nm it is below 16 (see Figure 2.3.1).

The amount of heat rejected from the system increases for increasing irreversibility, thus more moles of photons will be needed for the overall process. The straight line for the entropy balance also represents a reversible system for when the overall heat released by the system is $Q = 59.95\,kJ/mol$ of glucose (see equation 2.2.24). When considering the work that is put into the system, as the irreversibility increases the number of moles of photons will increase with greater process irreversibility ($S_{gen} > 0\,kJ/mol$). These extra photons will be turned into heat and any extra energy would need to be rejected. Thus, as the degree of irreversibility increases, more and more heat will need to be rejected by the process and hence by the organism.

2.3.2 What about the pressure assumption?

Since the process was found to be work limited, the pressure effects on the Gibbs free energy for the process ($\triangle G_{process}$) for the gases present in the system, namely: carbon dioxide and oxygen for the production of one mol of glucose will be analysed. The author assumed that the total temperature and pressure of the system was at 25°C (298.15 K) and 1 bar respectively, and the gases flowing into the system are ideal gases. The following equation 2.3.1 was derived from equation 2.2.19 to take into account the partial pressure of the gases present in the system. The full derivation can be found in Appendix C.1:

$$E_{photons}(\lambda) = \triangle G_{process} + RTln\left(\frac{X_{O_2}}{X_{CO_2}}\right) \tag{2.3.1}$$

One can see from this result that when the mole fractions of the CO_2 and the O_2 in the gas phase are equal the energy of photons required agrees with the previous calculation, where the gas phase effects were ignored.

The reader can see in Figure 2.3.2, that the graph of $E_{photons}(\lambda)$ versus the mole fraction of CO_2 was drawn for a process that includes the partial pressure effects where $X_{CO_2} = 1 - X_{O_2}$ (CO_2 and O_2 are the only gases present) as opposed to only considering the $\triangle G_{process} = E_{photons}(\lambda)$. The graph thus includes the different values of $RT\left(\ln\left(\frac{X_{O_2}}{X_{CO_2}}\right)\right)$ for different mole fractions (X) of pure O_2 and CO_2 at equilibrium in a system of 1 bar. Additionally, Figure 2.3.3 was drawn for a scenario when pure CO_2 is added with air resulting in a relationship of $X_{CO_2} = 1 - 5X_{O_2}$ (refer to Appendix C.1 for the derivation).

One can immediately see from Figure 2.3.2 that the partial pressure effects are small, that is less than 1%. Similar results are obtained in Figure 2.3.3 when the nitrogen from the air is included. As such we will not consider pressure effects any further.

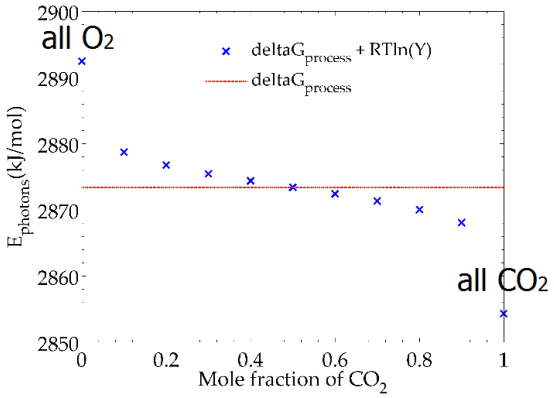


Figure 2.3.2: The adjusted $E_{photons}(\lambda)$, energy range when taking into consideration the effect of the partial pressures on the photosynthetic system for various mole fractions/partial pressures of CO_2 with oxygen. The graph is drawn at a total system pressure of 1 bar and temperature of 298.15 K, with only O_2 and CO_2. $E_{photons}(\lambda)$ changes with different mole fractions of CO_2 where $X_{CO_2} = 1 - X_{O_2}$.

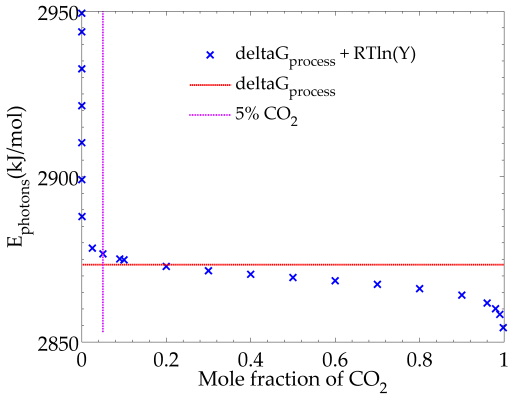


Figure 2.3.3: The adjusted $E_{photons}(\lambda)$ energy range when taking into consideration the effect of the pressure on the photosynthetic system for various mole fractions/partial pressures of CO_2 with air $X_{CO_2} = 1 - 5X_{O_2}$ (at 1 bar and 298.15 K).

2.3.3 Reality is irreversible and non-adiabatic

2.3.3.1 Contours of heat with the number of photons

It was calculated in this chapter that 9–17 moles of photons will be required to produce 1 mol of glucose from CO_2 and H_2O for a reversible process. The reports from other scientists in the field give between 24–90 photons (an average of 71.82 NP). Some of those results are shown in Table 2.3.1. Govindjee (1999) referred to the work by Warburg, where the researchers calculated the minimum amount of photons via a simple calculation where they obtained 2.8 as the quantum requirement to fix one CO_2 or produce one O_2. To get this result the researchers divided the enthalpy of the reaction (for the formation of carbohydrate and oxygen) by the energy of a red quanta. The values obtained in this chapter do not match that simple calculation that was done by Warburg (Govindjee, 1999), this is because in this chapter, the first and second law of thermodynamics were taken into consideration.

In this chapter's theoretical analysis, however, it was assumed that photon absorption efficiency was 100%, this was in order to obtain the thermodynamic limits of operation for the photosynthesis process, that is to find the boundary of the absolute minimum number of photons that will be required. Additionally, glucose in nature goes on to produce other products (within the metabolic cycle), so the process mass balance used does not represent the true mass balance occurring in nature. Therefore, it can be expected that in reality, more than the theoretically predicted amount of moles of photons will be required to produce one mole of glucose. The increasing amount of moles of photons relates to an inefficiency in the process, and as a consequence more heat will be released.

Therefore, for an inefficient process, reality will be irreversible and non-adiabatic, and so the calculations should take into account those cases (as well as an increasing number of photons) when considering the process. This means that the heat released, Q, should be calculated depending on the number of moles of photons and at which wavelength they are being fed into the system. To do this equation 2.2.8 can be rearranged into:

$$\mathrm{Q} = \mathrm{n_{photons}} \frac{hc\mathrm{N_A}}{\lambda} - \triangle \mathrm{H_{process}} \tag{2.3.2}$$

It can be seen that Q is directly proportional to NP but not to λ. This is illustrated in Figure 2.3.4 (drawn from equation 2.2.8 with different values of Q). The further away from reversibility (S_{gen} >0) the more photons will be required, resulting in more Q released (at a specific wavelength) from the process.

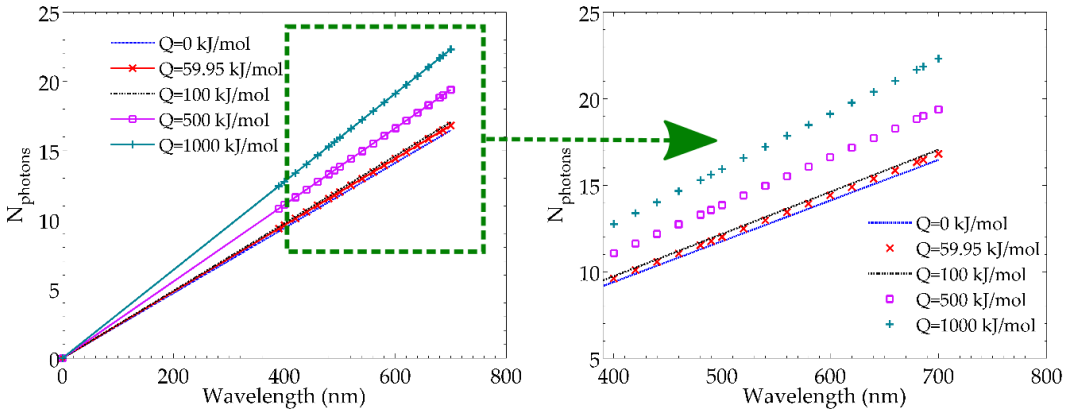


Figure 2.3.4: Contours of Q at various wavelengths and various numbers of moles of photons for the production of 1 mol of glucose (or the reduction of 6 mols of carbon dioxide). An increasing Q implies that it is increasing with irreversibility (for the case when S_{gen} >0).

Another way of presenting the results is when Q increases as the wavelength gets smaller, and is seen in Figure 2.3.5 (drawn from equation 2.3.2 with different values of NP). Therefore, if the photosynthesis process is about 35 % efficient, that is when 48 photons, or 8 photons for one mole of oxygen (Moroney and Ynalvez, 2001), will be required instead of 17 photons at 680 nm for one mole of glucose there will be a need to release close to 1000 kJ/mol of heat. This efficiency of 35 % also agrees with what Mauzerall (2013) reports from other researchers, which was a measured efficiency at the trap energy when using the accepted number of 8 photons at 680 nm. Thus, 1000 kJ/mol of heat is a large number, and a discussion on what plants do with the heat is found in Section 2.3.3.3.

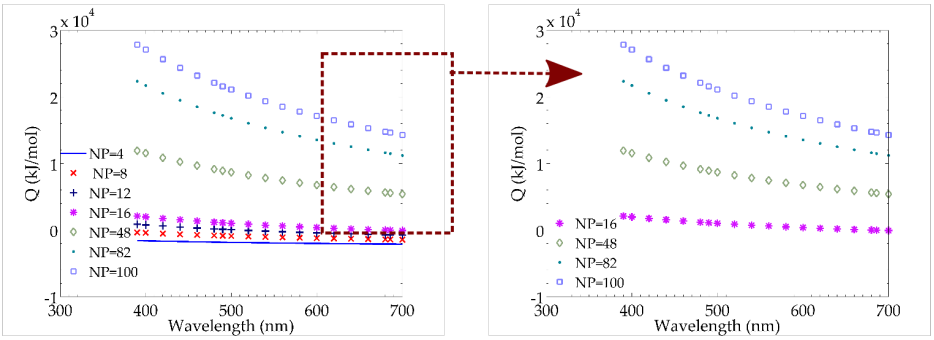


Figure 2.3.5: Contours of number of moles photons (NP) and resultant wavelength and Q, for the production of 1 mol of glucose or the reduction of 6 mols of carbon dioxide. If Q< 0 it implies that the overall photosynthesis process is endothermic and would not proceed. On the other hand, if Q>0 the process is overall exothermic and needs to reject energy to the environment.

Additionally, the following equation can be derived from equation 2.2.10, with the assumption that S_{photon} is still zero:

$$S_{gen} = \triangle S_{process} + \frac{Q}{T} \tag{2.3.3}$$

Since $\triangle S_{process}$ and T are quantities that are known at the basis, Q can be calculated from equation 2.3.2 for the intention of calculating S_{gen} from equation 2.3.3, resulting in the contours shown in Figure 2.3.6, which shows that S_{gen} is proportional to Q and NP, but decreases with increasing wavelength.

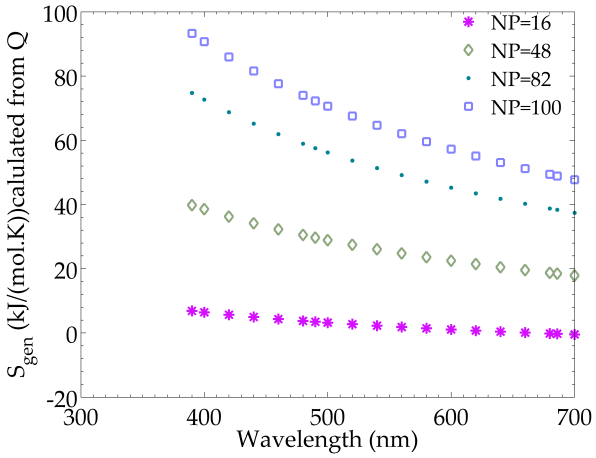


Figure 2.3.6: Contours of S_{gen} from the calculated Q at set number of moles of photons, using equation 2.3.3. for the production of 1 mol of glucose or the reduction of 6 mols of carbon dioxide, when Q>0.

In addition, the amount of heat rejected (Q) was calculated for the number of photons suggested in the literature and is shown in Table 2.3.1. This was done at a wavelength of 660 nm, using equation 2.3.2. If the values were calculated at a lower wavelength the amount of Q rejected would be higher, since the relationship of Q is inversely proportional to the wavelength (shown in Figure 2.3.5). Hence the amount of heat rejected using the photon yields given by other scientists and based on the equations derived in this chapter, represent the minimum amount of heat released when considering the wavelength of visible light at 660 nm (chlorophyll a).

If the reader compares the Q values, shown in Table 2.3.1, to the combustion of methanol (which is 726 kJ/mol), it suggests that the real photosynthesis process is a highly exothermic reaction. As mentioned earlier, in the report by Magee et al. (1939), they had shown that the amount of heat evolved within their calorimeter ranged between 0.013–0.015 Watts. The minimum Q for this chapter's photosynthetic process overall was calculated to be Q = 60 kJ/mol of glucose based on the enthalpy and entropy differences.

Therefore if these values, shown in Table 2.3.1, were to be correct, it suggests that the production of glucose is hugely irreversible. From the researcher's reports and generally in nature, there is no real evidence of the large amounts of heat being rejected. This suggests that the heat is being used within the system (the energy generated from the light reactions is distributed to other cellular processes, other than the Calvin cycle) or the plant is being cooled down via transpiration (an evaporative cooling system).

Furthermore, only one light wavelength is being considered in Table 2.3.1, naturally, a whole range of wavelengths are penetrating into a system, and as a consequence more Q will increase.

Table 2.3.1: List of some published results pertaining to the number of moles of photons involved in the photosynthesis reaction.

Researcher	Basic approach	$n_{photons}$ (per O_2)	$n_{photons}$ (per $C_6H_{12}O_6$)	Q_{min}* (kJ/mol)	S_{gen}** (kJ/mol · K)
Magee et al. (1939)	A photo-calorimeter with different light intensities.	12.5	75	10783	36
Ley and Mauzerall (1982)	Flashing light on *Chlorella Vulgaris.*	10	60	8064	27
Raven and Johnston (1991)	Calculated for an algae RUBISCO enzyme.	15	91	13720	46
Raven and Johnston (1991)	Computed for general RUBISCO.	8	48	5888	19
Warburg (Govindjee, 1999)	Measured by experiment with low intensities of light with algae.	12	72	10239	34
Warburg (Govindjee, 1999)	Measured by experimental manometry with algae.	4	24	1537	5
Raven and Geider (2004)	Stated algae photosynthesis proceeds with 8 photons.	8	48	5888	19
Albarrán-Zavala and Angulo-Brown (2007)	Reported from other researchers that 60 NP needed to produce 1 mol of glucose.	–	60	8064	27
Wilhelm and Jakob (2011)	Reported from other researchers that photon requirement differs (e.g Lipid) Theoretical vs Real.	12 vs. 28 per carbon	72 vs. 168	10239 vs. 27644	34 vs. 92
Present work (Theoretical)	Analysis at a specific wavelength, namely 680 nm	–	17	268	0.90

*The author calculated the value Q using equation 2.3.2, at 660 nm as the wavelength (one of the highest chlorophyll absorbance of red light) into the process, lower wavelengths can emit more Q. **This value was calculated using equation 2.3.3 and $\triangle S_{process} = -0.1997\,J/\,(mol \cdot K)$.

2.3.3.2 Contours of irreversibility

Another way (besides Figure 2.3.6) in which to represent how the number of moles of photons versus wavelength varies with changing values of S_{gen}, equation 2.3.3 can be substituted into equation 2.2.17 to get the following equation:

$$n_{photons} = (\triangle G_{process} + TS_{gen})\frac{\lambda}{hcN_A} \tag{2.3.4}$$

The increasing irreversibility (for different chosen values of S_{gen}) is shown in Figure 2.3.7, with the NP values from other researchers averaged. One can see that the average literature NP intersects at about $S_{gen} = 50\,kJ/\,(mol \cdot K)$ at the lower wavelengths (480–490 nm) of the visible light spectrum.

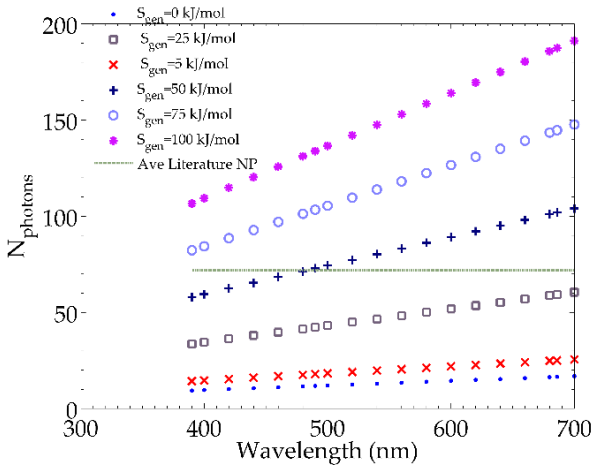


Figure 2.3.7: Minimum required amount of moles of photons for the production of 1 mol of glucose or the reduction of 6 mols of carbon dioxide. Number of moles of photons increases with increasing irreversibility.

2.3.3.3 What do plants do with the excess heat?

So what could be causing the difference from the minimum versus the experimental as shown in Table 2.3.1? A possibility is that the plants need water to cool themselves down, to prevent themselves from overheating, from the large amounts of energy that is being produced from the absorbed photon energy. This would suggest that one of the main functions of the plant's ability to transpire is to keep themselves cool. This is over and above the need to pump water to the leaves for photosynthesis.

One could then assume the amount of energy released from the photosynthetic

process could be dissipated by converting liquid water into gas, via transpiration. To illustrate this, we can obtain a new energy balance related to the photosynthetic mass balance. If not all the feed to the process is not 100% efficient, the energy from the photosynthetic process could be used to convert the unreacted liquid water into gas to cool the plant down.

Now we know that the enthalpy of vaporisation of water is $\triangle$Hvap $\sim 40\,\mathrm{kJ/mol}$. If the photosynthesis process is 100% efficient, to get rid of this heat that was calculated earlier (recall Q = 60 kJ/mol of glucose) we need to evaporate 1.5 moles of water per mole of glucose that we make. Clearly if the process is not 100% efficient we will need many more moles of water per mole of glucose. If we take some of the numbers from Table 2.3.1 we seem to need to get rid of 5,000–10,000 kJ/mole of glucose made. This means we would need to transpire between 125–250 moles of gaseous water per mole of glucose made. This suggests that the water needs of the plant are mainly for cooling, and relatively very little being needed for photosynthesis. Perhaps we might speculate that the limit of the growth rate of plants may be associated with their ability to keep cool?

Alternatively, as mentioned earlier in the literature review, it is accepted that a total of eight photons, to produce one mole of oxygen, are required for the light dependant reaction, since there are two photosystems where four electrons must be transported through each of them, and each electron transport requires one photon (Moroney and Ynalvez, 2001). Therefore 48 photons will be required to produce one mole of glucose, this implies that the process is about 35% efficient at 680 nm where the minimum limits of operation was at 17 photons. The ATP (the primary energy-supplying molecule for living cells) and NADPH generated from the electron transport chain can power different cellular processes and reactions, including the Calvin-cycle where CO_2 is converted into useful sugars (OpenStaxCollege, 2013). Therefore ATP (the stored energy) could be used for other cellular processes which could attribute to irreversibility of the photosynthesis system. Additionally, if ATP is not used quickly to perform work, 57 kJ/mol of free energy (in a living cell) is lost as heat if ATP spontaneously dissociates into ADP (OpenStaxCollege, 2013). This depends on the type of enzyme used in the reaction, as *hydrolases* will produce enthalpy, which is transferred to the medium as heat whereas *kinases* produce free energy (ATP) (Villadsen et al., 2011).

2.4 Conclusion

A heat and work limited approach towards theoretically calculating the photon yield for the intention of converting carbon dioxide and water into glucose, via a photosynthetic process, has been developed. The process was assumed to be a reversible process (100% efficient) and not affected by pressure, to obtain the minimum theoretical amount of moles of photons required, as well as the minimum energy rejected from the overall photosynthetic process at 25°C and 1 bar.

The photon values were evaluated at the wavelengths that had been estimated as being needed to activate the appropriate enzymes. The system was found to be work limited where work was supplied by the photons and heat needed to be rejected, even in a reversible process. Overall it was calculated that 9–17 photons were required to produce 1 mol of glucose (or to reduce 6 mols of carbon dioxide). These minimum boundary values are much lower when compared to values (50–150) reported in the literature. The literature values, if correct, would suggest that the rejection of heat is a big issue in photosynthesis and consequently the management of heat would be a very important issue in plant physiology. Furthermore, it suggests that photosynthetic process is very irreversible and inefficient (less than 35%) at utilizing photon energy. The numbers calculated corresponding to the literature values point to an extremely exothermic process which on the face of it conflicts with what some scientists say that the amount of heat released in the system is negligible. However, the calculations suggest the reason as to why plants transpire is to keep the system cool and this may be why the system does not appear have a highly exothermic reaction taking place. Additionally, not all of the heat may be dissipated, and the energy (ATP) generated from the photosynthesis process could have been used for other cellular reactions. However, large amounts of water will be required to satisfy the heat being released resulting in an apparently inefficient process (loss of heat) in terms of photosynthesis.

References

Albarrán-Zavala, E., Angulo-Brown, F., 2007. A simple thermodynamic analysis of photosynthesis. Entropy 9 (4), 152–168.

Alberty, R. A., Goldberg, R. N., 1992. Standard thermodynamic formation

properties for the adenosine 5'-triphosphate series. Biochemistry 31 (43), 10610–10615.

Andriesse, C., Hollestelle, M., 2001. Minimum entropy production in photosynthesis. Biophysical Chemistry 90 (3), 249–253.

Berg, J. M., Tymoczko, J. L., Stryer, L., 2002. The light reactions of photosynthesis. In: Biochemistry, 5th Edition. WH Freeman, New York, USA, Ch. 19.

Biocyclopedia, 2012. Transpiration. [Online]. Available at: http://www.biocyclopedia.com/index/transpiration.php, [accessed 12 May 2015].

Bisio, G., Bisio, A., 1998. Some thermodynamic remarks on photosynthetic energy conversion. Energy Conversion and Management 39 (8), 741–748.

Bolton, J. R., Hall, D. O., 1991. The maximum efficiency of photosynthesis. Photochemistry and Photobiology 53 (4), 545–548.

Brittin, W., Gamow, G., 1961. Negative entropy and photosynthesis. Proceedings of the National Academy of Sciences of the United States of America 47 (5), 724–727.

Carvalho, A. P., Silva, S. O., Baptista, J. M., Malcata, F. X., 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. Applied Microbiology and Biotechnology 89 (5), 1275–1288.

Covert, D., April 2015. Transpiration - water movement through plants. [Online] Available at: http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1092853841&topicorder=3, [accessed 12 May 2015].

Emerson, R., Lewis, C. M., 1943. The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light. American Journal of Botany 30 (3), 165–178.

Evers, E., 1991. A model for light-limited continuous cultures: Growth, shading, and maintenance. Biotechnology and Bioengineering 38 (3), 254–259.

Feng, C., Meng, Q., Zhang, Y., 2010. Theoretical and experimental analysis of the energy balance of extensive green roofs. Energy and Buildings 42 (6), 959–965.

Gouveia, L., 2011. Microalgae as a feedstock for biofuels. In: Microalgae as a Feedstock for Biofuels. SpringerBriefs in Microbiology. Springer Berlin, Heidelberg, Germany, pp. 1–69.

Govindjee, A., 1999. On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: A historical note. Photosynthesis Research 59 (2-3), 249–254.

Gudkov, N., 1998. Some thermodynamics of chemiluminescence. Journal of Luminescence 79 (2), 85–89.

Hall, D., Rao, K., 1987. Photosynthesis. In: New Studies in Biology, 4th Edition. E. Arnold, London, UK.

Hill, J. F., Govindjee, 2014. The controversy over the minimum quantum requirement for oxygen evolution. Photosynthesis Research 122 (1), 97–112.

Hoch, G., Kok, B., 1963. A mass spectrometer inlet system for sampling gases dissolved in liquid phases. Archives of Biochemistry and Biophysics 101 (1), 160–170.

Höxtermann, E., 2007. A comment on warburg's early understanding of biocatalysis. Photosynthesis Research 92 (1), 121–127.

Janssen, M., Patiño, R., von Stockar, U., 2005. Application of bench-scale biocalorimetry to photoautotrophic cultures. Thermochimica acta 435 (1), 18–27.

Janssen, M., Wijffels, R., von Stockar, U., 2007. Biocalorimetric monitoring of photoautotrophic batch cultures. Thermochimica Acta 458 (1), 54–64.

Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., Fawcett, P. J., 2013. Terrestrial water fluxes dominated by transpiration. Nature 496 (7445), 347–350.

Jennings, R. C., Belgio, E., Casazza, A. P., Garlaschi, F. M., Zucchelli, G., 2007. Entropy consumption in primary photosynthesis. Biochimica et Biophysica Acta-Bioenergetics 1767 (10), 1194–1197.

Jennings, R. C., Casazza, A., Belgio, E., Garlaschi, F., Zucchelli, G., 2006. Reply to "Commentary on Photosynthesis and Negative Entropy Production" by Jennings and coworkers by J. Lavergne. Biochimica et Biophysica Acta-Bioenergetics 1757 (11), 1460–1462.

Jennings, R. C., Engelmann, E., Garlaschi, F., Casazza, A. P., Zucchelli, G., 2005. Photosynthesis and negative entropy production. Biochimica et Biophysica Acta-Bioenergetics 1709 (3), 251–255.

Jeon, Y., Cho, C., Yun, Y., 2005. Measurement of microalgal photosynthetic activity depending on light intensity and quality. Biochemical Engineering Journal 27 (2), 127–131.

Johansson, P., Wadsö, I., 1997. A photo microcalorimetric system for studies of plant tissue. Journal of Biochemical and Biophysical Methods 35 (2), 103–114.

Kirwan, A. J., 2004. Intrinsic photon entropy? The darkside of light. International Journal of Engineering Science 42 (7), 725–734.

Knox, R. S., Parson, W. W., 2007a. Entropy production and the Second law in photosynthesis. Biochimica et Biophysica Acta-Bioenergetics 1767 (10), 1189–1193.

Knox, R. S., Parson, W. W., 2007b. On "Entropy consumption in primary photosynthesis" by Jennings et al. Biochimica et Biophysica Acta-Bioenergetics 1767 (10), 1198–1199.

Kok, H. W., 2011. A thermodynamic analysis of photosynthesis: Quantum requirement and theoretical biomass growth rates. MSc, Faculty of Engineering and the Built Environment, University of the Witwatersrand.

Lam, H., Bungay, H., Culotta, L., 1986. An engineer looks at photosynthesis. Applied Biochemistry and Biotechnology 13 (1), 37–73.

Lamprecht, I., Drong, K., Schaarschmidt, B., Welge, G., 1991. Some like it hot calorimetric investigations of voodoo lilies. Thermochimica Acta 187 (0), 33–40.

Lavergne, J., 2006. Commentary on: "Photosynthesis and negative entropy production" by Jennings and coworkers. Biochimica et Biophysica Acta-Bioenergetics 1757 (11), 1453–1459.

Leff, H., 2002. Teaching the photon gas in introductory physics. American Journal of Physics 70 (8), 792–797.

Ley, A., Mauzerall, D., 1982. Absolute absorption cross-sections for photosystem II and the minimum quantum requirement for photosynthesis in *Chlorella vulgaris*. Biochimica et Biophysica Acta-Bioenergetics 680 (1), 95–106.

Louw, T. M., Griffiths, M. J., Jones, S. M., Harrison, S. T., 2016. Techno-economics of algal biodiesel. Springer International Publishing, Cham, pp. 111–141.
URL http://dx.doi.org/10.1007/978-3-319-12334-9_7

Magee, J. L., DeWitt, T. W., Smith, E. C., Daniels, F., 1939. A photocalorimeter. The quantum efficiency of photosynthesis in algae. Journal of the American Chemical Society 61 (12), 3529–3533.

Mann, C. C., 1999. Genetic engineers aim to soup up crop photosynthesis. Science 283 (5400), 314–316.

Maskow, T., Kemp, R., Buchholz, F., Schubert, T., Kiesel, B., Harms, H., 2010. What heat is telling us about microbial conversions in nature and technology: from chip-to megacalorimetry. Microbial Biotechnology 3 (3), 269–284.

Mauzerall, D., 2013. Thermodynamics of primary photosynthesis. Photosynthesis Research 116 (2-3), 363–366.

McDonald, M., 2003. Photobiology of higher plants. John Wiley and Sons Ltd, Chichester, West Sussex, UK.

Millan-Almaraz, J. R., Guevara-Gonzalez, R. G., Romero-Troncoso, R., Osornio-Rios, R. A., Torres-Pacheco, I., 2009. Advantages and disadvantages on photosynthesis measurement techniques: A review. African Journal of Biotechnology 8 (25), 7340–7349.

Milo, R., 2009. What governs the reaction center excitation wavelength of photosystems I and II? Photosynthesis Research 101 (1), 59–67.

Moroney, J. V., Ynalvez, R. A., 2001. Algal photosynthesis. John Wiley & Sons, Ltd, USA.

Mukhanov, V., Kemp, R., 2009. Design and experience of using light-emitting diodes (LEDs) as the inbuilt light source for a customised differential photomicrocalorimeter. Journal of Thermal Analysis and Calorimetry 95 (3), 731–736.

Mussgnug, J. H., Thomas-Hall, S., Rupprecht, J., Foo, A., Klassen, V., McDowall, A., Schenk, P. M., Kruse, O., Hankamer, B., 2007. Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. Plant Biotechnology Journal 5 (6), 802–814.

OpenStaxCollege, 2013. Biology. [Online]. Available at: http://cnx.org/content/col11448/latest/>, [accessed 31 May 2016].

Osborne, B., Geider, R., 1987. The minimum photon requirement for photosynthesis. New Phytologist 106 (4), 631–644.

Patel, B., Hildebrandt, D., Glasser, D., Hausberger, B., 2005. Thermodynamics analysis of processes. 1. Implications of work integration. Industrial and Engineering Chemistry Research 44 (10), 3529–3537.

Patino, R., Janssen, M., von Stockar, U., 2007. A study of the growth for the microalga *Chlorella vulgaris* by photo-bio-calorimetry and other on-line and off-line techniques. Biotechnology and Bioengineering 96 (4), 757–767.

Perlman, H., 2014. Transpiration - the water cycle. [Online]. Available at: http://water.usgs.gov/edu/watercycletranspiration.html, [accessed 12 May 2015].

Petela, R., 2008. An approach to the exergy analysis of photosynthesis. Solar Energy 82 (4), 311–328.

Radakovits, R., Jinkerson, R., Darzins, A., Posewitz, M., 2010. Genetic en-

gineering of algae for enhanced biofuel production. Eukaryotic Cell 9 (4), 486–501.

Raven, J., Geider, R., 2004. Adaptation, acclimation and regulation in algal photosynthesis. Photosynthesis in Algae 14 (5), 385–412.

Raven, J., Johnston, A., 1991. Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnology and Oceanography 36 (8), 1701–1714.

Ruan, C., Shao, H., Teixeira da Silva, J., 2012. A critical review on the improvement of photosynthetic carbon assimilation in C_3 plants using genetic engineering. Critical Reviews in Biotechnology 32 (1), 1–21.

Schuhmann, H., Lim, D., Schenk, P., 2012. Perspectives on metabolic engineering for increased lipid contents in microalgae. Biofuels 3 (1), 71–86.

Simionato, D., Basso, S., Giacometti, G. M., Morosinotto, T., 2013. Optimization of light use efficiency for biofuel production in algae. Biophysical Chemistry 182 (0), 71–78.

Teixeira, C., Wadsö, I., 1994. Solution photocalorimeters. Netsu Sokutei 21 (1), 29–39.

Villadsen, J., Nielsen, J., Lidén, G., 2011. Bioreaction engineering principles. Springer US, Boston, MA, Ch. Chemicals from Metabolic Pathways, pp. 7–62.

von Stockar, U., Liu, J.-S., 1999. Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1412 (3), 191–211.

von Stockar, U., Marison, I., Janssen, M., Patino, R., 2011. Calorimetry and thermodynamic aspects of heterotrophic, mixotrophic, and phototrophic growth. Journal of Thermal Analysis and Calorimetry 104 (1), 45–52.

Wadsö, I., 1995. Microcalorimetric techniques for the investigation of living plant materials. Thermochimica Acta 250 (2), 285–304.

Wagman, Evans, Parker, Schumm, Halow, Bailey, Churney, Nuttall, 1982. The NBS tables of chemical thermodynamic properties. Journal of Physical and Chemical Reference Data 11 (Suppl. 2), 1–151.

Wilhelm, C., Jakob, T., 2011. From photons to biomass and biofuels: evaluation of different strategies for the improvement of algal biotechnology based on comparative energy balances. Applied Microbiology and Biotechnology 92 (5), 909–919.

Yourgrau, W., Van der Merwe, A., 1968. Entropy balance in photosynthesis.

Proceedings of the National Academy of Sciences of the United States of America 59 (3), 734–737.

Zeng, X., Danquah, M. K., Chen, X. D., Lu, Y., 2011. Microalgae bioengineering: From CO_2 fixation to biofuel production. Renewable and Sustainable Energy Reviews 15 (6), 3252 – 3260.

Zhu, X.-G., Long, S. P., Ort, D. R., 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology 19 (2), 153–159.

Chapter 3

A black-box experimental analysis on the growth of microalgae *Desmodesmus spp.* at specific light wavelengths

- In this chapter an experimental investigation into the affects of specific light wavelengths on growth rate is presented. This follows on from Chapter 2 to understand the actual limits of the growth of a local algae species, by implementing experiments at specific light wavelengths using a spectrophotometer.
- This chapter will be submitted for peer review. A portion of this chapter was also presented as a poster at the 2012 SAIChE Conference, Drakensberg.
- The contribution of the co-authors (Glasser, D.[†*]; Hildebrandt, D.[*]; Matambo, T.[*]) towards the work is primarily that of supervision or collaboration, and the work and write-up was conducted by the author of this thesis.

Abstract

A spectrophotometer is an analytical instrument that is normally used either to measure the absorption spectrum or to measure the absorbance (turbidity) of a sample. However, in this chapter, not only will the spectrophotometer device be used to obtain the absorption spectrum of microalgae (*Desmodesmus* spp.), but it will also be used to grow the small sample at a

specific light wavelengths. High carbon dioxide tolerance and high lipid content make this species suitable for mitigation of carbon dioxide and biofuels production. This simple experimental method demonstrated that a specific light wavelength in the blue region of the light spectrum (namely 440 nm) was preferred over a red wavelength (686 nm). Furthermore it highlighted that a spectrophotometer can be converted to an experimental device. These findings agreed with other reports, in which blue light encouraged the growth for lipid producing species. It was recommended that a specific light wavelength was preferred over a colour range, particularly when designing photobioreactors, for the intention to reduce the amount of heat released into the surroundings.

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3.1 Introduction

The following chapter is organized as follows: microalgae and its propagation in photobioreactors is first introduced, followed by a detailed review on different light sources. In Section 3.2 an overview of the specific objectives are given, which is followed by Section 3.3 detailing the experimental procedure. In Section 3.4, the results are presented and discussed, and where appropriate important observations are summarized throughout the text. The conclusions are provided in Section 3.5. Following on from Chapter 2, where we looked at specific light wavelengths when using the thermodynamic calculations, this chapter wishes to experimentally find the best specific wavelength in which a species of algae, for example *Desmodesmus* spp., could grow in. This is to understand the actual limits of the growth of a local algae species, by implementing experiments at specific light wavelengths using a spectrophotometer.

3.1.1 Microalgae

Microalgae are microorganisms (from fresh or marine water systems) which can be converted into useful products because of their lipid, protein and carbohydrate content. These manufactured items include food supplements, pharmaceutical and cosmetic goods, as well as biofuels and biogas (Leite et al.,

2013; Pignolet et al., 2013; Simionato et al., 2013; Zhao et al., 2013; Teo et al., 2014b). The main source of energy for microalgae to produce products, from the photosynthetic process, is visible light (Carvalho et al., 2011). Other uses of microalgae encompass photo-toxicity tests (Cleuvers and Ratte, 2002), wastewater clean-up (to take up nutrients such as nitrates and phosphates) and carbon dioxide (CO_2) sequestration (Soletto et al., 2008; Park et al., 2012; Kim et al., 2013; Leite et al., 2013; Blair et al., 2014).

The family of green algae (and marine algae—Kurano et al., 1995), can play an important role in reducing carbon dioxide emissions from an industrial plant. More specifically, Kativu and his colleagues*(2012) suggested that Desmodesmus spp.* (genus of *Scenedesmus*) is a good candidate for carbon dioxide sequestration. Algae can therefore help to reduce the amount of carbon dioxide released into the atmosphere, and in some cases the carbon source can be used to make lipids from the algal species to convert to biodiesel (Leite et al., 2013). In consequence, the conversion of CO_2 into biodiesel and other products could help to alleviate both the greenhouse gas problem and depletion of fossil fuels.

Open algal ponds or photobioreactors (PBR) are used to cultivate algae, and in some cases CO_2 is bubbled through the culture. Posten (2009) reports advantages of PBRs over open-ponds, such as a higher algae area production and reduction of water loss (from evaporation). Additionally, they can provide a controlled environment for microalgae growth; however, the costs of materials and contaminants (from wastewater) decrease the efficiency of PBRs (Zhang et al., 2014b). There are three types of PBRs: vertical column reactors, tubular reactors and flat panel reactors (Janssen et al., 2003).

Photobioreactors are required to have little energy input, and must produce large amounts of algae; however, high value algal commodities can offset the costs of PBRs (Pulz, 2001; Kommareddy and Anderson, 2003). When designing a PBR, light energy is very important for algae growth (Lee, 1999; Carvalho et al., 2011). Aspects with light such as intensity and spectral quality will need to match the photosynthetic pigments (such as chlorophyll) found in the algae for growth (Lee, 1999). Still, there needs to a balanced supply of light, as insufficient or excessive light can limit the performance of production (such as photo-oxidation/inhibition and growth limitations) (Carvalho et al., 2011). The sources of light in PBRs are mainly artificial as opposed to natural sun-

light (Kommareddy and Anderson, 2003).

There are still challenges faced in the commercialization of microalgae for biofuels, such as efficient photobioreactor designs (Ho et al., 2014d) and cultivation strategies (Zhang et al., 2014b). Teo et al. (2014c) emphasized that even though temperature, pH and CO_2 supply are resources that need optimization, in particular available light should be improved since it is a contributing component in the photosynthesis reaction. Factors limiting the growth of algae, with respect to light, include the increase in cell density, which decreases the ability of the light to penetrate through the solution (Lee, 1999; Simionato et al., 2013; Ho et al., 2014d). Additionally, low light intensity can affect algae growth (Sorokin and Krauss, 1958).

3.1.2 The effect of light

Hsieh and Wu (2009) cited studies where other opportunities to improve light utilization were the use of light and dark cycles (changing the length of time that light is shone on the sample) or by changing the angle of the illumination. Carvalho et al. (2011) also reviewed other studies since 1932, such as flashing lights (which mimics light switching on and off); he concluded that for cell densities above a critical cell density, it is only then that flashing light becomes useful.

Jeon et al. (2005) referred to Evers (1991), who recommended that it is important to understand and quantify the light dependence of the activity of microalgae. McLeod (1961) suggested that at low light intensities the rate of photosynthesis is directly determined by the intensity and colour of the light being absorbed. Photosynthetic microorganisms only use a portion of the solar spectrum, known as the photosynthetic active radiation (PAR), a range from 400 to 700 nm. Therefore PBRs need to emit light in the PAR region, more specifically, at the wavelengths that will be absorbed by algae pigments (Blair et al., 2014; Markou, 2014).

Janssen and his colleagues (2003) suggested that current PBR designs were poor for scale-up and suggested another PBR which utilized light more efficiently. However, since their review there have been many other researchers optimizing PBRs. Carvalho et al. (2011) reviewed two strategies to improve algae utilization of light, that is, genetic engineering (which will change the

algae) or light engineering (which will improve the source of light). Wang et al. (2014b) recommended that for successful scale-ups, light in PBRs and other related parameters such as intensity, a light/dark cycle, flashing light and the light source should be optimized. Therefore, when designing a PBR for cultivation of algae, how the light is utilized will be a requirement for optimization. Ho and his co-workers (2014a) reported from other scientists that the production of algae and accumulation of valuable components (such as fatty acids and polysaccharides) were enhanced with a specific light wavelength.

Concerning the study of light and specific colours to grow algae optimally, wide spread experiments are reported; such as:

- lamps using filters (McLeod, 1961; Aparicio et al., 1976);
- dyes and fluorescent paint (Prokop et al., 1984; Seo et al., 2014);
- different materials in a PBR (Hsieh and Wu, 2009; Michael et al., 2015; Ooms et al., 2015); and
- different sources of light (such as fluorescent lights and lasers) (Carvalho et al., 2011; Mohsenpour and Willoughby, 2013).

3.1.3 Monochromatic light

The experimental investigation conducted in this thesis relates specifically to *Desmodesmus* spp. However, in this section, a review is presented on various researchers who have found different results for a species of algae, for example, *Chlorella pyrenoidosa,* when grown under different sources of monochromatic light.

3.1.3.1 Early studies

Gordon and Polle (2007) referred to research by Engelmann, dating back to 1884, who obtained the first action spectrum of the pigments (chlorophyll a and b) in green algae. Engelmann, used a prism to split white light into the spectral colours, and concluded that photosynthesis was as efficient in white light as in blue and red light (Gordon and Polle, 2007). In a general study of the photosynthetic action spectra of marine-algae, Haxo and Blinks (1950) designed their own monochromator using an incandescent lamp as the light source into their reactor. Their monochromator had a 0.75 mm slit and set

wavelength settings as 300, 550, and 600 nm to analyse red algae (marine microalgae) oxygen formation. They concluded that compared to green algae, red algae are unique amongst the photosynthesis plants as it had reduced photosynthesis, where chlorophyll showed maximum absorption in the action spectrum.

3.1.3.2 Filters

McLeod (1961) studied different colours of light affecting photosynthetic rates of green algae (*Chlorella pyrenoidosa*) with five percent CO_2. The source of light in his study was a general projection and mercury lamp with interference and coloured glass filters, which provided the monochromatic light. He found that the rate of photosynthesis was dependent on the wavelength of the incident light in saturating light condition.

Jeon et al. (2005) investigated the effect of light on the *Haematococcus pluvialis* strain and concluded that the red light, which also simulated daylight, was suitable for the photosynthesis of that particular strain. Borodin (2008) studied the growth of the green algae (*Chlamydomonas reinhardtii*) using red (glass filters) and blue (plastic and plexi-glass filters) and supplied light via a tungsten-halogen lamp. His experiment revealed that there was no difference between red and blue light (the quality of light) on the algae growth under low conditions of CO_2.

Other researchers had considered different light sources and alternative technologies other than just dyes and filters to find the best light to grow algae in. A cheaper alternative to artificial light was recommended by Mohsenpour et al. (2012), who used Xenon arc lamps as a light source. They used luminescent acrylic sheets (painted with visible-emitting organic fluorescent dyes) as filters to obtain desired wavelengths when studying the growth rates of green algae and cyanobacteria (*Chlorella vulgaris* and *Gloeothece membranacea* respectively). They found that orange (for green algae) and violet light (for cyanobacteria) were the best, and red light was the least efficient. However, the study by Mohsenpour and Willoughby (2013) using luminescent PBRs found that red light was the best for both species. They suggested that the difference, from the previous study, was attributed to better mixing conditions and mass transfer improvements that resulted from the bubbling with air. Kumar et al. (2014)

used red and white florescent lights with variations in CO_2, and their results showed that red light improved the growth rates and lipid content of *Micractinium pusillum* and *Ourococcus multisporus*. Kang and his co-workers (2014) observed the nutrient removal from wastewater, and the growth and lipid content of microalgae (*Chlorella* spp. JK2*, Scenedesmus* spp. *JK10, Chlorella vulgaris* AG10032). They used florescent light and covered them with blue, red, green or clear cellophane papers. Their results revealed that blue light resulted in a better growth for all three strains (Kang et al., 2014). For the species *Nannochloropsis* spp., Vadiveloo et al. (2015) used different coloured halogen lamps and found that blue light had a better effect on the growth and productivity of their species.

Authors such as Hsieh and Wu (2009) used a novel design for a PBR, known as transparent chambers, to use light better in the growth of *Chlorella* spp. In the study by Michael and his co-workers (2015), they found that when using nano-materials to filter out natural light from the sun, it showed an improvement in the biomass yield and productivity. Even so, there was no difference in the growth of microalgae, *Chlorella vulgaris*, when using the red and blue filter. Amrei et al. (2015) compared an ultraviolet-stabilized polycarbonate sprayed with fluorescent dye (a spectral shifting material) in a PBR and a PBR without the material. The former showed an improvement in microalgae (*Chlorella* spp.) growth. As a consequence, the ultraviolet (UV)-A radiation was converted to visible light. For both experiments they used a metal halide lamp as a light source and bubbled air through the PBR. Most recently, the work of Ooms and his colleagues (2015) showed an improvement of algae growth in a PBR, which worked on the concept of plasmon resonance, where it had absorption peaks for the algae tuned in the plasmonic nanodisk arrays that were placed on the PBR.

3.1.3.3 Dyes

Scientists have considered using colour dyes to study the growth of algae. Prokop et al. (1984) investigated the use of single laser dyes, of specific colours, to grow algae with five percent CO_2 and used tungsten bulbs as a light source. Their results showed that when the dye was in the media, instead of obtaining any sort of propagation, they discovered that the dye was toxic to algae and thus growth inhibition occurred. Nonetheless, the algae managed to grow when the dye was separate from the reactor (made possible using a glass

unit). Seo et al. (2014) used a fluorescent paint solution surrounding an algae reactor, while bubbling five percent CO_2 at 50 mL/min. Their data showed that algae (*Chlorella* spp.) were best grown using a red colour, but a higher lipid content was obtained under a blue colour.

Seo and his co-workers (2014) used ultraviolet (UV) as the source of light, which mimicked the sunlight spectral composition and strong radiation, and therefore concluded that algae could grow in sunlight. Additionally, they concluded that UV light could be converted to visible light using fluorescent dyes. In another report by Seo et al. (2015) they used organic dyes separate from the algal suspension, with UV light as the light source. The researchers observed that *Chlorella vulgaris* grew best in a red dye but its lipid content was better in a blue dye. However, in this case, they achieved a better algae propagation when mixing the dyes.

3.1.3.4 Light emitting diodes (LED)

Currently, the most common source of light for targeting specific colours are LEDs (light emitting diodes). They are used in research, concerning the cultivation of microalgae in PBRs because of their low heat generation, low energy consumption, long life-cycle and narrow light emission (Matthijs et al., 1996; Leite et al., 2013; Markou, 2014; Schulze et al., 2014). Since LEDs have a specific (dominant) wavelength (near to monochromatic light but still exhibiting emission spectra—Schulze et al., 2014), they have been used in studies on the effect of the light wavelength and intensities on microalgae propagation. However, even though natural solar light is cost effective, LED algal systems can be affordable if they produce high value products, and LEDs were noted as the most important light sources of the future (Schulze et al., 2014). In the report by Kommareddy and Anderson (2003), they concluded that if a PBR were to operate for more than a year, LEDs were more cost effective than cool fluorescent, Gro-Lux, incandescent and halogen lamps. It is for the reasons mentioned above that the research using LEDs (and different light intensities) should be mentioned in this literature review. The research concerning LEDs is where a lot of colour-specificity studies of algal growth are found.

Matthijs et al. (1996) investigated the growth rates of the algal culture *Chlorella pyrenoidosa* in a bioreactor with flashing LEDs. They concluded that the red light was suitable enough to provide the necessary photons for the propagation

of the algae culture. Matthijs et al. (1996), cites Ruyters (1984) who suggested that blue light may play an important role in the metabolism and regulation of cell growth. Park and Lee (2000) compared the supply of continuous lighted LEDs with a high frequency of flashing lights mode (which mimics the on and off supply of the light source), and the latter improved the cell concentration, however, the improvements were observed more in algae cultures which had a higher cell density. Wang et al. (2007) studied the growth of *Spirulina platensis*, known for its protein content. Their experimental results revealed that this species of algae preferred to grow in red instead of blue LEDs. Additionally, their study showed that the higher the light intensity, the higher the biomass yield. Wang et al. (2007) suggested that within the photosynthetic process, different specific bio-chemical syntheses may be triggered by different wavelengths of LEDs, and this is worth investigating.

Kim and his co-workers (2013) studied wastewater treatment with microalgae (*Scenedesmus* spp.), using LEDs, supplying inorganic carbon via an air pump and a stone into the suspension. Their results revealed that when mixing red and blue light versus growing it in white light resulted in an improvement of microalgae production and nitrogen removal from wastewater. However, under blue light, the phosphorous content in wastewater was removed by algae. They also did not use green light as it consumed too much power with low algae productions rates. Yan et al. (2013a) found that when growing microalgae *Chlorella vulgaris* to purify domestic wastewater, under different LED lights, after six days red light was the best for algae propagation. Additionally, Yan et al. (2013b) also found that red light was the best for *Chlorella vulgaris* production and nutrient removal from wastewater, while green and blue were the least efficient for algae growth. Zhao et al. (2013) concluded that red light was effective when using microalgae *Chlorella* spp. to upgrade biogas and remove nutrients from the digestate. Choi (2014) mass cultivated *Chlorella vulgaris* using a PBR with light and dark cycles of LEDs and optical panels (which were different distances away from the PBR). The system was able to use the LEDs (range of 470–670 nm) more efficiently, and red light was better for wastewater treatment and algae growth while bubbling CO_2 through the PBR.

