Utilisation of High Rate Algal Ponds to Treat Secondary Lagoon Effluent and Enhance Biomass Production

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Environmental Engineering

Institute for Sustainable Industries & Liveable Cities/

College of Engineering and Science

Victoria University

By

Digby Wrede

February 2019

Declaration

I, Digby Wrede, declare that the PhD thesis entitled '**Utilisation of High Rate Algal Ponds to Treat Secondary Lagoon Effluent and Enhance Biomass Production**' is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes.

This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature:  February 2019

Journal publications, conference proceedings and conference presentation relevant to the scope of this thesis

Conference Presentations

1. Wrede, D., Hussainy, S. U., Gray, S., Rajendram, W. Presentation: Fungal Flocculation of single species and mixed algal communities. *In* The 8th International Conference on Algal Biomass, Biofuels and Bioproducts, June 11, 2018, Seattle, United States of America.

Refereed Journal Publications

1. Digby Wrede, Syed U. Hussainy, William Rajendram and Stephen Gray, “Investigation and modelling of high rate algal ponds utilising secondary effluent at Western Water, Bacchus Marsh Recycled Water Plant” in Water Science and Technology, 78, 20-30.

Abstract

High rate algal ponds (HRAPs) can be utilised as an efficient and economical wastewater treatment method while also producing algal biomass. This study focused on the use of HRAPs to assimilate nutrients from secondary lagoon effluent and investigated various methods in which to enhance the algal biomass productivity of the HRAPs. The natural operation, productive potential, biomass production, nutrient removal capacity and environmental conditions were observed. From these findings, three experiments were proposed to enhance biomass production and in turn, the nutrient removal of the HRAPs. The first experiment was the addition of three separate algal cultures to the HRAPs during winter. Two of the algal species enhanced biomass production, however, there was no significant difference in nutrient removal during any of these experiments. The second set of experiments controlled the pH of the HRAPs utilising an inorganic and organic acid to determine if it was solely the control of pH which enhanced biomass production or if the addition of carbon that played a significant role. It was found that under high algal productivity conditions utilising inorganic acid to control pH negatively impacted algal growth whereas utilising organic acid significantly enhanced algal growth. The third experiment compared secondary lagoon effluent and primary lagoon effluent as the media sources. Secondary lagoon effluent was found to have higher biomass productivity by 106mg/L. This was thought to be a result of the primary lagoon effluents high colloidal turbidity.

The results from the biomass enhancement experiments alongside the natural operation of the HRAPs were utilised to develop a simple and accurate algal growth model which utilised readily available data. The model aims to determine the biomass production of HRAPs in the south-eastern Australian climate which operated under elevated pH levels. The model was validated against the use of both secondary and primary lagoon effluent in the HRAPs and returned an R-squared value 0.98, suggesting a high accuracy.

Following this work, two algal harvesting methods were investigated; membrane filtration and fungal flocculation. Three different membrane filtration systems were trialled and compared; ceramic crossflow system,

polytetrafluoroethylene (PTFE) submerged system and a metal crossflow system. The PTFE membrane was found to be the most effective of the membranes tested for harvesting algae due to its low fouling tendency, low cost and relatively constant flux.

The flocculation capability of fungi to flocculate algae was examined. *Aspergillus oryzae* was found to be the most effective fungi species trialled for monoculture flocculation with over 95% removal for all algal species tested. The fungal flocculation of mixed algal communities in wastewater samples was also investigated and removal values of 70-100% were achieved.

Overall, the work conducted provides valuable information on the operation and enhancement of HRAPs. Furthermore, the simple model developed can be utilised to help identify the potential of an area for algal biomass production and the feasibility of incorporating HRAP systems into an existing wastewater treatment facility. The two harvesting techniques trialled offer new and vital insight into the often-difficult process of algal harvesting.

Acknowledgements

Firstly, I would like to express my sincere thanks and gratitude to my primary supervisor Professor Stephen Gray. Stephen has been helpful, supportive and encouraging. He was always willing to provide me with helpful feedback and push me to ensure I was doing my best possible work. His expertise and guidance helped me to develop and enhance my scholarly skills and knowledge.