Most recently Abiusi and his co-workers (2014) investigated the effect of light quality on algae growth, productivity and cell size. They found that for their algae species, *Tetraselmis suecica*, under white and red LED, the biomass pro-

ductivities were comparable, however, blue and green light reduced the species growth. Ho and his co-workers (2014a) found that white LED produced a better lutein content (used as a food additive for health benefits) in *Scenedesmus obliquus* over red, blue and green monochromatic light. Markou (2014) studied the biomass composition of *Arthrospira platensis* and found many results but not one conclusion. Consequently, what can be drawn from his research was that the lowest biomass productivity and protein content, but highest lipid content, was with blue LEDs. Yet, the highest biomass productivity was with red LEDs. However, white and green light produced the highest protein content.

3.1.3.5 Laser

On the other hand, the review by Carvalho et al. (2011) emphasized that laser diode technology is a promising technology, as it delivers a narrow wavelength with negligible heat. In the report by Kuwahara and her co-workers (2011), they suggest that lasers offer a true monochromatic light source. Their study, growing green algae (*Chlamydomonas reinhardtii*) revealed that the coupling of red and blue lasers (at the chlorophyll wavelength) increased algal cell count more than that of individual sources of white light and red laser. They did recommend that there should be more studies to isolate the specific light wavelength to what powers the processes for the algae propagation.

3.1.4 Effect of light on algal composition

3.1.4.1 Growth and lipid content

Blinks (1960) concluded that the best growth takes place with the pigments chlorophyll b and carotenoids, in green algae, when far red light is supplemented with other wavelengths. Govindjee and Thomas (1960) found that for certain algae species (such as *Chlorella pyrenoidosa*) extreme red light can inhibit photosynthesis. However, both blue light and red light might be necessary to break the chemical bonds of the compounds that take part in the photosynthesis reaction as there are two distinct light reactions within photosynthesis (Clayton, 1965). Faust et al. (1982) cited two authors that suggested that the growth rates, which depend on the species and the photon flux density, are generally higher in blue than in white light. They also found that growth rates are better in the blue and red light as opposed to white light. Rochet et al. (1986) mentions Faust et al. (1982) who found that other species

of algae grow better under blue light than white light; this was also confirmed by other investigators such as Wallen and Geen (1971); Yentsch (1974); Jeffrey and Vesk (1977) and Yago et al. (2011).

Furthermore, the review by Wang et al. (2014b) deduced that a mixture of blue and red is recommended for microalgae propagation. However, Wang et al. (2014b) refers to other researchers, who found that red light was better for growth (as it was a better carbon converter) but blue light was better for carbon assimilation and lipid production (as it triggers key enzymes for triglyceride production) of other algae species. On the other hand, it was also confirmed by Ho and his co-workers (2014a) and the reports from which they cited, that the light wavelength is species dependent for microalgae production.

When microalgae species are considered as a source of biodiesel, also known as fatty acid methyl esters (FAME), Das and his colleagues (2011) found that the specific growth rate under LED for *Nannochloropsis* sp, was better in the order of blue, white, green followed by red light. Park et al. (2012) found that blue light was suitable at the early stages for cultivation of marine microalgae (*Microcystis aeruginosa* and *Chlorella vulgaris*), however, introducing red light after four days can improve algae growth. They therefore recommended that for large scale production of microalgae, there should be a selection of red and blue LEDs. Atta et al. (2013) studied different light intensities (and light and dark cycles) on the growth of *Chlorella vulgaris*, and found that they were able to cultivate more algae in blue LED light than white fluorescent light.

Teo et al. (2014a) also that blue light was suitable for higher lipid production in the algae species, *Tetraselmis* spp. and *Nannochloropsis* spp. and Teo et al. (2014b) found that for the same microalgae, under LED light, the species growth was preferred under blue light as opposed to red light. Yang and Weathers (2015) found that at day 14, thirty minutes of red light was sufficient to increase the lipid content in *Ettlia oleoabundans*, matching data from other reports when grown with just red light. Choi and his co-workers (2015) achieved an improved growth of microalgae, *Acutodesmus obliquus* (previously *S. obliquus*), using red and blue LEDs flashing light effect. Wang et al. (2014b) showed that red light was the most preferred light source as it can penetrate denser well-mixed cultures, whereas blue light could cause photo-inhibition because of its high energy. Blue light is known to provide enzyme activation and

gene transcription regulation but is not used for energetic purposes (Matthijs et al., 1996).

The theoretical study by Lee et al. (2013) showed that the characteristics used to design PBRs depended on the wavelength, and from species to species, which can be determined by theory or experiments (which can be expensive). They use a theoretical approach (assuming the algae cells were spherical) by characterizing experimental absorption spectra to determine the optical properties (refraction and absorption indexes) of biofuel producing algae. In the review by Kim and his co-workers (2013), they report from researchers in the field that microalgae absorb different wavelengths depending on the species. It is therefore important to find which colour of light or specific light wavelength a species best grows in.

Yan et al. (2013a) and Yan et al. (2013b) found under LED lights, that *Chlorella vulgaris* was best grown under red light when using synthetic wastewater. Hultberg and her co-workers (2014) also used LED light but their results showed that yellow and then red light resulted in the higher growth of *Chlorella vulgaris* when in a standard growth media. However, Blair and his co-workers (2014) used compact fluorescent lamps (CFL) to determine the growth of *Chlorella vulgaris* in red, blue, green and clear light. They found that blue light yielded the best biomass productivity and growth rates when using basal salt media. On the other hand, Kim et al. (2014) found that at certain growth stages, by switching from blue LED light (430–465 nm) to red LED light (630– 665 nm) at the late exponential phase, one would enhance the growth and lipid content of *Chlorella vulgaris*. Teo et al. (2014c) also found that by mixing blue and red LED for the growth of the microalgae, *Nannochloropsis* spp., that the FAME qualities were improved. Thus, depending on the light source, one would need to determine the best wavelength for the specific species in and during the growing conditions (such as the growth medium, physiological conditions—Blair et al., 2014). It can also be seen from the above that different species will photosynthesize differently from varying light sources.

3.1.4.2 Algal growth with carbon dioxide and light

Ogasawara and Miyachi (1970) listed several authors who studied algal growth with specific colours of light using filters. A general conclusion was reached, namely, that fixed carbon atoms in red light gets transitioned into sugars and carbohydrates, but in blue light the atoms are used for protein and amino acids.

Pigmentation studies by Eley (1971) found that the pigment composition of the blue-green algae was caused by CO_2 concentration and not the light wavelength or intensity. Pulich Jr and Van Baalen (1974) found that blue light had an inhibitory effect on nitrogen metabolism and CO_2 fixation.

Additionally, Dring (1989) found that for brown algae blue light might have an effect on the uptake of inorganic carbon. He reports, from other researchers, that for green algae and higher plants, blue light has as a direct effect on the enzymes responsible for carbon metabolism, known as the 'blue-light syndrome'. In the Baba et al. (2012) report, they used monochromatic light, specifically red, blue and green, to study the growth and carbon fixation of algae (*Botryococcus braunii*), which produces oil. They found that red light was suitable for growth but blue was the best for carbon fixation, and green-light was the lowest in both experiments. Teo et al. (2014a) referred to reports that blue light activates the enzymes which effect CO_2 rates in microalgae growth, however, they do go on to add that different species requirement for light wavelength does vary.

It is important, however, to consider the interaction between temperature, irradiance and dissolved oxygen when designing for outdoor cultures (Ugwu et al., 2007). It is worth exploring other variations in CO_2 concentrations (Kumar et al., 2014).

It should be clear from all of the above that the factors that affect the growth of algae are very varied and complex. At present there appears to be no particular theory that can explain the growth of any particular species under specific conditions. Thus at this time it appears that the only way to get results for a particular situation is to do experiments.

3.2 Aims and objectives

Despite many studies focusing on light intensity and specific colours (dyes, laser, filters, LEDs) to improve the growth and composition of algae, to the author's knowledge, none of them provide information on the growth of *Desmodesmus* spp., using a specific light wavelength. The chapter will examine the growth of *Desmodesmus* spp. in a spectrophotometer while bubbling air or CO_2 through the sample.

A spectrophotometer can be used to measure the optical density (OD) (also known as absorbance) of a solution /suspension. It is a piece of equipment which has a monochromatic beam that passes through a cuvette containing the sample solution, with a slit (about 2 nm wide which controls the bandwidth of the beam) and the absorbance is then measured. Thus for a specific light wavelength, only light of that wavelength illuminates the sample (Dunne, 1999).

As seen in the previous chapter, a theoretical thermodynamic analysis was carried out on the photosynthesis process. This section deals with an experimental approach to the photosynthesis of microalgae, with respect to growing microalgae at a specific light wavelength. The work presented in this chapter by the author was to determine if a small experimental method served as a quick check for the wavelength specificity of a species. The aim was not to design a PBR but rather offer a small cost effective manner to experimentally test what specific light wavelength a species of microalgae (namely *Desmodesmus* spp.) best grows in, and to shed more light into the photosynthesis process at specific light wavelengths. The following were the objectives for the experimental analysis:

1. obtain the absorption spectrum of *Desmodesmus* spp. to determine the peaks at which it responds; and

2. compare the growth of the microalgae at the specific light wavelengths, using a spectrophotometer

    (a) Two sets of experiments were carried out using either pure CO_2 or air (maximum to minimum effects) on the growth of algae.

3.3 Experimental Section

3.3.1 Materials and Methods

Algae collection and storage

The algae strain, identified to be *Desmodesmus spp.* (Kativu, 2011 MSc dissertation), was collected by the author and Kativu from Johannesburg Zoo Lake in 2009, and Kativu cultivated the algae and isolated only *Desmodesmus spp.* after numerous filtering, autoclaving and CO_2 bubbling experiments; for a detailed explanation see Kativu (2011). The writer took a sample of the microalgae species from the Kativu (2011) back-up flask and stored it in the fridge to be used for future experiments. The author maintained a back-up supply of the algae and grew it under a mixture of Slovenia Gro-Lux and cool white fluorescent tubes, which covers the photosynthetic active radiation spectrum.

Beijerinck medium preparation

The writer used a modified Beijerinck medium (the recipe is shown in Table 3.3.1) to cultivate the algae (Kativu, 2011). Furthermore she prepared stock mediums one, two and three individually in a one litre Schott bottle, and but she prepared only 100 mL of micro-nutrients. Once the stock solutions and micro-nutrients were prepared, they were autoclaved (using a Hirayama HiClave; model HA–300MD) and stored at 4^{o}C. After autoclaving the micro-nutrients, precipitates were present in the medium. However, the writer ensured that precipitates were present in the final Beijerinck medium for all stock solutions and micro-nutrients by shaking the bottles (to ensure a homogeneous solution) well before measuring the required amount needed for the medium. For the purposes of this experiment, 100 mL of Beijerinck medium was prepared. All Schott bottles used in the experiment were autoclaved. General experimental precautions can be found in Appendix D.1.

Table 3.3.1: Modified Beijerinck Medium, from Kativu (2011).

Stock Solution 1 - Stock Mineral Solution	100 ml
Stock Solution 2 - Buffer A	40 ml
Stock Solution 3 - Buffer B	60 ml
Micro-nutrients - Trace Element Solution	1 ml
Distilled Water	799 ml

Stock Solution 1 - Stock Mineral Solution	g/l
NH_4Cl	0.68
KNO_3	2.23
K_2HPO_4	0.2
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.1

Stock Solution 2 - Buffer A	g/l
KH_2PO_4	4.535

Stock Solution 3 - Buffer B	g/l
K_2HPO_4	5.535

Micro-nutrients - Trace Element Solution	g/100ml
H_2BO_3	1
$CuSO_4.5H_2O$	0.15
EDTA	5
$ZnSO_4.7H_2O$	2.2
$MnCl_2.4H_2O$	0.5
$FeSO_4.7H_2O$	0.5
$CoCl_2.6H_2O$	0.15
$(NH_4)6Mo_7O_{24}.4H_2O$	0.1

Experimental preparation

For the initial trial runs the author mixed 10 mL mother algae (a suspension containing isolated Zoo Lake *Desmodesmus* spp.) with 90 mL modified Beijerinck medium. However, the author found that it was better to store them separately and mix the medium and algae on the day of the experiment in the following amounts: 400 μL *Desmodesmus* spp. and 4000 μL of the medium in a cuvette. Micropipettes (Biohit Oyj Pipetman Plastic Ejector, #P1000G, Helsinki, Finland) were used to measure the required amounts and dispense into a 2 mL cuvette. Additionally, the researcher prepared a blank, consisting of only Beijerinck medium, for all of the spectrophotometer readings (optical density (OD) or absorbance). This was to establish a difference between algae and

medium components, in order to monitor only the microalgae OD (growth).

Assumption

The algal cells were suspended in a liquid phase of a particular composition, and that a homogeneous distribution of algal cells throughout the volume of the suspension was assumed in this experiment. Thus assuming the homogeneous suspension will be just be a given with algae. This assumption was the same for the experiment carried out in Chapter 4.

Absorption spectrum

The author characterized the absorption spectrum (from 360 to 1000 nm) of *Desmodesmus* spp. using the UNICO 4802 UV/VIS double beam spectrophotometer (which has a pre-aligned halogen and deuterium lamp with a 1.82 nm bandwidth, and a photometric range of -0.3–3Abs). This process (which took less than two minutes) was to help indicate to the author at which wavelengths the algae was absorbing. The results of this process can be seen in the next section.

Specific light wavelength experiment

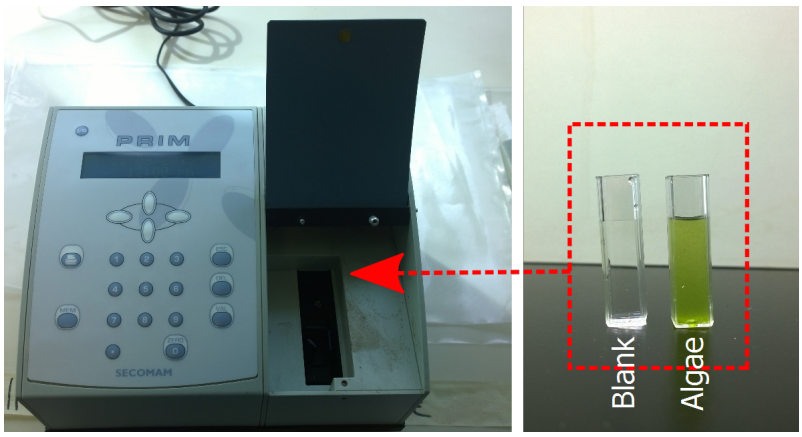


Figure 3.3.1: Secomam Prim advanced spectrophotometer as the experimental device, where the cuvette containing the blank or medium with algae was placed into the cell holder, for the specific light wavelength experiment.

The author carried out readings, for each experiment (at a specific light wavelength) for an average of 7.5 hours per day, for four days. Additionally the author carried out a dark control experiment, where only gas was bubbled through the suspension. The cuvette (containing the algae and medium) was shaken four

times a day. The experiment took place in the Secomam Prim Light spectrophotometer (which has a pre-aligned 10 Watt tungsten halogen lamp, with a 1 nm precision and a 10 nm bandwidth, and a photometric range of -0.3–2.5 Abs). The algae while in the spectrophotometer was allowed to grow at the specific light wavelength with gas (either air or CO_2) bubbling through it.

The author did not control pH, and used litmus paper to take readings before and after the experimental run. To measure the temperature surrounding the cuvette and spectrophotometer, two J-thermocouples were placed in the areas of measurement. Initially the surrounding temperature was not controlled at night, however, over the winter months it was deemed necessary to maintain a constant temperature surrounding the spectrophotometer (as the author noted slower growths) by using fan heaters in the section of the laboratory. Therefore the average daily recorded controlled temperature readings can be found in Appendix E.2.

The supply of inorganic carbon was bubbled at a flow of 3.75 mL/min (set points were calibrated using a soap bubble meter) into the cuvette suspension using a Pipe Parker Parflex (1/8 O.D and X 0.31 Wal, 500 W.P piping) using gas supplied from Afrox: air (Instrument Grade (IG) 8.5 kg containing $<$ 0.05 mole% of CO_2) and CO_2 (IG Cylinder 31.3kg). The gas flow was controlled by using a Brooks gas flow controller (read out and control electronics 0154,I/O: 0(4) -20 mA, supply output +24V) and a Smart mass flow controller. When the absorbance readings of the sample were recorded, the bubbling was stopped, this was for less than a minute.

3.3.2 Optical density (OD) and Relative growth rate (RGR)

By the Beer-Lambert Law the absorbance, also known as the optical density (OD), is directly proportional to the concentration of the solution (Swinehart, 1962; Aneja, 2007). In some instances, the writer chose to show the results in this chapter in the form of OD at 680 nm (OD_{680}), since dry weight is commonly correlated with optical density (OD) (Wang et al., 2007; Atta et al., 2013).

According to Fogg and Thake (1987) there are five stages of growth that algae cells follows:

1. lag phase: there is no apparent increase in cell growth;
2. exponential phase: the multiplication of cells are rapid;
3. declining growth rate of the cells;
4. stationary phase: the cell numbers are approximately constant; and
5. a death phase of the cells.

Specific growth rate (μ), also known as the relative growth rate (RGR), can be derived from a simple first order rate law (assuming exponential growth phase) and the result is shown in equation 3.3.1. The author found the correlation between dry mass (grammes) per litre and OD_{680} (the values of the OD and mass data are shown in Appendix E.1). The OD_{680} values for this experiment were between two points only, therefore a simple linear dependence of the OD_{680} values on the biomass concentration was used, which resulted in the following relationship: $C_A = 0.201OD_{680} - 0.0358$.

$$RGR = \frac{\ln\left(\frac{C_{A,n}}{C_{A,0}}\right)}{(t_n - t_0)} \tag{3.3.1}$$

where

C_A = dry mass concentration of the microalgae (mg/L);
t = time in days;
n = day n; and
0 = day one.

3.4 Results and Discussion

3.4.1 Absorption spectrum

The wavelengths at which the algae were grown are shown in Figure 3.4.1 below. Red light (600–700 nm) and blue light (400–500 nm) (Blair et al., 2014), are thus expected to be the major wavelengths for activity, that is where the microalgae will absorb light. This absorption spectrum is specific for this species. As can be seen, six specific light wavelengths were chosen at which to grow the algae, three of these at peaks in the spectrum and three away from these peaks. The chosen wavelengths are shown in Figure 3.4.1 as vertical lines.

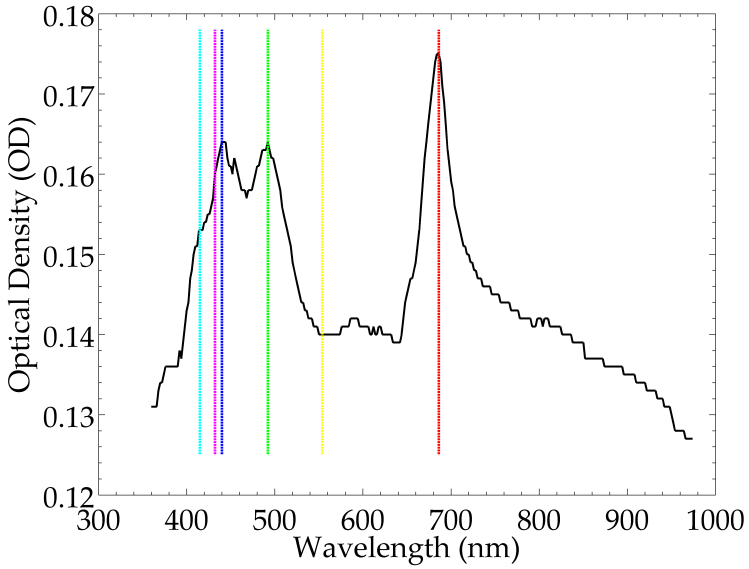
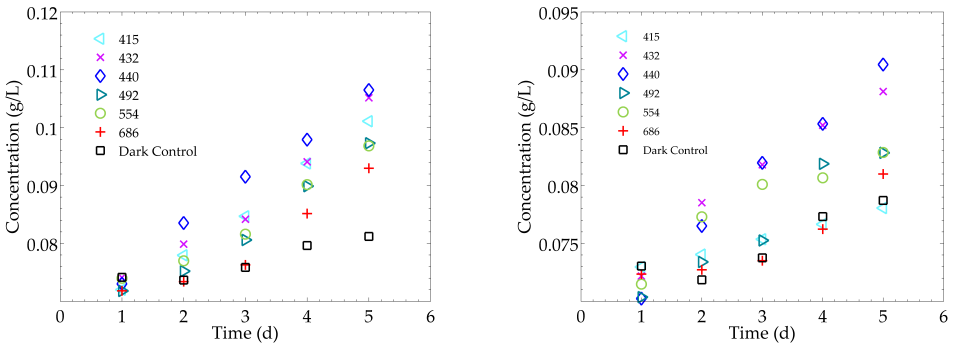


Figure 3.4.1: Absorption spectrum for microalgae *Desmodesmus* spp. From this spectrum, specific light wavelength experiments were carried out at the chosen wavelengths (415, 432, 440, 492, 554, 686 nm), which are represented by the vertical lines.

3.4.2 The growth at specific light wavelengths



(a) The air experiment with highest growth in light observed at 440 nm and lowest at 686 nm. Average temperatures were from 28º–32ºC. pH initial 6.5–7.0 and final pH 7.0–7.5.

(b) The growth of algae with CO_2 was highest at 440 nm and lowest at 686 nm. Average temperatures were from 26º–32ºC. pH initial 6.5–7.0 and final pH 6.0–6.5.

Figure 3.4.2: Concentration versus time for *Desmodesmus* spp. at specific light wavelengths of 415, 432, 440, 492, 554, 686 nm and the dark control.

After four days of experiments, it was shown by other researchers in the field (Park and Lee, 2000; Wang et al., 2007; Das et al., 2011; Seo et al., 2014; Teo et al., 2014a; Amrei et al., 2015; Seo et al., 2015) that clear conclusions could

be drawn, and this was seen in this experiment for the specific light of experimental data, with biomass or OD readings. The data from the experiments (which contain the OD values, concentration, temperature and RGR) can be found in Appendix E.2.

The comparison of the different wavelengths when the algae was grown in air is seen in Figure 3.4.2a. The growth of *Desmodesmus* spp. preferred to grow at 440 nm, followed closely by 432 nm. The reader can see from Figure 3.4.2b, the growth of algae at different wavelengths when grown in CO_2. Again, one can see that *Desmodesmus* spp. grew most strongly at 440 nm (blue wavelength). Furthermore the growth curve demonstrates a linear growth. If we draw specific growth rate as a function of the *Desmodesmus* spp. concentration for the blue wavelength (440 nm), we can see in Figure 3.4.3 that the growth follows a Monod equation, proceeding towards a maximum growth rate (Monod, 1949). Therefore, in Figure 3.4.2, the growth rate is proceeding towards maximum growth.

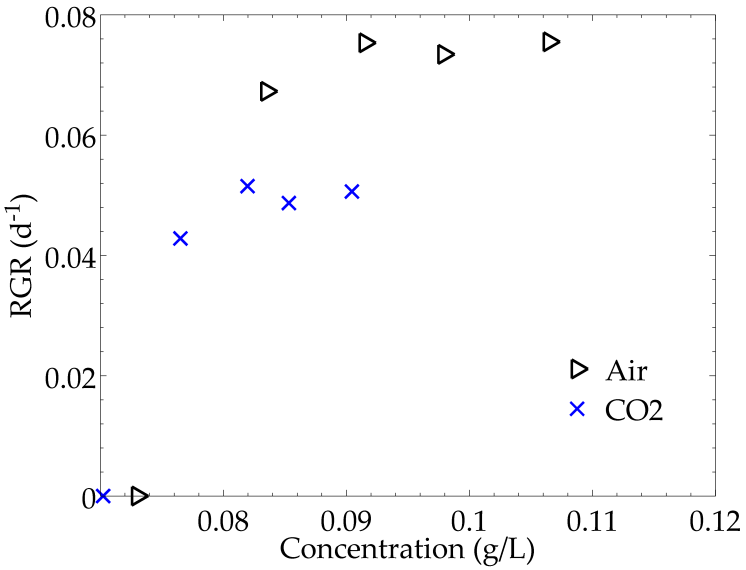


Figure 3.4.3: Specific growth as a function of the *Desmodesmus* spp. concentration for the blue wavelength (440 nm).

It is interesting to note that the results show that whether grown in air or a high CO_2 concentration, the specific light wavelength at which the maximum growth rate occurs is the same. It should be noted that the bubbling with air and CO_2, aided in mixing and mass transfer; as well as minimizing the absence of light and any other effects in the dark zone (Mohsenpour and Willoughby,

2013), since the algae managed to grow.

The growth in air with a blue wavelength (440 nm) was 14.5% higher than that in the red (686 nm) and 30% higher than that of the dark control growth. It can be seen that 100% CO_2 did not make the algae grow faster than in air; however it still grew. Kativu et al. (2012) found that at 5% CO_2, *Desmodesmus* spp. grew faster than that of air, and it would be worth doing further experiments with this CO_2 concentration.

The dark control experiments indicate that the growth of the *Desmodesmus* spp. was fairly slow and did not follow the same trend as when grown with a specific light wavelength. For the growth of microalgae in the dark control, it first decreased (declined in growth) and then increased after day two, indicating that they may first need to acclimatize to the environment with no light, but then were able to grow because of the presence of the source of carbon from air and CO_2.

Schulze et al. (2014) mentioned that even though red gave the highest absorption peak in the absorption spectrum, it did not necessarily match the optimal growth for the algae. This was also observed in the results from this experiment (as shown in Figure 3.4.4).

The RGR values in Figure 3.4.4 when compared to the absorption spectrum show very interesting results. In both cases, that is air or CO_2 bubbling, there is a single peak of growth rate at 440 nm the value of which corresponds to one particular peak in the absorption spectrum where it is absorbing light. There are no other growth rate peaks and in fact the growth rate decreases at wavelengths before and after this peak value. This brings us to our first insight: that the absorption spectrum does not give one a full picture for the growth rate of the algae species, even if it absorbs red light.

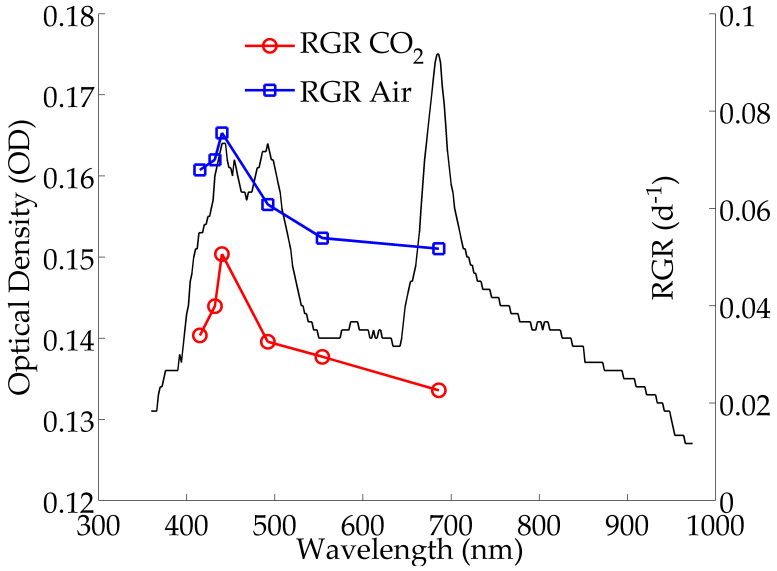


Figure 3.4.4: The RGR values calculated at day five for the growth of *Desmodesmus* spp. in CO_2 and air, shown with the absorption spectrum. The RGR when grown in the dark control was 0.01822 day^{-1} (Air) and 0.01494 day^{-1} (CO_2). It can be seen that the RGR peaks at 440 nm for both experiments.

It can also be seen that the algae species grew better in green than red wavelength of light. It may seem that red light is inactive for the photosynthesis process, which may be strange as the green chlorophyll pigments should reject the green light but absorb the red light (Douma, 2008). We can try to explain this phenomena, let us recall equation 2.2.23 from Chapter 2, $n_{photons} = (\triangle G_{process})\frac{\lambda}{hcN_A}$:

- If we assume that red light (686 nm) was at the same intensity as the blue light (440 nm), this could explain the observation that red light is almost inactive for photosynthesis. That is, when one assumes that the number of photons supplied by the spectrophotometer was constant, then that would imply that at lower wavelengths more energy is supplied to the algae species than at higher wavelengths. Hence a lower growth rate of the algae species is experienced at the red wavelength.

- On the other hand, if we assume that the number of photons were not constant, that is the intensity of the halogen lamp in the Secomam spectrophotometer followed a hyperbolic shape (a given for tungsten-halogen light source), then the blue light is less intense than the red light (Zaccheroni, 2012). Therefore, from equation 2.2.23, at lower wavelengths, less energy is required to activate the photosynthesis process, which is

seen in figure 2.3.1. As a result, the number of photons supplied at the blue wavelength was sufficient to activate the photosynthesis processes. However, at higher wavelengths, more photons were supplied and as a result, two things could have occurred, which could have potentially inhibited the growth of the algae species: (1) the dissipation of excess photons could have led to excess heat in the system; or (2) the algae grew too fast, and their temperature could have risen significantly above the temperature of the medium they were immersed in.

This brings us to insight two: this particular algae species grew best at a blue wavelength in the spectrophotometer, explained by equation 2.2.23. Additionally, insight three is now introduced, where the equation from Chapter 2 is only valid for certain wavelengths, that is wavelengths which are higher than 440 nm, because it asymptotes at this wavelength, and any other wavelengths which are lower than 440 nm (such as 415 nm, the violet wavelength) cannot be explained by the equation 2.2.23 presented in Chapter 2, because when more energy is supplied one would expect higher growth at lower wavelengths.

Therefore, one also can speculate then that this maximum growth rate (at 440 nm) corresponds to a particular frequency of some bond that is important in the photosynthesis reaction. The hypothesis presented here is: when photons of this frequency are used this is the most effective in causing growth or photosynthesis to occur. We can refer to this as the resonance frequency (RF), and discuss this more in Section 3.4.3. Photons of other frequencies will be less effective in stimulating this bond and so the growth rate is decreased. There is no evidence that any other bond takes place in the photosynthesis reaction.

It can be seen from Figure 3.4.4 that the RGR for the air 440 nm experimental run was 50 % higher than that of the CO_2 run. The RGR for air and CO_2 at 440 nm exhibited a normal distribution (refer to Appendix E.2.3.1), and thus the 50 % higher value was significant. This is confirmed when using ANOVA (analysis of variation) in Microsoft Excel implemented as a two factor without replication (see Appendix E.2.3 for the ANOVA results), the P-value = 0.016, which is less than an alpha value of 0.05, and therefore indicates a significant difference. The RGR for the air runs, 0.05 (red) to 0.075 (blue), are similar to the values found by Abiusi et al. (2014). They grew their microalgae (*Tetraselmis suecica*) in an air CO_2 mixture under red, green, blue and white

LED for nine days, and obtained resultant RGRs from 0.06 (blue) to 0.12 (red). Baba et al. (2012) bubbled sterilized air into their green algae (*Botryococcus braunii*) and their results for RGR were approximately 0.19 (red), 0.15 (blue) and 0.09 (green).

In this chapter, a spectrophotometer was used to ascertain what specific wavelengths the microalgae best grew in. A lower RGR was expected since this was a small scale experiment and the goal was not to compete for lab scale size RGR. However, the experiment itself was insightful as it was used to screen for the best wavelength regardless of the spectrophotometer size and light intensity. Sorokin and Krauss (1958) found that the growth rates are a function of the species genetic make-up and the surrounding temperature for different light intensities, so if one wished to scale the process up, a separate experiment should be done to investigate the light intensity responses of the specific species for that particular wavelength.

Note that in addition to growing the algae with light at a specific light wavelength, the algae was also grown without light (the dark control). Using ANOVA implemented as a two factor without replication, the experimental results show that *Desmodesmus spp.* RGR was significantly lower (P-value= $0.020 < 0.05$) in the dark control than at the blue wavelength. In fact RGR was respectively four and three times more growth with air and CO_2 than in the dark. These experiments show that the growth of the *Desmodesmus spp.* was affected by the specific light wavelengths, but more by the blue region as opposed to the red region. This finding is in agreement with Matthijs et al. (1996) and Bisio and Bisio (1998) where they both suggest that chlorophyll absorbs in these regions. This does not agree with the results quoted in the review by Gordon and Polle (2007), where they say red light drives photosynthesis five times more than the full spectrum of sunlight.

The literature review suggests that blue light worked best with lipid producing species and it activates the enzymes which affect CO_2 rates in microalgae growth (Teo et al., 2014a,b).

Note that Desmodesmus spp. former name was *Scenedesmus* spp. (Cleuvers and Ratte, 2002; Cleuvers and Weyers, 2003). The data presented here does not agree with Kim et al. (2013), who found that growing the species (*Scene-*

desmus spp.) was better in red than blue using an LED. However, a study by Kang et al. (2014) has shown that *Scenedesmus* spp. JK10 grown in blue light (achieved by covering a fluorescent light with blue cellophane) resulted in a higher growth rate and lipid content, as well as higher nutrient removal in wastewater. This is all important as *Desmodesmus* spp. F2 is a thermotolerant species and a candidate for biodiesel production (Ho et al., 2014b,c).

3.4.3 Growth rate with respect to the resonance frequency

In this section we will discuss the hypothesis that was presented in the previous section, namely the resonance frequency (RF). Let us assume that the distribution of energy of the bond that needs to be broken is governed by a Boltzmann distribution law. The Boltzmann distribution law, defined by Widom (2002), is the energy associated with some state or condition of a system defined as ϵ , then the probability of the occurrence is proportional to the following expression:

$$\exp\left(-\frac{\epsilon}{\mathrm{kT}}\right) \tag{3.4.1}$$

where

ϵ = some state or condition of a system (J);
k = Boltzmann's constant (J/K); and
T = Thermodynamic temperature (K).

If one takes the definition of frequency (F) as the speed of light (c) divided by wavelength (λ) ($\mathrm{F} = {}^{c}/_{\lambda}$), then it can be seen from Figure 3.4.4 that the microalgae cells in the sample still grew at other frequencies and not just the resonance frequency. If we designate 440 nm as $\mathrm{RF_{MAX}}$ for both experiments as the resonance frequency. This is where the growth rates are the maximum. The author is suggesting that the $\mathrm{RF_{MAX}}$ is the frequency where the bonds in molecules are broken for the process of photosynthesis to occur. In other words, RF is the some state or condition of a system(ϵ) as defined in equation 3.4.1.

Thus a proportion of these bonds will have the required resonant energy even when struck by photons not of the resonant energy. We can then write the Boltzmann distribution equation relating the growth rate (probability of occurrence) at any other frequency to that at the resonant frequency. Now we

remember that the algae grew even when there was no light (dark control). Let us assume this was via a different mechanism. In this case we can define a light induced growth rate to be the value at a particular frequency minus the one without light. We call these values RGR' and write the relationship accordingly to equation 3.4.1:

$$\mathrm{RGR}^{'} = \mathrm{A}\exp\left(-\frac{\mathrm{RF}}{\mathrm{kT}}\right) \tag{3.4.2}$$

$$\mathrm{RGR}^{'}_{\mathrm{max}} = \mathrm{A}\exp\left(-\frac{\mathrm{RF}_{\mathrm{max}}}{\mathrm{kT}}\right) \tag{3.4.3}$$

where

A = constant of proportionality (d^{-1}).

To express it by the Boltzmann distribution probability, we can divide state 1 (equation 3.4.2) by state 2 (equation 3.4.3, the maximum RGR' of the experiment):

$$\frac{\mathrm{RGR}^{'}}{\mathrm{RGR}^{'}_{\mathrm{max}}} = \frac{\mathrm{A}\exp\left(-\frac{\mathrm{RF}}{\mathrm{kT}}\right)}{\mathrm{A}\exp\left(-\frac{\mathrm{RF}_{\mathrm{max}}}{\mathrm{kT}}\right)} \tag{3.4.4}$$

$$\therefore \frac{\mathrm{RGR}^{'}}{\mathrm{RGR}^{'}_{\mathrm{max}}} = \exp\left(\frac{\mathrm{Abs}\left(\mathrm{RF}_{\mathrm{max}} - \mathrm{RF}\right)}{\mathrm{kT}}\right) \tag{3.4.5}$$

Log both sides of equation 3.4.5; then we obtain the following:

$$\log\left(\frac{\mathrm{RGR}^{'}}{\mathrm{RGR}^{'}_{\mathrm{max}}}\right) = \frac{1}{\mathrm{kT}}\left(\mathrm{Abs}\left(\mathrm{RF}_{\mathrm{max}} - \mathrm{RF}\right)\right) \tag{3.4.6}$$

Manipulating the above equation results in a constant C, and where the frequency has been normalized:

$$\log\left(\frac{\mathrm{RGR}^{'}}{\mathrm{RGR}^{'}_{\mathrm{max}}}\right) = \mathrm{C}\left(\frac{\mathrm{Abs}\left(\mathrm{RF}_{\mathrm{max}} - \mathrm{RF}\right)}{\mathrm{RF}_{\mathrm{max}}}\right) \tag{3.4.7}$$

where

$$C = \left(\frac{RF_{max}}{kT}\right)(\text{units of J}).$$

These results, from equation 3.4.7 and section 3.4.2, are plotted in Figure 3.4.5, and one can see that the graphs are good approximations of straight lines (air R-squared=0.9043; CO_2 R-squared= 0.923; refer to Appendix E.2.4 for the fit). It is really interesting that the points from both lower and higher frequencies lie on the same curve. This strongly suggests that the theory is a good approximation to what is happening.

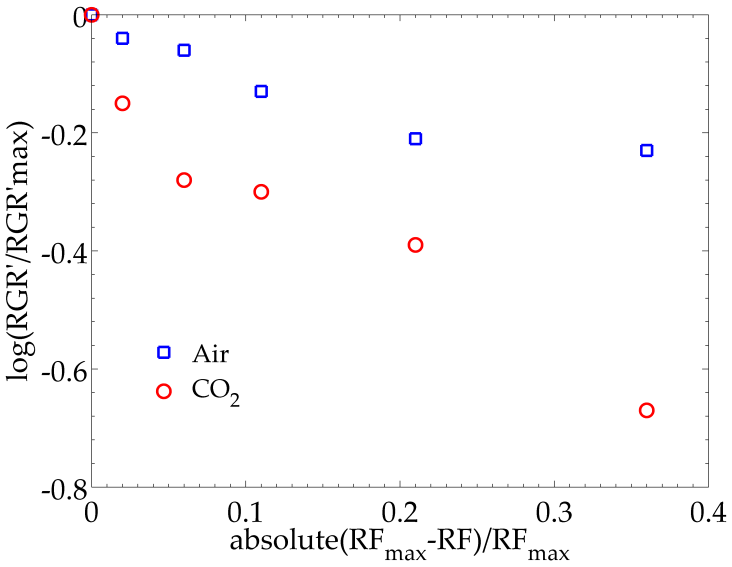


Figure 3.4.5: This graph shows how the relative growth rate changes with the difference of the frequency from the resonance frequency. Where the graphs are good approximations of straight lines, that is the air graph has an R-squared=0.9043, and the CO_2 graph has an R-squared= 0.923.

3.4.4 Photosynthesis process

3.4.4.1 Heat effects

It is easy to deduce that the photosynthesis process is very endothermic as it is just the reverse of the very exothermic complete combustion process. From the previous chapter we can say that the glucose photosynthesis formation process had a value of:

$$\triangle H_{process} = \triangle H_{reaction} = 2814\,kJ/mol$$

where we assume that the reaction proceeds as follows at 25ºC and 1 bar:

$$6CO_{2(g)} + 6H_2O_{(l)} + \text{light} \rightarrow C_6H_{12}O_{6(s)} + 6O_{2(g)}$$

Now if we take the maximum growth rate for the algae from the air experiment and the concentration of the algae in what is essentially water (from a heat capacity point of view), and the exothermic heat of the process above (assuming the product is glucose or something similar) we can show the adiabatic temperature rise in the water temperature is about 1/8 Kelvin. (Calculation shown in Appendix C.2). What this means is that in spite of the large exothermic overall reaction of the photosynthesis process, the temperature of the organism should be very close to the growing medium temperature. It would be interesting to devise a new experimental setup to try to measure and confirm this effect. Therefore to base future research on the insights gained from this chapter.

It is also interesting to speculate that the rate of growth of this species (and possibly others) could be limited by heat transfer; that is, if they tried to grow too fast their temperature could rise significantly above the temperature of the medium they are immersed in, thus perhaps damaging them.

3.5 Conclusions

A spectrophotometer turns out to be a very convenient device for studying the growth rates of algae over a range of different light wavelengths. Not only can one use it to measure the absorption spectrum of the species being studied (in this case *Desmodesmus* spp.), but one can then easily use the device to be the light source to enhance the growth rates at different wavelengths, and then finally use the device to measure the optical density of the material in the cuvettes over time.

It was found that even though the absorption spectrum had three major peaks (440nm, 492nm and 686nm) and the tests were conducted at six wavelengths (415, 432, 440, 492, 554, 686 nm), the only wavelength that showed enhanced growth rates was in the blue region (440nm). The same result was true whether the carbon dioxide needed for growth was supplied by bubbling air or pure carbon dioxide. The overall growth rates were, however, lower with the bubbled pure carbon dioxide. One could scale the experiment to a suitable size using 440 nm and a percentage of CO_2 acceptable for the algae species.

The growth rates at the different frequencies were then modelled using a Boltzman probability model assuming the probability of reaction with a certain wavelength was related to the difference of that frequency from that at the maximum growth rate (resonant frequency). This model seemed to correlate the results well suggesting that there is a particular bond with a particular bond energy that needs to be broken, and that the probability of breaking it decreases as the frequency of the incident light moves away from this resonant frequency.

References

Abiusi, F., Sampietro, G., Marturano, G., Biondi, N., Rodolfi, L., D'Ottavio, M., Tredici, M. R., 2014. Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-M33 grown with LEDs of different colors. Biotechnology and Bioengineering 111 (5), 956–964.

Amrei, H. D., Ranjbar, R., Rastegar, S., Nasernejad, B., Nejadebrahim, A., 2015. Using fluorescent material for enhancing microalgae growth rate in photobioreactors. Journal of Applied Phycology 27 (1), 67–74.

Aneja, K., 2007. Experiments in microbiology, plant pathology and biotechnology, 4th Edition. New Age International, New Delhi, India.

Aparicio, P. J., Roldán, J., Calero, F., 1976. Blue light photoreactivation of nitrate reductase from green algae and higher plants. Biochemical and Biophysical Research Communications 70 (4), 1071–1077.

Atta, M., Idris, A., Bukhari, A., Wahidin, S., 2013. Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*. Bioresource Technology 148 (0), 373–378.

Baba, M., Kikuta, F., Suzuki, I., Watanabe, M. M., Shiraiwa, Y., 2012. Wavelength specificity of growth, photosynthesis, and hydrocarbon production in the oil-producing green alga *Botryococcus braunii*. Bioresource Technology 109 (0), 266–270.

Bisio, G., Bisio, A., 1998. Some thermodynamic remarks on photosynthetic energy conversion. Energy Conversion and Management 39 (8), 741–748.

Blair, M. F., Kokabian, B., Gude, V. G., 2014. Light and growth medium effect on *Chlorella vulgaris* biomass production. Journal of Environmental Chemical Engineering 2 (1), 665–674.

Blinks, L., 1960. Action spectra of chromatic transients and the Emerson effect

in marine algae. Proceedings of the National Academy of Sciences of the United States of America 46 (3), 327–333.

Borodin, V., 2008. Effect of red and blue light on acclimation of *Chlamydomonas reinhardtii* to CO_2 -limiting conditions. Russian Journal of Plant Physiology 55 (4), 441–448.

Carvalho, A. P., Silva, S. O., Baptista, J. M., Malcata, F. X., 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. Applied Microbiology and Biotechnology 89 (5), 1275–1288.

Choi, H.-J., 2014. Effect of optical panel distance in a photobioreactor for nutrient removal and cultivation of microalgae. World Journal of Microbiology and Biotechnology 30 (7), 2015–2023.

Choi, Y.-K., Kumaran, R. S., Jeon, H. J., Song, H.-J., Yang, Y.-H., Lee, S. H., Song, K.-G., Kim, K. J., Singh, V., Kim, H. J., 2015. LED light stress induced biomass and fatty acid production in microalgal biosystem, *Acutodesmus obliquus*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 145 (0), 245–253.

Clayton, R. K., 1965. The biophysical problems of photosynthesis. Science 149 (3690), 1346–1354.

Cleuvers, M., Ratte, H. T., 2002. The importance of light intensity in algal tests with coloured substances. Water Research 36 (9), 2173–2178.

Cleuvers, M., Weyers, A., 2003. Algal growth inhibition test: does shading of coloured substances really matter? Water Research 37 (11), 2718–2722.

Das, P., Lei, W., Aziz, S. S., Obbard, J. P., 2011. Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. Bioresource Technology 102 (4), 3883–3887.

Douma, M., curator. 2008. Chlorophyll. In cause of color. [Online]. Available at: http://www.webexhibits.org/causesofcolor/7A.html, [accessed 31 May 2016].

Dring, M. J., 1989. Stimulation of light-saturated photosynthesis in *Laminaria* (phaeophyta) by blue light. Journal of Phycology 25 (2), 254–258.

Dunne, R. P., 1999. Spectrophotometric measurement of chlorophyll pigments: a comparison of conventional monochromators and a reverse optic diode array design. Marine Chemistry 66 (3), 245–251.

Eley, J., 1971. Effect of carbon dioxide concentration on pigmentation in the blue-green alga *Anacystis nidulans*. Plant and Cell Physiology 12 (2), 311–316.

Evers, E., 1991. A model for light-limited continuous cultures: Growth, shading, and maintenance. Biotechnology and Bioengineering 38 (3), 254–259.

Faust, M., Sager, J., Meeson, B., 1982. Response of *Prorocentrum Mariae-lebouriae (dinophyceae)* to light of different spectral qualities and irradiances: Growth and pigmentation. Journal of Phycology 18 (3), 349–356.

Fogg, G. E., Thake, B., 1987. Algal cultures and phytoplankton ecology, 3rd Edition. University of Wisconsin Press, Madison, Wisonsin, USA.

Gordon, J. M., Polle, J. E., 2007. Ultrahigh bioproductivity from algae. Applied Microbiology and Biotechnology 76 (5), 969–975.

Govindjee, E. R., Thomas, J. B., 1960. Inhibition of photosynthesis in certain algae by extreme red light. Biophysical Journal 1 (2), 91–97.

Haxo, F. T., Blinks, L., 1950. Photosynthetic action spectra of marine algae. The Journal of General Physiology 33 (4), 389–422.

Ho, S.-H., Chan, M.-C., Liu, C.-C., Chen, C.-Y., Lee, W.-L., Lee, D.-J., Chang, J.-S., 2014a. Enhancing lutein productivity of an indigenous microalga *Scenedesmus obliquus* FSP-3 using light-related strategies. Bioresource Technology 152 (0), 275–282.

Ho, S.-H., Chang, J.-S., Lai, Y.-Y., Chen, C.-N. N., 2014b. Achieving high lipid productivity of a thermotolerant microalga *Desmodesmus* sp. F2 by optimizing environmental factors and nutrient conditions. Bioresource Technology 156 (0), 108–116.

Ho, S.-H., Chen, C.-N. N., Lai, Y.-Y., Lu, W.-B., Chang, J.-S., 2014c. Exploring the high lipid production potential of a thermotolerant microalga using statistical optimization and semi-continuous cultivation. Bioresource Technology 163 (0), 128–135.

Ho, S.-H., Ye, X., Hasunuma, T., Chang, J.-S., Kondo, A., 2014d. Perspectives on engineering strategies for improving biofuel production from microalgae-A critical review. Biotechnology Advances 32 (8), 1448–1459.

Hsieh, C.-H., Wu, W.-T., 2009. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresource Technology 100 (17), 3921–3926.

Hultberg, M., Jönsson, H. L., Bergstrand, K.-J., Carlsson, A. S., 2014. Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*. Bioresource Technology 159 (0), 465–467.

Janssen, M., Tramper, J., Mur, L. R., Wijffels, R. H., 2003. Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. Biotechnology and Bioengineering 81 (2), 193–210.

Jeffrey, S., Vesk, M., 1977. Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis Turris*. Journal of Phycology 13 (3), 271–279.

Jeon, Y., Cho, C., Yun, Y., 2005. Measurement of microalgal photosynthetic activity depending on light intensity and quality. Biochemical Engineering Journal 27 (2), 127–131.

Kang, Z., Kim, B., Ramanan, R., Choi, J., Yang, J., Oh, H., Kim, H., 2014. A cost analysis of microalgal biomass and biodiesel production in open raceways treating municipal wastewater and under optimum light wavelength. Journal of Microbiology and Biotechnology 25 (1), 109–118.

Kativu, E., 2011. Carbon dioxide absorption using fresh water algae and identifying potential uses of algal biomass. MSc, Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, South Africa.

Kativu, E., Hildebrandt, D., Matambo, T., Glasser, D., 2012. Effects of CO_2 on South African fresh water microalgae growth. Environmental Progress and Sustainable Energy 31 (1), 24–28.

Kim, D. G., Lee, C., Park, S.-M., Choi, Y.-E., 2014. Manipulation of light wavelength at appropriate growth stage to enhance biomass productivity and fatty acid methyl ester yield using *Chlorella vulgaris*. Bioresource Technology 159 (0), 240–248.

Kim, T.-H., Lee, Y., Han, S.-H., Hwang, S.-J., 2013. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment. Bioresource Technology 130 (0), 75–80.

Kommareddy, A., Anderson, G., 2003. Study of light as a parameter in the growth of algae in a photo-bio reactor (PBR). In: ASAE Annual International Meeting Presentation. Vol. 34057. Las Vegas, USA.

Kumar, M. S., Hwang, J.-H., Abou-Shanab, R. A., Kabra, A. N., Ji, M.-K., Jeon, B.-H., 2014. Influence of CO_2 and light spectra on the enhancement of microalgal growth and lipid content. Journal of Renewable and Sustainable Energy 6 (6), 1–6.

Kurano, N., Ikemoto, H., Miyashita, H., Hasegawa, T., Hata, H., Miyachi, S., 1995. Fixation and utilization of carbon dioxide by microalgal photosynthesis. Energy Conversion and Management 36 (6), 689–692.

Kuwahara, S. S., Cuello, J. L., Myhre, G., Pau, S., 2011. Growth of the green

algae *Chlamydomonas reinhardtii* under red and blue lasers. Optics and Lasers in Engineering 49 (3), 434–438.

Lee, C., 1999. Calculation of light penetration depth in photobioreactors. Biotechnology and Bioprocess Engineering 4 (1), 78–81.

Lee, E., Heng, R.-L., Pilon, L., 2013. Spectral optical properties of selected photosynthetic microalgae producing biofuels. Journal of Quantitative Spectroscopy and Radiative Transfer 114 (0), 122–135.

Leite, G. B., Abdelaziz, A. E., Hallenbeck, P. C., 2013. Algal biofuels: challenges and opportunities. Bioresource Technology 145 (0), 134–141.

Markou, G., 2014. Effect of various colors of light-emitting diodes (LEDs) on the biomass composition of *Arthrospira platensis* cultivated in semi-continuous mode. Applied Biochemistry and Biotechnology 172 (5), 2758–2768.

Matthijs, H., Balke, H., Van Hes, U., Kroon, B., Mur, L., Binot, R., 1996. Application of light-emitting diodes in bioreactors: flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*). Biotechnology and Bioengineering 50 (1), 98–107.

McLeod, G., 1961. Action spectra of light-saturated photosynthesis. Plant Physiology 36 (1), 114–117.

Michael, C., del Ninno, M., Gross, M., Wen, Z., 2015. Use of wavelength-selective optical light filters for enhanced microalgal growth in different algal cultivation systems. Bioresource Technology 179 (0), 473–482.

Mohsenpour, S. F., Richards, B., Willoughby, N., 2012. Spectral conversion of light for enhanced microalgae growth rates and photosynthetic pigment production. Bioresource Technology 125 (0), 75–81.

Mohsenpour, S. F., Willoughby, N., 2013. Luminescent photobioreactor design for improved algal growth and photosynthetic pigment production through spectral conversion of light. Bioresource Technology 142 (0), 147–153.

Monod, J., 1949. The growth of bacterial cultures. Annual Reviews in Microbiology 3 (1), 371–394.

Ogasawara, N., Miyachi, S., 1970. Regulation of CO_2-fixation in *Chlorella* by light of varied wavelengths and intensities. Plant and Cell Physiology 11 (1), 1–14.

Ooms, M. D., Jeyaram, Y., Sinton, D., 2015. Wavelength-selective plasmonics for enhanced cultivation of microalgae. Applied Physics Letters 106 (6), 063902.

Park, J., Seo, J., Kwon, E. E., 2012. Microalgae production using wastewater:

effect of light-emitting diode wavelength on microalgal growth. Environmental Engineering Science 29 (11), 995–1001.

Park, K.-H., Lee, C.-G., 2000. Optimization of algal photobioreactors using flashing lights. Biotechnology and Bioprocess Engineering 5 (3), 186–190.

Pignolet, O., Jubeau, S., Vaca-Garcia, C., Michaud, P., 2013. Highly valuable microalgae: biochemical and topological aspects. Journal of Industrial Microbiology and Biotechnology 40 (8), 781–796.

Posten, C., 2009. Design principles of photo-bioreactors for cultivation of microalgae. Engineering in Life Sciences 9 (3), 165–177.

Prokop, A., Quinn, M., Fekri, M., Murad, M., Ahmed, S., 1984. Spectral shifting by dyes to enhance algae growth. Biotechnology and Bioengineering 26 (11), 1313–1322.

Pulich Jr, W. M., Van Baalen, C., 1974. Growth requirements of blue-green algae under blue light conditions. Archives of Microbiology 97 (1), 303–312.

Pulz, O., 2001. Photobioreactors: production systems for phototrophic microorganisms. Applied Microbiology and Biotechnology 57 (3), 287–293.

Rochet, M., Legendre, L., Demers, S., 1986. Photosynthetic and pigment responses of sea-ice microalgae to changes in light intensity and quality. Journal of Experimental Marine Biology and Ecology 101 (3), 211–226.

Ruyters, G., 1984. Effects of blue light on enzymes. In: Senger, H. (Ed.), Blue Light Effects in Biological Systems. Proceedings in Life Sciences. Springer Berlin Heidelberg, pp. 283–301.

Schulze, P. S., Barreira, L. A., Pereira, H. G., Perales, J. A., Varela, J. C., 2014. Light emitting diodes (LEDs) applied to microalgal production. Trends in Biotechnology 32 (8), 422–430.

Seo, Y. H., Cho, C., Lee, J.-Y., Han, J.-I., 2014. Enhancement of growth and lipid production from microalgae using fluorescent paint under the solar radiation. Bioresource Technology 173 (0), 193–197.

Seo, Y. H., Lee, Y., Jeon, D. Y., Han, J.-I., 2015. Enhancing the light utilization efficiency of microalgae using organic dyes. Bioresource Technology 181 (0), 355–359.

Simionato, D., Basso, S., Giacometti, G. M., Morosinotto, T., 2013. Optimization of light use efficiency for biofuel production in algae. Biophysical Chemistry 182 (0), 71–78.

Soletto, D., Binaghi, L., Ferrari, L., Lodi, A., Carvalho, J., Zilli, M., Converti, A., 2008. Effects of carbon dioxide feeding rate and light intensity

on the fed-batch pulse-feeding cultivation of *Spirulina platensis* in helical photobioreactor. Biochemical Engineering Journal 39 (2), 369–375.

Sorokin, C., Krauss, R. W., 1958. The effects of light intensity on the growth rates of green algae. Plant Physiology 33 (2), 109–113.

Swinehart, D., 1962. The beer-lambert law. Journal of Chemical Education 39 (7), 333–335.

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M., Idris, A., 2014a. Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. Bioresource Technology 162 (0), 38–44.

Teo, C. L., Idris, A., Wahidin, S., Lai, L. W., 2014b. Effect of different light wavelength on the growth of marine microalgae. Jurnal Teknologi 67 (3), 97–100.

Teo, C. L., Idris, A., Zain, N. A. M., Taisir, M., 2014c. Synergistic effect of optimizing light-emitting diode illumination quality and intensity to manipulate composition of fatty acid methyl esters from *Nannochloropsis* sp. Bioresource Technology 173 (0), 284–290.

Ugwu, C., Aoyagi, H., Uchiyama, H., 2007. Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. Photosynthetica 45 (2), 309–311.

Vadiveloo, A., Moheimani, N. R., Cosgrove, J. J., Bahri, P. A., Parlevliet, D., 2015. Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (*Eustigmatophyceae*). Algal Research 8 (0), 121–127.

Wallen, D., Geen, G., 1971. Light quality in relation to growth, photosynthetic rates and carbon metabolism in two species of marine plankton algae. Marine Biology 10 (1), 34–43.

Wang, C.-Y., Fu, C.-C., Liu, Y.-C., 2007. Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochemical Engineering Journal 37 (1), 21–25.

Wang, S.-K., Stiles, A. R., Guo, C., Liu, C.-Z., 2014b. Microalgae cultivation in photobioreactors: An overview of light characteristics. Engineering in Life Sciences 14 (6), 550–559.

Widom, B., 2002. Statistical mechanics: a concise introduction for chemists. Cambridge University Press.

Yago, T., Arakawa, H., Morinaga, T., Yoshie-Stark, Y., Yoshioka, M., 2011. Effect of wavelength of intermittent light on the growth and fatty acid pro-

file of the haptophyte *Isochrysis galbana*. In: Ceccaldi, H.-J., Dekeyser, I., Girault, M., Stora, G. (Eds.), Global Change: Mankind-Marine Environment Interactions: Proceedings of the 13th French-Japanese Oceanography Symposium. Springer Netherlands, Dordrecht, pp. 43–45.

Yan, C., Zhang, L., Luo, X., Zheng, Z., 2013a. Effects of various LED light wavelengths and intensities on the performance of purifying synthetic domestic sewage by microalgae at different influent C/N ratios. Ecological Engineering 51 (0), 24–32.

Yan, C., Zhao, Y., Zheng, Z., Luo, X., 2013b. Effects of various LED light wavelengths and light intensity supply strategies on synthetic high-strength wastewater purification by *Chlorella vulgaris*. Biodegradation 24 (5), 721–732.

Yang, Y., Weathers, P., 2015. Red light and carbon dioxide differentially affect growth, lipid production, and quality in the microalga, *Ettlia oleoabundans*. Applied Microbiology and Biotechnology 99 (1), 489–499.

Yentsch, C., 1974. Some aspects of the environmental physiology of marine phytoplankton: a second look. Oceanography and Marine Biology: An Annual Review 12, 41–75.

Zaccheroni, N., 2012. Spectrophotometry, Measurements in Solution. Springer Netherlands, Dordrecht, pp. 39–66.
URL http://dx.doi.org/10.1007/978-94-007-2042-8_3

Zhang, S., Liu, H., Fan, J., Yu, H., 2014b. Cultivation of *Scenedesmus dimorphus* with domestic secondary effluent and energy evaluation for biodiesel production. Environmental Technology 36 (7), 929–936.

Zhao, Y., Wang, J., Zhang, H., Yan, C., Zhang, Y., 2013. Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process. Bioresource Technology 136 (0), 461–468.

Chapter 4

The application of batch partial emptying and filling to improve the production rate of *Desmodesmus spp.* algae on a laboratory scale

- This chapter has been published in a peer-reviewed journal. Therefore, it is reprinted (and adapted) with permission from Low et al. (2015), titled "Batch partial emptying and filling to improve the production rate of algae". Copyright (2016) American Chemical Society. The permission/license can be found in Appendix B.
- A portion of this chapter was orally presented at the 2014 AIChE Annual Meeting in Atlanta, GA.
- This chapter presents the author experimentally testing a theoretical model to determine if the production rate of algae (the same species used in Chapter 3) can be improved using the same set of equipment by partial emptying and filling (PEF), a method used to retain a fraction of the algae suspension, and filling the remainder of the batch volume with the medium. By implementing the current theory without any significant extra cost we obtained an improved measured production rate and in the range predicted by theory.
- The contribution of the co-authors (Glasser, D.[‡]; Hildebrandt, D.[‡]; Ming,

D.[†]; Matambo, T.[‡]) towards the work is primarily that of supervision or collaboration, and the work and write-up was conducted by the author of this thesis. The author would like to acknowledge Ms F. Gunda and Mr A. Mokiti for collecting some of the data (during their vacation work) that is presented in this chapter.

Abstract

The objective of the research reported in this chapter was to determine how well predictions on the growth of algae, based on a theoretical model without needing to know the physiological conditions, would work when tested experimentally. These results predict that partial emptying (retaining a fraction of the algae suspension) and filling with fresh medium would improve the production rate of for example *Desmodesmus* spp. (subspicatus), a species of algae used as a source of biofuel. It was found that the method based on this graphical model is suitable for biological systems when the rate of production is an issue. The application of partial emptying and filling improved production by a factor of 1.28 and 1.26 (at product concentrations 1000 mg/L and 600 mg/L respectively) when retaining 40% of the algae suspension. This method may be particularly useful when large amounts of biomass are required, and to recommend an appropriate retained fraction for any desired exit concentration.
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4.1 Introduction

Batch cultures provide the simplest form of bioreactor operation. They can be used in a variety of different applications, for instance to make microbial products (Konopka et al., 1996) that can be used to mitigate carbon dioxide (Sayre, 2010) or to provide a source of biofuel (Wang et al., 2008; Gouveia and Oliveira, 2009; Brennan and Owende, 2010; Zeng et al., 2011; Cheah et al., 2015; Wang et al., 2014a). Palanisami and his co-workers (2015) reported that the most common microalgae found in soil and freshwater are of the genera *Desmodesmus* and *Scenedesmus*. Their investigation of the species *Desmodesmus communis* revealed that these algae were not only able to tolerate the toxic chemicals in flue gas, but grew well. *Desmodesmus* spp., on the other

hand, is a good candidate for the mitigation of carbon dioxide (CO_2) (Kativu et al., 2012; Solovchenko et al., 2014). Microalgae can also be used to remove nitrates from water (Urrutia et al., 1995) or for the bioremediation of wastewater (Woertz et al., 2009; Samorì et al., 2013; Gressler et al., 2014; Ji et al., 2014).

The range of benefits associated with cultivating algae extends even wider. They can provide the material for an array of byproducts such as protein, carbohydrates and lipids (depending on the species and growing conditions), and therefore are particularly well suited for use in microalgae biotechnology. The various applications of this technology result in products that range from biofuels and food (for both human and animals), to cosmetics and pharmaceutical goods (De la Noüe and de Pauw, 1988; Spolaore et al., 2006; Woertz et al., 2009; Brennan and Owende, 2010; Mata et al., 2010; Menetrez, 2012; Koller et al., 2014; Lin et al., 2015). All of the above should provide a compelling reason for the use of water to cultivate algae (Hu et al., 2013; Ho et al., 2014b), even when critics say that water, being a scarce commodity in South Africa, should be used to produce crops instead.

Although commercial production is in its early phase, De la Noüe and de Pauw (1988) pointed out that it was then unlikely that such enterprises could supply sufficient microalgae to meet the country's needs for fuel and electricity. Also, before any commercialization of biofuels from microalgae can begin, there are many technical issues that need to be to addressed (Abdelaziz et al., 2013), which is why continuous research is needed to obtain a sufficiently high production of microalgae to offer a feasible alternative source of energy (Woertz et al., 2009). In addition, the cost of the processes for cultivating microalgae on a large scale and extracting the lipids for biofuels are not at this point economically viable (Henrard et al., 2011; Ji et al., 2015). It has been suggested by Koller et al. (2014) that a range of experts should be working on the optimization of both the processes and the productivity of algal biomass (see also Park and Craggs 2010 and Elliott et al. 2012). Wijffels and Barbosa (2010) estimated that it will take 10 to 15 years to reach the point at which microalgae to biofuels will be commercialized in an economical and sustainable manner.

Consequently, on their own, individual alternative sources of energy and fuel will not be able to replace the demand that is now being met by fossil fuels

(Hoffert et al., 2002), which is why Williams et al. (2012) recommended that a mixed portfolio of all the technologies under development for sustainable means of producing energy and fuel should be pursued. However, this is not to say that the production of microalgae is unimportant. Rather it is to suggest that research into methodologies that could boost the production of algae as biofuel could be great of significance. All of the above provides motivation for the project described in this chapter.

The following chapter is organized as follows: we first address what repeated batch operation is. In Section 2.1 an overview of the graphical method is given that was used to predict improved production rate of the algae species. This is followed by the specific objectives and experimental procedure. In Section 3, the results are presented and discussed, and where appropriate the important observations are summarized throughout the text. The conclusions are provided in Section 4.

A repeated-batch operation is the term used in cases where most of the total volume of the batch solution is drained from the previous batch culture (from this point on designated as PC). The drained-off portion forms the product. The remainder, which acts as an inoculum for the next cycle, is topped up to the original level with fresh medium to start the next batch cycle. This technique is suited to the propagation of any microbial species, and has been found to be more effective than standard batch production where the batch is completely emptied (Bibal et al., 1991; Reinehr and Costa, 2006; Choi et al., 2009).

The research so far conducted into repeated-batch approaches/ modes of operation has investigated a number of different aspects:

- partial biomass recycle (De la Noüe and Ni Eidhin, 1988);
- cell-recycle (Bibal et al., 1991; Zeng et al., 1991);
- repeated-batch culture (Naritomi et al., 2002; Radmann et al., 2007; Zhao et al., 2010a; Pattanamanee et al., 2012; Abdel-Rahman et al., 2013b; Sánchez et al., 2013); and
- semi-continuous cultivation (Reichert et al., 2006; Kaewpintong et al., 2007; Henrard et al., 2011; Hu et al., 2013; Schlepütz and Büchs, 2013; Ho et al., 2014c).

Ways can be found to reduce the costs of repeated-batch operation. These savings which increase the production rate include fewer expenses incurred during downtime for cleaning of the bioreactor (Naritomi et al., 2002; Pattanamanee et al., 2012), and reductions in both the length of the batch cycle and the lag phase in the growth profile (Kosińska and Miśkiewicz, 2009; Schlepütz and Büchs, 2013).

In their reports on the production of bioethanol (Choi et al., 2009; Mussatto et al., 2009) and lactic acid (Zhao et al., 2010a), the researchers recycled a chosen percentage of cells in their repeated-batch experiments. During cell-recycle, some of these scientists recycled 100% of their cells in either a continuous flow reactor (Konopka et al., 1996) or in a repeated-batch fermentation mode, for example in the production of lactic acid (Zhao et al., 2010b; Abdel-Rahman et al., 2013a,b). However, even though all recycled cells provided advantages such as an increased substrate conversion rate (Konopka et al., 1996), the biomass growth rate was found to decline when a certain number of repeated-batch cycles had taken place (Mussatto et al., 2009; Abdel-Rahman et al., 2013b; Koti et al., 2013).

More specifically, the researchers (listed below with the processes) who had proved most successful in boosting algae production used the following processes:

- semi-continuous (Fábregas et al., 1996a; Hu et al., 2013);
- repeated-fed-batch (Hata et al., 2001; Matsudo et al., 2009; Xie et al., 2014);
- repeated-batch (Sánchez et al., 2013; Radmann et al., 2007); and
- biomass recycling (Weissman and Benemann, 1979; Samson and Leduy, 1985; Radway et al., 1998; Park et al., 2011b, 2013a,b).

High productivity was achieved when recycling a portion of the algae in the operations mentioned above, to a level far surpassing that of standard batch (discontinuous) operations (De la Noüe and Ni Eidhin, 1988; Reichert et al., 2006; Kaewpintong et al., 2007).

Table 4.1.1: Literature associated with PEF experiments.

Author	Product	Method	Conclusion	Mode
Fábregas et al. (1995a)	*Dunaliella tertiolecta*	Factorial design. Compared 10–50% daily renewal rates* of PC at stationery phase over 21 days.	Best daily renewal rate: 30%. ∴Φ=0.7	Semi-continuous cultivation
Naritomi et al. (2002)	Bacterial Cellulose	Broth exchange ratio = = 0.7;0.8;0.9;0.95;0.99 = ratio of volume drawn to the total volume.	Best = 0.9. Productivity 2.5 times more than their standard batch. Did four cycles. ∴Φ=0.1	Repeated-batch fermentation
Reinehr and Costa (2006)	*Spirulina Platensis*	Factorial design. Compared renewal rates* (0.25, 0.50) at specific concentrations (0.50, 0.75 g/L) over 90-days.	Best renewal rate: 50% at low biomass concentrations. Two fold increase over batch. Productivity 42.3 mg/L · d. ∴Φ=0.5	Repeated-batch cultivation
Radmann et al. (2007)	*Spirulina Platensis*	Raceway pond. Compared renewal rates (20, 40, 60 %) at blend concentrations** (0.4, 0.6, 0.8 g/L) with different medium dilutions.	The method was a feasible optimisation for the species. Renewal rate: 40–60% at 0.4 g/L and 20 % (v/v) medium. ∴Φ=0.4-0.6	Repeated-batch cultivation
Choi et al. (2009)	Bioethanol using cassava mash	Compared recycle ratios: 5, 10 & 50 %. Implemented a total of ten cycles.	Best recycle: 5%. Productivity in cells were ten fold than that of batch. ∴Φ=0.95	Repeated-batch fermentation
Matsudo et al. (2009)	*Spirulina*	Compared ratios of renewal volume to total volume (R): 0.2, 0.5, 0.8, 0.95 when the exponential phase was reached.	Best R: 0.8. Productivity: 219 mg/L · d. Three cycles. ∴Φ=0.2	Repeated-fed-batch cultivation
Zhao et al. (2010b)	*D-Lactic acid*	Used nine runs and recycled all cells at the end of each cycle of 48 h.	Best production of fewer batches. ∴Φ=1	Repeated-batch fermentation
Henrard et al. (2011)	*Cyanobium spp.*	Responses of 3 desired concentrations and renewal rates* (30, 40 or 50%) of PC.	Best concentration: 1 g/L. Renewal Rate: 30%, Productivity 71 mg/L · d. Did ten cycles. ∴Φ=0.7	Semi-continuous cultivation
Pattanamanee et al. (2012)	Hydrogen	At specified times, different volumes removed and replaced: 50, 70 & 90%.	Best replacement and renewal: 90% every 84 h. Did four cycles. ∴Φ=0.1	Repeated-batch fermentation
Ho et al. (2014c)	*Desmodesmus* spp. F2	At 4 g/L replaced PC with percentages: 10, 30, 50, 90% with fresh medium. Implemented a total of six cycles.	Best: 90% replacement for biomass and lipid productivity. ∴Φ=0.1	Semi-continuous cultivation

*Renewal rate = inverse of retained fraction. **Blend concentration= desired exit concentration

Table 4.1.1 summarizes the approaches used by researchers in the field to select different recycle ratios. Yet another team of scientists, Fábregas et al. (1995b), focused on choosing a specific recycle value for different medium concentrations while Fábregas et al. (1995a, 1996a) used factorial design to compare different daily renewal rates. Furthermore, once a desired cell concentration was reached, other scientists chose to recycle a random percentage value (Kaewpintong et al., 2007; Xie et al., 2014), or a range of percentages (Radway et al., 1994; Fábregas et al., 1996b). Therefore it appears that historically the recycle ratio for repeated-batch operation has been selected by means of reference, statistical design or guesswork.

Another method proposed to improve the productivity of a species in the batch (discontinuous) domain has been proposed by Ming et al. (2012), who showed in theory that when one plots a growth profile, determined experimentally for a particular species, the production rate can in some cases be improved for that batch (see the graphic in Figure 4.1.1 and in the experimental procedure, Figure 4.2.2). Using this result, predictions of the amount of PC to recycle and the production rate can be determined at desired exit concentrations. First, however, it is necessary to determine graphically whether the growth profile is convex or concave in concentration/time space.

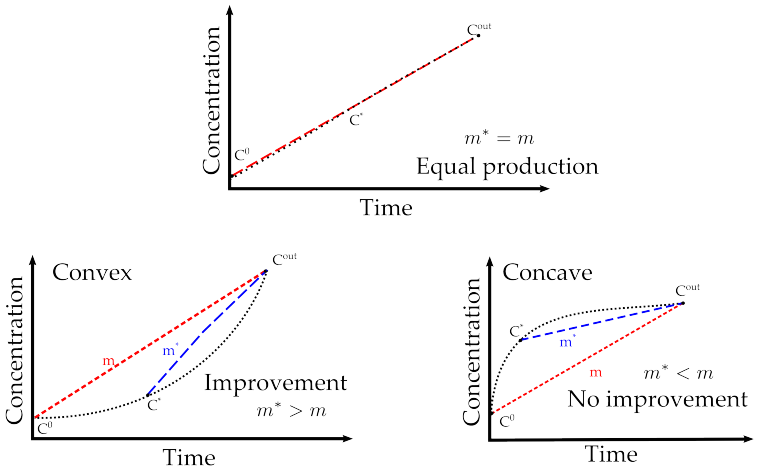


Figure 4.1.1: This figure is adapted from Ming et al. (2012). The shape of the batch profile (black dotted line) may be used to graphically identify opportunities for improving production rate, by comparing the gradients (red dashed line) in concentration/time space. The shape (black dotted line) is identified by being either a straight line, convex or concave.

4.2 Materials and Methods

4.2.1 Overview of the graphical method

The production rate for a volume of batch is defined by the change in concentration of a hypothetical component A (C_A) from final to initial stage, divided by the total time taken for a complete cycle of batch:

$$P = \frac{V_{tot}\left(C_A^{out} - C_A^0\right)}{t\left(C_A^{out}\right) - t\left(C_A^0\right)} \tag{4.2.1}$$

In the graphic representing concentration/time space (as shown in Figure 4.1.1), if a line between the initial (C_A^0) and final (C_A^{out}) concentration is drawn, the gradient of that straight line, multiplied by the volume of batch, represents the production rate for a standard batch.

Although there is a strong similarity between the definition of a repeated-batch operation and the suggestion put forward by Ming et al. (2012), in the latter case (and not in the former) it involves predicting an improved production rate and a retained fraction $\left(\phi = \frac{V_{retained}}{V_{total}}\right)$ of the PC. The researchers achieved this by defining partial emptying and filling (PEF), where the remainder of the PC is removed but replaced with fresh medium containing an initial inoculum. The PEF therefore changes the initial conditions of the next batch, resulting in an intermediate concentration (C_A^*). To predict improvements, one can use the same concentration/time space to plot an intermediate gradient, m^*, which is the slope of the line between C_A^* and a fixed C_A^{out}. This produces a PEF production rate, P^*:

$$P^* = \frac{(1-\phi)\,V_{total}\left(C_A^{out} - C_A^0\right)}{t\left(C_A^{out}\right) - t\left(C^*\right)} \tag{4.2.2}$$

P^* will be need to be greater than P to show whether the production rate can be improved. Since P^* is proportional to m^*this implies that if m^* is greater than m, then there is a convex shape in the concentration/time space profile, which suggests a potential for improving the standard batch production rate by retaining a certain fraction of the PC via PEF (Ming et al., 2012). On the other hand, concavities suggest that there are no opportunities for improving production. Therefore, via a simple graphical approach, the shape of the growth profile determines the conditions under which PEF is beneficial. It follows that the best production rate, related to a specific recommended Φ,

can be predicted at any chosen exit concentration. This is done by comparing the effects on the growth profile of the intermediate concentration (intermediate gradient) on production rate, between each experimental point via spline interpolation. An Φ of zero would result in a standard batch (discontinuous) production rate.

As mentioned previously, many authors explore different recycling ratios or percentages. However, thus far no actual experiments have been carried out applying the concepts suggested by Ming et al. (2012). Naritomi et al. (2002) utilized a similar model by using a broth exchange ratio in order to improve the production of bacterial cellulose. But Naritomi and his co-workers (2002) investigated values experimentally from 0.7 to 0.99 only, and offered no prediction of the production values or of the range of expected performance.

In this study, laboratory-scale work carried out over a 120-day period entailed obtaining a growth curve experimentally and then comparing the predictions with the results of the PEF experiments. The researcher's specific objectives were to:

1. apply the concepts formulated by Ming et al. (2012) directly to experimentally-measured data for the improvement of the production rate of algae. *Desmodesmus* spp. was chosen as an example;
2. optimize the retained fraction (Φ); and
3. verify that the recommended PEF retained fraction (Φ) was suitable for the algae growth data under the experimental conditions used, in order to check the improvements.

4.2.2 Experimental procedure: preparation

4.2.2.1 Microalgae species and Beijerinck medium

The researcher used Beijerinck medium (recipe is from Kativu, 2011 and is shown in Chapter 3) to cultivate *Desmodesmus* spp. This medium was made from stock solutions and micro-nutrients, which were decanted into 1 litre Schott bottles. In order to save time, the researcher (the present writer) premixed Beijerinck medium and stored in bottles in the refrigerator to use as a backup for the experiments. There were precipitates in the micronutrient

solution (after it had been autoclaved), however, when mixing the 1 mL micronutrient solution into the final medium mixture, it was ensured that it contained traces of the precipitate by shaking the bottle well in order to ensure a homogeneous solution. There could have been soluble precipitates in the stock solutions, however, it was ensured that the stock solutions were also well shaken before making the medium.

4.2.2.2 Batch growth profile

The author, with Kativu (2011), had collected *Desmodesmus* spp. from the Zoo Lake in Johannesburg, and cultivated it in the laboratory, following the procedure explained by Kativu (2011) dissertation. The latter had retained a backup supply of the mother algae suspension stored at 4°C, which the present researcher used in experiments.

In previous work by Kativu (2011) it was determined that growing the algae by bubbling 5 % CO_2 at 50 mL/min for 24 hours yielded the highest biomass of algae (1001.31 mg/L after 12 days). In order to test whether partial emptying and filling improves *Desmodesmus* spp. production, a worst case scenario condition using 100 % CO_2 bubbled at 50 mL/min for 1 hour was investigated. In previous work by Kativu (2011) this condition produced 279.46 mg/L after 12 days. The following conditions were used in this work: the algae cultures were grown in the laboratory under continuous light (consisting of a combination of Gro-Lux and normal fluorescent lamps, 4000–6000 lux), and bubbled with 100 % CO_2 (IG Cylinder 31.3kg) for one hour each day at 50 mL/min. Because of the uneven light intensity across the setup (see Figure 4.2.1), the flasks were rotated once a day, so that each flask would be exposed to similar amounts of light. The flasks were placed on magnetic stirrers (Heidolph MR Hei-Tec, #505-30081-00) set at 250 rpm.

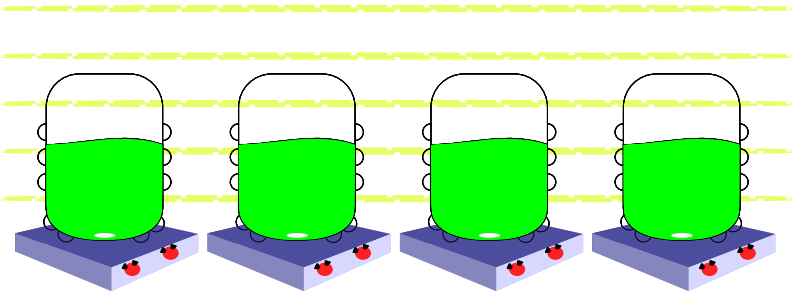


Figure 4.2.1: Example of the lab set-up to illustrate the light distribution.

To start a batch, the researcher placed 1 litre of Beijerinck medium in an autoclaved (using a Hirayama HiClave; model HA–300MD) flat-bottomed flask, and inoculated it with 5 mL of *Desmodesmus spp.* from the mother algae suspension (which had an absorbance of 2.294 Abs at 740 nm and a pH of 8.828). Since *Desmodesmus spp. is unicellular in nature,* the following assumption was made: since the algal cells were suspended in a liquid phase of a particular composition, and therefore a homogeneous distribution of algal cells throughout the volume of the suspension was assumed in this experiment. As a result, it was assumed that the homogeneous suspension will be just be a given with algae. This assumption was the same for the experiment carried out in Chapter 3. *This meant that,* using a Prim spectrophotometer, absorbance and cell dry mass could be correlated with one another to monitor the growth rate, (measured in dry weight in mg/L vs time in days) for a period of 57 days (shown in Appendix E.1).

The method for calibration of dry mass with absorbance was as follows: the researcher weighed a 0.45 μm nitrocellulose filter paper (Cat. # 11406–14–ACN, Sartorius Stedim Biotech, Germany) on the scale, transferred it to the glass filtration unit connected to a vacuum pump system using forceps, and then pipetted 15 mL of the algae suspension onto the paper. The filtrate remaining on the filter paper was then left to dry overnight. The following day the scientist weighed the air-dried filter paper with the filtrate. The difference between the mass of the filter with algae and without was then recorded. As mentioned above, the mass of the air-dried algae (from this point forward, referred to mg dry algae) corresponds with the absorbance value, and therefore can be taken as a direct measure of algal growth, when compared against the results taken the previous day. The temperature of this experiment was not controlled but was monitored, and were between 25º and 30ºC. These measurements are shown in Figure 4.2.2, in the form of a batch growth rate profile, with error bars (sometimes referred to as noise) to indicate duplicate runs. The data for the work done in this chapter can be found in Appendix E.3.

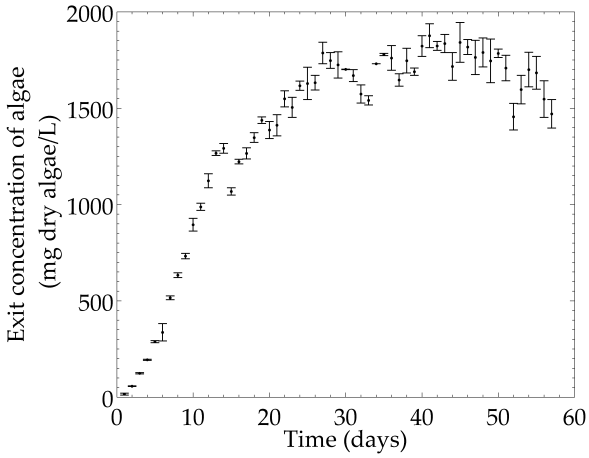


Figure 4.2.2: Average batch profile of the algae over 57 days (in order to identify any convexities), after bubbling with 50 mL/min of 100 % CO_2 for 1 hour each day, 7 days a week.

4.2.2.3 Sample analysis

The absorbance, pH and temperature were recorded every morning, seven days a week. In preparation for the absorbance analysis, a blank reading of the medium solution was made to provide a standard against which individual samples could be measured, in order to establish the difference. The absorbance measurements were taken from a 3 mL sample of the algae suspension. Once the reading was taken, the sample was returned to the flask containing the algae suspension. In this experiment, the pH and temperature were monitored but not controlled. The pH meter was calibrated daily, by checking with 4.0, 7.0 and 9.21 buffer solutions (Cat. #9463.99, #9464.99 and #9465.99 respectively, Crison, Germany), and the temperature of the algae suspensions in the flasks was measured with a thermometer.

4.2.3 Experimental procedure for partial emptying and filling (PEF) cultivation

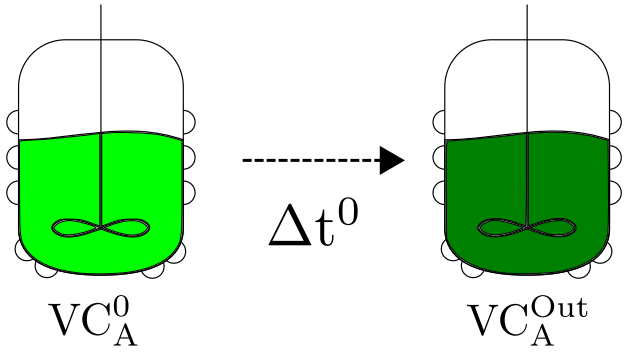


Figure 4.2.3: Schematic diagram of conventional batch operation which can be repeated n times.

A zero retained fraction was used as the standard batch control, as seen in Figure 4.2.3 above. The diagram that follows, Figure 4.2.4, shows how the standard batch was converted to PEF batches. Since the model derived from Ming et al. (2012) is based on an initial inoculation for all cycles, 2.5 mg/L of algal inoculum was placed in the flasks at the start of each standard and PEF batch. The algae flask was left to bubble with CO_2 if it had not reached the target algae concentration range. On the other hand, if the algae flask had achieved this range, the experimental procedure for PEF could be carried out. This entailed the retention of the appropriate ϕ of the PC and the addition of $1 - \phi$ of fresh medium with the initial algae inoculum (Table 4.2.1) at time Δt^0 so the final concentration was in the target range. After this, a fixed time Δt^* was chosen for that Φ so that the final concentration remains in the target range. Thereafter the researcher bubbled CO_2 through the flasks, and repeated the process n times (representing the number of cycles). However, n was a dissimilar number for the standard and PEF batches, depending on which Φ had been chosen for that flask. A series of experiments were conducted at a difference of Φ=0.1 starting from 0.3 and ending with 0.8.

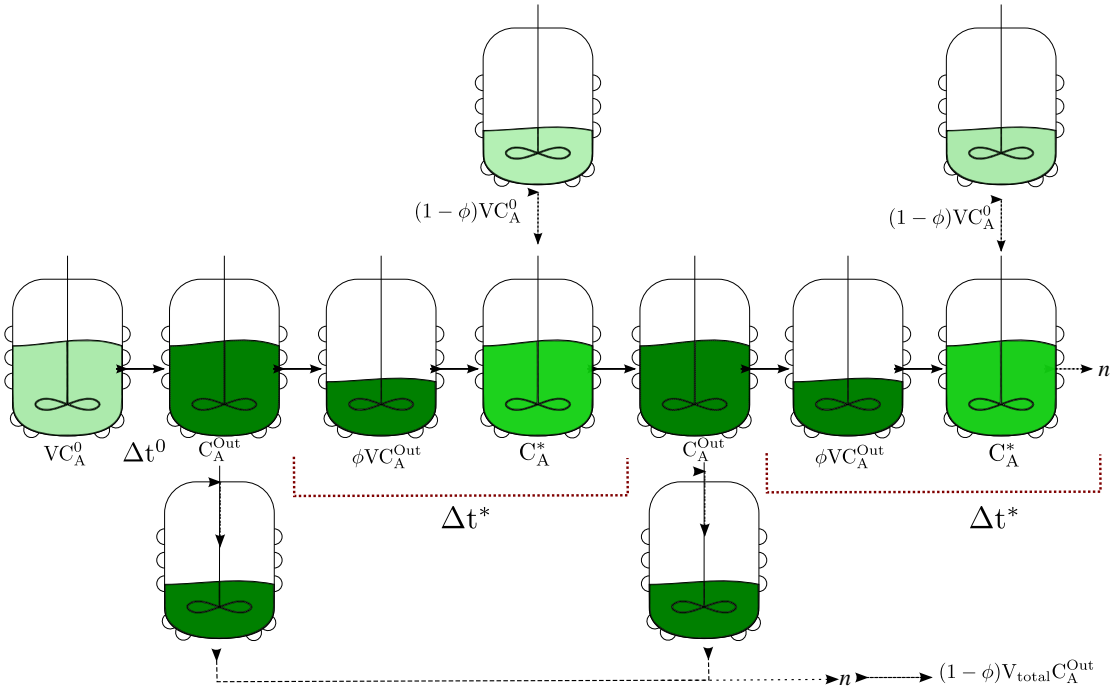


Figure 4.2.4: . Schematic diagram of the experimental partial emptying and filling n times. Figure adapted from the structure devised by Ming et al. (2012).

In both Figures 4.2.3 and 4.2.4, the symbols denote:

V = the working volume;

V_{total} = the final volume collected after n cycles;

C_A^0 = the initial concentration of the algae;

C_A^* = the intermediate concentration;

C_A^{Out} = the final concentration of the algae from the production;

Δt^0 = initial time for batch to reach desired end concentration;

Δt^* = time for each batch after refilling with fresh feed to reach desired end concentration;

n = number of cycles; and

Φ = retained fraction.

Table 4.2.1: Chosen retained fraction (Φ) and related volume of mother algae to add to the top-up Beijerinck medium (1-Φ).

(Φ)	**Mother algae (mL)**	**Beijerinck Medium (mL)**	(Φ)	**Mother algae (mL)**	**Beijerinck Medium (mL)**
0	5	1000	**0.60**	2	400
0.30	3.5	700	**0.70**	1.5	300
0.40	3	600	**0.80**	1	200
0.50	2.5	500			

4.3 Results and Discussion

4.3.1 Graphical approach

Before any PEF could be implemented, the researcher inspected the batch profile (see Figure 4.2.2) for any convexity by comparing the gradients in concentration/time space, as explained in Section 4.1 and Section 4.2.1. For the purposes of demonstrating the graphical method in concentration/time space, Figure 4.3.1 was drawn with gradients up to an exit concentration of 1000 mg/L. As the figure shows, convexities indicate the possibility of improving the production rate, as the intermediate gradient, m^* (blue dashed line), represented a higher value than the standard batch production rate, m (shown as a red dotted line). For the purposes of this experiment, the data for above 1000 mg/L were not considered, as the improvements after 1000 mg/L (and after 12 days) were tiny, as the convexity in the curve disappeared.

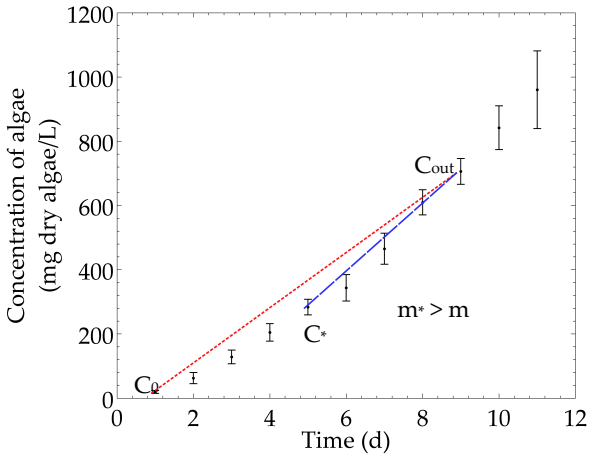
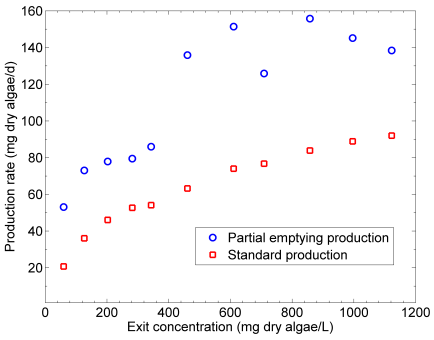


Figure 4.3.1: Identifying concavities in the algae batch profile in the concentration/time space; where m*> m implies convexity and thus production for *Desmodesmus* spp. could be improved under the experimental conditions used in this chapter.

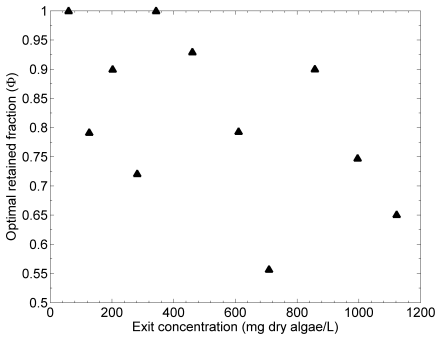
4.3.2 Predictions

The standard batch profile data for all the batch profiles obtained from the experimental runs were averaged and used to predict improved production rates. This was done by varying retained fractions for a fixed C_A^{out} (see Appendix *G.1*). This was repeated for all C_A^{out} to construct a concentration versus production profile, for which every improved production rate, shown in Figure 4.3.2a, depended on a specific Φ (Figure 4.3.2b).

Two sets of experiments were carried out for target exit concentrations of 1000 mg/L and 500 mg/L respectively. The first was over a period of 50 days, while the second lasted 20 days. The expected ranges the for the exit concentrations during the set of PEF experiments were 900–1000 mg/L and 500–600 mg/L, it was not possible to achieve an exact exit concentration.



(a) Predicted production rate with partial emptying and filling using raw experimental batch data.



(b) Predicted retained fractions used to calculate the improved predicted production rate (Figure 4.3.2a). The calculation was done for a stationary cycle, that is the result obtained after an infinite number of cycles.

Figure 4.3.2: Predictions for production rate and retained fractions based on all of the batch growth profiles.

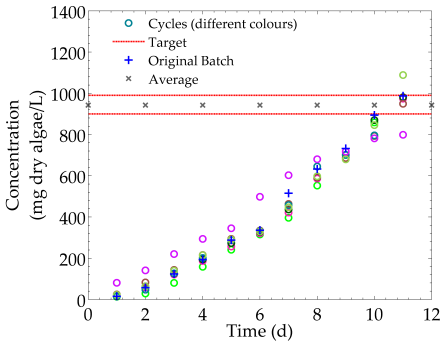
It can be seen that there is quite a large scatter in the results which is probably due to experimental errors. As seen in Figure 4.3.2, the prediction for an exit concentration of 1000 mg/L will yield an improved production rate from 88 mg/d to approximately 145 mg/d when retaining 0.75 of the working volume, which is about a 60% rise. Similarly, for an approximate exit concentration of 600 mg/L, the production is expected to improve from 74 mg/d to 151 mg/d which is about 100% rise.

4.3.3 The growth profiles and production rate of PEF

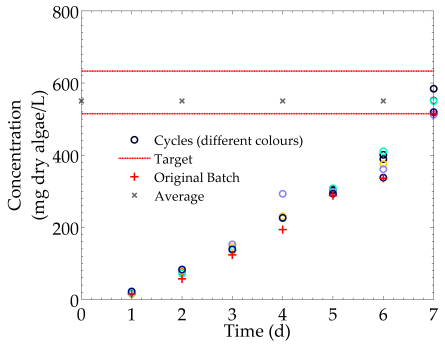
As mentioned earlier, the temperature and pH were monitored but not controlled (see results in Appendix G.1). If the pH values varied too much then the algae stopped thriving, and a PEF would be implemented. The average inlet temperature for the flasks were below room temperature, as the Beijerinck medium was taken from the refrigerator, and all suspensions were brought to an average inlet temperature of 15°C. The PEF exit temperatures were between 25° and 30°C. The average inlet and exit pH, for the 900–1000 mg/L suspensions, were around the same value pH 7 and pH 9 respectively, whereas for the 500–600 mg/L pH were 7 and pH 8.

The growth curves for the desired exit concentration of 900–1000 mg/L for the range of retained fractions carried out over a period of 50 days also followed the same growth profile. For Φ=0 the growth profile was expected to be the same as the initial batch profile on which the predictions were based.

This is seen in Figure 4.3.3a. The repeat-batch cycles had consistent profiles, which agreed with what Pattanamanee and his co-workers (2012) found when they were conducting multiple repeat-batches during their hydrogen production project. Similarly, for the desired exit concentration for 500–600 mg/L growth curves also followed that of the original batch profile, as shown in Figure 4.3.3b. From these curves one also gets an idea of the experimental errors involved.



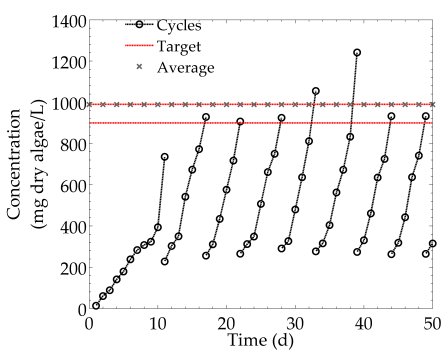
(a) Target exit concentration of 900–1000 mg/L averaging at approximately 940 mg/L.



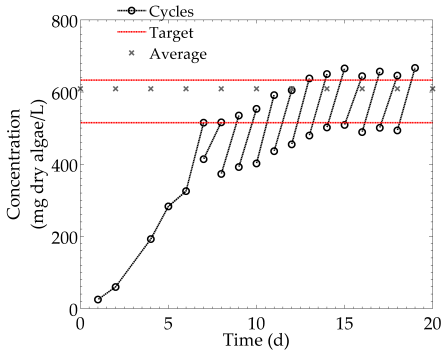
(b) Target exit concentration of 500–600 mg/L averaging at approximately 550.04 mg/L.

Figure 4.3.3: Zero Φ for both ranges, which show the results follow the original batch profile from which the predictions were based.

Only certain growth curves are shown in this chapter (although the full set of curves is shown in the Appendix G.1). Figure 4.3.4 is intended to demonstrate visually why, for all the Φ experiments, the initial and final runs were excluded from the analysis. For example, the initial Φ=0.3 experiment ended before the desired concentration range was reached (Figure 4.3.4a). It must be noted that, over a long period, that is, an infinite number of cycles, the initial and final runs will not be given much weight or significance, as it is the intermediate cycles that will represent the overall production rate. Therefore, in this chapter, the intermediate cycles will be used to calculate the overall production rate.



(a) The 0.3 for 9 cycles for 900–1000 mg/L range.

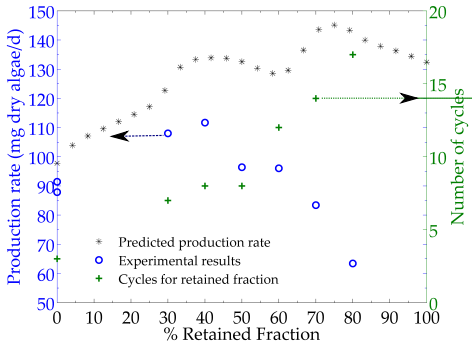


(b) The 0.8 for 13 cycles for the 500–600 mg/L range.

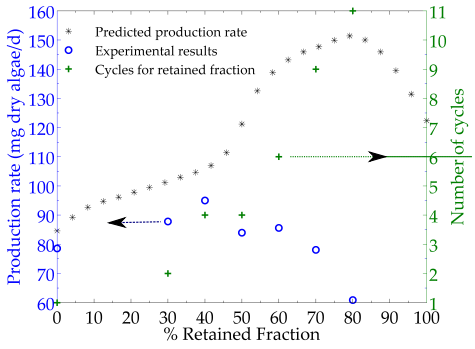
Figure 4.3.4: Growth curves for the exit concentration for target range of 900–1000 mg/L for Φ=0.3 and 500-600 mg/L Φ=0.8.