I am profoundly grateful to my associate supervisor Adjunct Professor Syed Hussainy. Syed was always available for a friendly chat or to discuss my work and provide invaluable insight and knowledge. Syed's constant support, patience and guidance was a blessing and I could not imagine the outcome of this research without his aid. Syed always ensured I questioned the research and encouraged me to broaden my research to ensure a more complete and thorough understanding. I would also like to thank Associate Professor Mary Millikan who along with Syed were the driving forces for the formation of this PhD project.

The technical staff at the Werribee campus, Ms Stacey Lloyd, Mr Joseph Pelle, Ms Mary Marshall, Dr Sarah Fraser, Ms Min Nuygen, Ms Charmaine DiQuattro and Mr Larruceo Bautista, supported me during the experimental and planning stages of my research. Without their support, training and help, this research would have been immensely difficult.

I am thankful to the staff at Western Waters' Melton Treatment Plant and those at the Bacchus Marsh Recycled Water Plant, Andy Bridson, Ryan Closter and the other staff for their help, technical support, maintenance and transport of primary lagoon effluent. I am thankful to Dean Phillips who provided me with the weather data for the Bacchus Marsh Recycled Water Plant

I am very thankful to the staff at Western Waters' Sunbury Offices, especially William Rajendram who was crucial in the setup of the project and made arrangements for the installation and maintenance of the HRAPs, operations shed and the surrounding areas. William was always eager to hear of the project's progress and to offer guidance.

I am eternally grateful to my fellow postgraduates, Mr Ikechukwu Anthony Ike, Ms Anbharasi Vanangamudi, Ms Inci Boztepe and Mrs Morley Muse for their ongoing assistance, stimulating discussions and company. My sincere thanks to the staff at the Institute for Sustainability and Innovations, Mr Noel Dow, Dr Peter Sanicolo, Dr Jianhua Zhang, Dr Bo Zhu, Dr Marlene Cran, Mrs Catherine Enriquez and Professor Mikel Duke for their valued assistance.

I am grateful to both Western Water and Victoria University for providing me with the opportunity to complete this PhD and for the scholarship.

I am extremely grateful to my fiancée Aimee Coulter, without whom this project would never have been started. She provided me with the courage to seek out the unknown and embark on this wonderful journey. Her patience, support and encouragement have been invaluable during this entire research period. I am thankful to my parents and family members for their endless support and encouragement for always being there and comforting me. I am very thankful to my friends who were always able to provide me with much-needed breaks and support during this project.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Al_2O_3	Aluminium Oxide
ATP	Adenosine Triphosphate
BBM	Bold's Basal Medium
BMRWP	Bacchus Marsh Recycled Water Plant
BOD	Biochemical Oxygen Demand
C	Carbon
CO_2	Carbon Dioxide
DB	Dark Bottle
DO	Dissolved Oxygen
EDTA	Ethylene-diamine-tetra-acetic acid
EOM	Extracellular Organic Matter
EPS	Extracellular Polymeric Substances
FAME	Fatty Acid Methyl Ester
FE	Flocculating Efficiency
FPA	Fungal Pellet Assisted
fPE	Filtered Primary Lagoon Effluent
FSA	Fungal Spore Addition
HCl	Hydrochloric Acid
HRAPs	High Rate Algal Ponds
LB	Light Bottle
LDO	Luminescent Dissolved Oxygen
MF	Microfiltration
N	Nitrogen
NATA	National Association of Testing Authorities
OD	Optical Density
P	Phosphorus

P/R	Photosynthesis to Respiration Ratio
PAN	Polyacrylonitrile
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PES	Polyethersulphone
PP	Productive Potential
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
PVDF	Polyvinyl difluoride
PVP	Polyvinylpyrrolidone
RMSE	Root Mean Square Error
RNA	Ribonucleic acid
RT	Room Temperature
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
Std. Dev	Standard Deviation
TIN	Total Inorganic Nitrogen
TIP	Total Inorganic Phosphorus
TN	Total Nitrogen
TP	Total Phosphorus
UF	Ultrafiltration
VCF	Volumetric Concentration Factor

Chapter 1 - Introduction

Western Water is one of Victoria's regional urban water corporations and provides water services to over 150,000 people in a rapidly growing region. Western Water is committed to providing the services in the highest quality water possible and doing so in an environmentally friendly way. They are continually exploring new methods for wastewater treatment as well as ways to reduce greenhouse gas emissions and energy consumption. This study investigated the use of high rate algal ponds (HRAPs) to treat urban wastewater and was supported by Western Water and Victoria University. The project utilised ambient microalgae in sewage effluent treatment lagoons and investigated methods to enhance their growth in the HRAP to optimise their nutrient removal capabilities. The project also developed a predictive algal growth model for the HRAPs utilised. Furthermore, this project investigated two microalgal harvesting techniques, membrane filtration and fungal flocculation. The harvested biomass could be utilised to produce biofuel or other products to help reduce Western Waters' greenhouse gas emissions, carbon footprint and/or operational costs.