The reader can observe in Figure 4.3.4b that, after a PEF process had been carried out, the intermediate concentration (C_A^*) was closer to the lower boundary of the range. Because measurements and decisions were only taken once a day, the desired exit concentration overshot the range for each incremental cycle. This also occurred for Φ=0.6 and Φ=0.7 (shown in Appendix G.1).

A more detailed summary of the average exit concentrations within each target range and the associated production rates is shown in Figure 4.3.5 and Table 4.3.1. An Φ=0.4 gave the highest production rate for the 900–1000 mg/L range, even though it did not necessarily have the highest average exit concentration. One should note that the number of cycles that could be done over the time periods was probably not enough to ensure final stationary state, but this will be discussed at a later stage. For the 1000-1200 mg/L the Φ=0.4 resulted in a production that was 1.27 better than that of the control flasks (Φ=0). As for the 500–600 mg/L range, although 0.4 did not have as high an average exit concentration as the Φ=0.8 ; it achieved a higher production rate. It can also be seen that as the retained fraction increased the discrepancy between the predicted production rate and the experimental results got worse. It needs to be noted that as the retained fraction increases the number of cycles possible increased, in the 50 and 20 days periods respectively, and so one would not necessarily expect to see the long-term benefits of Φ.



(a) The 900–1000 mg/L range estimated from the 990 mg/L concentration from the batch data, gives a predicted best production rate at a retained fraction of 0.75.



(b) The 500–600 mg/L range estimated for the 610 mg/L concentration from the batch data, where 0.8 was the best retained fraction.

Figure 4.3.5: Production rate versus retained fraction, using spline interpolation predictions (*) between the batch data points, at a set concentration of 990 mg/L and 610 mg/L respectively, assuming an infinite number of cycles. The experimental production curves (**o**) were generated considering only the intermediate runs, ignoring the initial and final runs. The specific number of cycles (+) relating to each experimental run is shown on the right hand side of the graph.

Table 4.3.1: Summary of the average exit concentrations and production rates for the target range of 900–1000 mg/L and 500–600 mg/L.

Φ	**0**	**0**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
Average values 900-1000 mg/L over 50 days								
Conc. (mg/L)	937.68	947.24	988.84	990.38	973.08	960.33	1001.02	1011.19
Prod Rate (mg/d)	87.84	91.42	107.99	111.70	96.42	96.03	83.42	63.41
No. cycles	3	3	7	8	8	12	14	17
Average values 500-600 mg/L over 20 days								
Conc. (mg/L)	550.04	550.04	560.87	590.50	582.55	600.55	568.48	608.91
Prod Rate (mg/d)	78.58	78.58	87.82	95.00	83.96	85.58	78.09	60.89
No. Cycles	1	1	2	4	4	6	9	11

4.3.4 Aspects of the production

4.3.4.1 The number of cycles needed to approximate a stationary cycle

PEF follows a periodic procedure, and assuming that there are sufficient nutrients and no product inhibitors, after an infinite number of cycles, one can expect repeated cyclic behavior (defined as a stationary cycle). The actual PEF experiments were carried out over a 50-day or a 20-day period respectively, and not at an infinite number of cycles. As a result, these tests did

not necessarily reach a stationary cycle . The highest experimental production rates were achieved for both cases with an Φ=0.4 while the predictions suggested an Φ=0.8. In fact for the higher Φs the experimental production rate was lower than the batch one.

The method proposed by Ming et al. (2012) forecasts production rates for an infinite number of cycles. Therefore, if one intends to compare the predicted production against the experimental one should do it for the actual number of cycles. We can derive a production rate P that depends on the number of cycles where all the symbols are as previously defined:

$$\mathrm{P}=\frac{\left[(n-1)\left(1-\phi\right)\mathrm{V_{total}}\mathrm{C_A^{Out}}+\mathrm{VC_A^{Out}}\right]-\left[(n-1)\left(1-\phi\right)\mathrm{V_{total}}\mathrm{C_A^{0}}+\mathrm{VC_A^{0}}\right]}{(n-1)\,\triangle\mathrm{t}^{*}+\triangle\mathrm{t}^{0}} \tag{4.3.1}$$

Thus, when n tends towards infinity, the number of cycles versus production rate can be solved for via the following equation:

$$\lim_{n\to\infty}\mathrm{P}=\frac{(n-1)\left(1-\phi\right)\mathrm{V_{total}}\left(\mathrm{C_A^{Out}}-\mathrm{C_A^{0}}\right)+\mathrm{V_{total}}\left(\mathrm{C_A^{Out}}-\mathrm{C_A^{0}}\right)}{(n-1)\,\triangle\mathrm{t}^{*}+\triangle\mathrm{t}^{0}} \tag{4.3.2}$$

This can be simplified to the following equation using L'Hopital's rule:

$$\lim_{n\to\infty}\mathrm{P}=\frac{\left(1-\phi\right)\mathrm{V_{total}}\left(\mathrm{C_A^{Out}}-\mathrm{C_A^{0}}\right)}{\triangle\mathrm{t}^{*}} \tag{4.3.3}$$

Equation 4.3.1 allows one to predict the theoretical instantaneous production (for the actual number of cycles done for the Φ) at the average exit concentration. One can then compare this with the experimental results.

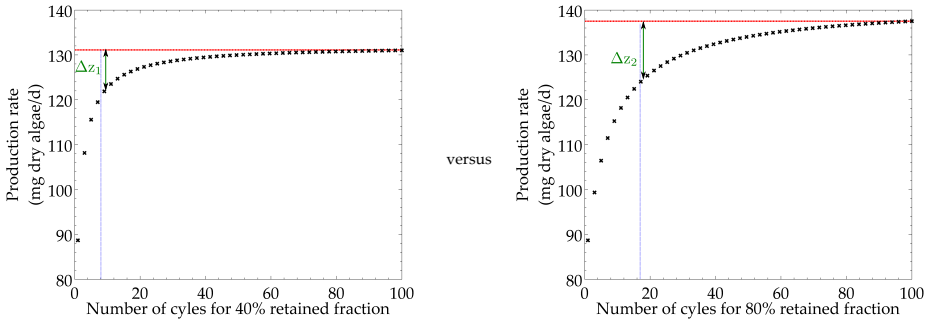
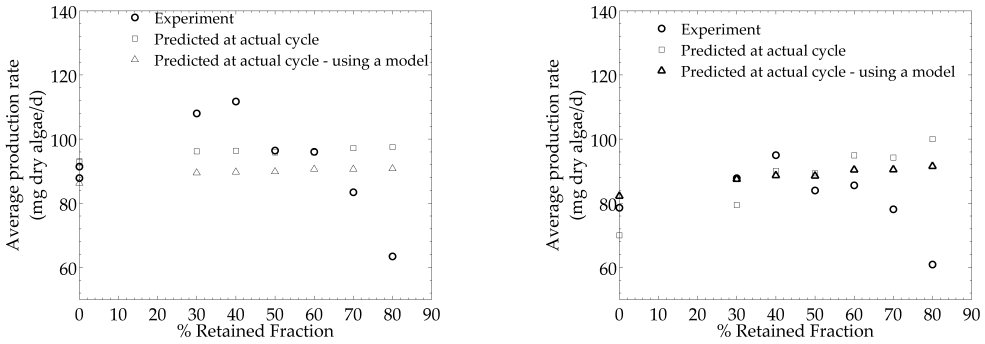


Figure 4.3.6: Production rate predictions. Comparison between Φ=0.4 and Φ=0.8 at their specific average exit concentration production rate predictions, shown for the 900–1000 mg/L range. The horizontal red dotted line is the stationary cycle production for that specific average exit concentration and retained fraction. The vertical blue dashed line is the number of cycles done for the actual experiment at that retained fraction.

One can readily see from Figure 4.3.6 that the predicted production rates for a limited number of cycles is significantly less than the final production for many cycles. We are now in a position to compare the experimental results with those predicted with a limited number of cycles.

These results are shown in Figure 4.3.7a and b for each experimental (shown as circles) average exit concentration and Φ pair. The 900–1000 mg/L target concentration range showed retained fractions of 0.3 and 0.4, exceeding the expected instantaneous production rate by 1.12 and 1.16 respectively, while 0.5 and 0.6 yielded the predicted instantaneous production rate for their average exit concentration. However, 0.7 and 0.8 had not yet reached their predicted instantaneous production rate (shown as squares), which were determined by using equations 4.3.1 and 4.3.2. Similarly, for the 500–600 mg/L range (Figure 4.3.7b), the Φ=0.4 was higher than the predicted production rate related to the instantaneous cycle, and 0.8 had not yet reached the expected production rate.



(a) From Table 4.3.1, for the 900–1000 mg/L range.

(b) From Table 4.3.1, for the 500–600 mg/L range.

Figure 4.3.7: Predicted instantaneous production rate from actual (□) and smoothed production (△) curves for various retained fractions versus the experimental values for the actual number of cycles completed (o).

Again, as we saw in the growth curves for the target ranges of 1000 mg/L and 500 mg/L, the fluctuations in the PEF growth profiles increased in step with the retained fractions. This led to exit concentrations overshooting the target range boundary, resulting in intermediate concentrations closer to the lower limit. An explanation of this phenomenon could be the experimental method itself. It is possible that, at higher retained fractions, one should alter the experimental method to PEF earlier and more frequently, as opposed to every 24 hours.

Clearly the experimental runs are subject to error relative to the calculations. For instance the time interval chosen ($\triangle t^*$) was based on the daily times that changes could be made and not the optimum times. Thus the agreement with the predicted results is reasonably good.

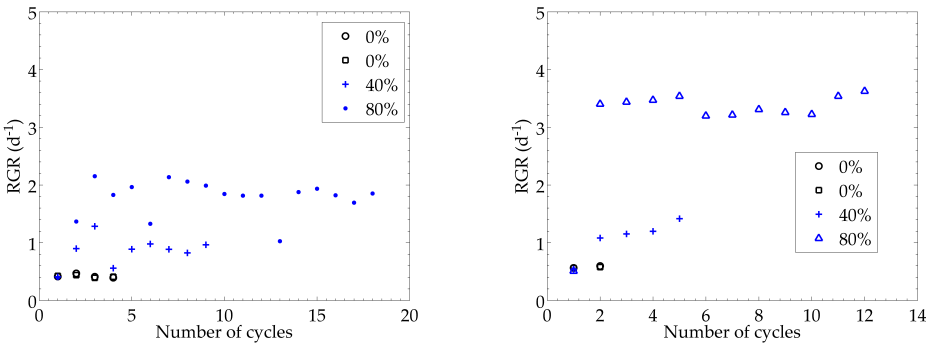
An attempt was made to see how the scatter of the experimental results affected the predictions. To do this a smooth curve (shown as triangles) was passed through the results in Figure 4.2.2 (shown in Appendix G.1), and the results also plotted in Figure 4.3.7. While the results are a bit smoother they do not really change the conclusions significantly.

4.3.5 Further remarks

In the summary (to be found in Table 4.3.1) the 900–1000 mg/L range showed Φ=0.4 as the highest (26%) improvement, but not 63% as predicted. The results do not correlate with the predictions where Φ=0.75 was recommended.

Additional experimental visual observations that might be relevant are that over the duration of the experiment it appeared that the Φ=0.8 was showing signs of deterioration, and the Φ=0.3 reached the desired exit concentration more easily. For the 900–1000 mg/L range, 0.5 and 0.8 became unhealthy sooner, but recovered over time. Bubbling with CO_2 enhanced the growth rate daily, but the production rate was improved further by retaining a certain fraction of PC. This could be attributed to an insufficient supply of nutrients that could be needed at higher retained fractions for necessary cell growth. Another possibility is that there could be an accumulation of by-products at higher retained fractions, which could be slowing down the growth of the algae.

A test of whether there was any deterioration of the algae (for example from nutrient deficiency and by-product inhibitors), as a result of the Φ policy or from the precipitation of salts from in the medium preparation, can be seen by evaluating the RGR for each cycle. These relative growth rates (RGR) are displayed in Figure 4.3.8. They match with what Radmann et al. (2007) reported; that RGR (also known as specific growth rate—μ_{max}) remained approximately constant over repeated-batch experiments. This suggests that PEF is suitable for *Desmodesmus* spp. growth experiments. Notice also as the retained fraction increased so did the RGR. This is what one would expect but at the higher values of Φ, based on the experimental results, this was clearly not enough of an increase to give an improved production rate.



(a) RGR for 1000 mg/L. (b) RGR for 500 mg/L.

Figure 4.3.8: Relative growth rates (RGR) of the retained fractions, excluding the last cycle. As the retained fraction increased, so did the RGR.

Historically researchers have used a one-third rule as a guide based on their experience of working with the system. This entailed removing 30% and re-

taining 70% of the biomass. Wu and Merchuk (2002) used one-third of the algae seed inoculum to prevent contamination and to avoid lag time. Blánquez et al. (2006) and Blánquez et al. (2008) used the one-third rule to remove that amount of the initial inoculated biomass, and concluded that it was good for processes that are intended to involve long-term continuous operation. These researchers also emphasized that this method, known to them as the biomass purge and renovation methodology, helped to ensure microbial activity that would maintain a young culture.

What has been done historically in regard to Φ is summarized in Table 4.1.1. The PEF production rates in some cases corresponded to the growth rates from researchers in the field growing *Desmodesmus* spp. (Kativu et al., 2012; Ji et al., 2014, 2015; Solovchenko et al., 2014). Even though the author of this chapter was able to bring about better growth rates by means of PEF, this did not happen with the higher Φ values predicted by the theory. It is suggested that this is probably caused by the difficulties of controlling the cycle lengths as well as the need for a large number of cycles to reach the stationary cycle. In fact if one contemplates using high values of Φ one should rather think of continuous feed and removal, that is continuous operation rather than batch operation. However, one has to balance the predicted gains against the experimental difficulties in achieving these.

The theoretical tools used in this chapter do however provide a useful method to see whether Φ is a useful option and how good this is likely to be. One can readily assess this from the concavity of the batch curve.

4.4 Conclusion

It has been shown that, based on a measured batch profile and using the Ming et al. (2012) method, one can predict the amount of Retained Fraction (Φ) that should give the best production rate of algae for a Partial Filling and Emptying production approach for different exit algal concentrations. In practice it was shown that this worked well for lower values of Φ but was not so successful for higher values of the Φ. It was speculated that this was because of the difficulty of doing experiments at high Φ. These were thought to be because these entailed short cycles and many emptying and filling actions, which were difficult to manage properly. In fact for these high Φ experiments it is suggested one

rather converts to continuous operation.

It was established that at lower Φ values the batch profile remained reasonably constant, and the measured production rate was comparable with the predicted production rate. It was further shown that the shortened batch profile in each cycle did not change in relation to that part of the original batch profile. For the lower Φ it was shown within experimental variation, which tends to be large for biological experiments, the Ming et al. (2012) method was reasonably able to predict the improvements in production rate. The production increased by a factor of 1.26 at approximately 1000 mg/L and by 1.28 at approximately 600 mg/L for the experimental conditions used in this chapter at an Φ=0.4. The Φ=0.3 used by many previous experimenters is therefore probably a reasonable compromise if no other information is available.

In conclusion, as we are dealing with a biological species, which will behave differently in different experiments, we need to be careful about making predictions without a large amount of experimental data. Despite this, the author observed improvements over the standard batch production rate, using the Ming et al. (2012) method to identify a retained fraction. The author managed to apply theory to laboratory work and increased the production rate of the algae species with the given experimental setup. This technique offered a practical structured method to obtain predictions of operating performance in an expected range. It therefore seems effective for algal propagation, and can also be used as an application for a variety of other biological systems that exhibit a growth curve with convexity. Using this one should be able to improve production, save cultivation costs and overcome the stationary growth phase.

References

Abdel-Rahman, M. A., Tashiro, Y., Sonomoto, K., 2013a. Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances 31 (6), 877–902.

Abdel-Rahman, M. A., Tashiro, Y., Zendo, T., Sonomoto, K., 2013b. Improved lactic acid productivity by an open repeated batch fermentation system using *Enterococcus mundtii* QU 25. Royal Society of Chemistry Advances 3 (22), 8437–8445.

Abdelaziz, A. E., Leite, G. B., Hallenbeck, P. C., 2013. Addressing the chal-

lenges for sustainable production of algal biofuels: I. Algal strains and nutrient supply. Environmental Technology 34 (13-14), 1783–1805.

Bibal, B., Vayssier, Y., Goma, G., Pareilleux, A., 1991. High-concentration cultivation of *Lactococcus cremoris* in a cell-recycle reactor. Biotechnology and Bioengineering 37 (8), 746–754.

Blánquez, P., Sarrà, M., Vicent, M., 2006. Study of the cellular retention time and the partial biomass renovation in a fungal decolourisation continuous process. Water Research 40 (8), 1650–1656.

Blánquez, P., Sarrà, M., Vicent, T., 2008. Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions. Process Biochemistry 43 (1), 1–7.

Brennan, L., Owende, P., 2010. Biofuels from microalgae–A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews 14 (2), 557–577.

Cheah, W. Y., Show, P. L., Chang, J.-S., Ling, T. C., Juan, J. C., 2015. Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. Bioresource Technology 184 (0), 190 – 201, advances in biofuels and chemicals from algae.

Choi, G.-W., Moon, S.-K., Kang, H.-W., Min, J., Chung, B.-W., 2009. Simultaneous saccharification and fermentation of sludge-containing cassava mash for batch and repeated batch production of bioethanol by *Saccharomyces cerevisiae* CHFY0321. Journal of Chemical Technology and Biotechnology 84 (4), 547–553.

De la Noüe, J., de Pauw, N., 1988. The potential of microalgal biotechnology: a review of production and uses of microalgae. Biotechnology Advances 6 (4), 725–770.

De la Noüe, J., Ni Eidhin, D., 1988. Improved performance of intensive semicontinuous cultures of *Scenedesmus* by biomass recirculation. Biotechnology and Bioengineering 31 (5), 397–406.

Elliott, L. G., Feehan, C., Laurens, L. M., Pienkos, P. T., Darzins, A., Posewitz, M. C., 2012. Establishment of a bioenergy-focused microalgal culture collection. Algal Research 1 (2), 102–113.

Fábregas, J., Patiño, M., Arredondo-Vega, B. O., Tobar, J., Otero, A., 1995a. Renewal rate and nutrient concentration as tools to modify productivity and biochemical composition of cyclostat cultures of the marine microalga *Dunaliella tertiolecta*. Applied Microbiology and Biotechnology 44 (3-4), 287–292.

Fábregas, J., Patiño, M., Morales, E. D., Cordero, B., Otero, A., 1996a. Optimal renewal rate and nutrient concentration for the production of the marine microalga *Phaeodactylum tricornutum* in semicontinuous cultures. Applied and Environmental Microbiology 62 (1), 266–268.

Fábregas, J., Patiño, M., Morales, E. D., Dominguez, A., Otero, A., 1996b. Distinctive control of metabolic pathways by *Chlorella autotrophica* in semicontinuous culture. Canadian Journal of Microbiology 42 (11), 1087–1090.

Fábregas, J., Patiño, M., Vecino, E., Cházaro, F., Otero, A., 1995b. Productivity and biochemical composition of cyclostat cultures of the marine microalga *Tetraselmis suecica*. Applied Microbiology and Biotechnology 43 (4), 617–621.

Gouveia, L., Oliveira, A. C., 2009. Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology and Biotechnology 36 (2), 269–274.

Gressler, P., Bjerk, T., Schneider, R., Souza, M., Lobo, E., Zappe, A., Corbellini, V., Moraes, M., 2014. Cultivation of *Desmodesmus* subspicatus in a tubular photobioreactor for bioremediation and microalgae oil production. Environmental Technology 35 (2), 209–219.

Hata, N., Ogbonna, J. C., Hasegawa, Y., Taroda, H., Tanaka, H., 2001. Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic-photoautotrophic culture. Journal of Applied Phycology 13 (5), 395–402.

Henrard, A., De Morais, M., Costa, J. A. V., 2011. Vertical tubular photobioreactor for semicontinuous culture of *Cyanobium* sp. Bioresource Technology 102 (7), 4897–4900.

Ho, S.-H., Chang, J.-S., Lai, Y.-Y., Chen, C.-N. N., 2014b. Achieving high lipid productivity of a thermotolerant microalga *Desmodesmus* sp. F2 by optimizing environmental factors and nutrient conditions. Bioresource Technology 156 (0), 108–116.

Ho, S.-H., Chen, C.-N. N., Lai, Y.-Y., Lu, W.-B., Chang, J.-S., 2014c. Exploring the high lipid production potential of a thermotolerant microalga using statistical optimization and semi-continuous cultivation. Bioresource Technology 163 (0), 128–135.

Hoffert, M. I., Caldeira, K., Benford, G., Criswell, D. R., Green, C., Herzog, H., Jain, A. K., Kheshgi, H. S., Lackner, K. S., Lewis, J. S., Lightfoot, H. D., Manheimer, W., Mankins, J. C., Mauel, M. E., Perkins, L. J., Schlesinger, M. E., Volk, T., Wigley, T. M. L., 2002. Advanced technology paths to

global climate stability: energy for a greenhouse planet. Science 298 (5595), 981–987.

Hu, B., Zhou, W., Min, M., Du, Z., Chen, P., Ma, X., Liu, Y., Lei, H., Shi, J., Ruan, R., 2013. Development of an effective acidogenically digested swine manure-based algal system for improved wastewater treatment and biofuel and feed production. Applied Energy 107 (0), 255–263.

Ji, F., Liu, Y., Hao, R., Li, G., Zhou, Y., Dong, R., 2014. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresource Technology 161 (0), 200–207.

Ji, F., Zhou, Y., Pang, A., Ning, L., Rodgers, K., Liu, Y., Dong, R., 2015. Fed-batch cultivation of *Desmodesmus* sp. in anaerobic digestion wastewater for improved nutrient removal and biodiesel production. Bioresource Technology 184 (0), 116 – 122.

Kaewpintong, K., Shotipruk, A., Powtongsook, S., Pavasant, P., 2007. Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. Bioresource Technology 98 (2), 288–295.

Kativu, E., 2011. Carbon dioxide absorption using fresh water algae and identifying potential uses of algal biomass. MSc, Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, South Africa.

Kativu, E., Hildebrandt, D., Matambo, T., Glasser, D., 2012. Effects of CO_2 on South African fresh water microalgae growth. Environmental Progress and Sustainable Energy 31 (1), 24–28.

Koller, M., Muhr, A., Braunegg, G., 2014. Microalgae as versatile cellular factories for valued products. Algal Research 6, Part A (0), 52 – 63.

Konopka, A., Zakharova, T., Oliver, L., Camp, D., Turco, R., 1996. Biodegradation of organic wastes containing surfactants in a biomass recycle reactor. Applied and Environmental Microbiology 62 (9), 3292–3297.

Kosińska, K., Miśkiewicz, T., 2009. Performance of an anaerobic bioreactor with biomass recycling, continuously removing COD and sulphate from industrial wastes. Bioresource Technology 100 (1), 86–90.

Koti, B. A., Shinde, M., Lalitha, J., 2013. Repeated batch production of agaro-oligosaccharides from agarose by an amberlite ira-900 immobilized agarase system. Biotechnology and Bioprocess Engineering 18 (2), 333–341.

Lin, J.-H., Lee, D.-J., Chang, J.-S., 2015. Lutein in specific marigold flowers and microalgae. Journal of the Taiwan Institute of Chemical Engineers 49, 90 – 94.

Low, M., Glasser, D., Ming, D., Hildebrandt, D., Matambo, T., 2015. Batch partial emptying and filling to improve the production rate of algae. Industrial & Engineering Chemistry Research 54 (50), 12492–12502.
URL http://dx.doi.org/10.1021/acs.iecr.5b03093

Mata, T. M., Martins, A. A., Caetano, N. S., 2010. Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews 14 (1), 217–232.

Matsudo, M. C., Bezerra, R. P., Sato, S., Perego, P., Converti, A., Carvalho, J. C. M., 2009. Repeated fed-batch cultivation of *Arthrospira*(*Spirulina*) *platensis* using urea as nitrogen source. Biochemical Engineering Journal 43 (1), 52–57.

Menetrez, M. Y., 2012. An overview of algae biofuel production and potential environmental impact. Environmental Science and Technology 46 (13), 7073–7085.

Ming, D., Hildebrandt, D., Glasser, D., 2012. A graphical method of improving the production rate from batch reactors. Industrial and Engineering Chemistry Research 51 (42), 13562–13573.

Mussatto, S. I., Rodrigues, L., Teixeira, J., 2009. β-Fructofuranosidase production by repeated batch fermentation with immobilized *Aspergillus japonicus*. Journal of Industrial Microbiology and Biotechnology 36 (7), 923–928.

Naritomi, T., Kouda, T., Yano, H., Yoshinaga, F., Shigematsu, T., Morimura, S., Kida, K., 2002. Influence of broth exchange ratio on bacterial cellulose production by repeated-batch culture. Process Biochemistry 38 (1), 41–47.

Palanisami, S., Lee, K., Balakrishnan, B., Nam, P., 2015. Flue-gas-influenced heavy metal bioaccumulation by the indigenous microalgae *Desmodesmus communis* LUCC 002. Environmental Technology 36 (4), 463–469.

Park, J., Craggs, R., 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Science and Technology 6 (3), 633–639.

Park, J., Craggs, R., Shilton, A., b 2011b. Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology 102 (1), 35–42.

Park, J., Craggs, R., Shilton, A., a 2013a. Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. Water Research 47 (13), 4422–4432.

Park, J., Craggs, R., Shilton, A., b 2013b. Investigating why recycling gravity harvested algae increases harvestability and productivity in high rate algal ponds. Water Research 47 (14), 4904–4917.

Pattanamanee, W., Choorit, W., Kantachote, D., Chisti, Y., 2012. Repeated-batch production of hydrogen using *Rhodobacter sphaeroides* S10. International Journal of Hydrogen Energy 37 (21), 15855–15866.

Radmann, E. M., Reinehr, C. O., Costa, J. A. V., 2007. Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. Aquaculture 265 (1), 118–126.

Radway, J., Weissman, J., Benemann, J., Wilde, E., 1998. Practical considerations in cyanobacterial mass cultivation. In: Zaborsky, O., Benemann, J., Matsunaga, T., Miyake, J., San Pietro, A. (Eds.), BioHydrogen. Springer US, pp. 467–473.

Radway, J., Weissman, J., Wilde, E., Benemann, J., 1994. Nutrient removal by thermophilic *Fischerella Mastigocladus laminosus* in a simulated algaculture process. Bioresource Technology 50 (3), 227–233.

Reichert, C., Reinehr, C., Costa, J., 2006. Semicontinuous cultivation of the cyanobacterium *Spirulina platensis* in a closed photobioreactor. Brazilian Journal of Chemical Engineering 23 (1), 23–28.

Reinehr, C. O., Costa, J. A. V., 2006. Repeated batch cultivation of the microalga *Spirulina platensis*. World Journal of Microbiology and Biotechnology 22 (9), 937–943.

Samorì, G., Samorì, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. Water Research 47 (2), 791–801.

Samson, R., Leduy, A., 1985. Multistage continuous cultivation of blue-green alga *Spirulina maxima* in the flat tank photobioreactors with recycle. The Canadian Journal of Chemical Engineering 63 (1), 105–112.

Sánchez, Á., Maceiras, R., Cancela, Á., Pérez, A., 2013. Culture aspects of isochrysis galbana for biodiesel production. Applied Energy 101, 192–197.

Sayre, R., 2010. Microalgae: the potential for carbon capture. Bioscience 60 (9), 722–727.

Schlepütz, T., Büchs, J., 2013. Investigation of vinegar production using a novel shaken repeated batch culture system. Biotechnology Progress 29 (5), 1158–1168.

Solovchenko, A., Gorelova, O., Selyakh, I., Semenova, L., Chivkunova, O., Baulina, O., Lobakova, E., 2014. *Desmodesmus* sp. 3Dp86E-1 - A novel symbiotic chlorophyte capable of growth on pure CO_2. Marine Biotechnology 16 (5), 495–501.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering 101 (2), 87–96.

Urrutia, I., Serra, J. L., Llama, M. J., 1995. Nitrate removal from water by *Scenedesmus obliquus* immobilized in polymeric foams. Enzyme and Microbial Technology 17 (3), 200–205.

Wang, B., Li, Y., Wu, N., Lan, C. Q., 2008. CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology 79 (5), 707–718.

Wang, J., Yang, H., Wang, F., 2014a. Mixotrophic cultivation of microalgae for biodiesel production: Status and prospects. Applied Biochemistry and Biotechnology 172 (7), 3307–3329.

Weissman, J. C., Benemann, J. R., 1979. Biomass recycling and species competition in continuous cultures. Biotechnology and Bioengineering 21 (4), 627–648.

Wijffels, R. H., Barbosa, M. J., 2010. An outlook on microalgal biofuels. Science(Washington) 329 (5993), 796–799.

Williams, J. H., DeBenedictis, A., Ghanadan, R., Mahone, A., Moore, J., Morrow, W. R., Price, S., Torn, M. S., 2012. The technology path to deep greenhouse gas emissions cuts by 2050: the pivotal role of electricity. Science 335 (6064), 53–59.

Woertz, I., Feffer, A., Lundquist, T., Nelson, Y., 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. Journal of Environmental Engineering 135 (11), 1115–1122.

Wu, X., Merchuk, J. C., 2002. Simulation of algae growth in a bench-scale bubble column reactor. Biotechnology and Bioengineering 80 (2), 156–168.

Xie, Y.-P., Ho, S.-H., Chen, C.-Y., Chen, C.-N. N., Liu, C.-C., Ng, I.-S., Jing, K.-J., Yang, S.-C., Chen, C.-H., Chang, J.-S., Lu, Y.-H., 2014. Simultaneous enhancement of CO_2 fixation and lutein production with thermo-tolerant *Desmodesmus* sp. F51 using a repeated fed-batch cultivation strategy. Biochemical Engineering Journal 86 (0), 33 – 40.

Zeng, A.-P., Biebl, H., Deckwer, W.-D., 1991. Production of 2, 3-butanediol in a membrane bioreactor with cell recycle. Applied Microbiology and Biotechnology 34 (4), 463–468.

Zeng, X., Danquah, M. K., Chen, X. D., Lu, Y., 2011. Microalgae bioengineering: From CO_2 fixation to biofuel production. Renewable and Sustainable Energy Reviews 15 (6), 3252 – 3260.

Zhao, B., Wang, L., Li, F., Hua, D., Ma, C., Ma, Y., Xu, P., 2010a. Kinetics of d-lactic acid production by *Sporolactobacillus* sp. strain CASD using repeated batch fermentation. Bioresource Technology 101 (16), 6499–6505.

Zhao, B., Wang, L., Ma, C., Yang, C., Xu, P., Ma, Y., 2010b. Repeated open fermentative production of optically pure l-lactic acid using a thermophilic *Bacillus* sp. strain. Bioresource Technology 101 (16), 6494–6498.

Chapter 5

Comparison between different media used for duckweed propagation: steps toward optimized scale-up production of duckweed biomass

- This chapter is important as it provides an investigation into an alternative aquatic plant, that is duckweed. This species has one of the fastest growth rates, is easier to harvest than algae and is a candidate for biofuel because of its high starch content. This chapter shows that one can propagate the duckweed species in a cost effective media instead of using the expensive laboratory media, achieving the same or better growth rates and starch content. This experimental work was done at Lam Lab, Rutgers New Jersey as part of the IGERT Research Scholar program.
- This chapter will be submitted for peer review. A portion of this chapter was orally presented at the Rutgers-IGERT NSF Project Seminar in 2013 as well as The Second International Conference on Duckweed Research and Applications (2013).
- The contribution of the co-authors (Lam, E.[‡]; Glasser, D.[†*]; Hildebrandt, D.[*]) towards the work is primarily that of supervision or collaboration, and the work and write-up was conducted by the author of this thesis.

Abstract

Duckweed, when studied in the laboratory, is normally cultivated on a basal medium such as Schenk and Hildebrandt (SH). Alternatively, in larger-scale applications, it can be grown in wastewater. In such a case, the propagation of this plant species is used to treat industrial effluent, but duckweed is also capable of another valuable function: being convertible to biofuel. In this chapter, the objective of the research described was to determine whether duckweed could be cultivated in media less expensive than the basal laboratory medium, so as to find an economical and reliable alternative to bridge the scale gap between laboratory and industrial production. The investigation entailed measuring the growth rates for multiple strains of duckweed in a variety of media, and a cost comparison between the different media used to cultivate them. The duckweed strains cultivated in media containing commercial fertilizers realized a growth rate of between 300% and 700% higher than that obtained from cultivation in the basal media (which was 400–600%). These results indicated that duckweed can be cultivated more efficiently, and in a more cost-effective manner, in the alternative media types, while maintaining a starch content comparable with that obtained with the conventional laboratory media.

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5.1 Introduction

5.1.1 *Lemnaceae*

There are five genera (*Spirodela*, *Landoltia*, *Lemna*, *Wolffia* and *Wolffiella*) and 37 accepted species of *Lemnaceae*, also known as duckweed (Appenroth et al., 2015). The vegetative propagation of duckweed typically occurs when a 'daughter' frond grows out of pockets (meristem regions) in the 'mother' frond. The sizes of the fronds range from 1 mm to 15 mm, making these plants the smallest known angiosperms (Oron et al., 1986). Duckweed also has the

fastest doubling time of all but one of the similar species of aqueous plants (Hillman and Culley Jr, 1978; Zhao et al., 2012a). It is able to grow in air or in water that is enriched with carbon dioxide (Satake and Shimura, 1983; Björndahl and Nilsen, 1985), and various other kinds of wastewater.

Xu et al. (2012a) provide examples from the published literature in which researchers report that the starch content in duckweed is as low as three percent. On the other hand, Zhao et al. (2012a) and Su et al. (2014) have obtained a starch content of up to 50%. As the amount of starch is crucial to the usefulness of duckweed as a source of food or biofuel, researchers in the field are focusing on ways to culture duckweed to obtain a starch accumulation of up to 60–70% (Cheng and Stomp, 2009; Xu et al., 2011; Xiao et al., 2013). Other researchers report that carbon dioxide in the water can help to stimulate photosynthesis, which brings about an increase in starch content (Cui and Cheng, 2015).

Cheng and Stomp (2009) claim that the growth of duckweed can be optimized to increase either the starch or protein content. For example, the starch content will rise when nutrient starvation is implemented (Zhao et al., 2012a; Cui and Cheng, 2015). Seeing that duckweed contains high percentages of protein and starch, it can be used as a food supplement for fish, cattle, swine and poultry (Mbagwu and Adeniji, 1988; Bekcan et al., 2009; Cheng and Stomp, 2009; Xu et al., 2012a).

Not only does duckweed have nutritional value. *Lemna* is known for its bioremediation qualities in wastewater, as it can take up heavy metals and suspended solids (Jain et al., 1990; Rahmani and Sternberg, 1999; Bekcan et al., 2009; Axtell et al., 2003). Allam and his co-workers (2014) investigated the use of *Lemna gibba* to treat drainage water which comprised a combination of domestic and food industry wastewater, to make it fit for re-use in agricultural applications. The systems for which duckweed is commonly known to digest ammonium, nitrogen-nitrate and phosphates, include the following:

- swine-farming effluent (Bergmann et al., 2000; Cheng et al., 2002a,b; Cheng and Stomp, 2009; Xu and Shen, 2011b; Ge et al., 2012; Zhao et al., 2014b);
- domestic wastewater (Oron, 1994; Awuah et al., 2004; Nalbur et al., 2004; Verma and Suthar, 2014); and

- sewage lagoons (Al-Nozaily et al., 2000; Yu et al., 2014).

Xu and his co-workers (2012a), noting that duckweed has a rapid growth rate and can have a high starch content, proposed that duckweed could be used for the production of fuel ethanol. Baliban et al. (2013) used a model to demonstrate that it would be economical for duckweed refineries to compete with petroleum plants in making a variety of energy products. Owing to *Lemna's* high potential starch content (Cheng and Stomp, 2009) it can be used to generate biomass energy for the production of:

- bioethanol (Chen et al., 2012; Ge et al., 2012; Yu et al., 2014);
- pyrolysis products such as biochar (Muradov et al., 2010, 2012);
- diesel biofuel and green gasoline precursor bio-oil components (Muradov et al., 2014);
- higher alcohols such as butanol (Su et al., 2014); and
- biogas (Cui and Cheng, 2015).

Lemna gibba and *Spirodela polyrhiza* have also been used in laboratory and space studies to supply edible protein-rich food for space missions (Gale et al., 1989). These are known as Controlled Ecological Life Support Systems (CELSS). *Lemna gibba* has been used in phytotoxicity tests (Wang and Freemark, 1995; Mkandawire et al., 2004; Fulton et al., 2009), whereas Rival et al. (2008) investigated *Spirodela* as a source of aprotinin for the pharmaceuticals industry. There are already some companies and groups that are cultivating duckweed on a commercial scale for wastewater remediation or for food (Lam et al., 2014).

In their editorial for *V Plant Biology 17*, Appenroth and his co-workers (2015) summarize the many variations in duckweed usage. Zhao et al. (2014a) reports that in their study, duckweed (*Lemna japonica*) was more versatile than water hyacinth. However, even though water hyacinth grows even faster than duckweed, researchers (such as Zhao et al. (2014a); Appenroth et al. (2015)) offer compelling reasons as to why duckweed should be cultivated in preference to other macrophytes.

The body of work presented here focuses on lab-bench scale experiments that will provide insight into, first, finding the best strain from each of the three

genera of duckweed, specifically, six species (*Spirodela polyrhiza* 8483, *Spirodela polyrhiza* 9509, *Lemna gibba* 8428, *Lemna minor* DWC 112, *Wolffia cylindracea* 7340, *Wolffia globosa* 9527) to be studied for cultivation purposes, and identifying the medium in which they grow most rapidly. The media used in these experiments were SH (Schenk and Hildebrandt), MS (Murashige and Skoog) and commercially-available fertilizers that have a certain NPK (nitrogen, phosphorous and potassium) content. The results of these trials of duckweed samples in these different media were then compared in terms of the starch content of the duckweed and the relative cost of the medium. The main purpose of these experiments was to establish the practicality of cultivating duckweed in a 'seeding-stock' facility, which could supply enough duckweed for wastewater treatment or biofuel purposes. Thus this research was intended to provide information that would be useful when scaling up duckweed production in cost-effective media.

5.1.2 Cultivation and Harvesting

With regard to the propagation and harvesting conditions of duckweed cultures, the recommendations of scientists currently working in the field are briefly reviewed in the sections that follow.

Roijackers et al. (2004) and Szabó et al. (2005) investigated the growth of duckweed in competition with that of algae, and their reports both emphasize the importance of monitoring the pH of the growing medium, and that the area of the pond covered by duckweed should be shaded to discourage invasive growth from algae. Awuah et al. (2004) found that when algal-based and macrophyte wastewater stabilization ponds were used, both resulted in the removal of nutrients and pathogens from wastewater (Ennabili et al., 1998). Also, because duckweed plants are small, and occur on the surface of the water (unlike algae), they are easily harvested. They are also more readily processed as their root systems are not extensive (Hillman and Culley Jr, 1978; Oron et al., 1986; van der Steen et al., 1998; Xu et al., 2012a). Continual harvesting of duckweed is desirable (Cheng and Stomp, 2009), since crowding limits its growth (Driever et al., 2005). Van der Steen et al. (1998) harvested 50% of the duckweed surface once a week, and Xu et al. (2011) maintained two layers (a 'double thickness' of the mat or blanket) of duckweed fronds on the surface of the water by harvesting three times a week (Xu et al., 2012a). The growth rates they obtained were comparable with those of corn when grown

on swine wastewater (Xu et al., 2012a). Zhao et al. (2012a) quoted Lam as saying that continued improvement in strain cultivation will be important for the establishment and optimization of the duckweed platform.

5.1.2.1 Nutrients

Nutrients are an important factor in duckweed growth. According to Cheng and Stomp (2009), when placed in wastewater duckweed stores nutrients (such as nitrogen (N) and phosphorus (P)) in its tissues. Seth et al. (1970) found that *Wolffia microscopica* did not grow well when iron was omitted from the medium. Huebert and Shay (1991) grew *Lemna triscula* in a standard medium with different concentrations of external phosphorus, nitrogen and calcium, in order to determine more exactly the conditions under which the duckweed fronds would survive or even thrive. They found that the growth rate of the duckweed fell when nitrogen was excluded from the medium, or when more calcium was added to the media.

Reid and Bieleski (1970) reported a 75% starch content in the tissue dry weight of *Spirodela oligorrhiza* cultivated in a phosphorus-deficient medium. Ge and his co-workers (2012) managed to achieve a higher starch content by two methods: either nutrient starvation or the addition of glucose when the duckweed was grown in the dark. Zhao et al. (2014b) investigated *Lemna minor* and *Landoltia punctata* growth and starch content in both mixed and monocultures of duckweed under different temperatures and lighting, and media containing variable concentrations of N and P. Zhao et al. (2014b) found that the growth rate of duckweed increased when the light intensity changed from 2000 lux to 5000 lux, although they also discovered that a lower light intensity encouraged a higher starch content.

The research carried out by Zhang et al. (2014a) entailed varying the amount of total Kjedahl nitrogen (TN) for one surface area, and maintaining a constant TN for a different surface area. They reported that no difference in growth of the duckweed could be observed.

Bitcover and Sieling (1951) reported that when duckweed is grown in non-sterile water, one cannot be certain as to what form of nitrogen the species is assimilating. These researchers therefore investigated the growth of *Spirodela polyrhiza* in sterile media. They found that nitrate (NO_3^-), as opposed to

ammonium nitrogen (NH_4^+), was the preferable form of nitrogen to use for duckweed cultivation, as the latter was toxic at high concentrations. The findings of Wang et al. (2014c) agreed with that point, but added that at lower concentrations NH_4^+ was more effective than NO_3^-.

It is through fertilizers that the macro-nutrients N, P, and K (potassium) are provided to boost crop growth. There is an additional benefit in that NPK can increase the nutrient uptake of the plant (Rengel et al., 1999). It is therefore important to consider the NPK content when cultivating any type of plant, including duckweed.

5.1.2.2 Wastewater as a cultivation medium

One method of providing the necessary nutrients for duckweed growth is to cultivate it on wastewater, where the plants use the chemical pollutants as nutrients while remediating the water. Reddy and DeBusk (1985) were among the first to recommend that duckweed should be used in wastewater treatment, to take up the toxic chemicals and to proliferate. Their research (which was carried out in Florida, USA) into this medium for duckweed cultivation found that the warmer months were more conducive to growth than the cooler ones, while on the other hand they reported that high plant densities hindered the propagation of *Spirodela polyrhiza* and *Lemna minor*.

Bergmann et al. (2000) carried out an in-vitro experiment on a selection of duckweed strains from different geographical locations, using swine artificial medium (SAM). Out of the 41 species, they found that *Lemna gibba* 8678, *Spirodela punctata* 7776, and *Lemna minor* 8627 were the types that grew best in SAM, and that the resultant biomass had a high protein content. Cheng et al. (2002b) experimented with *Lemna minor* in both a controlled environment and a swine lagoon located out of doors, in both cases with varying dilutions of SAM. Those researchers found that in the controlled environment, the duckweed growth was similar for all degrees of dilution, whereas growth was inhibited at higher wastewater concentrations in the outdoor experiment. However, there was little difference in the growth rates for the two setups. Cheng and his co-workers (2002b) therefore concluded that the wastewater most suited to duckweed cultivation should be diluted to 100 mg/L of total Kjeldahl nitrogen (which is the combined total organic and ammonia nitro-

gen) and 50 mg/L of total phosphates. This is achieved by starting to dilute untreated wastewater with recycled treated wastewater. In an investigation by Chaiprapat and his co-workers (2005) into the propagation of *Spirodela punctata* in SAM, they found that it continued to grow even after the nutrients in the water had been depleted, which they explained by suggesting that the plant species was probably using nutrients from within itself to develop. Cheng and Stomp (2009) carried out a duckweed selection process to determine which species grew best in swine wastewater. Of the twelve species they tested, *Spirodela punctata*, *Lemna gibba* and *Lemna minor* were the most responsive to this medium.

Verma and Suthar (2014) conducted a study of *Lemna gibba* growth in different concentrations of wastewater. They found that their duckweed biomass was 24.6–52.8% higher than the original feed, and that *Lemna gibba* had taken up the nitrate, phosphate and calcium content in the wastewater. Furthermore, they found that the harvested weed had a high carbohydrate and protein yield, making it suitable for both bioethanol production and animal feed.

Zhao et al. (2015) made a pilot scale investigation of four different genera of duckweed to assess their relative values for nutrient recovery from wastewater, and for what purpose their biomass production could be used. They found that even though *Spirodela* and *Wolffia* grew only in the summer months, they were more suitable for use as flavonoids than for starch production. *Lemna* and *Landoltia*, on the other hand, could be cultivated the whole year round. Furthermore, *Lemna* was most effective for wastewater treatment, whereas *Landoltia*, which had a higher starch content, should be the recommended genus for biofuels.

Therefore studies on wastewater treatment by means of duckweed have enabled the research community to gain insight into what species should be used for particular purposes like remediation, and what type is best for each of the post-harvesting applications under development.

5.1.2.3 Commercial fertilizers

Commercial fertilizers/agrochemicals have also been used to supply the necessary nutrients to cultivate duckweed. Guy et al. (1990b) grew the duckweed in a commercial liquid fertilizer, Nutrical-3, which had an N-P-K content weight

percentage of 7.5–3.0–6.5. They reported that *Lemna gibba* could be propagated in an open pond under desert conditions for periods up to 12 months, provided that fresh water was available.

Some researchers (such as Rahmani and Sternberg, 1999 and Axtell et al., 2003) used minute amounts of commercial N-P-K fertilizer once a week (that is, two grammes of fertilizer to one litre of water) to cultivate duckweed in a laboratory with the purpose of carrying out heavy metal tests. Each set of researchers found that the duckweed could reduce the lead content in the water by 70–80%. Bekcan et al. (2009) grew their duckweed (*Lemna minor*) in a chemical fertilizer using an image analysis technique to monitor its growth.

5.1.2.4 Mediums

The more common types of medium (basal salt or artificial medium) used in the laboratory to cultivate or maintain duckweed stocks for various research studies are:

- Hoagland (Miranda and Ilangovan, 1996; Moon and Stomp, 1997; Frédéric et al., 2006; Njambuya et al., 2011; Xiao et al., 2013; Huang et al., 2014; Zhao et al., 2012b);
- Murashige and Skoog (MS) (Stefaniak et al., 2002; Rahman et al., 2007; Vunsh et al., 2007; Ko et al., 2011);
- Schenk and Hildebrandt (SH) (Bergmann et al. (2000); Rival et al. (2008); Cheng and Stomp (2009); Cheng and Cheng (2012); Ge et al. (2012); Khvatkov et al. (2015); Borisjuk et al. (2015); Ziegler et al. (2015) and more than 20 other authors involved in duckweed cultivation), who grow duckweed (see Schenk and Hildebrandt—1972); and
- Hutner (Chang and Hsing, 1978; Vermaat and Hanif, 1998; Caicedo et al., 2000; Szabó et al., 2005; Xu et al., 2012b).

5.1.3 Comparisons between different media

A great deal of recent research into duckweed cultivation has entailed experiments that render it possible to make comparisons between the growth obtained using basal salt media, commercial fertilizer or wastewater, or the combinations of the two in different concentrations.

Smith and Castle (1960) studied the growth of *Spirodela polyrhiza* in four different variations of a medium. These scientists noticed that the duckweed propagated faster and appeared healthier when glucose was added to the medium. They also commented post hoc that adding ammonia salts (as opposed to ammonium ions) as a source of nitrogen would have helped the growth rate.

In the study reported by Hammouda et al. (1995), the propagation of *Lemna gibba,* in five different dilutions of the Nile River and wastewater, was better in a diluted solution of wastewater as opposed to pure river water. The duckweed system was also able to reduce algae in the same pond, and proved to have a higher protein content (47%) in wastewater than when grown in Nile water, and had a very good uptake of metal. Vermaat and Hanif (1998) investigated the growth of five different duckweed species on three different types of wastewater (two artificially made and one comprising domestic wastewater). They concluded that *Lemna gibba* and *Spirodela polyrhiza* were the species best able to grow in wastewater when compared with *Lemna minor*, *Lemna triscula* and *Wolffia arrhiza* (and a water hyacinth). Caicedo and his co-workers (2000) investigated the growth of *Spirodela polyrhiza* in three different media (domestic waste water, anaerobic sludge and a modified Hutner medium) to determine the effects of ammonia nitrogen concentration on the growth rates (and pH) of the duckweed. They found that levels of ammonia should be below 50 mg/L, because there was a decline in the rate with an increase in the level of total ammonia.

Not many authors have compared the growth of duckweed in SH and MS media. To the author's knowledge no-one has compared the growth of duckweed in MS, commercial fertilizer and homemade hydroponic medium (namely, in this chapter, tomato hydroponic medium was used). Moon and Stomp (1997) studied the different effects of basal salt media, (such as MS and SH) for frond regeneration, callus induction and growth on *Lemna gibba.* They found that MS and SH promoted flowering equally, but when benzyladenine was added to the medium, SH and Gamborg (another basal medium) caused a rise in duckweed proliferation. Furthermore, they found that a sucrose concentration of 3% also encouraged growth, but that at 8% the frond size decreased.

Ge and his co-workers (2012) cultivated *Lemna minor* in swine lagoon wastewa-

ter and SH media, and raised the starch content by implementing two changes to the system: the addition of glucose and nutrient starvation (by growing the duckweed in the dark). Yu et al. (2014) studied the cultivation of *Lemna aequinoctialis* in sewage water and SH media for use as a bioethanol precursor. They commented that SH medium is not economical when growing duckweed on a large scale. Ziegler and his co-workers (2015) suggested a strategy that helps to screen growth potential under standard conditions, and can be employed in any future research when determining growth rates. Their research involved studying under a controlled environment 39 clones from 13 species from all five genera of duckweed. The duckweed's RGR (relative growth rate) matched those reported in the literature, which led them to conclude that the clones adapt to specific local conditions. However, that research was carried out under conditions that were good for duckweed screening but not necessarily for any particular application.

5.1.4 Growth rates

The RGR (relative growth rate) of *Lemnaceae* can be defined by equation 5.1.1 (Körner and Vermaat, 1998; Driever et al., 2005; Wendeou et al., 2013). This is assuming that the duckweed follows the exponential growth of unicelluar algae (Leng et al., 1995).

$$\mathrm{RGR} = \frac{(\ln \mathrm{M}_2 - \ln \mathrm{M}_1)}{\mathrm{t}_2 - \mathrm{t}_1} \tag{5.1.1}$$

where

RGR = Relative Growth Rates—normally shown as day^{-1} but also g/(g · day)

M = Measured parameter (Frond area or dry weight); and

t = Time (day).

When Björndahl and Nilsen (1985) carried out their study, the highest RGR was 0.45/day. Oron (1994) reported that the longer the residence time, the greater the decrease in the RGR of the species. He observed that while the RGR he obtained was 0.31/day at day three, it had reduced to 0.24 per diem by day 10. Bekcan et al. (2009) had an RGR of 0.09–0.15 a day for *Lemna minor* L over 28 days when they were cultivating it in different concentrations of commercial fertilizer in a 15-litre aquarium, replenishing water that

had evaporated. Njambuya et al. (2011) studied the effect of different nutrient concentrations of Hoagland medium to reduce the competition between an invasive duckweed, *Lemna minuta,* and *Lemna minor*. They obtained a higher RGR for *Lemna minor* in low concentrations (0.09/day) than for *Lemna minuta.* However, at higher nutrient concentrations, *Lemna minuta* had a higher RGR (0.13/day). This suggests that lower nutrient concentration could help in reducing the competition with the less desirable species. In the research conducted by Ziegler and his co-workers (2015), duckweed was cultivated in 300 mL SH under continuous light for seven days. Their results showed that *Wolffia globosa* 9527 (one of the clones studied in this work) had an RGR of 0.457/day and that the genus *Lemna* RGR presented a range of 0.315–0.476/day. However, an RGR of 0.168–0.386/day was obtained for the species *Spirodela polyrhiza* when grown in a modified SH medium. Ziegler and his colleagues (2015) therefore concluded that the growth rate is a property of the clone rather than the species or genus. In Table 5.1.1, the reader can find a summary of biomass yield (BY) and RGR values reported by other scientists growing duckweed on larger surface areas. An attempt has been made to find RGR of the clones used in this chapter, however, *Wolffia globosa* 9527 was the only one that was found.

Van der Steen et al. (1998) emphasized that optimizing the growth rate of duckweed per unit area is more important than nutrient removal, but that longer retention times diminish the supply of nutrients and thus retard duckweed production. Driever and his co-workers (2005) found that when overcrowding increased, the RGR of *Lemna minor* started to decay from 0.30/day to 0.08/day. Xu and Shen (2011a) reported that a 20% removal of duckweed from swine wastewater twice a week led to higher growth rates and hence nutrient removal, as opposed to harvesting 80% of the mat every four weeks. However, Alahmady et al. (2012) recommended harvesting the weed every five days. Xu et al. (2012a) proposed that when working in ponds, researchers should cover 60% of the surface of the water. Zhang et al. (2014a) reported that variations in the surface area covered by the plant may cause variations in duckweed propagation. Therefore, depending on the system and process conditions, anyone aiming to cultivate duckweed would need to find a harvesting rate that will ensure sufficient duckweed coverage, growth and uptake of the necessary nutrients.

Table 5.1.1: Comparison with literature in larger ponds.

Author	**Species**	**Method**	**BY (g/m^2d)[a]**	**RGR(d^{-1})**	**Starch (g/m^2d)**
Reddy and DeBusk (1985)	*Spirodela polyrhiza* *Lemna minor*	Grown in 700 L of medium in an outdoor tank. Once a week medium was replaced and duckweed removed. Grown over different seasons.	-	0.081 to 0.237 (*Spirodela*) 0.118 to 0.723 (*Lemna*)	-
Oron et al. (1986)	*Spirodela polyrhiza* (Struggled to grow *Wolffia arrhiza)*	Grown in raw domestic sewage waste water in an outdoor experiment in varying concentrations of $NH4^+$ and COD. 10 vs. 20 days.	6.47 (10 d) 15.30 (20 d)	0.035 to 0.216 (10 d) 0.135 to 0.158 (20 d)	-
	Lemna gibba		3 (10 d) 12.70 (20 d)	0.031 to 0.237 (10 d) 0.162 to 0.238 (20 d)	-
Guy et al. (1990b)	*Lemna gibba*	Grown in a $12m^2$ shallow pond for 12 months in increasing amounts of Nutrical-3 commercial fertilizer. Harvested 4 times a month.	2.6 (Jan) to 7.0 (May)	0.081 to 0.191	-
Edwards et al. (1992)	*Wolffia arrhiza*	Extrapolated results from literature. Grown in different concentrations of sewage in outdoor septage-loaded tanks. (4.0–8.4 t/ha//year)	0.011–0.023	-	-
Xu et al. (2012a)	*Spirodela polyrhiza*	Grown in swine wastewater under pilot conditions in a pond for 26 weeks	10.1 (9 months)	-	1.88

[a]BY = Biomass Yield =Total dry weight produced/ (Total time multiplied by the Surface area of the medium)

5.2 Materials and methods

5.2.1 Duckweed reserve

All of the duckweed strains used in this study were sampled from the Rutgers Duckweed Stock Cooperative (RDSC; http://www.ruduckweed.org) collection. As suggested by Borisjuk et al. (2015), the strains were stored on 0.8 %(w/v) agar containing 0.5X SH salts (that is 1.6 g/L, Cat. #S6765, Sigma–Aldrich, USA) and 0.5 %(w/v) sucrose, with a pH of 5.7–6.0, at 15°C. Rutgers Lam Lab would, on occasion, also use 0.5X MS (that is 2.2 g/L, Cat. #2610024, MP Biomedicals, USA) salts to cultivate the duckweed. The species that were chosen for this experiment are listed in Table 5.2.1 below, and represent the best-performing species that were available from the RDSC. The objective of the study reported here was to screen for the strain from each genus best suited for the purpose of producing large amounts of duckweed biomass with a high starch content. The monitoring of each species was carried out on monocultures (rather than grown together in competition with one another). (See Clatworthy and Harper, 1962 and Tipping et al., 2009).

Table 5.2.1: Species for experiment.

Genus	Species	Clone	Continent	Country
Spirodela	*Polyrhiza*	8483	N.America	USA
	Polyrhiza	9509	Europe	Germany
Lemna	*Gibba*	8428	Europe	Switzerland
	Minor	DWC 112	Europe	United Kingdom
Wolffia	*Cylindracea*	7340	Africa	Tanzania
	Globosa	9527	Asia	India

Prior to use, all glassware and media were autoclaved (using an AMSCO 3021-S Gravity Autoclave) to ensure that work was done under sterile conditions. When the duckweed was plated onto the agar plates or into the flasks, hands were disinfected with 70 %(v/v) ethanol. Furthermore, all plating and transferring of species took place in the laminar flow hood, with forceps sterilized in a flame and stored in a 95 % (v/v) ethanol solution.

5.2.2 Pre-cultivation and cultivation

5.2.2.1 Sterilization

A few of the duckweed species on the agar plates could be observed to be surrounded by bacteria and/or fungi, which presented as a cloudy or mucus substance or cuts on the surface of the agar. This visual identification was confirmed by the use of a microscope. In such cases, separate steps were required to sterilize the duckweed prior to using the culture for the experiments: either surface sterilization to remove bacteria, or the addition of antibiotics to the agar plate to combat the fungus. Each of these methods is set out below.

Surface sterilization Surface sterilization of the duckweed strains had to be performed when bacteria were present on the agar plate. The procedure for surface sterilization involved collecting duckweed fronds from the RDSC plates by means of a sterile inoculation loop. The fronds were placed on a plate containing a 20 % bleach solution for two to five minutes. The time would vary depending on the genus and species. However, once the edges of the leaves started to turn white, they were transferred from the bleach solution to plates containing sterilized water, to prevent the whole duckweed frond from turning white (as recommended by Huebert et al., 1990). The bud or centre of the frond is where the meristematic zone, which is responsible for the regrowth of the duckweed, is located. The frond will die if bleaching continues. After having rinsed the duckweed twice, it was relocated to a Petri dish containing agar and 0.5X SH with no sucrose (-S). The dish was sealed with clear Parafilm M, Bemis (Cat. #52858-000, VWR, USA) before being removed from the working space in the hood. Thereafter the growth of the duckweed had to be monitored daily. It was noticed that the weed grew more slowly than usual probably because it was recovering from the surface sterilization process.

Antibiotics If fungus was found on the agar plate, the fronds were transferred to another agar plate with 50 mg/L cefotaxime (#Cat.104-25, GoldBio, USA). To prepare the plates, 500 μL of 100 mg/mL stock cefotaxime solution was added to 1 Litre of 0.5X SH media with 1 %(w/v) sucrose and with 0.8 %(w/v) agar, and in order to sterilize the solution, it was autoclaved (using an AMSCO 3021-S Gravity Autoclave). Thereafter, the solution was poured into the Petri dishes. Once the duckweed had regained a healthy appearance, it would be transferred to the agar plates with 0.5X SH. (See more in Appendix

D.2.2).

5.2.2.2 Preparation and start of experiment

Duckweed Reserve About six fronds of duckweed species from the RDSC collection were placed onto separate agar plate, using the forceps, and were gently pressed down onto the agar. After cultivating the chosen species on 0.5X SH and 0.5 %(w/v) sucrose for two weeks without any sign of contamination (by bacteria or fungus), the fronds were transferred from the solid agar plates to 200 mL of liquid media containing 0.5X SH and 0.5 %(w/v) sucrose medium in a 500 mL flask, and left to propagate for two weeks before moving it to a 2000 mL flask which contained 300 mL of 1 %(w/v) sucrose and 0.5X SH and leaving it for a further 10 days.

Commercial fertilizer preparation The recipe for the homemade hydroponic medium (denoted as HP hereafter) consisted of 1 litre of water and 3.302 ml of each of two stocks, made from the ingredients set out in Table 5.2.2.

Table 5.2.2: Homemade tomato fertilizer mixture to make 500 mL of each stock.

Stock A	**Mass (g)**
Calcium Nitrate	47.88
Potassium Nitrate	11.97
Iron Chelate (Sprint)	0.5283
Stock B	**Mass (g)**
Mono Potassium Phosphate	11.97
Potassium Sulphate	26.42
Magnesium Sulphate	23.94
STEM (Soluble Trace Element Mix)	0.5283

Sucrose was not added to the medium for the experiment. The amounts of each fertilizer added to one litre of water are shown in Table 5.2.3. The Results and Discussion section (Section 5.3 below) explains how the values were found.

All of the media, except SH and MS (which served as the controls for the experiments), were used in both sterile and non-sterile scenarios when there was enough duckweed reserve to allow it. The 0.5X SH (which is 1.6 g/L) and 0.5X MS (which is 2.2 g/L) media were autoclaved, and the HP and NPK fertilizers were sterilized by vacuum filtration using Nalgene® Rapid-Flow™ Tissue

Culture Filter Units (0.45 μm, Surfactant- Free Cellulose Acetate, Sterile). From this point, the basal salt medium and fertilizers will be referred to as medium/media.

The pH of the HP and NPK fertilizers were not altered from the initial pH, and were recorded between 6 and 6.6. The MS and SH media were modified to pH 6 using KOH.

Table 5.2.3: Composition of NPK media for experiment based on MS N of 418.0 mg/kg yielding a NPK of 420.5 mg/kg.

Type	**1 Litre**
20-20-20	2.103g
20-10-20	2.103 g
18-08-17	2.336 g
17-03-17	2.474 g
*** 05-11-26 (91 mg/kg)**	1.815 g
05-11-26 Ca	+ 0.6491 mg Ca

*based on MS K

The day before the experiment was carried out, the strain assay tubes (OD: 25 mm, ID: 23 mm, H: 150 mm; SA: 4.15E-04 m^2), with their white lids were autoclaved, and then 30 mL of well-shaken sterile and non-sterile homogeneous medium was pipetted into the tubes under the laminar flow hood. The sterilized media served as a control for the purpose of this assay, since the long-term aim was to cultivate the duckweed on unsterilized media in the 'seeding-stock' facility.

Start of the experiment The duckweed fronds were placed in the tubes containing the media, so as to cover the surface of the medium with one layer of duckweed. The trays holding the tubes of duckweed on the media (see Figure 5.2.1) were placed in growth chambers, maintained at a temperature of 26°C and supplied continuously with light at approximately 2800–4100 lux. The trays were rotated every second day. The medium was not renewed over the 21 days of the experiment conducted for each genus.

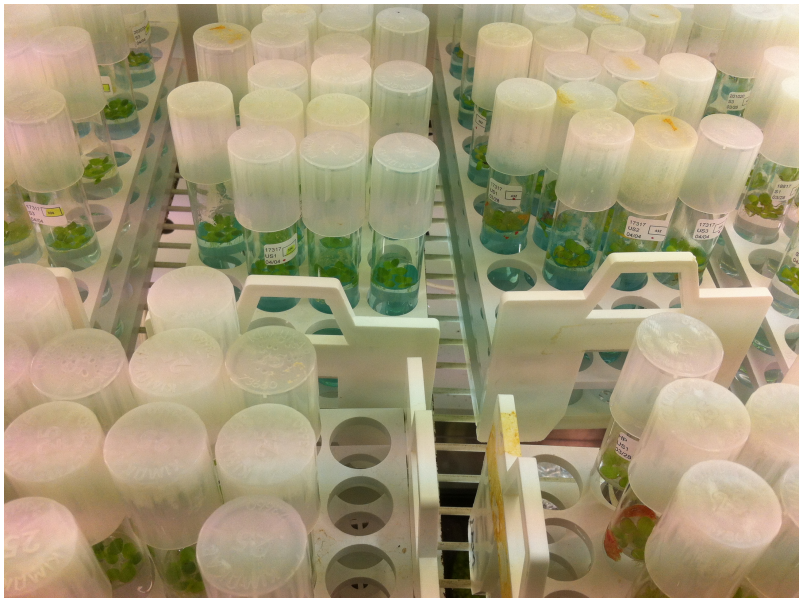


Figure 5.2.1: Trays holding tubes containing duckweed in different media.

A summary of the preparation for each species discussed above can be seen in Figure 5.2.2.

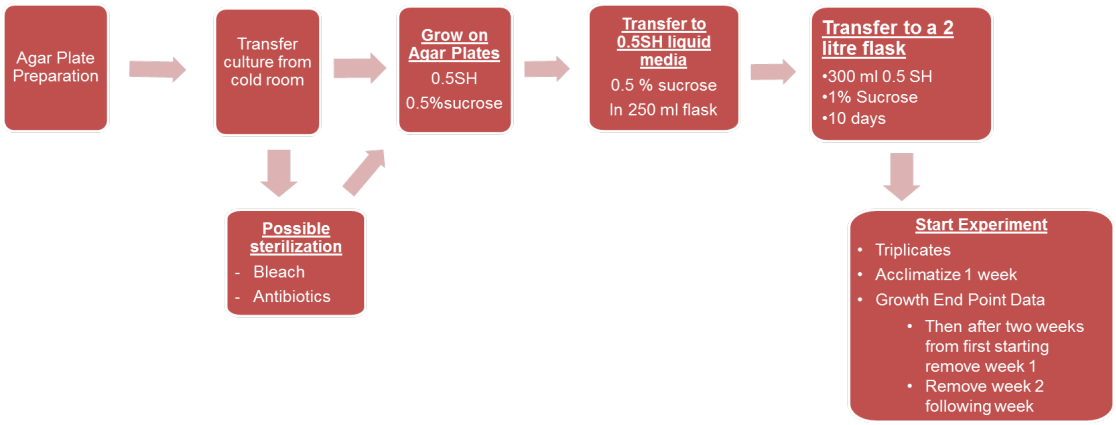


Figure 5.2.2: Process to prepare the duckweed.

5.2.2.3 Growth Rates

The method used to measure the growth rate was growth end-point analysis using destructive sampling. The species was left to acclimatize in the media for one week, after which the researcher removed three tubes of a particular species (triplicates) at week two, and another triplicate at week three. Thus two time points were generated, and 72 strain assay tubes (maximum) were needed for each duckweed species: that is, 3 (a triplicate) x 2 (two weeks of extraction) x 12 (types of media: sterile and non-sterile). Enough duckweed

had to be prepared for days one, 14 and 21, as at these times the duckweed in the tubes would be removed and processes according to the method are explained below.

The duckweed was separated from the media using a sieve. The forceps was then used to transfer the duckweed from the sieve to a paper towel. After removing droplets of the media to dry the weed, they were placed on pre-weighed boats, each of which was labelled, and placed in an oven at 45ºC for four days, after which the total mass was measured. The mass of each boat was subtracted to obtain the dry mass of duckweed. (All masses reported in this paper are dry weight.) The growth profiles shown in this paper were calculated using equation 5.2.1:

$$\text{Mass at day t} = \text{Mass measured at t} - \text{Initial mass at time 0} \tag{5.2.1}$$

Air-drying duckweed can take from five to eight days depending on the mixing of the duckweed, the amount of sunlight and the air temperatures (Bell, 1998). On the other hand, artificial drying of duckweed can be expensive (INRA, CIRAD, AFZ and FAO, 2016). In order to be balance cost and time, the oven temperature of 45ºC was chosen to dry the duckweed. Marín and Oron (2007) and Alvarado et al. (2008) had used 70ºC for 48 hours to oven dry *Lemna gibba* and *Lemna minor* respectively. Other researchers, such as Holshof et al. (2009) used 45ºC for 30 hours and found that the percent moisture of the duckweed decreased from 95% to 10%. Therefore, 45ºC for 96 hours was a mid-point chosen between air and oven drying temperatures, in relation to the different times used for drying the duckweed, this was in an attempt to balance cost and time.

In order to obtain a standard against which to measure the growth of the duckweed mass, an additional six tubes were prepared with a layer of duckweed at the start of each experiment, and this established the initial dry mass weight according to the procedure outlined above. The average starting masses ranged from 3–5 mg, depending on the species. This amount was subtracted from the mass of duckweed measured at subsequent stages of the experiment.

5.2.2.4 Starch Content

The starch content protocol was obtained from the Rutgers Lam Lab. (Details on can be found in Appendix D.2.1.) Either five or 10 mg of the dry duckweed was used in this analysis. Furthermore, the control (cornstarch) was included to monitor the protocol. To measure the dextrose concentration, a YSI 2700 Select Biochemistry Analyser was used. Thereafter a conversion factor from dextrose to starch as 0.9 was used (as per the manufacturer's manual). The total starch content (% Starch per mass dry duckweed) was calculated using the following equation 5.2.2:

$$\%\,\text{Starch} = \frac{\text{dextrose concentration}}{\text{new concentration}} \times 0.9 \times 100 \tag{5.2.2}$$

5.2.2.5 Water Test

The water test method was based on the supplier's (Hach Company) manual test kits. The test kits were used to verify the initial amount of nitrate nitrogen (Cat. #nitrate 146803; NI-11, Hach, USA, Loveland, Colorado), ammonia-nitrogen (Cat. #224100; NI-8, Hach, USA, Loveland, Colorado) and phosphate (Cat. #orthophosphate 224800; PO-19, Hach, USA, Loveland, Colorado) content in the media. The method involved taking a 5 mL sample and adding the necessary reagent for each test to the sample. The following reagents were supplied by Hach Company and used for each respective test as per the supplier's instructions:

- nitrate nitrogen: NitraVer® 5 Nitrate reagent powder;
- ammonia-nitrogen: Nessler reagent; and
- phosphate: PhosVer® 3 Phosphate reagent powder.

The solution would change colour and that colour change would correspond to a concentration of either the nitrate nitrogen, ammonia-nitrogen or phosphate content. Colour wheels were used to determine the nutrient concentration.

5.2.2.6 Statistics

The growth data points are presented as an average from the triplicates, except for the *Wolffia cylindracea*, which was carried out in duplicates. A statistical test ANOVA (analysis of variation) implemented as a two factor with replication was used on the percentage growth rate and % Starch data in Microsoft

Excel. A significant difference in the growth was identified when (P-value < (= 0.05)) and ($F > F_{critical}$). Furthermore, a t-Test: Two-Sample Assuming Unequal Variances $|t_{stat}| > t_{critical}$ and P-value < 0.05, was done for each species and its control. These results are shown in Appendix E.4.

5.3 Results

5.3.1 Mass balance

A theoretical analysis of the components present in 1X SH, 1X MS and 1X HP media was carried out before starting the experiment. The composition of the recipes can be found in Appendix F.1 and the values from the calculations can be found in Appendix E.4. The SH and MS quantity of mineral components for plant tissue and cell culture were obtained from Gamborg et al. (1976). The sources of NPK in the macro-nutrients were N (NH_4^+, NH_2^-, NO_3^-), P ($H_2PO_4^-$) and K (K^+). The micro-nutrients were ignored as they were present in minute amounts. The number of moles for all dissociating compounds containing NPK in the recipes for the SH, MS and HP media at full strength medium was calculated using molecular weights to convert them to mass. The mass percentage of NPK was arrived at by adding together each element obtained from the elemental balance of the NPK compounds. The final mg/kg of N were calculated for the following: SH (118 mg/kg), MS (419 mg/kg) and HP (65 mg/kg).

Table 5.3.1: Component NPK ratios within the macro-nutrients, of the mineral salt media for 1 L of stock solution at full strength.

(a) MS.

Source	mass %
N	19.04
P	0.8750
K	17.74

(b) SH.

Source	mass %
N	11.78
P	2.481
K	29.73

(c) HP.

Source	mass %
N	7.91
P	2.19
K	16.03

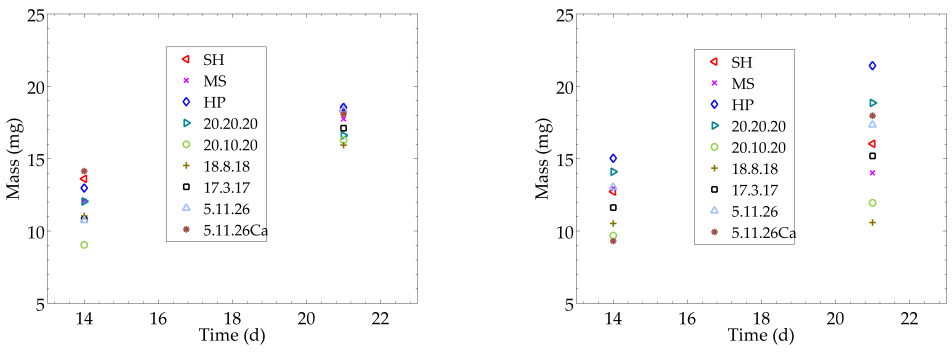
Table 5.3.1 above shows that the nitrogen and potassium were present in larger amounts when compared to the phosphate. Moon et al. (1998) found that MS was the best for duckweed growth, and therefore the composition of the NPK fertilizers was based on the mg/kg of the nitrogen content found in MS, which resulted in 420 mg/kg of nitrogen (a recommendation on the NPK bag), which represented the highest nitrogen content of the three. Using

mass %, the final mass calculations were based on the N source of MS 841 mg/L non-diluted, which resulted in a slightly higher mass than that of the P and K requirements, but met that of the N source. After that, the sample size of the commercial fertilizers needed was calculated in grammes per litre. The final amounts for a 0.5X commercial fertilizer are shown in Table 5.2.3, and are close to the dilute fertilizer to water ratio (2 g in 1 L of water) used by Axtell et al. (2003) dilute fertilizer to water ratio. An additional advantage is that a 25 lb (11.34 kg) bag at undiluted strength can provide more working volume (2200–2700 L) than the mere 2 L in one MS or SH bottle.

5.3.2 *Spirodela polyrhiza* 9509 and 8483

Spirodela was the first genus to recover from the surface sterilization process. As can be seen in Figure 5.3.1 below, the growth profiles *for Spirodela polyrhiza* 9509 and 8483 are shown for both unsterilized media and the controls. The reader can refer to Appendix E.4 for the %growth rates and %starch data for all the duckweed species, Appendix G.2 for the growth rates of all of the strains cultivated in sterilized media, and Appendix E.4.6 for the statistical data.

Spirodela polyrhiza 8483 had a faster growth rate in HP and 20-20-20 than compared to *Spirodela polyrhiza* 9509 (see Figure 5.3.1a and b).



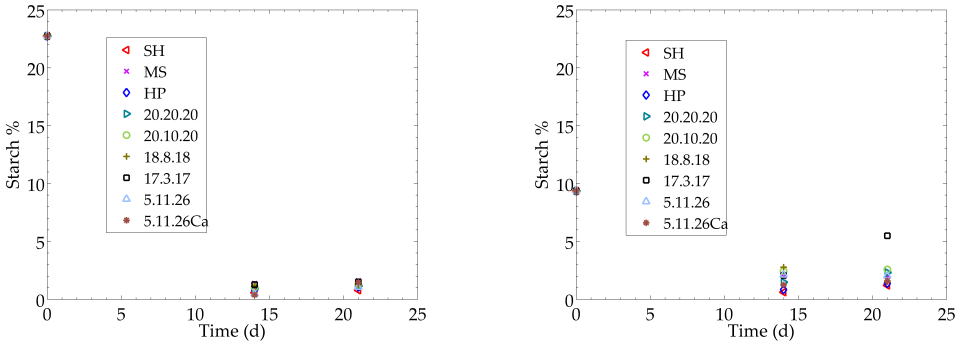
(a) *Spirodela polyrhiza* 9509. (b) *Spirodela polyrhiza* 8483.

Figure 5.3.1: Growth profile for *Spirodela polyrhiza* in non-sterile media. In each these species there was no significant difference (p> 0.05) when comparing growth in sterilized and unsterilized media at day 21. However, there was a difference (p <0.05) between the species growth rates when grown in different media at day 21.

The graphic in Figure 5.3.2, which compares the percentage of starch con-

tent in *Spirodela polyrhiza* 8483 and 9509, shows that *Spirodela polyrhiza* 8483 had a higher starch content in a NPK 17-3-17 medium. Thus, if one were interested in obtaining a higher starch content for ethanol production, 17-3-17 would be the medium of choice media. The t-test for each species showed that sterilization did not affect the growth for 9509 and 8483, but that cultivating the weed in different media did.

The t-test for the starch content of *Spirodela polyrhiza* 9509 showed that sterilization did not affect it, although the variations in medium did. As for *Spirodela polyrhiza* 8483, the MS medium and sterilization made no difference to the starch content, but cultivation in SH reduced it.



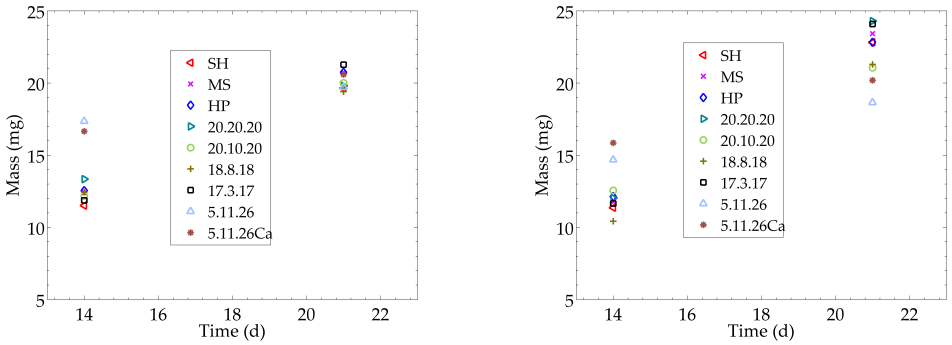
(a) *Spirodela polyrhiza* 9509. (b) *Spirodela polyrhiza* 8483.

Figure 5.3.2: Comparison of % starch content in unsterilized media for *Spirodela polyrhiza*.

When comparing the two clones at day 21, the researcher found that there was a difference (P-value=0.0009 <0.05) in growth rates when comparing the two species. There was a difference (P-value=0.004 <0.05) for starch content between the species. More precisely, a higher growth rate and starch were observed for *Spirodela polyrhiza* 8483 in HP and 17-3-17 respectively.

5.3.3 *Lemna gibba* 8428 and *Lemna minor* DWC 112

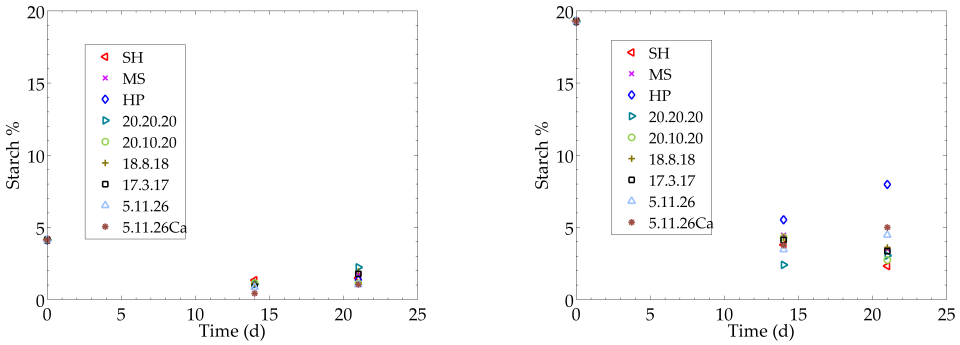
For the comparison between the controls and unsterilized media for *Lemna gibba* 8428 and *minor* DWC 112, the reader is referred to the growth profiles in Figure 5.3.3, and the starch content in Figure 5.3.4. The t-test showed that sterilization made no difference to the growth rates of each species, whether cultivated in MS or in SH.



(a) *Lemna gibba* 8428. (b) *Lemna minor* DWC 112.

Figure 5.3.3: Growth profile comparison for *Lemna* in unsterilized media and controls. For both species, at day 21 there was no difference (p>0.05) in growth in unsterilized media and 1) sterilized media or 2) the SH and MS controls.

As for the starch t-test for 8428, the medium made no difference to the starch content, although sterilization did as higher amounts of starch were measured in sterilized and 20-10-20 media. The DWC 112 t-test showed the differences in media affected the starch content, but sterilization did not. The starch obtained in SH was lower than that of duckweed grown in unsterilized medium, which indicated that SH is unsuitable for propagation of this species.



(a) *Lemna gibba* 8428. (b) *Lemna minor* DWC 112.

Figure 5.3.4: Comparison of % starch content in non-sterile media and controls for *Lemna*.

Comparing the two species, *Lemna minor* DWC 112 had the highest % starch content compared with *Lemna gibba* 8428 (P-value= 3E-12 <0.05; F>Fcrit) and also the best growth rate (P-value=1E-10 <0.05; F>Fcrit) in 20-20-20 or 17-3-17.

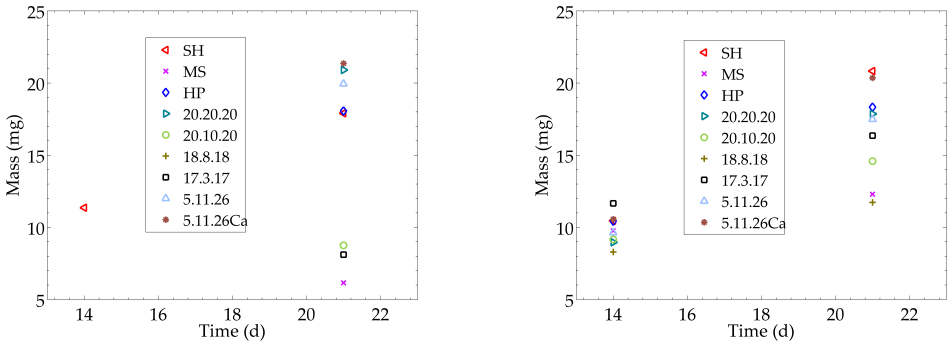
Lemna minor DWC 112 looked healthy in 17-3-17, even though it had a slightly higher growth rate in 20-20-20. It is thus recommended that *Lemna*

minor DWC 112 should be cultivated in 17-3-17 as opposed to 20-20-20.

5.3.4 *Wolffia cylindracea* (7340) and *globosa* (9527)

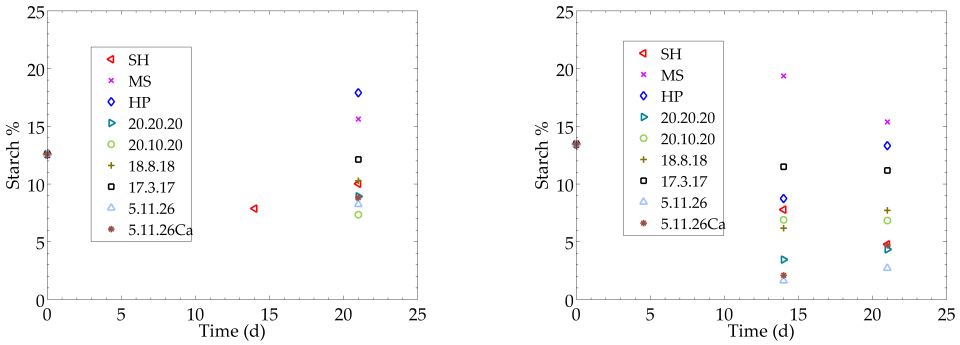
Wolffia was the most difficult genus to produce in enough quantity to provide reserve stock for the experiment. *Wolffia Globosa* 9527 was grown in two sterilized media, SH and MS, and in the available unsterilized media. The results can be seen in Figure 5.3.5b. However, there was sufficient stock to grow for *Wolffia cylindracea* 7340 only in duplicates, and one set of sterilized SH medium, while in the case of unsterilized media there was only enough duckweed for one time-point. These results are presented in Figure 5.3.5a. However, *Wolffia cylindracea* grew much better in the unsterilized media than in sterilized conditions. The resulting % starch analysis for *globosa* and *cylindracea* can be seen in Figure 5.3.6.

The t-test for both of these species showed that while MS as the medium produced good starch content, it was not conducive to species growth. The starch reading for *Wolffia Globosa* 9527 was low when cultivated In SH, although the growth rate was high. *Wolffia cylindracea* 7340 did not grow in MS, but in the same medium had a higher starch content.



(a) *Wolffia cylindracea* (7340). (b) *Wolffia globosa* (9527).

Figure 5.3.5: Comparison of the growth profiles for two strains of *Wolffia* in unsterilized media and controls. For each species, at day 21, the t-test showed that there was a difference in the growth rate ($p<0.05$) between the unsterilized media and the controls: MS and SH.



(a) *Wolffia cylindracea* (7340). (b) *Wolffia globosa* (9257).

Figure 5.3.6: Comparison of % Starch for *Wolffia* in unsterilized and control media.

A comparison between *Wolffia globosa* and *cylindracea* highlighted there was a difference between the media used to grow the duckweed (P-value=6E-09<0.05; F>Fcrit). However, a comparison between the two with regard to the starch content indicated that *cylindracea* produced a higher starch content (P-value=1E-07<0.05; F>Fcrit) in HP than in 17-3-17. *Wolffia cylindracea* grew best in 5-11-26-Ca, followed by 20-20-20, and had a high starch content in both HP and 17-3-17. The difficulty the writer experienced in cultivating enough *cylindracea* for the experiment resulted in a time loss that favoured using *globosa* over *cylindracea,* even though the latter had a higher growth rate. Nonetheless, the starch content and growth rates of *cylindracea* are most suitable for cultivation of *Wolffia*, despite the difficulties attendant on its cultivation.

The reader can refer to Appendix G.2 for other figures that were not shown in this chapter.

5.4 Discussion

5.4.1 Comparison of different NPK compositions

The Hach Company water test (explained in Section 5.2.2.5) was carried out before starting cultivation of the duckweed, to confirm that the concentrations of ammonia-nitrogen, nitrate-nitrogen and phosphorous matched the values stated on the fertilizer's guaranteed analysis chart. (This can be found in Appendix G.2).

The potassium levels were similar to those of NPK and MS, SH and HP. However, the 17-3-17 or 18-8-17 Peat-Lite High Mag special both contained lower amounts of phosphorous than 20-20-20 water-soluble fertilizer. Using the results of the water test and the details on the supplier sheet, it was established that 17-3-17 held high levels of nitrate-nitrogen; medium levels of ammonia-nitrogen; low levels of phosphorous, and medium levels of potassium. From the same sources, it was confirmed that 20-20-20 contained low levels of nitrate-nitrogen; medium levels of ammonia-nitrogen; high levels of phosphorous and medium levels of potassium.

Wang et al. (2014c) investigated the effect of cultivating duckweed in different amounts of ammonium-N. They found that the duckweed could grow in 7-84 mg/L but not 840 mg/L, and was optimal at 28 mg/L. However, the research of Cheng et al. (2002a) studied the successful growth of duckweed (*Spirodela punctata)* in levels as high as 240 mg/L (of ammonium-N) and 31 mg/L (of phosphorus). The nutrients in NPK are similar to those in swine-wastewater (Cheng et al., 2002a), the main components of which are 262 mg/L (TKN) and 88 mg/L (TP) (Cheng and Stomp, 2009). The concentrations were fairly high, but the duckweed had already shown its ability to grow in high concentrations of nitrogen (which agrees with the findings of Xu and Shen, 2011b on their use of ammonia-nitrogen).

5.4.2 SH and MS as controls

SH and MS served as suitable controls, even though Huebert et al. (1990) warn that unknown precipitates may be produced when autoclaved. The formation of precipitates is dependent to some extent on the concentration of salts and the pH, however, precipitates should not form at relatively acidic pH (a ph 6.0 was used) and/or low concentrations of 1X or less concentration (for this chapter, SH and MS concentrations were at 0.5X) (Lam, 2016). Furthermore, to ensure a homogeneous solution was used in the experiment (in case precipitates formed), the mediums used were well shaken before pipetting them into the strain assay tubes.

As mentioned earlier some differences were found between growth in the unsterilized NPK fertilizers and in either SH or MS. For example, in some cases the growth rates of the duckweed species were much higher than those achieved in SH and MS.

When considering the controls *per se*, if one wished to cultivate duckweed in the laboratory, one would need to determine whether SH or MS was suitable. Table 5.4.1 below compares the percentage growth (which is the final mass divided by the initial mass) obtained in the six species cultivated in both SH and MS. A significant difference, where $p<0.05$ can be seen when one compares the results.

To sum up the information given in this section, *Spirodela* 9509 and *Lemna* DWC 112 are the species most suited to propagation in the laboratory, in either SH or MS ($p>0.05$; F<Fcrit). For faster growth rates, *Wolffia Cylindracea* and *globosa* should be grown in SH ($p<0.05$). However, for a high starch content *Spirodela* 9509, *Wolffia* 7340 and 9527 should be cultivated in MS ($p<0.05$; F>Fcrit). The remaining species studied, 8483, 8428, and DWC 112, can be grown in either SH or MS. Refer to Appendix E.4.8 for the tables listing the statistical values.

It is also important, when cultivating duckweed in the laboratory, to consider the optimal medium for the clones. The lowest percentage growth calculated for *Wolffia* is below 300% in MS. The unsterilized NPK, however, provided higher growth rates and starch contents than duckweed cultivated in to MS and SH. The SH medium does not yield high starch content in duckweed but does stimulate its growth. The MS medium, on the other hand, results in a high starch content for 7340 and 9527 but low growth. As can be seen, therefore, when comparing MS and SH, the growth rate is the inverse of starch content, a conclusion also reached by Xiao et al. (2013).

Table 5.4.1: Comparison of duckweed grown in controls at 21 days.

	MS	**SH**	**MS**	**SH**
	% Growth		**% Starch**	
Spirodela polyrhiza 8483	448	498	1.54	1.348
Spirodela polyrhiza 9509	512	524	2.072	0.7291
Lemna gibba 8428	472	452	1.697	1.484
Lemna minor DWC 112	600	587	3.49	2.31
Wolffia cylindracea 7340	270	597	15.6	10
Wolffia globosa 9527	401	610	15.73	4.763

5.4.3 Relative growth rates

For the amount of total ammonium available in the medium, the RGR matched that reported by Caicedo et al. (2000) (see Table 5.4.2). However, the RGRs presented in this chapter are much lower than those shown in the table. A possible remedy could be to increase the surface of the experimental area to improve the growth rates. Table 5.1.1 provides an example of the RGRs reported by researchers who had larger areas/ volumes in which to cultivate duckweed, which would raise the growth rate, as would enrichment with a carbon source and continual harvesting of the duckweed. For example, when comparing the researchers in Table 5.1.1 with the data obtained from this chapter's experiment, the surface area that was used in this chapter was a test tube size of 4.15E-04 m^2 with a percent growth rate of 618-690%. However, Yu et al. (2014) propagated duckweed in SH media with sucrose using an area of 0.24 $m^2$and obtained a 1500 % growth. The difference could be attributed to the larger surface area, and the source of carbon.

Wolffia globosa had one of the highest growth rates, but even though *Wolffia cylindracea* was the most difficult species to cultivate and prepare, it had the highest starch content. Most of the duckweed strains did not look healthy after being grown in 18-8-17 or 20-10-20 or 5-11-26 without Ca. However, most of the species enjoyed growing in HP and 5-11-26 with calcium (which is like HP in terms of both composition and cost).

Spirodela polyrhiza 9509 (P-value=4.2E-12<0.05) and 8483 (P-value=1.7E-12<0.05) had a higher growth percentage in HP, which contained low levels of nitrate-nitrogen, ammonia-nitrogen, phosphate and potassium. The starch content of both was higher when grown in 17-3-17, and it was statistically different for 9509 (P-value=2.5E-03<0.05). However the starch content was not statistically different for *Spirodela polyrhiza* 8483 (P-value=2.9E-01>0.05), and that of *Lemna gibba* in 20-20-20 (P-value=1E-04<0.05). On the other hand, *Lemna minor* (P-value=0.0404<0.05) had a raised starch content in HP medium, including *Wolffia globosa* and *cylindracea* (however, there is no statistical data available since additional experiments could not be carried out, as mentioned earlier in Section 5.3.4). As for *Lemna gibba*, no difference could be observed between cultivation in sterilized and sterile media (P-value=0.053>0.05). The reader can refer to Appendix E.4.8 for the tables listing the statistical values.

Table 5.4.2: Comparison with literature of duckweed grown in the laboratory.

Author	**Species**	**Method**	**% Growth[a]**	**BY (g/m^2d)[b]**	**RGR (day^{-1})[c]**	**Starch (g/m^2d)**
Caicedo et al. (2000)	*Spirodela polyrhiza*	Fed-batch renewal over 14- days. in 250 mL containers. Compared three different mediums with different concentrations of NH_4-N (100–3.5 mg).	-	-	0.1–0.30	-
Cheng et al. (2002b)	*Lemna minor* 8627	In vitro test in 25.8cm^2container with 150 mL: four dilutions of SAM. Adjusted pH; destructive sampling every 48 h. Lasted 22–24 days.	-	28.6	-	-
Wang et al. (2014c)	*Lemna minor* L.	Hoagland solution with different concentrations of ammonium. Medium replaced every day for 7 days.	-	-	0.2 to 0.3	-
Ge et al. (2012)	*Lemna minor*	Compared SH with 10 g/L sucrose (27 days) and swine lagoon wastewater (15–18 days) in 100 mL of water.	2250* (Swine) 375* (SH)	3.5 (Swine) 14.1 (SH)	-	115(w/w) Swine 13%(w/w) SH
Yu et al. (2014)	*Lemna aequinoctialis* 6000	Compared diluted sewage water (SW) and 10 g/L sucrose in SH for 4-weeks in 60x40x10 cm tank; harvested every 6 days.	1500 * (SH) 750* (swine)	10 (SH) 4.3 (swine)	-	39%(w/w) SH 34%(w/w) SW
Zhao et al. (2015)	*Wolffia globosa* 0222	Compared for 6 months in parallel pilot-scale wastewater treatment system (WTS). *Wolffia* and *Spirodela* did not grow well in the winter months. Also compared *Lemna* and *Landoltia* (not shown) for a full year. Conducted accumulation of starch in duckweed (nutrient starvation) tests for 8 days in the lab.	-	3.30 (WTS) 1.96 (Starch Exp)	-	0.95
	Spirodela polyrhiza 0225		-	5.24 (WTS) 2.55 (Starch Exp)	-	1.44
This study	Compared 5 species from three different genera in strain assay tubes 30 mL. Non-renewal of medium. Over 21 days. Compared growth in MS, SH and commercial NPK, with a surface area of 4.15E0-4m^2.					
	Spirodela polyrhiza 8483	Best growth homemade HP media. Best Starch 17 - 3 -17	633.2	2.454	0.11 (day 14) 0.088 (day 21)	0.0956 (5.30%(w/w))
	Lemna minor DWC 112	Best growth in commercial fertilizer 20-20-20. Best High Starch HP	618.5	2.783	0.091 (day 14) 0.087 (day 21)	0.208 (7.97%(w/w))
	Wolffia cylindracea 7340	Best growth in commercial fertilizer 5-11-26 Ca. Best High Starch HP	693.1	2.447	0.092 (day 21)	0.37 (17%(w/w))

[a]%Growth is the final mass divided by the initial mass. [b]BY = Biomass Yield = (Total dry weight produced)/ (Total time.Surface area of the medium). [c]RGR = Relative Growth Rate = [ln(Mass final) - ln(Mass initial)]/(Time final - Time Initial) based on dry weight. *Calculated by this chapter.

5.4.4 Cost comparison of the media used to grow the duckweed

The cost comparison of the media can be seen in Table 5.4.3, which takes into account the cost of the media only. It excludes the start-up/ laboratory costs, such as consumables, since this calculation is a cost comparison to find the most cost-effective media. The costing is based on a one-time preparation per litre. If those additional costs were to be included into the cost comparison, it would increase all of the values shown in Table 5.4.3 by a constant value. A source of carbon, such as sucrose was not included. However, if sucrose were to be added at 1 %(w/v), all the values shown in the table would increase by $0.53 (1 litre) and $5,250 (10000 litres).

MS was the most expensive medium to use in the laboratory, as opposed to SH, and the cheapest commercial fertilizer was 20-10-20.

Table 5.4.3: Cost of the types of media based on volume with 0.5 strength, with no sucrose or carbon source, at 2013 prices.

Media	**SH**	**MS**	**20-20-20**	**20-10-20**
1 litre	$ 2.20	$ 4.75	$0.01	$0.01
10 000 litres	$22,000.00	$47,500.00	$67.42	$66.76

Media	**18-8-17**	**17-3-17**	**5.11.26 Ca***	**5.11.26****	**HP**
1 litre	$0.01	$0.01	$0.07	$0.01	$0.08
10 000 litres	$81.07	$80.52	$667.47	$67.47	$ 805.97

*if based on N cost $912 or **$312

For costing purposes, the following matches are made to achieve the best conditions for growing specific strains of duckweed in a cost-effective manner with some level of starch content to prepare for the seeding-stock facility, instead of using MS and SH. The growth rates were the focus of the list of cultivation conditions to propagate particular duckweed species most efficiently:

- *Spirodela polyrhiza* 8483: in HP with a small amount of starch, followed by 20-20-20. Or 17-3-17 for a high starch content.
- *Lemna minor* DWC 112: in 17-3-17 followed by HP for high starch and growth rate or just 20-20-20 for a high growth rate.
- *Wolffia cylindracea* 7340: 5-11-26 Ca followed by 20-20-20, but HP for a high starch content.

5.4.5 Experimental recommendations

The author acknowledges the following should be addressed to expand the research described in this chapter:

1. Since the aim of this chapter was to compare the growth in different media, it would be interesting, to carry out a Hach Company water test (testing the NPK) on the media after the completion of the experiment. This recommendation for future work would be useful if one wanted to test the assumption if growth increases, the nutrients decrease.

2. An application of duckweed is to be used in the secondary treatment of waste water treatment cleaning. If there is concern to grow duckweed in municipal water, the nutrient will be fairly constant as humans will be most likely to excrete the same waste weekly. Further experiments can be carried out to alter the composition of the NPK fertilizers and thus optimize the growth even more. Investigate varying concentrations of NPK such as based on SH nitrogen ppm (or mg/kg).

3. Increase the starch content by transferring to medium poor solution as done by Tao et al. (2013) who suggested that duckweed had a relatively low starch percentage in nutrient-rich growth. As a result, when the researchers transferred their *Landoltia punctata* from Hoagland medium to distilled water after 168 h the starch content increased from 3% to 48%.

4. Supply a source of carbon for growth and additional nutrients.

5.5 Conclusion

Even though some critics say that commercial fertilizers (agrochemicals) are costly and that some of them may contribute to environmental pollution, duckweed can grow in them. Thus it can be used as an alternative feed to wastewater treatment when one would like to propagate large amounts of duckweed in order to cover the surface of the water in stock facilities. This stock could then be used for either cleaning wastewater or using the weed as a biofuel or a food source. In the research described in this chapter it was found that duckweed is able to grow in high levels of nutrients, and in diluted amounts of fertilizer to achieve higher growth and starch content than in basal salt media.

The duckweed strains demonstrated their potential to be used as 'seeding-stock' for a facility (in the kilogram range) to convert duckweed to either biofuels or food on the metric tonne scale. The writer found that different fertilizers yielded varying growth rates and starch content. However, an NPK of 20-20-20 achieved one of the three best growth rates for all three strains. Some media did not propagate the duckweed well, as the lowest values of the percentage growth were close to 300%. However, the top three strains all had a growth rate closer to 700%.

This research has contributed to the study of media, because it shows that it is possible to grow duckweed in commercial fertilizers in the laboratory (or even on a much larger scale) in a cost-effective manner. On the other hand, a discussion of the best basal salt media to use when cultivating duckweed species in the laboratory has been included. Although the RGR values of the best species were similar, it was the starch content that was the most variable from one strain to another. A source of carbon or addition of nutrients (at an appropriate time in the process) is recommended for future experimental studies, so as to provide a sufficient quantity of duckweed to use it in the food and biofuel sectors.

References

Al-Nozaily, F., Alaerts, G., Veenstra, S., 2000. Performance of duckweed-covered sewage lagoons-II. Nitrogen and phosphorus balance and plant productivity. Water Research 34 (10), 2734–2741.

Alahmady, K. K., Stevens, K., Atkinson, S., 2012. Effects of hydraulic detention time, water depth, and duration of operation on nitrogen and phosphorus removal in a flow-through duckweed bioremediation system. Journal of Environmental Engineering 139 (2), 160–166.

Allam, A., Tawfik, A., El-Saadi, A., Negm, A., 2014. Potentials of using duckweed (*Lemna gibba*) for treatment of drainage water for reuse in irrigation purposes. Desalination and Water Treatment 0 (0), 1–9.

Alvarado, S., Guédez, M., Lué-Merú, M. P., Nelson, G., Alvaro, A., Jesús, A. C., Gyula, Z., 2008. Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*Eichhornia crassipes*) and lesser duckweed (*Lemna minor).* Bioresource technology 99 (17), 8436–8440.

Appenroth, K.-J., Crawford, D., Les, D., 2015. After the genome sequencing of

duckweed–how to proceed with research on the fastest growing angiosperm? Plant Biology 17 (s1), 1–4.

Awuah, E., Oppong-Peprah, M., Lubberding, H., Gijzen, H., 2004. Comparative performance studies of water lettuce, duckweed, and algal-based stabilization ponds using low-strength sewage. Journal of Toxicology and Environmental Health, Part A 67 (20-22), 1727–1739.

Axtell, N. R., Sternberg, S. P., Claussen, K., 2003. Lead and nickel removal using *Microspora* and *Lemna minor*. Bioresource Technology 89 (1), 41–48.

Baliban, R. C., Elia, J. A., Floudas, C. A., Xiao, X., Zhang, Z., Li, J., Cao, H., Ma, J., Qiao, Y., Hu, X., 2013. Thermochemical conversion of duckweed biomass to gasoline, diesel, and jet fuel: process synthesis and global optimization. Industrial and Engineering Chemistry Research 52 (33), 11436–11450.

Bekcan, S., Atar, H., Beyaz, A., 2009. Measurement of the effects of liquid fertilizers at the different levels on duckweed (*Lemna Minor L.*) growth using image analysis technique. Biotechnology and Biotechnological Equipment 23 (2), 1205–1209.

Bell, R., 1998. Duckweed–a potential high protein feed source for domestic animals and fish. Rural industries research and development corporation, Pub (98/148).

Bergmann, B., Cheng, J., Classen, J., Stomp, A.-M., 2000. In vitro selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation. Bioresource Technology 73 (1), 13–20.

Bitcover, E. H., Sieling, D. H., 1951. Effect of various factors on the utilization of nitrogen and iron by *Spirodela Polyrhiza* (L.) Schield. Plant Physiology 26 (2), 290–303.

Björndahl, G., Nilsen, S., 1985. Growth potential of *Lemna gibba*: Effect of CO_2 enrichment at high photon flux rate. Aquatic Botany 22 (1), 79–82.

Borisjuk, N., Chu, P., Gutierrez, R., Zhang, H., Acosta, K., Friesen, N., Sree, K., Garcia, C., Appenroth, K., Lam, E., 2015. Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. Plant Biology 17 (s1), 42–49.

Caicedo, J., Van der Steen, N., Arce, O., Gijzen, H., 2000. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Water Research 34 (15), 3829–3835.

Chaiprapat, S., Cheng, J., Classen, J., Liehr, S., 2005. Role of internal nutrient

storage in duckweed growth for swine wastewater treatment. Transactions of the American Society of Agricultural Engineers 48 (6), 2247–2258.

Chang, W.-C., Hsing, Y.-i., 1978. Callus formation and regeneration of frond-like structures in *Lemna perpusilla* 6746 on a defined medium. Plant Science Letters 13 (2), 133–136.

Chen, Q., Jin, Y., Zhang, G., Fang, Y., Xiao, Y., Zhao, H., 2012. Improving production of bioethanol from duckweed (*Landoltia punctata*) by pectinase pretreatment. Energies 5 (8), 3019–3032.

Cheng, J., Bergmann, B. A., Classen, J. J., Stomp, A. M., Howard, J. W., 2002a. Nutrient recovery from swine lagoon water by *Spirodela punctata*. Bioresource Technology 81 (1), 81–85.

Cheng, J., Landesman, L., Bergmann, B., Classen, J., Howard, J., Yamamoto, Y., 2002b. Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. Transactions of the American Society of Agricultural Engineers 45 (4), 1003–1010.

Cheng, J., Stomp, A., 2009. Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. Clean–Soil, Air, Water 37 (1), 17–26.

Cheng, L.-J., Cheng, T.-S., 2012. Oxidative effects and metabolic changes following exposure of greater duckweed (*Spirodela polyrhiza*) to diethyl phthalate. Aquatic Toxicology 109 (0), 166–175.

Clatworthy, J., Harper, J. L., 1962. The comparative biology of closely related species living in the same area v. inter-and intraspecific interference within cultures of *Lemna* spp. and *Salvinia Natans*. Journal of Experimental Botany 13 (2), 307–324.

Cui, W., Cheng, J., 2015. Growing duckweed for biofuel production: a review. Plant Biology 17 (s1), 16–23.

Driever, S. M., van Nes, E. H., Roijackers, R. M., 2005. Growth limitation of *Lemna minor* due to high plant density. Aquatic Botany 81 (3), 245–251.

Edwards, P., Hassan, M., Chao, C., Pacharaprakiti, C., 1992. Cultivation of duckweeds in septage-loaded earthen ponds. Bioresource Technology 40 (2), 109–117.

Ennabili, A., Ater, M., Radoux, M., 1998. Biomass production and NPK retention in macrophytes from wetlands of the Tingitan Peninsula. Aquatic Botany 62 (1), 45–56.

Frédéric, M., Samir, L., Louise, M., Abdelkrim, A., 2006. Comprehensive mod-

eling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication. Water Research 40 (15), 2901–2910.

Fulton, B. A., Brain, R. A., Usenko, S., Back, J. A., King, R. S., Brooks, B. W., 2009. Influence of nitrogen and phosphorus concentrations and ratios on *Lemna gibba* growth responses to triclosan in laboratory and stream mesocosm experiments. Environmental Toxicology and Chemistry 28 (12), 2610–2621.

Gale, J., Smernoff, D., Macler, B., MacElroy, R., 1989. Carbon balance and productivity of *Lemna gibba*, a candidate plant for CELSS. Advances in Space Research 9 (8), 43–52.

Gamborg, O., Murashige, T., Thorpe, T., Vasil, I., 1976. Plant tissue culture media. In Vitro Cellular and Developmental Biology-Plant 7 (7), 473–478.

Ge, X., Zhang, N., Phillips, G. C., Xu, J., 2012. Growing *Lemna minor* in agricultural wastewater and converting the duckweed biomass to ethanol. Bioresource Technology 124 (0), 485–488.

Guy, M., Granoth, G., Gale, J., 1990b. Cultivation of *Lemna gibba* under desert conditions. I: Twelve months of continuous cultivation in open ponds. Biomass 21 (2), 145–156.

Hammouda, O., Gaber, A., Abdel-Hameed, M., 1995. Assessment of the effectiveness of treatment of wastewater-contaminated aquatic systems with *Lemna gibba*. Enzyme and Microbial Technology 17 (4), 317–323.

Hillman, W., Culley Jr, D., 1978. The uses of duckweed: The rapid growth, nutritional value, and high biomass productivity of these floating plants suggest their use in water treatment, as feed crops, and in energy-efficient farming. American Scientist 66 (4), 442–451.

Holshof, G., Hoving, I., Peeters, E., 2009. Eendenkroos: van afval tot veevoer= duckweed from waste to animal feed. Rapport/Wageningen UR Livestock Research (Rapport 306), 1–31.

Huang, M., Fang, Y., Xiao, Y., Sun, J., Jin, Y., Tao, X., Ma, X., He, K., Zhao, H., 2014. Proteomic analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation. Industrial Crops and Products 59 (0), 299–308.

Huebert, D. B., McIlraith, A., Shay, J., Robinson, G., 1990. Axenic culture of *Lemna trisulca* L. Aquatic Botany 38 (2), 295–301.

Huebert, D. B., Shay, J. M., 1991. The effect of external phosphorus, nitrogen and calcium on growth of *Lemna trisulca*. Aquatic Botany 40 (2), 175–183.

INRA, CIRAD, AFZ and FAO, 2016. Feedipedia Animal feed resources inform-

ation system: Duckweed. [Online]. Available at: http://www.feedipedia.org/node/15306, [accessed 22 January 2016].

Jain, S., Vasudevan, P., Jha, N., 1990. *Azolla pinnata* R. Br. and *Lemna minor* L. for removal of lead and zinc from polluted water. Water Research 24 (2), 177–183.

Khvatkov, P., Chernobrovkina, M., Okuneva, A., Shvedova, A., Chaban, I., Dolgov, S., 2015. Callus induction and regeneration in *Wolffia arrhiza* (L.) Horkel ex Wimm. Plant Cell, Tissue and Organ Culture 120 (1), 263–273.

Ko, S.-M., Sun, H.-J., Oh, M., Song, I.-J., Kim, M.-J., Sin, H.-S., Goh, C.-H., Kim, Y.-W., Lim, P.-O., Lee, H.-Y., Kim, S., 2011. Expression of the protective antigen for PEDV in transgenic duckweed, *Lemna minor*. Horticulture, Environment, and Biotechnology 52 (5), 511–515.

Körner, S., Vermaat, J., 1998. The relative importance of *Lemna gibba* l., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. Water Research 32 (12), 3651–3661.

Lam, E., 2016. Autoclaving SH and MS. Personal Communication, 18 February 2016.

Lam, E., Appenroth, K. J., Michael, T., Mori, K., Fakhoorian, T., 2014. Duckweed in bloom: the 2nd international conference on duckweed research and applications heralds the return of a plant model for plant biology. Plant Molecular Biology 84 (6), 737–742.

Leng, R., Stambolie, J., Bell, R., 1995. Duckweed-a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development 7 (1), 36.

Marín, C. M. D.-C., Oron, G., 2007. Boron removal by the duckweed *Lemna gibba*: a potential method for the remediation of boron-polluted waters. Water research 41 (20), 4579–4584.

Mbagwu, I., Adeniji, H., 1988. The nutritional content of duckweed (*Lemna paucicostata* Hegelm.) in the Kainji Lake area, Nigeria. Aquatic Botany 29 (4), 357–366.

Miranda, M., Ilangovan, K., 1996. Uptake of lead by *Lemna gibba* L.: Influence on specific growth rate and basic biochemical changes. Bulletin of Environmental Contamination and Toxicology 56 (6), 1000–1007.

Mkandawire, M., Lyubun, Y. V., Kosterin, P. V., Dudel, E. G., 2004. Toxicity of arsenic species to *Lemna gibba* L. and the influence of phosphate on arsenic bioavailability. Environmental Toxicology 19 (1), 26–34.

Moon, H., Rajbhandari, N., Stomp, A., 1998. Effect of media components and

phytohormones on in vitro frond proliferation of *Lemna gibba* G3 and 24 additional *Lemna gibba* strains. Plant Resources 1 (2), 98–104.

Moon, H., Stomp, A., 1997. Effects of medium components and light on callus induction, growth, and frond regeneration in *Lemna gibba* (duckweed). In Vitro Cellular and Developmental Biology-Plant 33 (1), 20–25.

Muradov, N.and Fidalgo, B., Gujar, A., T-Raissi, A., 2010. Pyrolysis of fast-growing aquatic biomass *Lemna minor* (duckweed): Characterization of pyrolysis products. Bioresource Technology 101 (21), 8424–8428.

Muradov, N., Fidalgo, B., Gujar, A., Garceau, N., T-Raissi, A., 2012. Production and characterization of *Lemna minor* bio-char and its catalytic application for biogas reforming. Biomass and Bioenergy 42 (0), 123–131.

Muradov, N., Taha Elfouly, M., Miranda, A., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A., Mouradov, A., 2014. Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. Biotechnology for Biofuels 7 (1), 1–17.

Nalbur, E., Aka, L., Bayhan, H., 2004. Nitrogen removal during secondary treatment by aquatic systems. Water Science and Technology 48 (11), 355–361.

Njambuya, J., Stiers, I., Triest, L., 2011. Competition between *Lemna minuta* and *Lemna minor* at different nutrient concentrations. Aquatic Botany 94 (4), 158–164.

Oron, G., 1994. Duckweed culture for wastewater renovation and biomass production. Agricultural Water Management 26 (1), 27–40.

Oron, G., Porath, D., Wildschut, L. R., 1986. Wastewater treatment and renovation by different duckweed species. Journal of Environmental Engineering 112 (2), 247–263.

Rahman, M. A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M. M., 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. Chemosphere 69 (3), 493–499.

Rahmani, G. N. H., Sternberg, S. P., 1999. Bioremoval of lead from water using *Lemna minor*. Bioresource Technology 70 (3), 225–230.

Reddy, K., DeBusk, W., 1985. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: II. Azolla, Duckweed, and Salvinia. Economic Botany 39 (2), 200–208.

Reid, M., Bieleski, R., 1970. Response of *Spirodela oligorrhiza* to phosphorus deficiency. Plant Physiology 46 (4), 609–613.

Rengel, Z., Batten, G., Crowley, D. D., 1999. Agronomic approaches for im-

proving the micronutrient density in edible portions of field crops. Field Crops Research 60 (1), 27–40.

Rival, S., Wisniewski, J.-P., Langlais, A., Kaplan, H., Freyssinet, G., Vancanneyt, G., Vunsh, R., Perl, A., Edelman, M., 2008. *Spirodela* (duckweed) as an alternative production system for pharmaceuticals: a case study, aprotinin. Transgenic Research 17 (4), 503–513.

Roijackers, R., Szabo, S., Scheffer, M., 2004. Experimental analysis of the competition between algae and duckweed. Archiv für Hydrobiologie 160 (3), 401–412.

Satake, K., Shimura, S., 1983. Carbon dioxide assimilation from air and water by duckweed *Spirodela polyrrhiza* (L.) Schleid. Hydrobiologia 107 (1), 51–55.

Schenk, R. U., Hildebrandt, A., 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canadian Journal of Botany 50 (1), 199–204.

Seth, P., Venkataraman, R., Maheshwari, S., 1970. Studies on the growth and flowering of a short-day plant, *Wolffia microscopica*. Planta 90 (4), 349–359.

Smith, D., Castle, J., 1960. Production of auxotrophs in a duckweed, *Spirodela polyrhiza*. Plant Physiology 35 (6), 809–815.

Stefaniak, B., Woźny, A., Budna, I., 2002. Callus induction and plant regeneration in *Lemna minor* L. Biologia Plantarum 45 (3), 469–472.

Su, H., Zhao, Y., Jiang, J., Lu, Q., Li, Q., Luo, Y., Zhao, H., Wang, M., 2014. Use of duckweed (*Landoltia punctata*) as a fermentation substrate for the production of higher alcohols as biofuels. Energy and Fuels 28 (5), 3206–3216.

Szabó, S., Roijackers, R., Scheffer, M., Borics, G., 2005. The strength of limiting factors for duckweed during algal competition. Archiv f r Hydrobiologie 164 (1), 127–140.

Tao, X., Fang, Y., Xiao, Y., Jin, Y. L., Ma, X. R., Zhao, Y., He, K. Z., Zhao, H., Wang, H. Y., 2013. Comparative transcriptome analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation. Biotechnol Biofuels 6 (1), 1–15.

Tipping, P. W., Bauer, L., Martin, M. R., Center, T. D., 2009. Competition between *Salvinia minima* and *Spirodela polyrhiza* mediated by nutrient levels and herbivory. Aquatic Botany 90 (3), 231–234.

van der Steen, P., Brenner, A., Oron, G., 1998. An integrated duckweed and algae pond system for nitrogen removal and renovation. Water Science and Technology 38 (1), 335–343.

Verma, R., Suthar, S., 2014. Synchronized urban wastewater treatment and biomass production using duckweed *Lemna gibba* L. Ecological Engineering 64 (0), 337–343.

Vermaat, J. E., Hanif, M. K., 1998. Performance of common duckweed species (*Lemnaceae*) and the waterfern *Azolla filiculoides* on different types of waste water. Water Research 32 (9), 2569–2576.

Vunsh, R., Li, J., Hanania, U., Edelman, M., Flaishman, M., Perl, A., Wisniewski, J.-P., Freyssinet, G., 2007. High expression of transgene protein in spirodela. Plant Cell Reports 26 (9), 1511–1519.

Wang, W., Freemark, K., 1995. The use of plants for environmental monitoring and assessment. Ecotoxicology and Environmental Safety 30 (3), 289–301.

Wang, W., Yang, C., Tang, X., Gu, X., Zhu, Q., Pan, K., Hu, Q., Ma, D., 2014c. Effects of high ammonium level on biomass accumulation of common duckweed *Lemna minor* L. Environmental Science and Pollution Research 21 (24), 14202–14210.

Wendeou, S. P. H., Aina, M. P., Crapper, M., Adjovi, E., Mama, D., 2013. Influence of salinity on duckweed growth and duckweed based wastewater treatment system. Journal of Water Resource and Protection 5 (10), 993–999.

Xiao, Y., Fang, Y., Jin, Y., Zhang, G., Zhao, H., 2013. Culturing duckweed in the field for starch accumulation. Industrial Crops and Products 48 (0), 183–190.

Xu, J., Cheng, J. J., Stomp, A.-M., 2012a. Growing *Spirodela polyrrhiza* in swine wastewater for the production of animal feed and fuel ethanol: a pilot study. CLEAN–Soil, Air, Water 40 (7), 760–765.

Xu, J., Cui, W., Cheng, J., Stomp, A., 2011. Production of high-starch duckweed and its conversion to bioethanol. Biosystems Engineering 110 (2), 67–72.

Xu, J., Shen, G., 2011a. Effects of harvest regime and water depth on nutrient recovery from swine wastewater by growing spirodela oligorrhiza. Water Environment Research 83 (11), 2049–2056.

Xu, J., Shen, G., 2011b. Growing duckweed in swine wastewater for nutrient recovery and biomass production. Bioresource Technology 102 (2), 848–853.

Xu, J., Zhao, H., Stomp, A.-M., Cheng, J. J., 2012b. The production of duckweed as a source of biofuels. Biofuels 3 (5), 589–601.

Yu, C., Sun, C., Yu, L., Zhu, M., Xu, H., Zhao, J., Ma, Y., Zhou, G., 2014.

Comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol. PloS One 9 (12), 1–15.

Zhang, K., Chen, Y.-P., Zhang, T.-T., Zhao, Y., Shen, Y., Huang, L., Gao, X., Guo, J.-S., 2014a. The logistic growth of duckweed (*Lemna minor*) and kinetics of ammonium uptake. Environmental Technology 35 (5), 562–567.

Zhao, H., Appenroth, K., Landesman, L., Salmeán, A., Lam, E., 2012a. Duckweed rising at Chengdu: summary of the 1st international conference on duckweed application and research. Plant Molecular Biology 78 (6), 627–632.

Zhao, X., Elliston, A., Collins, S., Moates, G., Coleman, M., Waldron, K., 2012b. Enzymatic saccharification of duckweed (*Lemna minor*) biomass without thermophysical pretreatment. Biomass and Bioenergy 47 (0), 354–361.

Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., Wang, M., Zhao, H., 2015. Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production. Plant Biology 17 (s1), 82–90.

Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., Zhao, H., 2014a. Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: A pilot-scale comparison with water hyacinth. Bioresource Technology 163 (0), 82–91.

Zhao, Z., Shi, H., Liu, Y., Zhao, H., Su, H., Wang, M., Zhao, Y., 2014b. The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. Bioresource Technology 167 (0), 383–389.

Ziegler, P., Adelmann, K., Zimmer, S., Schmidt, C., Appenroth, K.-J., 2015. Relative in vitro growth rates of duckweeds (*Lemnaceae*)–the most rapidly growing higher plants. Plant Biology 17 (s1), 33–41.

Chapter 6

Determining the partial emptying and filling (PEF) potential for optimizing production rate of *Spirodela polyrhiza* 8483

- This chapter is an extension of Chapter 4 but using duckweed. As we saw from the literature review in Chapter 5, duckweed has one of the fastest growth rates and is a potential source of biofuel. Thus, this chapter theoretically investigated if its propagation can be improved via PEF by examining its growth profile. This experimental work was done at the Lam Lab, Rutgers New Jersey as part of the IGERT Research Scholar program.
- This chapter will be submitted for peer review.
- The contribution of the co-authors (Glasser, D.[†*]; Hildebrandt, D.[*]; Lam, E.[‡]) towards the work is primarily that of supervision or collaboration and the experimental work and write-up was conducted by the author of this thesis. The author would like to acknowledge Ms G. Figueira and Ms S. Govender for insights into the preliminary studies mentioned in later in this chapter.

Abstract

The objective of the research reported in this chapter was to determine whether it was possible by partial emptying and filling (PEF) to improve the

production rate of duckweed, more specifically, *Spirodela polyrhiza* 8483, and if so what retained fraction would be best. From the experimental results it only proved possible to improve production of duckweed when the medium was enriched with carbon. Theoretically, the best production would result from retaining 67% of the duckweed crop density (mat), when grown in 0.5X SH (Schenk and Hildebrandt) medium and enriched with a carbon source, such as 0.5%(w/v) sucrose. This chapter demonstrates the use of a theoretical technique that enables one, through monitoring the growth rate of a species, to ascertain whether its propagation through PEF can be improved. The theoretical retained value found in this chapter is consistent with the harvesting frequencies empirically determined by other researchers.

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6.1 Introduction

Of the flowering species, the smallest, known as duckweed (*Lemnaceae*), has one of the fastest known growth rates. Researchers from around the world, working on a scale extending from laboratories to pilot-plants, have studied the cultivation of duckweed, mostly to produce animal feedstock or as source of a biofuel (Appenroth and Lam, 2012). Most of these investigations have involved cultivating duckweed on wastewater, and harvesting it as part of the propagation process.

A brief review of the debate on methods of cultivation and harvesting follows. Guy et al. (1990a) harvested the duckweed weekly, and then reinoculated their ponds with 5 kg of the weed that had been removed. Journey et al. (1993) emphasized that regular harvesting of duckweed is needed to maintain the health of the culture and the quality of the product. As a guideline, the duckweed mat should be maintained in a minimum height of 20 cm of water, even though

it has been observed that duckweed can grow in a height of one cm of water. However, Iqbal (1999) took a height of one metre as an acceptable value. The mat should be grown in a pond that allows sufficient space for the duckweed to propagate, but allows complete coverage of the surface of the water. Iqbal reports harvesting frequencies that have large variations, from daily and weekly to bi-weekly extractions, and quantities which range between 10 and 25% of the standing crop. In practice other researchers also found that if an excessive amount (more than 80%) of duckweed was harvested, algae would start to bloom (Iqbal, 1999). This caused a reduction in duckweed growth (Roijackers et al., 2004). Xiao et al. (2013) maintained 100% coverage, but they found that they obtained different growth rates with the same biomass composition when harvesting at different frequencies (between seven and 28 days). Allam et al. (2014) grew duckweed in a continuous system to clean canal drainage water (consisting of food industry and domestic wastewater). These researchers removed 50% of the surface area of the duckweed every four days, basing their regimen on the doubling time of duckweed, as reported by previous researchers.

Accompanying these researches into growing and harvesting conditions are investigations that focus on duckweed production. Rejmankova and her coworkers (1990) used a strategy (developed by Elizarov and Svirezhev, 1972) to cultivate duckweed, on the assumption that if the duckweed species followed the logistical growth function, a production rate could be predicted and compared with the experimental data. Ramanova and her team found a 27.2% difference between their experimental results and their predictions. (More about this strategy can be found in some strategies for harvesting a single species, by Swan, 1975.) On the other hand, the concepts outlined by Ming et al. (2012) rely on obtaining the individual growth profile of the species before determining the optimum retained fraction (Φ) that will result in improved production. These researchers proposed a graphical method for determining whether an improvement in production is possible. This involves the researcher in plotting the concentration/time (or mass/time) growth of a species, and using this to define the production rate (mass/day). One can then find the potential for improvement by drawing a line between the initial, intermediate and final concentrations (see Figure 6.1.1). Provided that the batch growth profile is convex, and not concave, this will correspond with the best production rate. The question that follows is: how can that potential growth be realized?

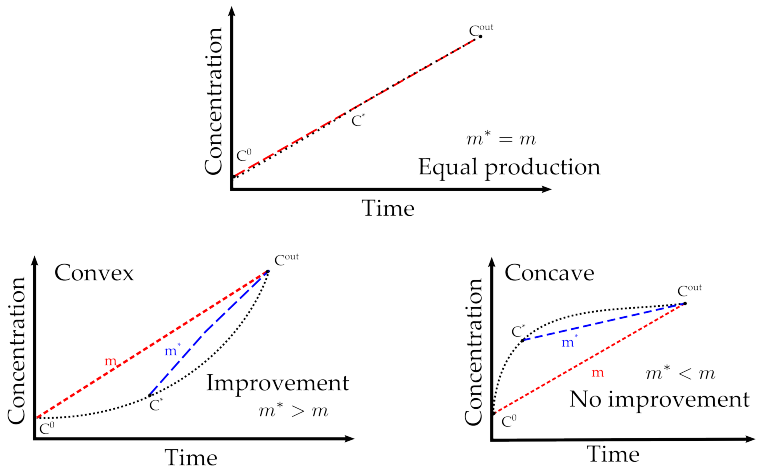


Figure 6.1.1: The figure is adapted from Ming et al. (2012). The shape of the batch profile may be used to graphically identify opportunities for improving production rate, by comparing the gradients in concentration.

The technique addressed by Ming et al. (2012) is known as partial emptying and filling (PEF). The experiment in which this method is applied entails starting a batch and monitoring its growth until a desired exit concentration is reached. This is done by frequent testing of samples in order to weigh the dried duckweed mass. These results are used to plot the graph equivalent to those in Figure 6.1.1. On these graphs the slope between the initial and endpoint m^* can be shown to be the production rate. The basis of the method is to choose the initial point C^* to get a particular slope m^*. This initial concentration is achieved by retaining a fraction of the reacted material and mixing it with fresh material. The theoretically determined Φ $\left(\text{defined as } \phi = \frac{\text{Volume}_{\text{retained}}}{\text{Volume}_{\text{total}}}\right)$ is calculated to determine the fraction of the previous culture (PC) that is to be retained, which will result in an improved production rate. Clearly, m^* (for the convex curve) is a maximum as Φ tends to one. This is the equivalent to continuous operation. In order to use the method, if that is the batch graph obtained, one needs to decide on an initial concentration in terms of how often one wants to carry out the emptying and filling operation. As these take time it might be useful to define a production rate that takes these procedures into account. Furthermore, the growth curve may be more complex than this one, which might give rise to a different answer. We will, however, not take the matter further in this chapter.

In the experiments, the researcher starts the next batch by introducing fresh

medium and a new dose of inoculum to the remainder of the PC, while the harvested duckweed is removed as product. The PEF method therefore changes the initial conditions (which results in an intermediate concentration) under which the next batch starts to grow. Provided that the growth profile of the species proves convex on the diagram and the experimental conditions are the same, the method (retaining a fraction of the original crop and refilling the pond with fresh medium) should result in an accurate prediction of improved production rate.

The experiments described below represent an attempt to apply the Ming theory to determine whether the cultivation of *Spirodela polyrhiza* 8483 is suitable for the PEF method, either with or without a source of carbon. By using the PEF method, the aim is to use the same experimental conditions that are used to obtain the growth profile by improving the operation of the process. This is instead of optimising the intrinsic growth parameters and incurring additional significant expenses, such as a different light sources / intensities, however, for the purpose of this experiment 2800-4100 lux was used. This screening process has not been undertaken before on duckweed. Thereafter the predictions will be compared with the findings of other scientists in the field of duckweed growth.

6.2 Experimental Setup

6.2.1 Sample and media preparation for the growth profile

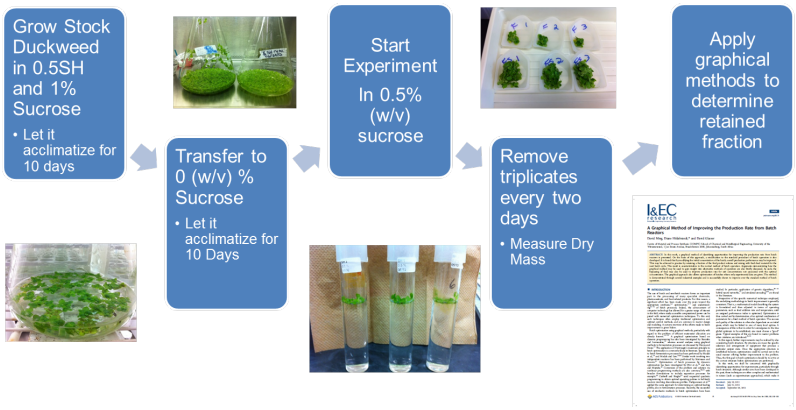


Figure 6.2.1: Process diagram of work flow for producing the growth rate curve, standard batch profile.

The *Spirodela polyrhiza* 8483 was sourced from the Rutgers Duckweed Stock Cooperative (RDSC; http://www.ruduckweed.org) collection. The strain was maintained on 0.8%(w/v) agar containing 0.5X SH salts (Cat. #S6765, Sigma–Aldrich, USA) and 0.5%(w/v) sucrose, pH 5.7–6.0, at 15°C (Borisjuk et al., 2015).

The author started by preparing the duckweed working stock, as follows: *Spirodela polyrhiza* 8483 was transferred from the stock plates and grown in a two-litre flask with 300 mL 0.5X SH and 1%(w/v) sucrose for 10 days. Thereafter, the duckweed was transferred to another two-litre flask containing 300 mL of 0.5X SH but with no sucrose. The researcher let the duckweed acclimatize to a non-sucrose environment for a further 10 days. The day before the start of the growth profile experiment, the researcher cleaned the strain assay tubes and autoclaved them, then transferred 30 mL of 0.5X SH media, both with 0.5%(w/v) sucrose or without sucrose, into the individual glass tubes by means of a sterilized pipette in a laminar flow hood. About 78 strain assay tubes were used in this experiment, since the method for measuring dry mass involved destructive sampling. The medium in each tube, which was sealed using 3M™ Micropore™ surgical tape (Cat. #1530-0, VWR, USA) wound around the lid of the tube to allow air but not microorganisms to flow in, and was left to stand overnight. The experiment began when the researcher transferred one layer of the duckweed into each tube (by means of a sterilized loop) and resealed the lid and tube with micro-tape again. The experiment was carried out in a growth chamber, which was controlled at a temperature of 26°C and a 2800–4100 lux continuous supply of light. The tubes were rotated every second day. A diagrammatic overview is shown in Figure 6.2.1.

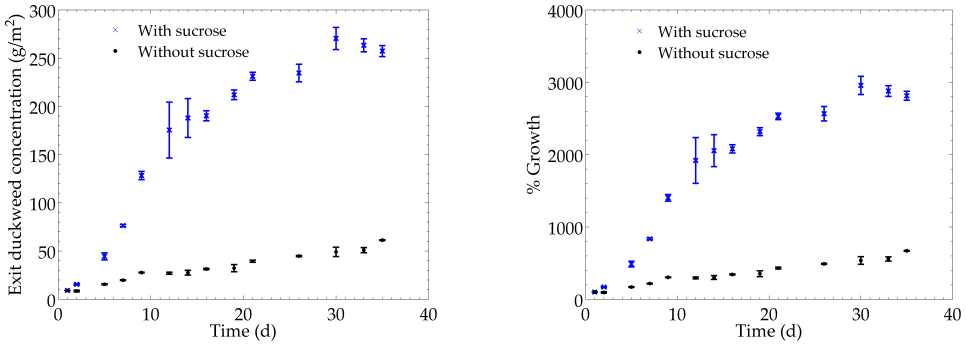
6.2.2 Dry mass and starch content

At the start of the experiment, the initial mass was weighed and the starch content of six samples measured to establish a baseline for the profile. This could also be used as a standard against which all the subsequent samples could be measured. All of the mass weights reported in this chapter are recorded on a dry weight basis. To obtain the dry mass points on the growth profile, as explained earlier, the researcher removed duckweed from the tubes by means of a sieve (to separate the duckweed from the medium), after which the liquid was returned to the tube for pH measurements. Tissue paper was used to dry the

duckweed, which was then placed on plastic weighing boats, which had been weighed empty beforehand. After the duckweed had been loaded into them, the author placed the boats with the duckweed in an oven at 45°C. After four days the mass of the boat with dried duckweed was measured, subtracting the weight of the boat itself, and recording the weight and day for the growth profile. The starch analysis using a YSI 2700 Select Biochemistry Analyser, as described in Appendix *D.2.1,* was carried out. Normally, duckweed is not well mixed with the medium but forms a layer on the surface of the water. Thus, the concentration of duckweed in a batch will be expressed in grams per surface area instead of grams per volume of medium.

6.3 Results and Discussion

The growth batch profile and percentage growth of *Spirodela polyrhiza* 8483 are shown in Figure 6.3.1a and 6.3.1b below, both with and without a carbon source. It can be seen that the addition of sucrose (a carbon source), accelerated the growth by 289% and 427% at days five and 35 respectively. The batch profile of *Spirodela polyrhiza* 8483 grown in a medium enriched with sucrose was convex. The growth of duckweed, in a medium containing no sucrose was concave in nature (see Figure 6.3.1a), and therefore no prediction in improvement was possible (see Appendix G.3).

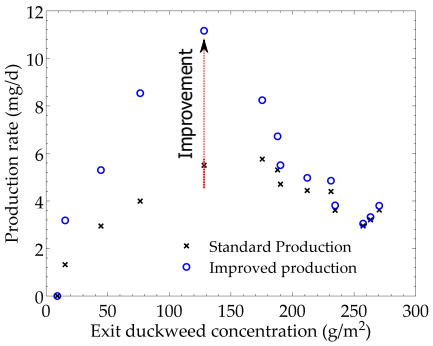


(a) Batch profile of *Spirodela polyrhiza* 8483.

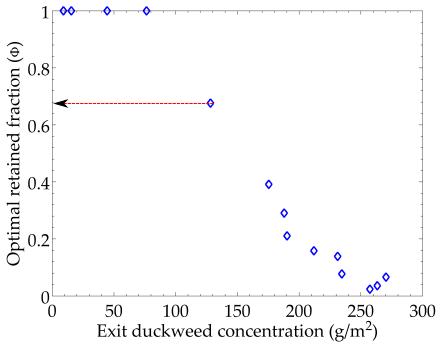
(b) %Growth profile of *Spirodela polyrhiza* 8483. Calculated by dividing the final by the initial mass.

Figure 6.3.1: Batch and growth percentage for sucrose and no sucrose.

The resultant of the predictions for the batch profile of duckweed grown in a medium enriched with sucrose are shown in Figure 6.3.2. A 210% improvement is predicted at a 0.67 retained fraction, at a mass concentration of 128 g/m^2, to yield the best production rate of 11.6 mg/d.



(a) Production rate of *Spirodela polyrhiza* based on a surface area of 4.15cm^2.



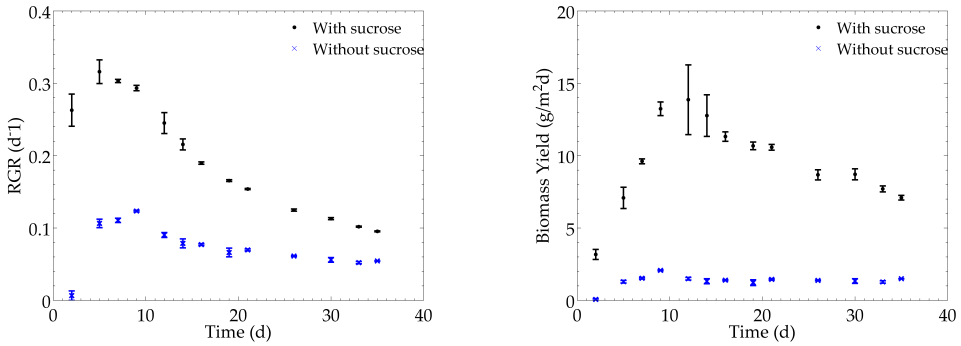
(b) The predicted retained fraction of *Spirodela polyrhiza* related to the production rate versus an exit duckweed concentration.

Figure 6.3.2: Prediction of production rate and retained fraction for *Spirodela polyrhiza* 8483, grown with sucrose, based on the assumption of 1 litre of medium.

The predicted retained fraction is close to some of the harvesting frequencies reported by other researchers, such as the 25% listed by Iqbal (1999). Edwards et al. (1992) initially covered the water surface area with 50% of duckweed and then removed 25% during their experiments. However, the theoretical value found in this chapter agrees most with the harvesting value of 33% achieved by Said et al. (1979), who obtained a maximum growth of duckweed when stocking their tank with 505 g/m^2 of fresh duckweed and harvesting at 33%. Journey et al. (1993) found that when starting with a complete cover of 600 g/m^2, it resulted in a production of 0.5–1.5 tons of fresh duckweed per hectare per day.

Additionally, when the relative growth rate (RGR) or biomass yield (BY) obtained in these experiments are compared with the results of other researchers, it seems that sucrose, or another source of carbon, is needed to achieve the higher values. The percentage growth, as can be seen in Figure 6.3.1b above, is much higher with sucrose, and the values without sucrose correspond to the findings from a previous study, which are recorded in full in Appendix E.4. At the recommended mass concentration of duckweed grown in sucrose, the RGR of 0.29 day^{-1} BY of 13 g/(m^{2}day) are comparable with the values reported by other researchers. Guy et al. (1990a) recorded RGR and BY in two different growing conditions, one in an open pond and the other in a pond that was covered and supplied with air enriched with carbon dioxide. They recorded an RGR of 0.112–0.178 day^{-1} and BY from 4.54–7.78 g/(m^{2}day) respectively.

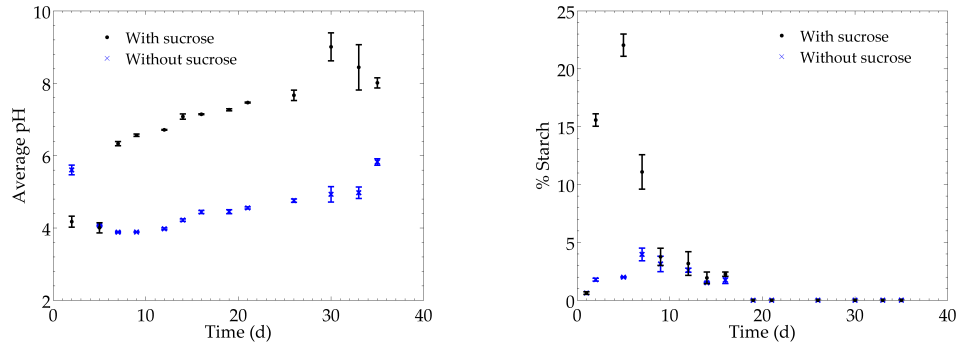
These results were derived from duckweed grown in open pond, and a pond that was covered and the air was enriched with carbon dioxide respectively. Under different environmental conditions, Mestayer et al. (1984) obtained a BY between 12 and 19 g/(m^{2}day) when the duckweed was grown on swine manure.



(a) Relative growth rate for *Spirodela* 8483. (b) Biomass yield of *Spirodela* 8483.

Figure 6.3.3: Biomass yield and RGR of *Spirodela*, with and without sucrose.

The pH was monitored, as seen in Figure 6.3.4a, and the RGR, shown in Figure 6.3.3a above, was found to be similar to the results reported by Caicedo et al. (2000). These scientists investigated the RGR of *Spirodela polyrhiza* at different ammonium concentrations, and discovered that as the pH increased, the RGR decreased.



(a) pH with and without sucrose. (b) %Starch content with and without sucrose.

Figure 6.3.4: %Starch and pH for media containing sucrose and no sucrose for *Spirodela polyrhiza* 8483.

The starch content, without nutrient starvation, which increases the starch content (Zhao et al., 2012a), but enriched with a carbon source, was highest at day five, and within the ranges mentioned in other scientists' published

work at day nine. The starch content decreased as the duckweed grew further, implying that the duckweed may have used the starch for growth. It was approaching stationary phase after day 20, with starch percentages much lower than 0.01%. Therefore, the duckweed can be harvested between day five and day six, and used for high starch content purposes, such as biofuel. Temperature is an important factor in duckweed cultivation, because it affects harvesting frequency and intrinsic growth (Lasfar et al., 2007; Xiao et al., 2013). However, the experiments described in this chapter were carried out under a constant temperature environment.

The data presented in this chapter suggest that the growth rate of *Spirodela polyrhiza* 8483 was carbon-limited. Because organic carbon (sucrose) with light for propagation was used, it was assumed that duckweed follows a photoheterotrophic growth pattern. Most biological plants use sucrose (one of the main products of photosynthesis) as a source of carbon and energy for growth (Lunn, 2001; Mello et al., 2001). In the study by Oota (1972), he found that sucrose could reduce the flowering in *Lemna gibba* G3. However, Frick (1994) reported that sucrose could support growth in darkness and in light for *Lemna minor* L. and *Wolffia brasiliensis* Weddell. Vidaković-Cifrek et al. (2013) investigated the response of *Lemna minor* L. to differences in sucrose concentration and light supply. They concluded that in low light conditions, the higher concentrations of sucrose prompted better growth of the duckweed. Also, Pérez et al. (2000) found that the best carbon sources for berry growth were sucrose and glucose but not fructose. Duckweed can also grow in inorganic carbon such as CO_2. In the reports published by Andersen et al. (1985); Björndahl and Nilsen (1985) and Guy et al. (1990a), the growth of the duckweed (*Lemna gibba*) increased with a rise in carbon dioxide enrichment. A research study of the effect of carbon dioxide and *Spirodela polyrhiza* has yet to be undertaken.

However, preliminary studies revealed that with carbon dioxide (in 0.5X SH) the growth of *Landoltia punctata* 2019 improved over four days by a factor of 5.14 (100% CO_2 versus 100% air for 30 minutes and 50 mL/minutes 1900–2300 lux). This was compared with the same preliminary studies, which showed a 9.98 difference from 50% CO_2 and air (60 mL/min for 15 minutes). In the research study carried out by Kativu et al. (2012) with algae (at 100%CO_2 vs 100% air for 50 mL/minutes a day, 24 hours and 10000 lux), the growth differ-

ence was 1.5 on day four. The concentration best suited to algae propagation (5% CO_2) achieved a difference at day four of 11.6, but at day 12 the difference was 3.3.

In this work the researcher compared the growth of duckweed in media with (WS) and without (-S) sucrose, over five days the growth achieved WS was 2.86 better than that of -S; but at day 35 the difference was 4.19 (at 2800–4100 lux). During photosynthesis, the rate of carbon dioxide fixation is linked to sucrose synthesis (Lunn, 2001). This suggests that in the production of duckweed, specifically *Spirodela polyrhiza* 8483, a carbon source (inorganic or organic) will aid in the growth, especially with the addition of CO_2. This would not only produce a source of feed for biofuel but can assist greenhouse gas reduction. If one wanted to optimise the intrinsic growth rate, along with improving the operation via PEF, one apply the method (by getting different growth rates) at different light intensities.

6.4 Conclusion and recommendations

By carrying out the experiments described in this chapter it was established that, provided there is an enrichment of carbon in the growth medium, the growth profile of *Spirodela polyrhiza* 8483 is suitable for optimization using the technique described by Ming et al. (2012). If one assumes that the batch profile, under the experimental conditions already described, is consistent between batch cycles, then production can be improved, either by harvesting 33% of the duckweed or by keeping 67% of the surface covered with duckweed. However, in order to improve the predictions further it is recommended that readings should be taken daily to obtain more data points. Additionally, enrichment by means of a carbon source (such as carbon dioxide and/or sucrose, for economic purposes, preferably an inorganic source) introduced into the batch has been shown to accelerate the growth of duckweed. The actual PEF experiments should now be carried out to confirm the predictions obtained. The value of the work, while done specifically on *Spirodela polyrhiza* 8483, is to be able to generalize the Ming approach to other plant forms, species and clones under different experimental conditions. This could expand the range of methods by which plants to be used as sources of biofuel can be assessed and production rates possibly optimized so that they can be produced in the quantity and at the speed required for large-scale production.

Nonetheless, the researcher has shown that the potential to improve the production of *Spirodela polyrhiza* 8483 can be predicted, and that the retained fraction identified by means of the growth profile agrees with the harvesting values reported in previous studies by other researchers. The importance of duckweed as a focus of research is that its propagation is much faster than that of algae and it is easier to harvest. However, in South Africa duckweed is classified as belonging to an invasive alien species, so this is currently not a viable option for large-scale research.

This is the first occasion in which the concepts proposed by Ming et al. (2012) have been carried out experimentally on the aquatic plant duckweed. By making it possible for scientists to evaluate whether the intrinsic growth behaviour of this species has the potential for improved production, the PhD candidate has contributed a very promising technique to systematically improve the propagation of duckweed, without incurring additional significant costs, by means of using the same experimental setup.

References

Allam, A., Tawfik, A., El-Saadi, A., Negm, A., 2014. Potentials of using duckweed (*Lemna gibba*) for treatment of drainage water for reuse in irrigation purposes. Desalination and Water Treatment 0 (0), 1–9.

Andersen, I., Dons, C., Nilsen, S., Haugstad, M., 1985. Growth, photosynthesis and photorespiration of *Lemna gibba*: response to variations in CO_2 and O_2 concentrations and photon flux density. Photosynthesis Research 6 (1), 87–96.

Appenroth, K.-J., Lam, E., 2012. Wasserlinsen als nutzpflanzen. Biologie in unserer Zeit 42 (3), 181–187.

Björndahl, G., Nilsen, S., 1985. Growth potential of *Lemna gibba*: Effect of CO_2 enrichment at high photon flux rate. Aquatic Botany 22 (1), 79–82.

Borisjuk, N., Chu, P., Gutierrez, R., Zhang, H., Acosta, K., Friesen, N., Sree, K., Garcia, C., Appenroth, K., Lam, E., 2015. Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. Plant Biology 17 (s1), 42–49.

Caicedo, J., Van der Steen, N., Arce, O., Gijzen, H., 2000. Effect of total

ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Water Research 34 (15), 3829–3835.

Edwards, P., Hassan, M., Chao, C., Pacharaprakiti, C., 1992. Cultivation of duckweeds in septage-loaded earthen ponds. Bioresource Technology 40 (2), 109–117.

Elizarov, E. Y., Svirezhev, Y. M., 1972. Optimal productivity of biosystem. Zhurnal Obshchei Biologii 33 (3), 251–260.

Frick, H., 1994. Heterotrophy in the lemnaceae. Journal of Plant Physiology 144 (2), 189–193.

Guy, M., Granoth, G., Gale, J., 1990a. Cultivation of *Lemma gibba* under desert conditions. II: The effect of raised winter temperature, CO_2 enrichment and shading on productivity. Biomass 23 (1), 1–11.

Iqbal, S., March 1999. Duckweed aquaculture: Potentials, possibilities and limitations, for combined wastewater treatment and animal feed production in developing countries. SANDEC 6/99, EAWAG, Duebendorf, Switzerland.

Journey, W. K., Skillicorn, P., Spira, W., 1993. Duckweed Aquaculture, a new Aquatic Farming System for Developing Countries. Washington DC, USA.

Kativu, E., Hildebrandt, D., Matambo, T., Glasser, D., 2012. Effects of CO_2 on South African fresh water microalgae growth. Environmental Progress and Sustainable Energy 31 (1), 24–28.

Lasfar, S., Monette, F., Millette, L., Azzouz, A., 2007. Intrinsic growth rate: a new approach to evaluate the effects of temperature, photoperiod and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication. Water Research 41 (11), 2333–2340.

Lunn, J. E., 2001. Sucrose metabolism. In: Encyclopedia of life sciences (ELS). John Wiley and Sons, Ltd, Chichester, UK.

Mello, M. O., Dias, C. T. S., Amaral, A. F. C., Melo, M., 2001. Growth of *Bauhinia forficata* Link, *Curcuma zedoaria* Roscoe and *Phaseolus vulgaris* L. cell suspension cultures with carbon sources. Scientia Agricola 58 (3), 481–485.

Mestayer, C. R., Culley, D. D., Standifer, L. C., Koonce, K. L., 1984. Solar energy conversion efficiency and growth aspects of the duckweed, *Spirodela punctata* (GFW Mey.) Thompson. Aquatic Botany 19 (1), 157–170.

Ming, D., Hildebrandt, D., Glasser, D., 2012. A graphical method of improving the production rate from batch reactors. Industrial and Engineering Chemistry Research 51 (42), 13562–13573.

Oota, Y., 1972. A possible mechanism for sugar inhibition of duckweed flowering. Plant and Cell Physiology 13 (1), 195–199.

Pérez, F. J., Meza, P., Berti, M., Pinto, M., 2000. Effect of carbon source and sucrose concentration on growth and hexose accumulation of grape berries cultured in vitro. Plant cell, Tissue and Organ Culture 61 (1), 37–40.

Rejmankova, E., Rejmanek, M., Kvet, J., 1990. Maximizing duckweed (lemnaceae) production by suitable harvest strategy. In: Wetland Ecology and Management: Case Studies. Vol. 25 of Tasks for vegetation science. Springer Netherlands, pp. 39–43.

Roijackers, R., Szabo, S., Scheffer, M., 2004. Experimental analysis of the competition between algae and duckweed. Archiv für Hydrobiologie 160 (3), 401–412.

Said, M. Z. M., Culley, D. D., Standifer, L. C., Epps, E. A., Myers, R. W., Boney, S. A., 1979. Effect of harvest rate, waste loading, and stocking density on the yield of duckweeds. In: Proceedings of the World Mariculture Society. Vol. 10. Wiley Online Library, pp. 769–780.

Swan, G., 1975. Some strategies for harvesting a single species. Bulletin of Mathematical Biology 37 (6), 659–673.

Vidaković-Cifrek, Ž., Sorić, S., Babić, M., 2013. Growth and photosynthesis of *Lemna minor* L. exposed to different light conditions and sucrose supplies. Acta Botanica Croatica 72 (2), 211–219.

Xiao, Y., Fang, Y., Jin, Y., Zhang, G., Zhao, H., 2013. Culturing duckweed in the field for starch accumulation. Industrial Crops and Products 48 (0), 183–190.

Zhao, H., Appenroth, K., Landesman, L., Salmeán, A., Lam, E., 2012a. Duckweed rising at Chengdu: summary of the 1st international conference on duckweed application and research. Plant Molecular Biology 78 (6), 627–632.

Chapter 7

Discussion and concluding remarks

7.1 General discussion

The research in this thesis makes several contributions to the body of knowledge in the field of biomass growth and production. Firstly, the author shows that the photosynthesis process has a thermodynamic limit. More specifically, the process, which governs the number of photons supplied to a system, is work-limited. That number (9–17 moles of photons depending on the wavelength of the light) is much lower than had been recorded in previous experimental reports. Analysis of the former result suggests that there is a large amount of heat that needs to be rejected by the plants, or the heat is being used for other cellular processes within the plant cell. For plants such as algae that are immersed in water, this need not be a problem because of its high heat capacity, but for land-based plants this may account for a large part of their transpiration and may in fact limit their growth rate. This apparent photon inefficiency also suggests that photosynthesis is highly irreversible.

Secondly, the researcher discovered that a spectrophotometer can be used not only for its traditional purpose, but it can also be used to grow a small sample of algae in the measuring cuvette. This allowed the writer to determine the most efficient light wavelength in which algae can grow using a spectrophotometer, in this one 440 nm. This is a departure from the convention of choosing a specific colour with a dominant wavelength, such as an LED (a light-emitting diode). Additional insights were shown into the equations derived in chapter one.

The third contribution to improving the photosynthesis process is that the writer was able to increase the batch production of an algal species. This was done by using a theoretical model, partial emptying and filling (PEF) that no one has used before in algae experiments. By means of this approach she obtained a higher production rate than that of the standard batch, under the same experimental conditions. Additionally, these experiments showed that if a sufficient number of cycles were completed, continuous batch PEF would allow the growth rate of the algae to attain the predicted production rate.

Fourthly, the writer showed that the PEF method can also be applied to other aquatic plants, such as duckweed. However, it was further shown that in order to make improvements on the standard batch profile, the duckweed species examined needed to be fed with a carbon source in the growing medium.

Fifthly and lastly, previous research has focused on growing duckweed on wastewater, but this scientist, following on from the work described above, compared the cultivation of duckweed in different commercial fertilisers. The purpose of these experiments was to reduce expenses (by using a cheaper growing medium), and to identify those duckweed strains that have good propagation rates and high starch content. This fifth contribution shows that the duckweed species can be cultivated on cheaper media, while achieving a better %percentage growth rate and starch content than the conventionally-used and expensive basal salt medium.

All of these results constitute contributions to knowledge in the area of biomass growth and production, with implications for biofuel feedstock production.

7.2 Conclusion

The photosynthesis process is interesting because it uses carbon dioxide to propagate biomass and is fuelled by light energy. The researcher's thermodynamic and experimental study on the photosynthesis reaction, using algae as a case study, suggests that it is possible to help the cultivation process to operate more efficiently. There are thermodynamic limits that govern the process, but one can use a specific light wavelength to boost species growth. In addition, PEF (partial emptying and filling) can be a useful tool for biological

systems because it allows the researcher to check whether improvements in biomass accumulation are possible. Furthermore, one is no longer limited in the attempt to cultivate large amounts of duckweed (or algae) by having to use the recommended basal medium, but can produce it at a fraction of the cost.

The National Development Plan (NDP) 2030 (NPC, 2013) for South Africa announces the Government's intention of making a significant reduction in the country's greenhouse gas emissions by 2025. The mitigation of carbon production is being made the responsibility of a mixed portfolio of processes that include a biofuels sector. However, the NDP also acknowledges that the costs associated with the supply of alternative sources of fuel and energy, such as biomass, are at present too high to be practicable. On the other hand, the NDP notes that, since South Africa is predominantly a water-scarce country, the cultivation of plants to be used as biofuel in water is counter-intuitive, although it may be a possibility for countries situated elsewhere in the southern African region, or for coastal areas, or in brackish water. However, this researcher has shown that the biofuel feed could be produced in South Africa if the photobioreactor designs she has outlined are optimized, taking into consideration the thermodynamic and experimental limitations of the species used. Moreover, within the next 20 years the country will see the start of a shift to electric cars (NPC, 2013), which might save on the demand for fossil-based fuel, but biofuels remain the most likely option to supply the fuel and energy a growing economy requires.

7.3 Final remarks and future work

The practical difficulties experienced by the researcher suggest the direction for further work. The thermodynamic analysis and spectrophotometer experiment suggested the possibility that heat could be released during the production of algae. An experiment worth considering would be to measure the actual amount of heat released by the photosynthesis reaction, using a simple photo-calorimeter at a specific light wavelength. Laser technology offers that specific wavelength and emits less heat, so would interfere less with the measurement. It would therefore be a much better option than LED lights, and give the researcher greater control over the heat released in the photobioreactors. During further PEF experiments, more regular readings that begin earlier need

to be recorded. Furthermore, additional PEF experiments should be carried out with a greater number of cycles, to assess to what extent these help the growth rate to reach the predictions. If one is not restricted by capital costs, one can consider improving the production rate by improving the growth rate, by means of changing the experimental setup (such as different light intensities, temperatures and nutrient concentrations), and thereafter implement PEF to the improved growth rate, and as a result further improve the growth rate, via changing the operational method .

While the present research focused on improving the production of algae species, further research should include using the same methods on other plant types, to see which specific light wavelengths each one prefers. The PEF experimental method improved the growth of algae; one should conduct similar experiments on duckweed to ascertain whether an acceleration of growth can be achieved, and whether that can match in actual terms the predictions obtained from the profile.

Although the methods discussed in this thesis used algae as the main case study, the end purpose of this research was to investigate means of producing a feedstock that will supply the next generation of biofuels and to gain insight into the photosynthesis process. This can be done only if a sufficient amount of the feedstock can be produced in a sufficiently short space of time and in a continuous manner. Keys to the solutions suggested in this document are the roles of the photosynthesis process and of the PEF technique in improving the production rate of that feedstock.

References

NPC, 2013. National Development Plan Vision 2030. [Online]. Available at: http://policyresearch.limpopo.gov.za/handle/123456789/941, [accessed 31 May 2015].

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Derivations

C.1 Related to Chapter two

C.1.1 Derivation of the adjusted $E_{photons}$

Derivation of the adjusted $E_{photons}$ when taking into account the pressure effects:

$$E_{photons} = G_{out} - G_{in}$$

$$E_{photons} = G_{CH}\,(solid) + G_{O_2}\,(P_{O_2}) - G_{H_2O}\,(liquid) - G_{CO_2}\,(P_{CO_2})$$

where $G_{O_2}\,(P_{O_2})$is the Gibbs energy of oxygen which is a function of the partial pressure of oxygen and similarly for carbon dioxide.

Add and subtract $G_{O_2}\,(1\,bar)$ and $G_{CO_2}\,(1\,bar)$ resulting in:

$$E_{photons} = [G_{CH}\,(solid) + G_{O_2}\,(1\,bar)] - [G_{H_2O}\,(liquid) + G_{CO_2}\,(1\,bar)] + ...$$
$$...+ [G_{O_2}\,(P_{O_2}) - G_{O_2}\,(1\,bar)] - [G_{CO_2}\,(P_{CO_2}) - G_{CO_2}\,(1\,bar)]$$

$$E_{photons} = \triangle G_{process} + G_{O_2}\,(P_{O_2}) - G_{O_2}\,(1\,bar) - G\,(P_{CO_2}) + G_{CO_2}\,(1\,bar)$$

but

$$RTlnX_i = \frac{G_i}{n_i}$$

where R is the ideal gas constant, T is temperature, X_i is the mole fraction of componenti, G is the Gibbs free energy of component i per mol.

For the purposes of easy manipulation let:

$G_{O_2}(P_{O_2}) \sim 1$

$G_{O_2}(1\,\text{bar}) \sim 2$

$G(P_{CO_2}) \sim 3$

$G_{CO_2}(1\,\text{bar}) \sim 4$

$$\therefore E_{photons} = \triangle G_{process} + RT\,(\ln X_1 - \ln X_2 - \ln X_3 + \ln X_4)$$

$$E_{photons} = \triangle G_{process} + RT\left(\ln\left(\frac{X_1 \cdot X_4}{X_2 \cdot X_3}\right)\right)$$

and from Raoult's Law

$$P_i = X_i p_m$$

where P_i is the partial pressure of component i and p_m is the pressure of the mixture in the system, which is 1 bar.

Therefore, after substitution the following equation is formed:

$$E_{photons} = \triangle G_{process} + RT\left(\ln\left(\frac{P_{O_2} \cdot 1\,\text{bar}}{P_{CO_2} \cdot 1\,\text{bar}}\right)\right)$$

Which results in the adjusted $E_{photons}$ when taking into consideration the pressure effects:

$$E_{photons} = \triangle G_{process} + RT\left(\ln\left(\frac{P_{O_2}}{P_{CO_2}}\right)\right)$$

Substituting in for Raoult's law

$$E_{photons} = \triangle G_{process} + RT\ln\left(\frac{X_{O_2}}{X_{CO_2}}\right)$$

and the mole fraction should total 1; so with just CO_2 and O_2

$$X_{CO_2} + X_{O_2} = 1$$

But with CO_2 and air

$$X_{CO_2} + X_{O_2} + X_{N_2} = 1$$

However, the relationship of nitrogen to oxygen when introduced with air is $X_{N_2} = 3.75X_{O_2}$; so

$$X_{CO_2} + X_{O_2} + 3.75X_{O_2} = 1$$

becomes

$$X_{CO_2} = 1 - 5X_{O_2}$$

C.2 Related to Chapter three

C.2.1 Adiabatic temperature

Using the maximum growth from the air experiment and assuming all energy goes to heating up the temperature of the water:

$$\triangle H_{process} = \triangle H_{reaction} = 2814\,kJ/mol$$

$$\triangle H_{process} = C_{p,water} \times \triangle T = 2814\,kJ/mol$$

$$\triangle T = \frac{\triangle H_{process}}{C_p}$$

$$\triangle T = 2814\,\frac{kJ}{mol} \times \frac{1}{4.181}\,\frac{L{\cdot}K}{kJ} \times 0.033\frac{g}{L} \times \frac{1}{180}\,\frac{mol\,glucose}{g}$$

$$\triangle T = 0.125\,K$$

Appendix D

Experimental Protocols

D.1 Microalgae

All equipment and lab consumables were purchased in the Republic of South Africa.

D.1.1 Precaution for Experiments

D.1.1.1 Using the autoclave

Before starting to use the autoclave, water must cover the bottom element and the exhausts should be closed. Once the autoclave has finished autoclaving the medium or apparatus, one must wait until the gauge pressure in the autoclave is down to zero (which is one atmospheric pressure) in order to remove the contents. If one does not wait, hot steam will certainly burn the user.

D.1.1.2 Using the spectrophotometer

There are three practical considerations to be taken into account for the use of a spectrophotometer.

1. The optical density of the culture depends on the wavelength.

2. Subtract the blank, place the cuvette in the same position each time when you take a reading, don't touch the clear part of the cuvette and fingerprints must be removed.

3. The relationship between cell density and Optical Density values are only valid for numbers below 2.5. This is the photometric upper limit of

the Secomam Prim Light spectrophotometer (according to the manufacturer's manual).

D.1.1.3 Using the gas lines

1. Ensure that there are no leakages in the gas lines when installing, by spraying a solution of soap over the connections while the gas line is open.

2. At the end of the bubbling of gas into the suspension, ensure that the valve for the gas is closed (V-) and that the gas pressure is at zero.

D.2 Duckweed

All equipment and lab consumables were purchased in the United States of America.

D.2.1 Starch content protocol

D.2.1.1 Starch Content

- This is an extended version of the protocol obtained from the Rutgers Lam Lab.
- Read all instructions first.
- Few days before: prepare the SAB (Sodium Acetate Buffer) and enzyme if you do not have have them already
- SAB: 50 mM sodium acetate which is 4.10g $C_2H_3NaO_2$ + 1 litre water heated up to 55^{o}C and pH adjusted to 4.5 using acetic acid.
- Enzyme: prepare 1mg/mL amyloglucosidase. Adjust accordingly. e.g. 250 mg in 250 mL. 0.25g. Find and read data sheet of that enzyme as it might get activated/ incubated at a different temperature used here.
- Label all tubes used.

D.2.1.2 Grinding Sample Stage

- An Autogen Autogrinder 48 was used for this stage of the process.

- Record all masses and keep track of how much sample is placed in the Autogen grinding tube and which Autogen grinding tube it is placed in.
- Use 5-10mg for the final dry masses. Record each individual masses as it effects the conversion factor.
- Use a blank as cornstarch (approx 0.003-0.004g is enough. When the final calculations are done it will give a %starch of 80-100%)
- Weigh out the samples and place into the Autogen Grinder Tubes. Using a pipette transfer 500 uL of SAB add a ball to each tube.
- Tape the tubes with the lid.
- Place tubes into the Autogen Grinder and grind for 10 minutes (800 seconds) at maximum speed.

D.2.1.3 Solubilizing the Starch - Autoclaving Stage

- Transfer each sample to a labelled glass tube (i.e. strain assay tube) and dilute with further 1.5mL SAB
- Change the pipette tip each time a sample is transferred.
  - Rather transfer the remaining 1.5mL SAB to the Autogen tube to clean out the tube.
  - Then transfer that solution to the glass tube
  - Once a autogen tube set is finished, soak the Autogen grinding tube and lid in water to prevent staining
- Close lids on all glass tubes. Autoclave for 30minutes.
- Allow to cool

D.2.1.4 Enzyme Activation Stage

- Switch on water bath (Fisher Scientific Co., stability and accuracy ±0.5°C, New Hampshire, United States) to 60°C a few hours before starting the whole starch analysis.
- Add 250 μL to labelled 1.5 mL micro-centrifuge tube.

- Add 1 mL of the solubilized starch from the glass tube to a labelled micro-centrifuge tube and close lid.
- Place samples in the water-bath for 30 minutes, but halfway through, turn the tube up and down to mix the mixture.
- Wash the glass tubes and use ethanol to wipe off the permanent marker which may have been used to label the glass tubes.

D.2.1.5 Supernatant

- After incubation, place the tubes in a centrifuge (an Eppendorf centrifuge 5417 R, Hamburg, Germany) to spin the mixture (14 000 rpm for 7-8 min)
- Pipette 1 mL of the supernatant and place into a collection tube.
- Place the collection tube in the YSI 2700 Select Biochemistry analyser sample bay in order to measure the dextrose concentration.
- Refrigerate samples if need to read it the next day

D.2.1.6 How to use the YSI 2700 Select Biochemistry analyser to sample dextrose concentration

- Switch on at the back
- Let the YSI 2700 Select Biochemistry analyser stabilize current
  - [Menu] - [Diagnostic] - [Probe]
    * It will read the probe current, it will probably be at 220 nA or lower; let it come down to approx 7nA and then exit
- To edit the number of samples
  - [Menu] - [Setup] - [Run Mode] - [Sample Protocol] - [Turntable] - [Enter start position] - [Enter number of samples] - [Push Enter for Yes fluid detection] - [Menu] or [Back] to go back.
- Start the Run or analyzing
  - [Run]: YSI 2700 Select Biochemistry analyser will display “ready to sample” - this should happen within 10minutes.

    * Else troubleshoot by checking the sipper position, probe current or clean membrane.
  - push [Sample]
  - YSI 2700 Select Biochemistry analyser will asks the user for replicates [enter the number]
  - Analysis begins
  - It calibrates every five readings

- Switch off or to change settings
  - [STANDBY]
  - Switch off at the back or change settings etc.

- Maintenance
  - Replace enzyme membrane every three weeks
  - Prepare buffer when needed, two packets mix in 500 mL reagent water (distilled or pure), then add a further 500 mL.
  - Remove waste liquid when the bottle is full
  - Check thermal paper supply

D.2.1.7 Calculating the starch content

- As per the manufacturer's instructions, 0.9 is a conversion factor from dextrose to starch to take into account hydrolysis. It is the molecular weight of the starch polymer, dextrose ($C_6H_{10}O_5$) divided by the molecular weight of glucose ($C_6H_{12}O_6$). i.e. 162g/mol divided by180g/mol results in a value of 0.9.

- Total starch content (% starch per mass dry duckweed)

1. $\%\,\text{starch} = \frac{\text{dextrose concentration(mg/mL)}}{\text{new concentration(mg/mL)}} \times 0.9 \times 100\%$
2. $\text{new concentration} = \frac{\text{Old concentration(mg/mL)} \times \text{volume pipette into microcentrifudge tube(mL)}}{\text{enzyme volume(mL)} + \text{volume pipette into microcentrifudge tube(mL)}}$
3. $\text{old concentration} = \frac{\text{mass of dry duckweed(mg)}}{\text{volume of buffer(mL)}}$

D.2.2 Preparing cefotaxime

To prepare a stock solution of antibiotics (cefotaxime), 100 mg of cefotaxime (from GoldBio; catalogue #C-104-25, USA) was prepared with 1 mL of water to make 100 mg/mL of cefotaxime stock solution, placed in micro-centrifuge 500 μL tubes and frozen. When needed, one 100 mg/mL of cefotaxime was added to 1 L of 0.5X SH media with 1 %(w/v) sucrose 0.8 %(w/v) agar and sterlized using the autoclave.

Appendix E

Data from experiments

E.1 General calibration curves

E.1.1 Optical density (absorbance) and air-dried mass concentration of *Desmodesmus* spp.

Table E.1.1: Calibration between the OD at 680 and 740 nm with air-dried mass concentration. Bubbled with CO_2 for seven days a week.

Day	**Mass concentration (g/l)**	**Std Dev**	**Ave OD_{680}**	**Ave OD_{740}**	**Day**	**Mass concentration (g/l)**	**Std Dev**	**Ave OD_{680}**	**Ave OD_{740}**
1	0.0153	0.0047	0.0655	0.0700	29	1.7240	0.0680	2.3375	2.1860
2	0.0570	0.0017	0.1055	0.1145	30	1.7010	0.0010	2.3415	2.1915
3	0.1233	0.0033	0.4360	0.4025	31	1.6690	0.0310	2.3435	2.1995
4	0.1937	0.0030	0.8845	0.7365	32	1.5730	0.0470	2.3385	2.1990
5	0.2877	0.0057	1.2815	1.0335	33	1.5400	0.0240	2.3375	2.2050
6	0.3360	0.0450	1.6935	1.2810	34	1.7300	0.0000	2.3350	2.2005
7	0.5150	0.0100	2.0465	1.4800	35	1.7780	0.0060	2.3170	2.1905
8	0.6325	0.0125	2.2555	1.5940	36	1.7600	0.0640	2.3240	2.1940
9	0.7319	0.0144	2.4435	1.7335	37	1.6460	0.0320	2.3000	2.1775
10	0.8944	0.0331	2.5700	1.8470	38	1.7460	0.0640	2.2805	2.1670
11	0.9875	0.0187	2.6120	1.9150	39	1.6890	0.0190	2.2775	2.1710
12	1.1225	0.0362	2.6005	1.9190	40	1.8220	0.0540	1.2600	2.1555
13	1.2660	0.0120	2.5800	1.9455	41	1.8760	0.0620	2.2460	2.1480
14	1.2910	0.0250	2.5750	1.9765	42	1.8230	0.0230	2.2415	2.1475
15	1.0670	0.0190	2.5295	1.9905	43	1.8350	0.0470	2.2390	2.1530
16	1.2220	0.0120	2.4675	2.0885	44	1.7160	0.0720	2.2330	2.1395
17	1.2650	0.0290	2.4020	2.0060	45	1.8410	0.1030	2.2260	2.1405
18	1.3470	0.0250	2.3250	2.0045	46	1.8170	0.0390	2.2115	2.1315
19	1.4370	0.0170	2.2975	2.0260	47	1.7630	0.0890	2.2045	2.1265
20	1.3860	0.0440	2.2830	2.0460	48	1.7890	0.0750	2.1990	2.1215
21	1.4110	0.0550	2.2945	2.0790	49	1.7450	0.1130	2.1920	2.1195
22	1.5480	0.0420	2.3200	2.1120	50	1.7840	0.0220	2.1840	2.1105
23	1.5040	0.0520	2.3310	2.1390	51	1.7080	0.0660	2.1750	2.1040
24	1.6160	0.0240	2.3295	2.1485	52	1.4550	0.0690	2.1695	2.0945
25	1.6280	0.0840	2.3385	2.1660	53	1.5960	0.0740	2.1940	2.0880
26	1.6320	0.0380	2.3660	2.1735	54	1.7000	0.0900	2.1575	2.0805
27	1.7860	0.0560	2.3385	2.1790	55	1.6830	0.0850	2.1475	2.0765
28	1.7470	0.0410	2.3325	2.1790	56	1.5470	0.0950	2.1455	2.0780

E.2 Chapter three

E.2.1 Summarized data for Air

Table E.2.1: Data for 432 nm air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1907	0.0033	31	3.8877	0.0741	0.0000
2	0.2194	0.0080	30	0.3775	0.0799	0.0374
3	0.2409	0.0095	32	2.2411	0.0842	0.0425
4	0.2903	0.0137	33	1.6177	0.0941	0.0597
5	0.3454	0.0000	30	3.2000	0.1052	0.0700

Table E.2.2: Data for 440 nm air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1853	0.0115	31	4.4632	0.0730	0.0000
2	0.2376	0.0163	35	0.3559	0.0835	0.0673
3	0.2775	0.0147	35	1.7512	0.0915	0.0753
4	0.3094	0.0097	32	1.1557	0.0979	0.0734
5	0.3521	0.0000	30	0.0000	0.1065	0.0755

Table E.2.3: Data for 492 nm air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1792	0.0051	35	3.3827	0.0718	0.0000
2	0.1961	0.0038	35	1.5642	0.0752	0.0232
3	0.2231	0.0075	33	0.3700	0.0806	0.0386
4	0.2694	0.0051	33	1.0670	0.0899	0.0562
5	0.3063	0.0000	31	0.0000	0.0973	0.0608

Table E.2.4: Data for 554 nm air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1901	0.0036	29	3.6114	0.0740	0.0000
2	0.2052	0.0058	31	2.3069	0.0770	0.0200
3	0.2281	0.0072	28	1.4020	0.0816	0.0327
4	0.2707	0.0117	29	1.8348	0.0902	0.0494
5	0.3041	0.0000	30	0.0000	0.0969	0.0539

Table E.2.5: Data for 686 nm air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1793	0.0014	32	4.0003	0.0718	0.0000
2	0.1872	0.0029	34	2.2819	0.0734	0.0109
3	0.2016	0.0057	31	0.6944	0.0763	0.0202
4	0.2458	0.0084	33	0.4918	0.0852	0.0426
5	0.2848	0.0000	33	0.0000	0.0930	0.0517

Table E.2.6: Data for dark control with air.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1909	0.0024	27	2.1839	0.0741	0.0000
2	0.1886	0.0008	28	0.5500	0.0737	-0.0031
3	0.1994	0.0071	27	4.0610	0.0758	0.0076
4	0.2183	0.0024	28	1.6991	0.0796	0.0179
5	0.2262	0.0000	31	0.6500	0.0812	0.0182

E.2.2 Summarized data for Carbon Dioxide

Table E.2.7: Data for 432 nm CO_2.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1811	0.0021	33	3.2614	0.0722	0.0000
2	0.2127	0.0021	33	3.0274	0.0785	0.0421
3	0.2290	0.0050	33	1.7545	0.0818	0.0417
4	0.2460	0.0049	32	1.3075	0.0852	0.0415
5	0.2605	0.0000	32	0.0000	0.0881	0.0399

Table E.2.8: Data for 440 nm CO_2.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1714	0.0042	35	3.0166	0.0702	0.0000
2	0.2027	0.0071	33	2.2624	0.0765	0.0428
3	0.2299	0.0053	34	1.8433	0.0820	0.0515
4	0.2466	0.0054	34	0.2500	0.0853	0.0487
5	0.2721	0.0000	28	0.0000	0.0904	0.0506

Table E.2.9: Data for 492 nm CO_2.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1720	0.0048	27	2.1922	0.0704	0.0000
2	0.1872	0.0038	28	1.0377	0.0734	0.0212
3	0.1965	0.0065	29	1.7988	0.0753	0.0225
4	0.2294	0.0051	30	0.1633	0.0819	0.0379
5	0.2342	0.0000	31	0.0000	0.0828	0.0326

Table E.2.10: Data for 554 nm CO_2.

Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1777	0.0037	25	3.5926	0.0715	0.0000
2	0.2067	0.0060	27	3.7811	0.0773	0.0391
3	0.2206	0.0045	22	5.2669	0.0801	0.0379
4	0.2234	0.0041	24	4.6384	0.0807	0.0302
5	0.2343	0.0003	23	2.5500	0.0829	0.0295

Table E.2.11: Data for 686 nm CO_2.

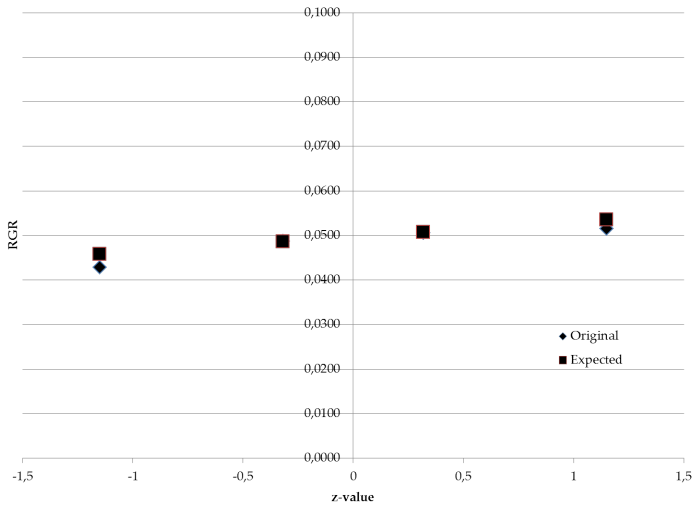
Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1820	0.0018	31	2.8614	0.0724	0.0000
2	0.1838	0.0025	33	1.3695	0.0727	0.0025
3	0.1877	0.0017	29	4.6338	0.0735	0.0039
4	0.2013	0.0034	31	1.7299	0.0762	0.0130
5	0.2250	0.0000	21	0.0000	0.0810	0.0226

Table E.2.12: Data for dark control with CO_2.

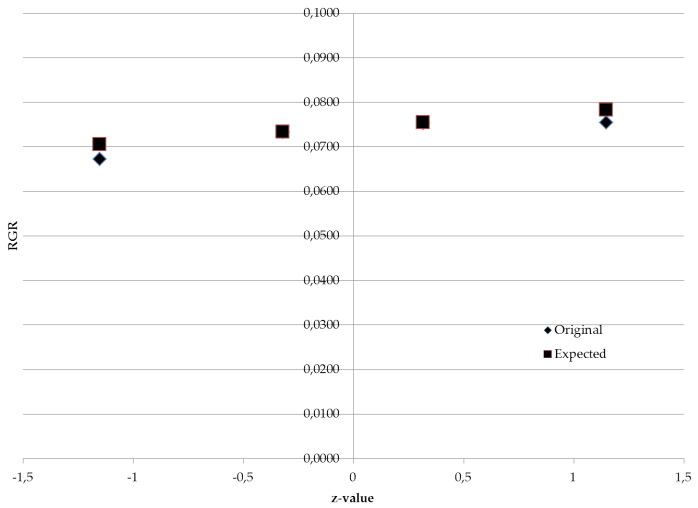
Day	Ave OD_{680}	Std Dev	Ave T (ºC)	Std Dev	Mass conc (g/L)	RGR
1	0.1854	0.0013	25	2.4475	0.0730	0.0000
2	0.1795	0.0015	29	3.8361	0.0719	-0.0082
3	0.1890	0.0027	27	1.1586	0.0738	0.0032
4	0.2067	0.0080	26	1.6045	0.0773	0.0142
5	0.2137	0.0000	26	0.0000	0.0787	0.0149

E.2.3 Stats for Chapter three

E.2.3.1 Statistical Testing for Normality for RGR at 440 nm



(a) CO_2 , where skew < 2*(sqrt(6/count); (i.e. 1.5<2.5) implying not significantly skewed.



(b) Air, where skew < 2*(sqrt(6/count); (i.e. 1.7<2.5) implying not significantly skewed.

Figure E.2.1: Normal Probability Plot for RGR at 440 nm from day two-five.

E.2.3.2 ANOVA test for the RGR between CO_2 and air at the blue wavelength (440 nm)

Table E.2.13: ANOVA two factor between the RGR of dark control and blue wavelength over the 5 days. For a significant difference, F> Fcrit and P-value < 0.05.

	F	P-value	F-crit
Source of Variation	**Source of light**		
Days	24.895	0.004	6.388
RGR (d^{-1})	15.979	0.016	7.709

E.2.3.3 ANOVA test for the RGR between dark control and blue wavelength

Table E.2.14: ANOVA two factor between the RGR of dark control and blue wavelength over the 5 days. For a significant difference, F> Fcrit and P-value < 0.05. No variation between the days, however a difference is observed between the RGR of the air and CO2 blue wavelength and dark control.

	F	P-value	F-crit
Source of Variation	**Source of light**		
Days	1.791	0.293	6.388
RGR (d^{-1})	13.895	0.020	7.709

E.2.4 Linear regression on the Boltzmann Distribution RGR

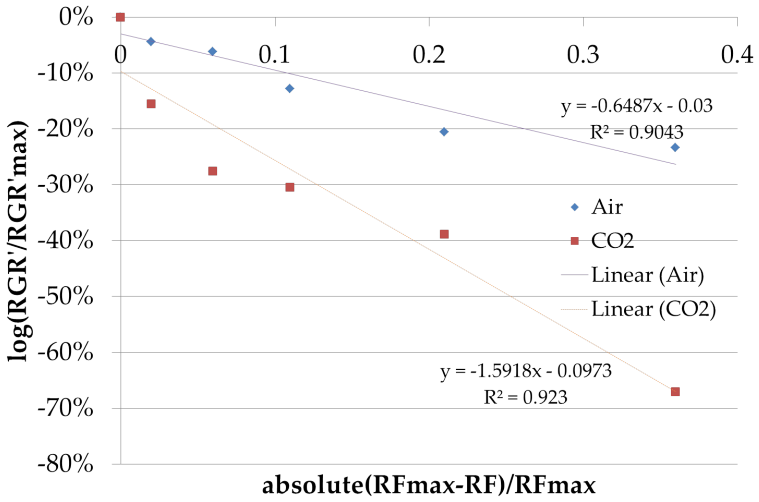


Figure E.2.2: Linear Regression applied to Figure 3.4.5 using Microsoft Excel.

E.3 Chapter four

E.3.1 Absorbance and concentration

Table E.3.1: Average absorbance and concentration relationship. Obtained from the growth profile over 16 days. In order to find the concentration of the sample (variable y_3) correlating to the absorbance (variable x_3) , the researcher did a linear interpolation between the points using the following generalized equation: $\frac{y_2-y_1}{x_2-x_1} = \frac{y_2-y_3}{x2-x_3}$.

Absorbance (740 nm) [x]	**Concentration (g /L) [y]**
0	0
0.0060	0.01533
0.0505	0.057
0.3385	0.12333
0.6725	0.19367
0.9695	0.28767
1.217	0.336
1.416	0.515
1.53	0.6325
1.6695	0.73188
1.783	0.89438
1.851	0.9875
1.855	1.1225
1.8815	1.266
1.9125	1.291
1.962	1.437

E.3.2 Production values per cycle

Table E.3.2: Microalgae production rates 900-1000 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
2	85.856	79.56	92.86	91.22	85.29	88.72	70.98	46.23
3	88.52	98.98	105.78	115.26	42.65	89.44	66.00	63.00
4	89.15	95.74	92.45	90.43	105.17	92.04	68.52	58.77
5			123.08	110.61	95.60	95.33	97.16	58.29
6			124.16	108.80	88.72	96.56	96.16	56.12
7			108.82	113.90	117.12	112.25	89.47	82.05
8			108.82	128.74	121.45	92.32	94.91	70.33
9				134.70	115.39	92.04	71.60	64.46
10						94.78	72.72	63.18
11						91.35	90.81	64.55
12						112.25	87.43	52.75
13						95.32	91.22	50.91
14							76.59	70.33
15							94.37	64.01
16								62.91
17								65.28
18								84.75

Table E.3.3: Microalgae production rates 500-600 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
2	83.43	78.72	93.01	88.07	76.74	82.82	80.34	51.60
3	78.58	78.58	82.63	90.39	80.75	85.85	63.82	53.46
4	73.18	74.16		98.72	90.81	99.22	85.75	55.31
5				102.81	87.55	74.57	80.34	59.13
6						85.00	83.74	60.46
7						86.04	77.25	63.75
8							65.46	64.96
9							87.14	66.52
10							78.95	64.39
11								65.67
12								64.53

E.3.3 Concentration values of the microalgae per cycle

Table E.3.4: Microalgae concentration 900-1000 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
2	858.59	795.59	928.62	912.18	852.86	887.22	946.42	924.51
3	973.81	1088.75	906.70	768.39	938.20	894.38	880.06	945.05
4	980.65	957.37	924.51	1055.00	841.41	920.40	913.55	881.49
5			1055.00	921.77	956.00	953.26	1295.42	874.34
6			1241.63	906.70	887.22	965.59	961.48	1122.50
7			932.72	949.16	1171.24	1122.50	1192.90	1230.80
8			932.72	1287.37	1214.56	923.14	949.16	1055.00
9				1122.50	923.14	920.40	954.63	966.96
10						947.79	969.70	947.79
11						913.55	908.07	968.33
12						1122.50	1165.82	791.30
13						953.26	912.18	1272.85
14							1021.25	1055.00
15							943.68	960.11
16							0.00	943.68
17								979.28
18								1271.24

Table E.3.5: Microalgae concentration for 500-600 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
1	584.06	551.07	593.33	520.15	523.25	561.38	596.43	515.00
2	550.04	550.04	531.49	587.15	613.95	621.16	535.61	516.03
3	512.30	519.12	590.24	602.61	646.04	643.90	638.20	534.58
4				658.15	544.89	496.11	571.69	553.14
5				514.10	525.31	559.32	535.61	591.27
6						637.49	558.29	604.67
7						645.32	515.00	637.49
8							654.58	649.60
9							580.96	665.27
10							526.34	643.90
11								656.72
12								645.32

E.3.4 Days between cycle

Table E.3.6: The residence time.

Concentration	**0**	**0**	**30**	**40**	**50**	**60**	**70**	**80**
900–1000 mg/L	9.75	9.3	5.43	4.38	4.5	3	2.64	2.24
500–600 mg/L	6	6	3.67	2.75	2.5	1.83	1.2	1

E.3.5 Relative growth rates

Table E.3.7: Microalgae RGR 900-1000 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
1	0.415	0.425	0.405	0.407	0.403	0.426	0.422	0.394
2	0.468	0.439	0.800	0.898	1.005	1.488	1.605	1.366
3	0.409	0.391	0.745	1.285	0.395	1.283	1.350	2.152
4	0.394	0.411	0.631	0.559	1.152	1.309	1.204	1.827
5	0.369	0.320	0.823	0.887	1.004	1.338	1.406	1.964
6			0.629	0.978	0.844	1.343	1.870	1.327
7			0.759	0.885	1.055	1.393	1.280	2.135
8			0.712	0.824	1.015	1.310	1.954	2.058
9				0.964	1.220	1.246	1.130	1.988
10						1.336	1.166	1.843
11						1.230	1.802	1.816
12						1.299	1.371	1.816
13						1.218	1.910	1.027
14							1.216	1.877
15							1.785	1.935
16								1.822
17								1.693
18								1.853

Table E.3.8: Microalgae RGR for 500-600 mg/L.

	0	**0**	**30**	**40**	**50**	**60**	**70**	**80**
1	0.562	0.561	0.454	0.543	0.488	0.581	0.591	0.506
2	0.597	0.577	1.162	1.083	1.192	1.742	3.335	3.401
3			0.858	1.151	1.158	1.650	1.864	3.436
4				1.197	1.629	3.165	3.400	3.471
5				1.415	1.658	1.642	3.335	3.537
6					2.801	1.755	3.081	3.198
7						1.761	3.248	3.214
8							1.478	3.308
9							3.279	3.256
10							3.477	3.224
11								3.539
12								3.625

E.4 Chapter five

E.4.1 Growth rates

Table E.4.1: % Growth rates unsterilized at 21-days.

	Spirodela		***Lemma***		***Wolffia***	
	9509	**8483**	**8428**	**DWC 112**	**9527**	**7340**
HP	532.56	590.04	468.86	617.08	563.73	619.44
	532.56	669.71	499.40	550.89	524.51	583.33
	527.91	639.83	449.10	593.59	558.82	-
2.2.2.	486.05	582.57	450.90	612.81	536.76	691.67
	513.95	595.02	431.14	576.51	529.41	669.44
	458.14	530.29	485.03	666.19	546.57	-
2.1.2.	530.23	418.26	459.88	546.62	495.10	319.44
	451.16	363.49	495.81	527.40	448.53	366.67
	453.49	410.79	422.16	574.38	428.92	-
1.8.1	467.44	356.02	449.10	546.62	470.59	200.00
	462.79	356.02	441.92	533.81	352.94	236.11
	481.40	378.42	454.49	582.92	338.24	-
1.3.1	497.67	455.60	492.22	619.22	473.04	325.00
	493.02	495.44	467.07	606.41	558.82	325.00
	502.33	482.99	486.83	617.08	470.59	-
5.11.26	530.23	417.72	323.17	295.37	551.47	641.67
	523.26	444.30	346.93	281.99	517.16	666.67
	527.91	425.32	332.67	298.46	517.16	-
5.11.26Ca	486.05	438.61	357.62	285.08	598.04	702.78
	527.91	455.70	329.11	305.66	610.29	683.33
	546.51	429.11	346.93	332.42	588.24	-

E.4.2 Starch

Table E.4.2: % Starch unsterilized at 21-days.

	Spirodela		***Lemma***		***Wolffia***	
	9509	**8483**	**8428**	**DWC 112**	**9527**	**7340**
HP	1.05%	1.44%	2.80%	8.23%	13.61%	19.19%
	1.08%	1.39%	1.02%	8.00%	13.95%	16.61%
	1.04%	1.28%	0.25%	7.67%	12.38%	-
2.2.2.	1.11%	3.08%	1.92%	2.53%	4.90%	8.39%
	0.89%	1.68%	2.48%	3.86%	4.60%	9.45%
	1.09%	2.17%	2.25%	2.71%	3.50%	-
2.1.2.	1.14%	1.91%	1.39%	2.78%	7.69%	7.75%
	1.57%	1.36%	1.92%	2.80%	6.84%	6.93%
	1.21%	4.46%	0.00%	2.62%	5.98%	-
1.8.1	1.09%	1.35%	1.98%	2.93%	7.46%	9.70%
	1.18%	1.61%	1.63%	4.90%	7.89%	10.85%
	1.18%	1.60%	2.23%	2.98%	7.80%	-
1.3.1	0.99%	9.32%	2.47%	2.07%	12.12%	12.11%
	1.77%	1.90%	1.44%	2.73%	10.22%	12.15%
	1.74%	5.26%	1.38%	5.23%	11.19%	-
5.11.26	0.44%	1.70%	1.04%	4.39%	0.43%	8.45%
	0.63%	1.41%	0.73%	4.82%	3.80%	8.03%
	3.29%	1.71%	1.31%	4.19%	3.92%	-
5.11.26Ca	1.16%	2.79%	0.72%	5.30%	5.60%	9.57%
	1.30%	1.10%	0.86%	5.24%	4.63%	8.08%
	0.72%	2.20%	1.53%	4.43%	3.83%	-

E.4.3 RGR

Table E.4.3: RGR 2 samples per row.

	Spirodela	***Lemma***	***Wolffia***	**370**	**494**	**180**
	9509	**8483**	**8428**	**DWC 112**	**9527**	**7340**
HP	0.080	0.085	0.074	0.087	0.082	0.087
	0.080	0.091	0.077	0.081	0.079	0.084
2.2.2.	0.075	0.084	0.072	0.086	0.080	0.092
	0.078	0.085	0.070	0.083	0.079	0.091
2.1.2.	0.079	0.068	0.073	0.081	0.076	0.055
	0.072	0.061	0.076	0.079	0.071	0.062
1.8.1	0.073	0.060	0.072	0.081	0.074	0.033
	0.073	0.060	0.071	0.080	0.060	0.041
1.3.1	0.076	0.072	0.076	0.087	0.074	0.056
	0.076	0.076	0.073	0.086	0.082	0.056
5.11.26	0.079	0.068	0.056	0.052	0.081	0.089
	0.079	0.071	0.059	0.049	0.078	0.090
5.11.26Ca	0.075	0.070	0.061	0.050	0.085	0.093
	0.079	0.072	0.057	0.053	0.086	0.092

E.4.4 MS, SH and HP NPK calculation

Elemental mass relationships used:

- $\mathrm{N} = 0.226\,\mathrm{NO_3^-}$;
- $\mathrm{P} = 0.319\,\mathrm{H_2PO_4^-}$;
- $\mathrm{N} = 0.777\,\mathrm{NH_4^+}$;
- $\mathrm{N} = 0.874\,\mathrm{NH_2^-}$;
- $\mathrm{P} = 0.436\,\mathrm{P_2O_5}$; and
- $\mathrm{K} = 0.83\,\mathrm{K_2O}$.

Table E.4.4: Mineral salt media for plant tissue and cell culture from Gamborg et al. (1976), calculating the number of mols.

1 Litre		**MS full strength = 4.53 g**					**SH = 3.1 g**				
		mg/L	**mg**	**g**	**MW (g/mol)**	**mol**	**mg/L**	**mg**	**g**	**MW (g/mol)**	**mol**
NH_4NO_3		1650.00	1650.00	1.65	80.04	2.06E-02	-	-	-	-	-
	NH_4^+					1.03E-02					
	NH_2^-					1.03E-02					
	H^+					2.06E-02					
	NO_3^-					2.06E-02					
KNO_3		1900.00	1900.00	1.9	101.10	1.88E-02	2500.00	2500.00	2.50	101.10	2.47E-02
	K^+					1.88E-02					2.47E-02
	NO_3^-					1.88E-02					2.47E-02
$CaCl_2.2H_2O$		440.00	440.00	0.44	147.01	2.99E-03	200.00	200.00	0.20	147.01	1.36E-03
	Ca^{2+}					2.99E-03					1.36E-03
	Cl^-					5.99E-03					2.72E-03
	H^+					2.99E-03					1.36E-03
	OH^-					2.99E-03					1.36E-03
$MgSO_4.7H_2O$		370.00	370.00	0.37	246.47	1.50E-03	400.00	400.00	0.40	246.47	1.62E-03
	Mg^{2+}					1.50E-03					1.62E-03
	SO_4^{2-}					1.50E-03					1.62E-03
	H^+					1.50E-03					1.62E-03
	OH^-					1.50E-03					1.62E-03
KH_2PO_4		170.00	170.00	0.17	136.09	1.25E-03	-	-	-	-	-
	K^+					1.25E-03					
	$H_2PO_4^-$					1.25E-03					
$NH_4H_2PO_4$		-	-	-	-	-	300.00	300.00	0.30	115.03	2.61E-03
	NH_4^+										2.61E-03
	$H_2PO_4^-$										2.61E-03

Table E.4.5: MS mass percent from Table E.4.4.

MS Ratios	mols	MW	mass	% fraction	g	mass%
NH_4^+	0.01031	18.03846	0.18592	0.77700	0.14446	4.20907
NH_2^-	0.01031	16.02258	0.16514	0.87400	0.14434	3.73869
NO_3^-	0.03941	62.00490	2.44340	0.22600	0.55221	55.31615
$H_2PO_4^-$	0.00125	96.98724	0.12116	0.31900	0.03865	0.87498
K^+	0.02004	39.09830	0.78360	1.00000	0.78360	17.74004
H^+	0.02511	1.00794	0.02531			0.57293
Ca^{2+}	0.00299	40.07800	0.11995			2.71554
Cl^-	0.00599	35.45300	0.21221			4.80434
OH^-	0.00449	17.00734	0.07643			1.73035
Mg^{2+}	0.00150	24.30500	0.03649			0.82601
SO_4^{2-}	0.00150	96.06260	0.14421			3.26469
		Total Mass	**4.42**			

Table E.4.6: SH mass percent from Table E.4.4.

MS Ratios	mols	MW	mass	% fraction	g	mass%
NH_4^+	0.00261	18.03846	0.04705	0.77700	0.03656	1.45E+00
NH_2^-	0.00000	16.02258	0.00000	0.87400	0.00000	0.00E+00
NO_3^-	0.02473	62.00490	1.53321	0.22600	0.34651	4.71E+01
$H_2PO_4^-$	0.00261	96.98724	0.25295	0.31900	0.08069	2.48095
K^+	0.02473	39.09830	0.96679	1.00000	0.96679	29.72484
H^+	0.00298	1.00794	0.00301			0.09245
Ca^{2+}	0.00136	40.07800	0.05452			1.67634
Cl^-	0.00272	35.45300	0.09646			2.96578
OH^-	0.00298	17.00734	0.05074			1.55998
Mg^{2+}	0.00162	24.30500	0.03944			1.21275
SO_4^{2-}	0.00162	96.06260	0.15590			4.79324
		Total Mass	3.25247			

Table E.4.7: MS and SH NPK mass percent from Table E.4.5 and E.4.6.

	mass %	mass (g)	mass%	mass (g)
	MS		**SH**	
N	19.040	0.841	11.778	0.383
P	0.875	0.039	2.481	0.081
K	17.740	0.784	29.725	0.967

Table E.4.8: Hydroponic media moles.

		Litre of stock	0.003302125	
Stock A		**g**	**MW (g/mol)**	**mol**
Calcium Nitrate		0.31622	164.08	0.00193
	NO_3^-			0.00385
	Ca^{2+}			0.00193
Potassium Nitrate		0.07905	101.10	0.00078
	K^+			0.00078
	NO_3^-			0.00078
Iron Chelate (Sprint)		0.00698		
Stock B		**g**	**MW (g/mol)**	**mol**
Mono Potassium Phosphate		0.07905	136.08	0.00058
	K^+			0.00058
	$H_2PO_4^-$			0.00058
Potassium Sulphate		0.17446	174.25	0.00100
	K^+			0.00200
	SO_4^{2-}			0.00100
Magnesium Sulphate		0.15811	120.37	0.00131
	Mg^{2+}			0.00131
	SO_4^{2-}			0.00131
STEM (Soluble Trace Element Mix)		0.00698		
	Total Mass	**0.8209**		

Table E.4.9: HP mass percent from Table E.4.8.

HP Ratios	**mols**	**MW**	**mass**	**mass%**
NO_3^-	4.64E-03	62.00	0.29	35.02
$H_2PO_4^-$	5.81E-04	96.99	0.06	6.86
K^+	3.37E-03	39.10	0.13	16.03
Ca^{2+}	1.93E-03	40.08	0.08	9.41
SO_4^{2-}	2.31E-03	96.06	0.22	27.09
Mg^{2+}	1.31E-03	24.31	0.03	3.89
		Total Mass	0.8209	

Table E.4.10: HP NPK mass percent from Table E.4.9.

HP Ratios	**mass% total**	**%**	**Element%**	
NO_3^-	35.020	0.226	7.915	N
$H_2PO_4^-$	6.864	0.319	2.190	P
K^+	16.029	1.000	16.029	K

E.4.5 Commercial fertilizer NPK

The following equation was used to calculate the sample size needed from each NPK based on 0.841 g of full strength MS based on N of MS: $\text{mass}\,\% = \frac{\text{mass N}}{\text{sample size NPK}}$. Except for of 5-11-26 and 5-11-26 Ca which was based on full strength K (0.784 g).

Table E.4.11: How much grammes needed for a full strength NPK from MS.

	mass%	**actual**	**mass (kg)**	**Calculated Sample size (g)**	**% off**	**Amount of litres**
17-3-17						
N	17.000	17.000	1.927	4.947	0%	2291.83
P	3.000	1.308	0.340		-67%	
K	17.000	14.110	1.927		11%	
18-8-17						
N	18.00	18.00	2.04	4.672	0%	2426.64
P	8.00	3.49	0.91		-322%	
K	17.00	14.11	1.93		16%	
20-10-20						
N	20.00	2.268	0.841	4.205	0%	2696.27
P	4.36	1.134	0.039		-374%	
K	16.60	2.268	0.784		11%	
20-20-20						
N	20.00	2.27	0.841	4.205	0%	2696.27
P	8.72	2.27	0.0387		-849%	
K	16.60	2.27	0.7836		11%	
5-11-26 (with and without Ca) based on K of MS						
N	5.00	0.5669	0.8410	3.6312	78%	3122.38
P	4.80	1.2472	0.0386		-351%	
K	21.58	2.9478	0.7836		0%	

Table E.4.12: How much in grams to add to different litres of water based on 0.5 strength of commercial media.

	0.1	**0.5**	**1**	**5**	**10**	**100**	**10000**
MS	0.22	1.10	2.20	11.00	22.00	220.00	22000.00
SH	0.16	0.80	1.60	8.00	16.00	160.00	16000.00
20-20-20	0.21	1.05	2.10	10.51	21.03	210.25	21025.09
20-10-20	0.21	1.05	2.10	10.51	21.03	210.25	21025.09
18.8.17	0.23	1.17	2.34	11.68	23.36	233.61	23361.21
17.3.17	0.25	1.24	2.47	12.37	24.74	247.35	24735.40
5.11.26	0.18	0.91	1.82	9.08	18.15	181.50	18150.00

Table E.4.13: Cost of items in 2013.

Name	**Cost/unit**
Schenk and Hildebrandt Basal Salt Mixture	$4.40
Murashige and Skoog Basal Medium	$9.50
17.3.17 Peat-Lite Neutral Cal-Mag	$36.90
18.8.17 Peat-Lite Neutral Cal-Mag	$39.35
20.20.20 General Purpose	$36.35
5.11.26 General Purpose	$42.15
20.10.20	$35.99

E.4.6 Stats for all species

Duckweed ANOVA two factor replication using two samples for ANOVA as *Wolffia cylindracea* 7340 only had two sets. Unsterilized Media: HP; 2.2.2; 2.1.2.; 1.8.1;1.3.1; 5.11.26 and 5.11.26 Ca. As for the ANOVA test on the RGR, it can be seen that the F value was greater than the F-crit as well as the P-value was greater than the alpha value (0.05). Hence the variation between the RGR content in the media as well as the duckweed is significant. Not shown is 14 days ANOVA RGR which also showed a significant difference in values.

Table E.4.14: ANOVA two factor between all species (2 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

	F	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Unsterilized Media	49.93	1.51E-17	2.32
Species	17.02	3.55E-09	2.44
Interaction	30.52	3.89E-20	1.73
Source of Variation	**% Starch**		
Unsterilized Media	24.53	2.95E-12	2.32
Species	183.23	2.23E-27	2.44
Interaction	7.57	2.83E-09	1.73
Source of Variation	**RGR**		
Unsterilized Media	50.74	1.1E-17	2.32
Species	16.21	6.9E-09	2.44
Interaction	32.83	9.3E-21	1.73

E.4.7 Stats between each genus

E.4.7.1 *Spirodela*

Table E.4.15: ANOVA two factor between unsterilized *Spirodela* 9509 and 8483 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

	F	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	29.75	7E-11	2.45
Unsterilized vs Sterilized Media	13.94	9E-04	4.20
Interaction	18.85	1E-08	2.45
Source of Variation	**% Starch**		
Type of Media	2.44	5E-02	2.45
Unsterilized vs Sterilized Media	10.09	4E-03	4.20
Interaction	1.76	1E-01	2.45

E.4.7.2 *Lemna*

Table E.4.16: ANOVA two factor between unsterilized *Lemna* 8428 and DWC 112 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

	F	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	103.04	9E-18	2.45
Unsterilized vs Sterilized Media	99.53	1E-10	4.20
Interaction	16.75	4E-08	2.45
Source of Variation	**% Starch**		
Type of Media	7.10	1E-04	2.45
Unsterilized vs Sterilized Media	136.66	3E-12	4.20
Interaction	10.01	6E-06	2.45

E.4.7.3 *Wolffia*

Table E.4.17: ANOVA two factor between unsterilized *Wolffia* 7340 and 9527 (2 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

	F	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	55.29	6E-09	2.85
Unsterilized vs Sterilized Media	0.76	4E-01	4.60
Interaction	20.89	3E-06	2.85
Source of Variation	**% Starch**		
Type of Media	81.10	5E-10	2.85
Unsterilized vs Sterilized Media	99.62	1E-07	4.60
Interaction	5.32	5E-03	2.85

E.4.8 Stats for each species

E.4.8.1 *Spirodela polyrhiza* 9509

Table E.4.18: ANOVA two factor between unsterilized and sterilized *Spirodela* 9509 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

Spirodela 9509	**F**	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	5.381	4.2E-03	2.866
Unsterilized vs Sterilized Media	0.223	6.4E-01	4.351
Interaction	1.829	1.6E-01	2.866
Source of Variation	**% Starch**		
Type of Media	5.968	2.5E-03	2.866
Unsterilized vs Sterilized Media	0.013	9.1E-01	4.351
Interaction	3.017	4.2E-02	2.866

Table E.4.19: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH

Spirodela 9509	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	-2.546		-4.150
P(T<=t) one-tail	0.012		0.000
t Critical one-tail	1.761		1.761
P(T<=t) two-tail	0.023		0.001
t Critical two-tail	2.145		2.145
	% Starch		**% Starch**
t Stat	-4.300		7.108
P(T<=t) one-tail	3.66E-04		2.64E-06
t Critical one-tail	1.761		1.761
P(T<=t) two-tail	0.001		0.000
t Critical two-tail	2.145		2.145

E.4.8.2 *Spirodela polyrhiza* 8483

Table E.4.20: ANOVA two factor between unsterilized and sterilized *Spirodela* 8483 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

Spirodela 8483	**F**	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	90.209	1.7E-12	2.866
Unsterilized vs Sterilized Media	0.146	0.7063	4.351
Interaction	1.971	0.1379	2.866
Source of Variation	**% Starch**		
Type of Media	1.350	2.9E-01	2.866
Unsterilized vs Sterilized Media	0.593	0.4504	4.351
Interaction	2.504	0.0748	2.866

Table E.4.21: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH.

Spirodela 8483	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	1.412		0.3753
P(T<=t) one-tail	0.090		0.3565
t Critical one-tail	1.761		1.7613
P(T<=t) two-tail	0.180		0.7130
t Critical two-tail	2.145		2.1448
	% Starch		**% Starch**
t Stat	-1.354		-2.504
P(T<=t) one-tail	0.099		0.013
t Critical one-tail	1.771		1.761
P(T<=t) two-tail	0.199		0.025
t Critical two-tail	2.160		2.145

E.4.8.3 *Lemna Gibba* 8428

Table E.4.22: ANOVA two factor between unsterilized and sterilized *Lemna* 8428 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

Lemna 8428	**F**	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	2.299	0.094	2.866
Unsterilized vs Sterilized Media	4.227	0.053	4.351
Interaction	0.869	0.499	2.866
Source of Variation	**% Starch**		
Type of Media	0.633	0.644	2.866
Unsterilized vs Sterilized Media	5.136	0.035	4.351
Interaction	2.847	0.051	2.866

Table E.4.23: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH.

Lemna 8428	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	-1.427		1.755
P(T<=t) one-tail	0.088		0.051
t Critical one-tail	1.761		1.761
P(T<=t) two-tail	0.175		0.101
t Critical two-tail	2.145		2.145
	% Starch		**% Starch**
t Stat	-0.096		0.939
P(T<=t) one-tail	0.463		0.182
t Critical one-tail	1.761		1.761
P(T<=t) two-tail	0.925		0.364
t Critical two-tail	2.145		2.145

E.4.8.4 *Lemna Minor* DWC 112

Table E.4.24: ANOVA two factor between unsterilized and sterilized *Lemna* DWC 112 (3 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

Lemna DWC 112	**F**	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Type of Media	5.0155	0.0555	5.3177
Unsterilized vs Sterilized Media	1.3705	0.2754	5.3177
Interaction	0.0933	0.7679	5.3177
Source of Variation	**% Starch**		
Type of Media	5.9659	0.0404	5.3177
Unsterilized vs Sterilized Media	0.0234	0.8822	5.3177
Interaction	0.0709	0.7967	5.3177

Table E.4.25: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH.

Lemna DWC 112	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	-0.205		-1.262
P(T<=t) one-tail	0.424		0.131
t Critical one-tail	2.132		2.015
P(T<=t) two-tail	0.848		0.263
t Critical two-tail	2.776		2.571
	% Starch		**% Starch**
t Stat	1.929		2.981
P(T<=t) one-tail	0.056		0.015
t Critical one-tail	2.015		2.015
P(T<=t) two-tail	0.112		0.031
t Critical two-tail	2.571		2.571

E.4.8.5 *Wolffia Globosa* 9527

Table E.4.26: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH.

Wolffia 9527	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	4.6327		-6.7272
P(T<=t) one-tail	0.0002		0.0000
t Critical one-tail	1.7613		1.7613
P(T<=t) two-tail	0.0004		0.0000
t Critical two-tail	2.1448		2.1448
	% Starch		**% Starch**
t Stat	-7.7105		4.5016
P(T<=t) one-tail	0.0000		0.0002
t Critical one-tail	1.7613		1.7613
P(T<=t) two-tail	0.0000		0.0005
t Critical two-tail	2.1448		2.1448

E.4.8.6 *Wolffia Cylindracea* 7340

Table E.4.27: t-Test: Two-Sample Assuming Unequal Variances for 0%. Unsterilized vs MS and SH.

Wolffia 7340	**MS**		**SH**
	% Growth Rate		**% Growth Rate**
t Stat	2.756		-2.771
P(T<=t) one-tail	0.011		0.011
t Critical one-tail	1.833		1.833
P(T<=t) two-tail	0.022		0.022
t Critical two-tail	2.262		2.262
	% Starch		**% Starch**
t Stat	-3.478		1.048
P(T<=t) one-tail	0.003		0.161
t Critical one-tail	1.833		1.833
P(T<=t) two-tail	0.007		0.322
t Critical two-tail	2.262		2.262

E.4.9 Stats for MS and SH

E.4.9.1 Between all species

Table E.4.28: ANOVA two factor between unsterilized and sterilized (2 rows per sample) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

	F	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Species	13.74	1.3E-04	3.11
MS vs SH	64.78	3.5E-06	4.75
Interaction	25.58	5.2E-06	3.11
Source of Variation	**% Starch**		
Species	402.59	6.3E-13	3.11
MS vs SH	220.17	4.4E-09	4.75
Interaction	64.74	2.9E-08	3.11

E.4.9.2 Within each species

Table E.4.29: ANOVA: Single Factor between SH and MS (3 rows per sample, except for 7340 (2 rows per sample)) at 21 days. For significant difference, F> Fcrit and P-value < 0.05.

MS vs SH	**F**	**P-value**	**F-crit**
Source of Variation	**% Growth Rate**		
Spirodela Polyrhiza 9509	0.31	6.1E-01	7.71
Spirodela Polyrhiza 8483	2.96	1.6E-01	7.71
Lemna Gibba 8428	3.30	1.4E-01	7.71
Lemna Minor DWC 112	0.46	5.4E-01	7.71
Wolffia Globosa 9527	104.36	5.2E-04	7.71
Wolffia Cylindracea 7340	101.33	9.7E-03	18.51
Source of Variation	**% Starch**		
Spirodela Polyrhiza 9509	49.36	2.2E-03	7.71
Spirodela Polyrhiza 8483	2.32	2.0E-01	7.71
Lemna Gibba 8428	0.25	6.4E-01	7.71
Lemna Minor DWC 112	5.85	7.3E-02	7.71
Wolffia Globosa 9527	132.82	3.2E-04	7.71
Wolffia Cylindracea 7340	160.19	6.2E-03	18.51

Appendix F

Medium Recipe

F.1 Duckweed

F.1.1 MS and SH

The 0.5X MS per litre or SH is used with 1% sucrose and a pH of 5.7. The MS bottle ordered comes in 4.4 g/L while the SH is generally 3.2 g/L. Therefore half of the contents were used in the medium.

Table F.1.1: SH and MS mineral components for plant tissue and cell culture (Gamborg et al., 1976).

Salts	**MS**	**SH**
Macro-nutrients	**mg/L**	**mg/L**
NH_4NO_3	1650.00	
KNO_3	1900.00	2500.00
$CaCl_2.H_2O$	440.00	200.00
$MgSO_4.7H_2O$	370.00	400.00
KH_2PO_4	170.00	
$NH_4H_2PO_4$		300.00
Micro-nutrients	**mg/L**	**mg/L**
KI	0.83	1.00
H_3BO_3	6.20	5.00
$MnSO_4.H_2O$	22.30	
$MnSO_4.H_2O$		10.00
$ZnSO_4.7H_2O$	8.60	1.00
$Na_2MoO_4.2H_2O$	0.25	0.10
$CuSO_4.5H_2O$	0.03	0.20
$CoCl_2.6H_2O$	0.03	0.10
Na_2.EDTA	37.30	20.00
$FeSO_4.7H_2O$	27.80	15.00

F.1.2 Tomato fertilizer mixture for hydroponic culture

The hydroponic media is a slightly different recipe. The amounts are the necessary amounts needed to produce a working media of 36 300 litres from 121 litres of stock medium. Thus the ratio of stock hydroponic culture to actual water needed for the medium is 0.00330 : 1.

Table F.1.2: Tomato fertilizer mixture for hydroponic culture of 121 litres of stock to make 36 300 litres.

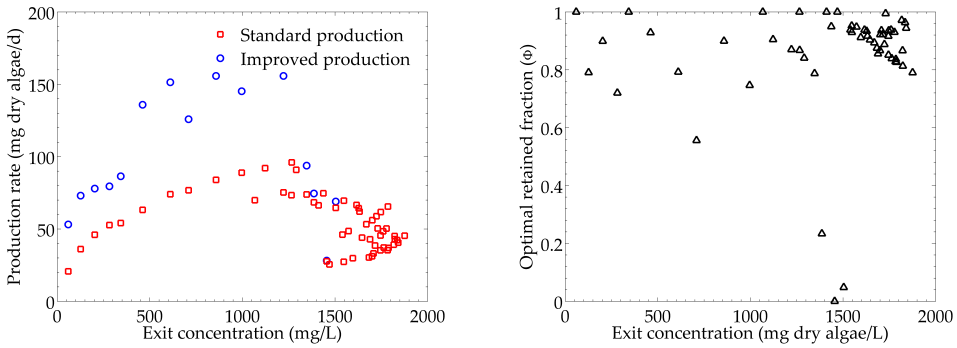
Stock A	**kg**	**Concentration (kg/litre)**
Calcium Nitrate	11.6	0.09576
Potassium Nitrate	2.9	0.02394
Iron Chelate (Sprint)	0.128	0.001057
Stock B	**kg**	**Concentration (kg/litre)**
Mono Potassium Phosphate	2.9	0.02394
Potassium Sulphate	6.4	0.05283
Magnesium Sulphate	5.8	0.04788
STEM (Soluble Trace Element Mix)	0.128	0.001057

Appendix G

Figures not shown

G.1 Chapter four

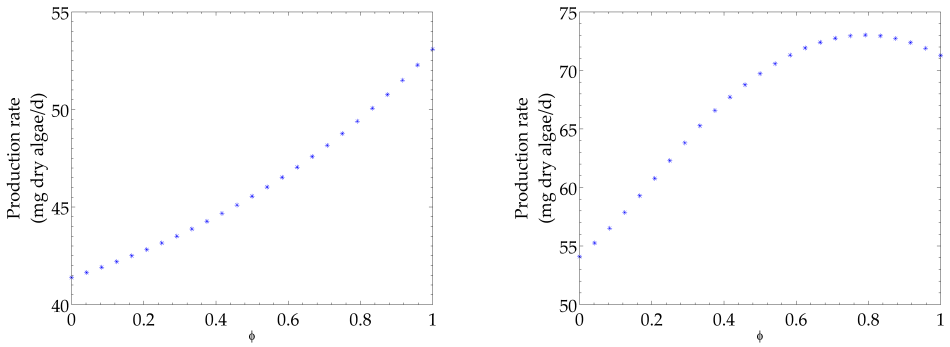
G.1.1 Predictions



(a) Predictions on production rate.

(b) Retained fraction predictions.

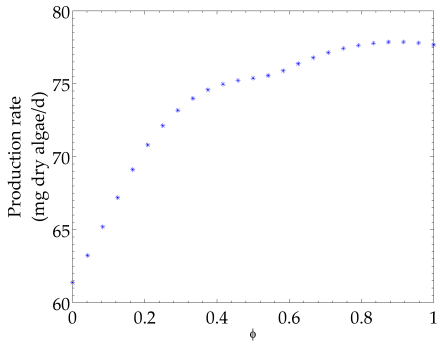
Figure G.1.1: Predictions based on the batch profile over all 57 days.



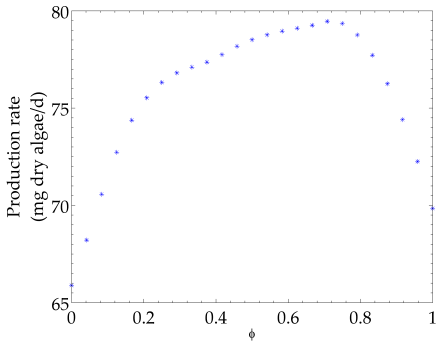
(a) Predictions for exit concentration of 59.90 mg/L.

(b) Predictions for exit concentration of 126.69 mg/L.

Figure G.1.2: Predictions for each exit concentration 59.90 mg/L and 126.69 mg/L.

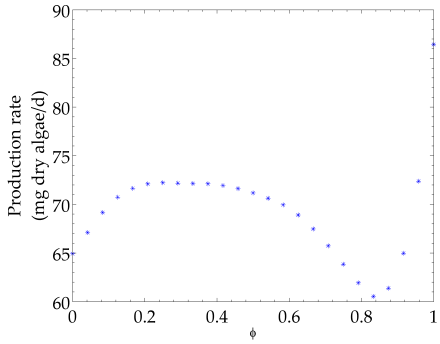


(a) Predictions for exit concentration of 202.64 mg/L.

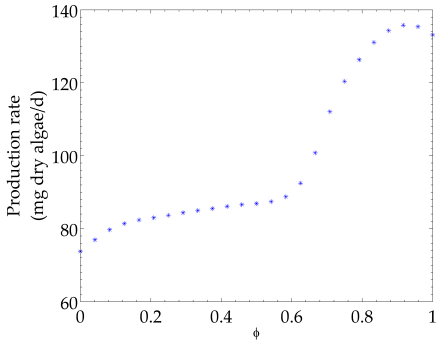


(b) Predictions for exit concentration of 282.85 mg/L.

Figure G.1.3: Predictions for each exit concentration 202.64 mg/L and 282.85 mg/L.

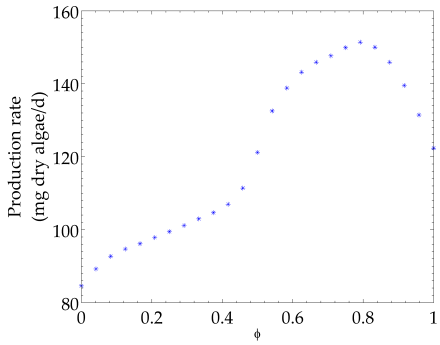


(a) Predictions for exit concentration of 343.12 mg/L.

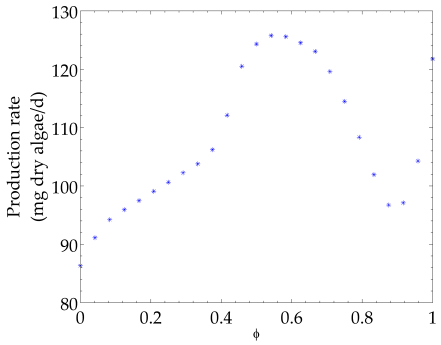


(b) Predictions for exit concentration of 460.93 mg/L.

Figure G.1.4: Predictions for each exit concentration 343.12 mg/L and 460.93 mg/L.

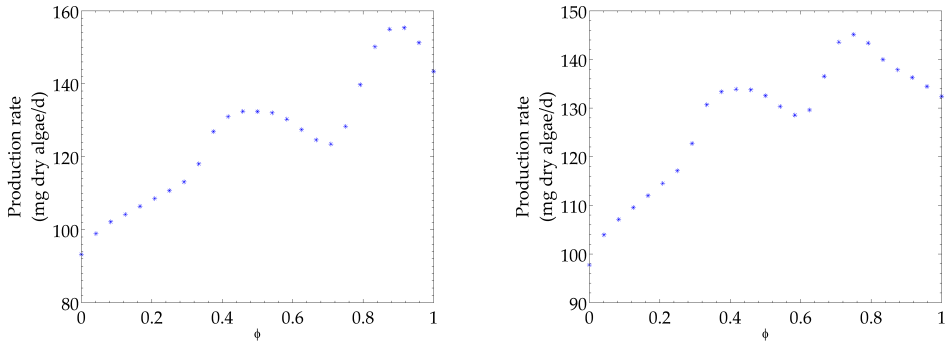


(a) Predictions for exit concentration of 610.63 mg/L.



(b) Predictions for exit concentration of 709.13 mg/L.

Figure G.1.5: Predictions for each exit concentration 610.63 mg/L and 709.13 mg/L.



(a) Predictions for exit concentration of 857.51 mg/L.

(b) Predictions for exit concentration of 995.97 mg/L.

Figure G.1.6: Predictions for each exit concentration 857.51 mg/L and 995.97 mg/L.

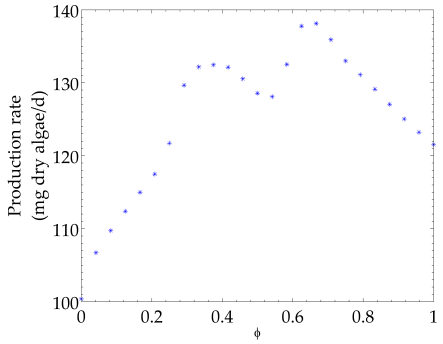
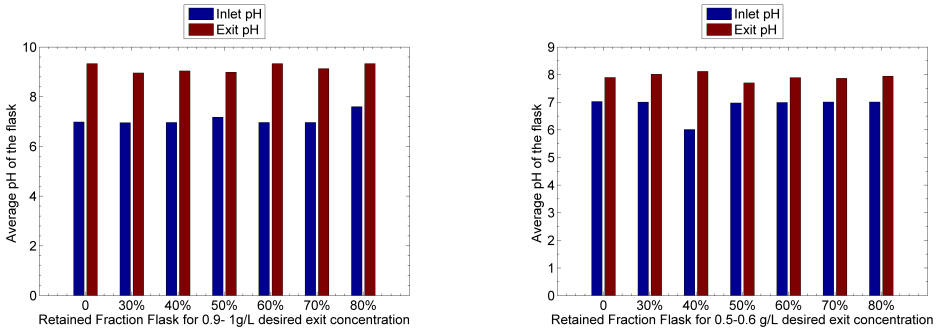


Figure G.1.7: Predictions for exit concentration of 1122.5 mg/L.

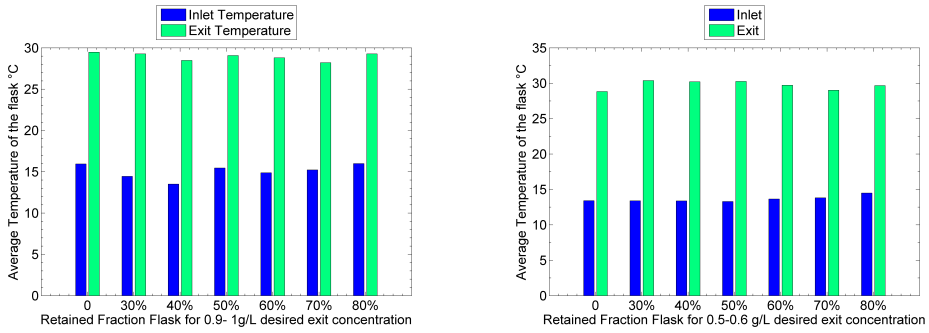
G.1.2 Monitored variables: pH and temperature



(a) 900–1000 mg/L range over 57 days.

(b) 500–600 mg/L range over 20 days.

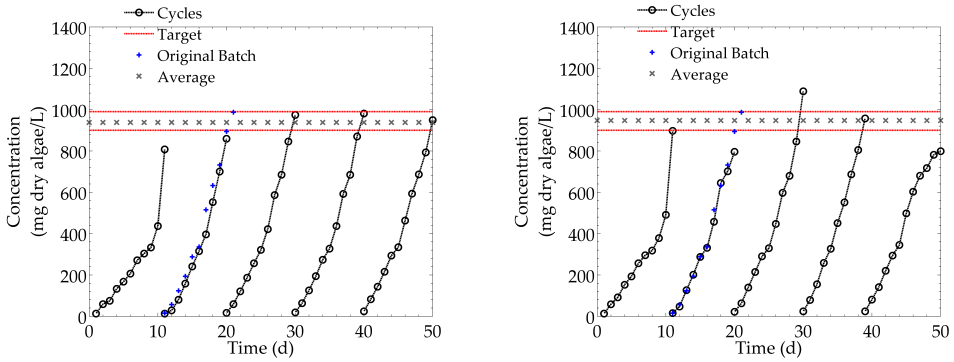
Figure G.1.8: pH of the experiment over the respective days.



(a) 900–1000 mg/L range over 50 days. (b) 500–600 mg/L range over 20 days.

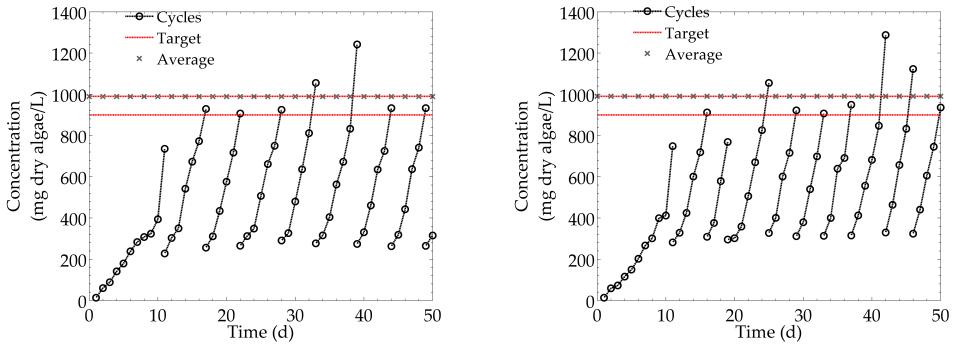
Figure G.1.9: Temperature of the experiment over the respective days.

G.1.3 Individual growth profiles



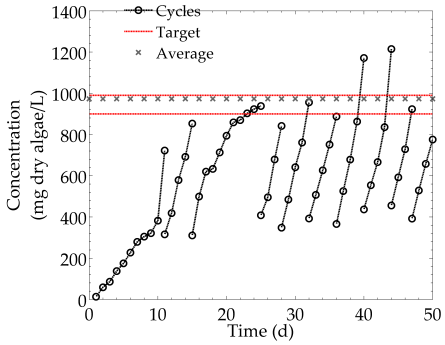
(a) 0 % retained fraction. (b) 0 % retained fraction.

Figure G.1.10: Growth profile 0% Φ at 900–1000 mg/L range.

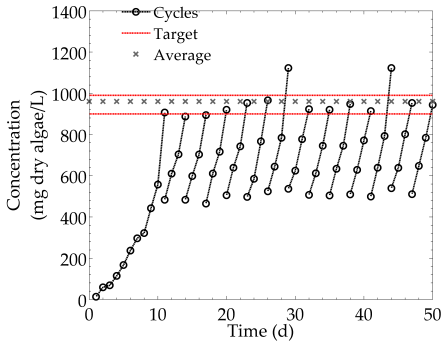


(a) 30 % retained fraction. (b) 40 % retained fraction.

Figure G.1.11: Growth profile 30–40% Φ at 900–1000 mg/L range.

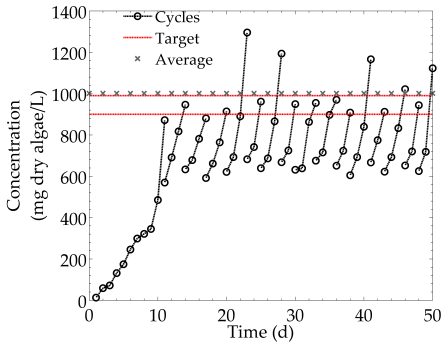


(a) 50 % retained fraction.

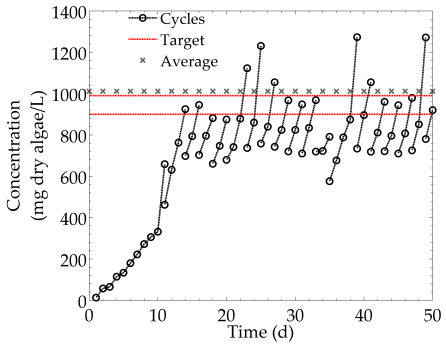


(b) 60 % retained fraction.

Figure G.1.12: Growth profile 50–60% Φ at 900–1000 mg/L range.

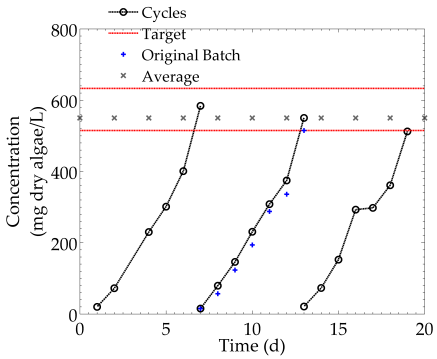


(a) 70 % retained fraction.

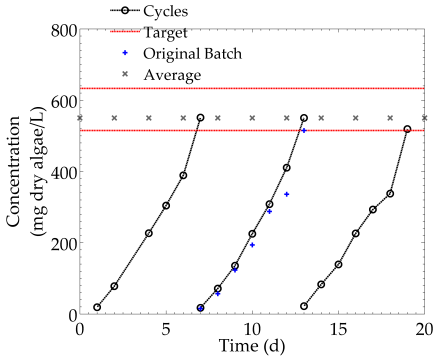


(b) 80 % retained fraction.

Figure G.1.13: Growth profile 70–80% Φ at 900–1000 mg/L range.

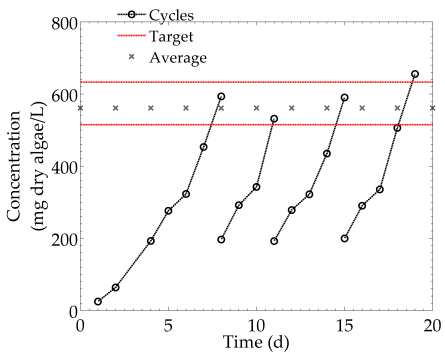


(a) 0 % retained fraction.

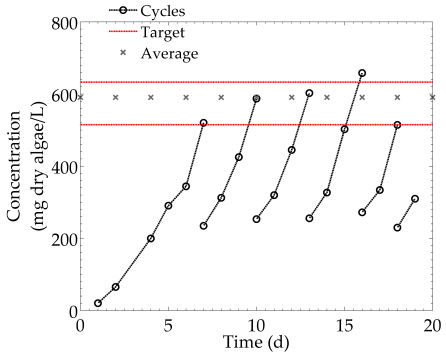


(b) 0 % retained fraction.

Figure G.1.14: Growth profile 0% Φ at 500–600 mg/L range.

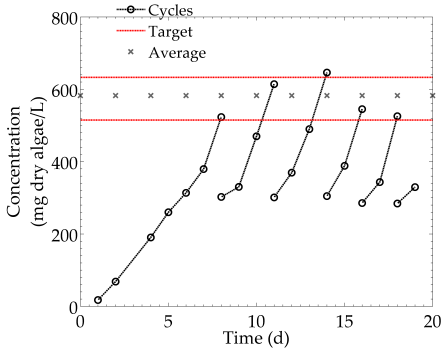


(a) 30 % retained fraction.

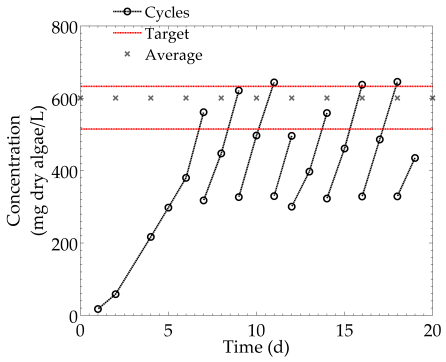


(b) 40 % retained fraction.

Figure G.1.15: Growth profile 30–40% Φ at 500–600 mg/L range.

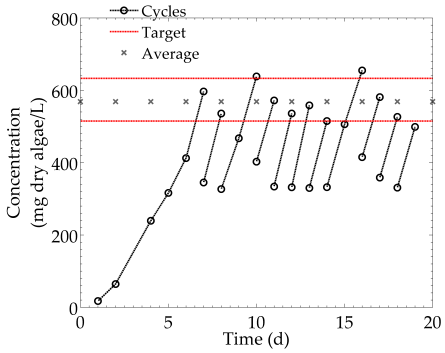


(a) 50 % retained fraction.

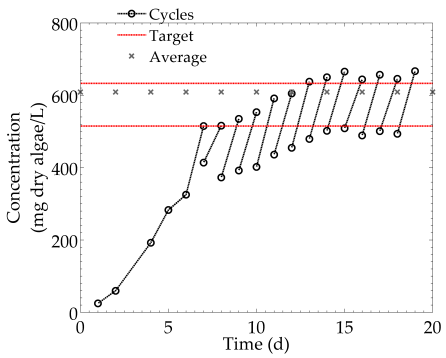


(b) 60 % retained fraction.

Figure G.1.16: Growth profile 50–60% Φ at 500–600 mg/L range.



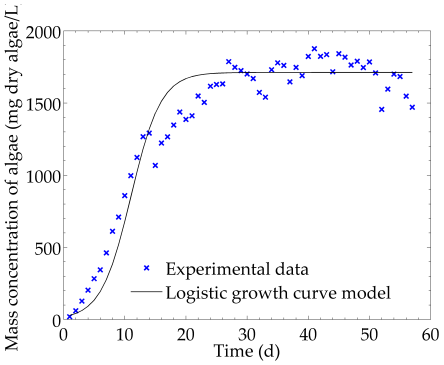
(a) 70 % retained fraction.



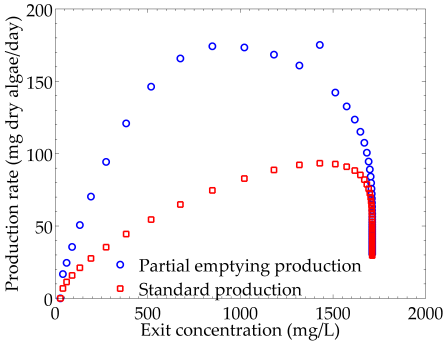
(b) 80 % retained fraction.

Figure G.1.17: Growth profile 70–80% Φ at 500–600 mg/L range.

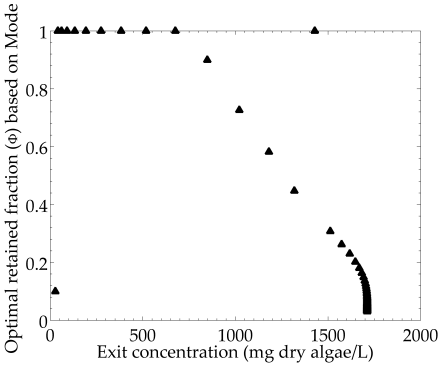
G.1.4 Modelling other fits to the growth profile



(a) Growth profile.

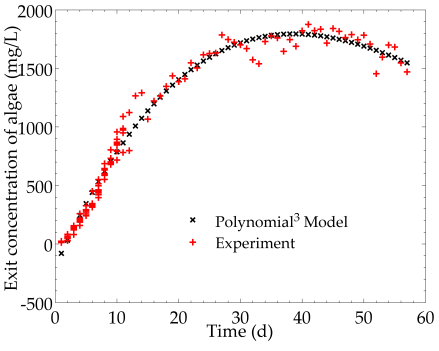


(b) Predicted production.



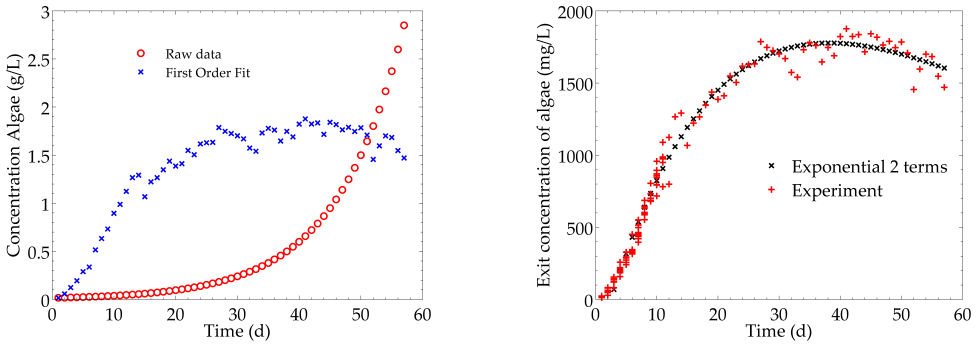
(c) Predicted retained fraction.

Figure G.1.18: Logistic model fitting on the initial growth profile where k = 1711.58 mg/L which was the average concentration from day 27 to day 57 on real experimental data.



(a) Growth profile.

Figure G.1.19: Polynomial to the power of three fitting towards the real data.



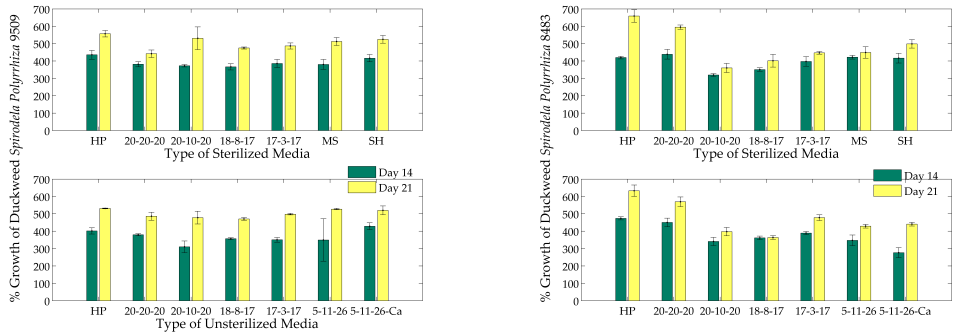
(a) First order.

(b) Exponential 2 terms.

Figure G.1.20: Attempt at fitting a models towards the real data.

G.2 Chapter five

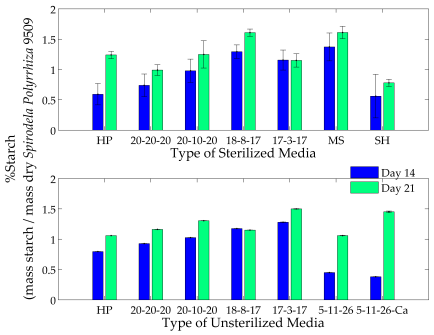
G.2.1 *Spirodela*



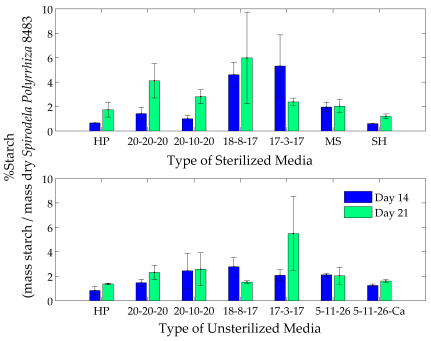
(a) *Spirodela polyrhiza* 9509. t-test shown that there was a difference between the growth rates in unsterilized media versus average of SH and MS control at 21 days.

(b) *Spirodela polyrhiza* 8483. t-test shown that there was no difference between the growth rates in unsterilized media when compared to an average of MS and SH control.

Figure G.2.1: % Growth rates for *Spirodela* in sterile and non-sterile media.



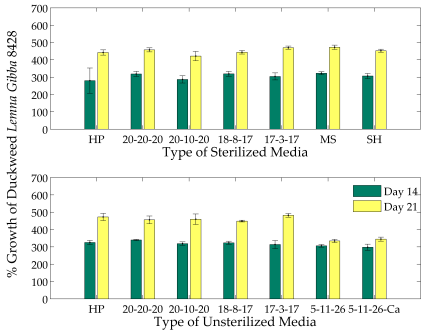
(a) *Spirodela polyrhiza* 9509. Difference ($p<0.05$) between the starch content when grown in different media but no difference for %Starch for unsterilized and sterilized conditions. t-test had shown there was a difference between the unsterilized media and controls (MS,SH) at day 21.



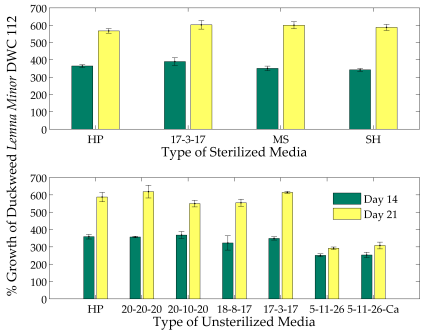
(b) *Spirodela polyrhiza* 8483. No significant difference for %Starch content when grown in different medium, as well as in unsterilized and sterilized conditions. No difference for starch content between unsterilized medium and MS control at day 21 but difference was shown with the other control SH in the t-test.

Figure G.2.2: Comparison of % Starch content in sterile and unsterilized media for *Spirodela polyrhiza*.

G.2.2 *Lemna*

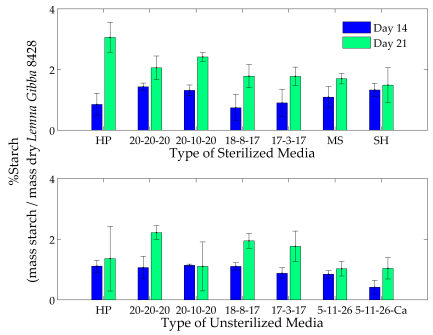


(a) *Lemna gibba* 8428.

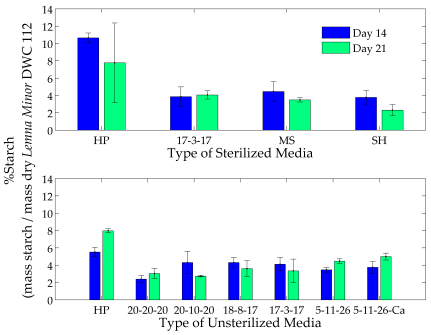


(b) *Lemna minor* DWC 112.

Figure G.2.3: % Growth rate comparison for *Lemna* in sterilized and unsterilized media. For each species, at day 21, there was no difference ($p>0.05$) when grown in unsterilized media with: 1) sterilized media 2) SH and MS control.



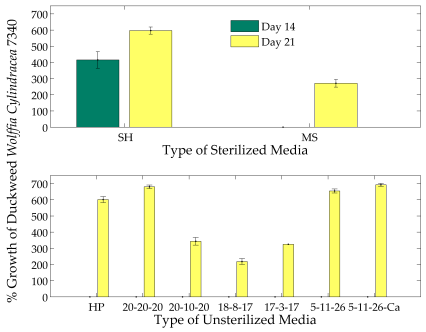
(a) *Lemna gibba* 8428. There was no significant difference for the starch content between the different unsterilized media but there was a difference ($p<0.05$) between sterilized and unsterilized conditions at day 21. t-test had shown there was no difference between the unsterilized media and controls (MS,SH) at day 21.



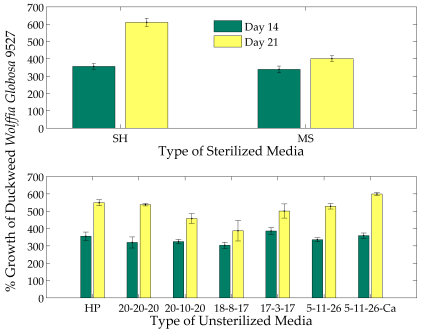
(b) *Lemna minor* DWC 112. There was a difference of starch content when grown in different unsterilized medium at day 21 but no difference between unsterilized or sterilized. t-test shown no difference between unsterilized media and MS control but there was difference with SH control.

Figure G.2.4: Comparison of %Starch content in sterile and non-sterile media for *Lemna*.

G.2.3 *Wolffia*

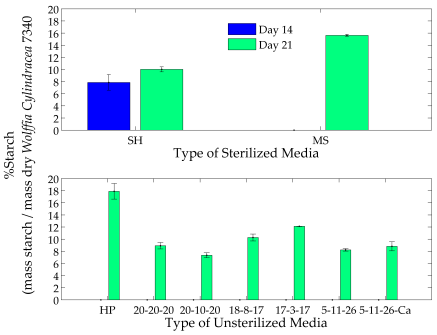


(a) *Wolffia cylindracea* (9527).

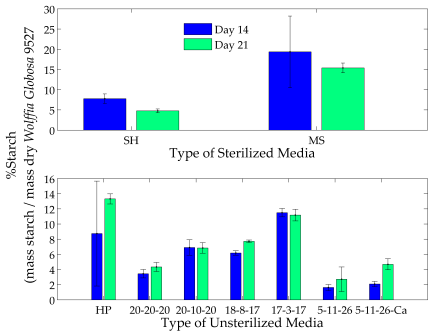


(b) *Wolffia globosa* (7340).

Figure G.2.5: Comparison of the %Growth rate for *Wolffia* in sterilized and unsterilized media. For each species, at day 21, t-test shown that there was a difference for the growth rate ($p<0.05$) between the unsterilized media and controls: MS and SH.



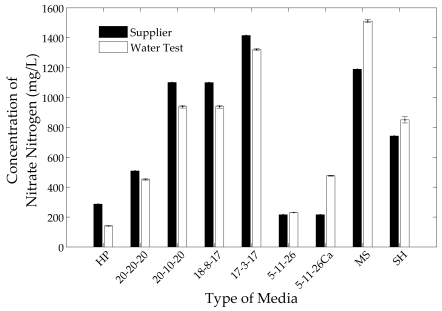
(a) *Wolffia cylindracea* (9527). There was a difference in starch content ($p<0.05$) between the unsterilized media and control MS but not SH control.



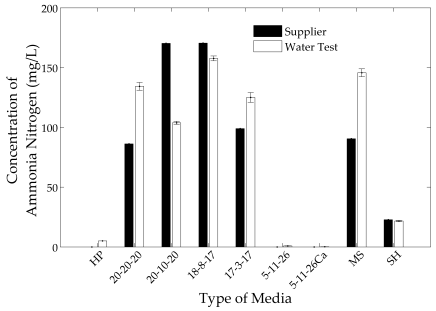
(b) *Wolffia globosa* (7340). There was a difference in starch content ($p<0.05$) between the unsterilized media and control MS and SH.

Figure G.2.6: Comparison of % Starch for *Wolffia* in sterilized and unsterilized media.

G.2.4 Water test

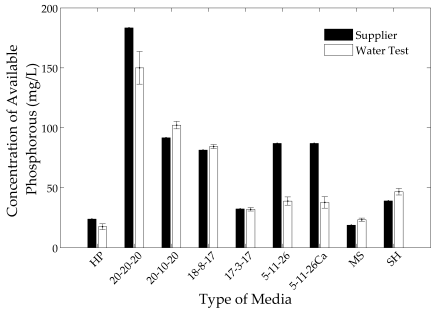


(a) Nitrate-Nitrogen.

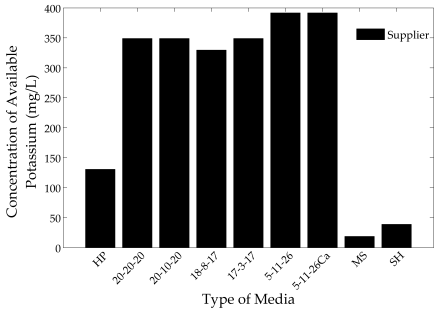


(b) Ammonia-Nitrogen.

Figure G.2.7: Compounds available in the media and fertilizers, comparison with the supplier and water test.



(a) Phosphorous.



(b) Potassium available in the media and fertilizers, no water test.

Figure G.2.8: Compounds available in the media and fertilizers.

G.3 Chapter six

G.3.1 No viable prediction

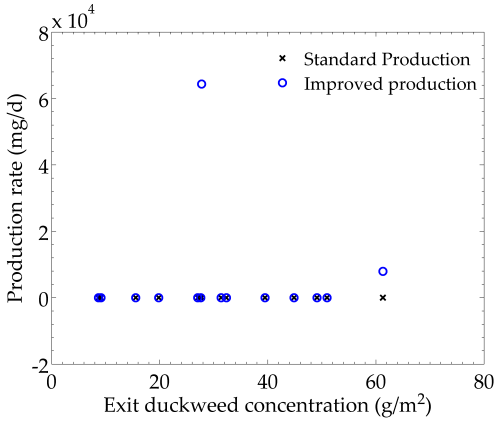


Figure G.3.1: *Spirodela* polyrhiza 8483 without sucrose prediction.