1.1 Microalgae

An important question to ask is, why use microalgae? Microalgae are believed to be the most productive source of photosynthetic biomass (Rogers et al., 2014). They are generally microscopic, can be unicellular, multicellular or colonial in structure. They can grow rapidly, with some species doubling 1-3 times per day and can grow in almost any aquatic environment ranging from fresh oligotrophic to eutrophic water, from non-saline to hypersaline water as well as wastewaters (Mata et al., 2010, Wrede et al., 2014). This is due to the enormous species diversity and their simple growth requirements. Microalgae are generally photosynthetic with some species solely relying on carbon dioxide (CO_2) as their carbon source while others are heterotrophic and can utilise CO_2, carbonic acids and sugars in the aquatic environment and many are mixotrophic meaning they are photosynthetic and can utilise carbon sources from the environment. Microalgae are either free-swimming

(planktonic) or attached (periphytic) and in addition to carbon, they require simple nutrients and sunlight to grow (Mata et al., 2010). Microalgae commonly require carbon (C), nitrogen (N) and phosphorus (P) in a ratio of 106:16:1 respectively, and this is known as the Redfield ratio (Redfield, 1958). The cost of providing nutrients to a sizeable algal culture can be expensive and finding alternative nutrient sources would help the production of algal biomass become more economical. One such alternative is wastewater.

1.2 Wastewater Treatment

Microalgae can assimilate nutrients from wastewater and utilise them for growth. Wastewaters are high in nitrogen, phosphorus and other nutrients providing a nutrient-rich medium for algae, which is generally not being utilised, at little to no extra cost (Beuckels et al., 2015). Microalgae have been utilised to treat wastewater since before the 1950s (Brennan and Owende, 2010, Golueke et al., 1957, Oswald et al., 1957, Hussainy, 1979). A joint approach to algal biomass production and wastewater treatment enhances the economic viability of algal biomass production. With wastewater providing the required nutrients, the microalgae, in turn, treat the wastewater and this helps to partially offset the downstream production costs. Microalgal cultures have been shown to reduce the concentration of nutrients in wastewaters significantly (Abdel-Raouf et al., 2012, Woertz et al., 2009, Fallowfield et al., 2016). Microalgae are also able to sequester heavy metals and other harmful chemicals from the surrounding media into their cells (Lizarralde et al., 2014). Park et al. (2011) found that the use of algal treatment was a less energy-intensive process than the conventional treatment processes and determined that their operational costs could be approximately one-fifth of activated sludge treatment systems.

Algal production and harvesting for low-value products such as biofuels are generally considered uneconomical due to the high cost of infrastructure, supplying nutrients, harvesting and the extraction of the target compound (Mehrabadi et al., 2015, Rogers et al., 2014, Brennan and Owende, 2010, Fallowfield et al., 2016). However, utilising wastewater as the

growth medium can help to offset these costs by supplying the nutrients at little to no cost (Christenson and Sims, 2011, Cai et al., 2013). The use of algae could reduce the operating costs of the wastewater treatment facility, which would further offset the algal production and harvesting costs (Craggs et al., 2014). Additionally, the use of algae in wastewater treatment could help to reduce the amount of energy and chemicals used in wastewater treatment and minimise greenhouse gas emissions (Park et al., 2014). The harvesting of nutrients and harmful chemicals such as heavy metals, in addition to the potential biofuel production, make microalgae treatment very attractive for wastewater treatment industries. A proper understanding of the algae's optimal growth conditions is required to provide economical and feasible wastewater treatment. An optimised process can minimise energy use, greenhouse gas emissions and operational costs while maximising nutrient removal and biomass production.

1.3 Primary Production

It is crucial to determine how well the wastewater can support algal growth. This can be done by examining the primary production in wastewater and general ecology of the system. Primary production is the production of organic matter from energy and inorganic compounds, the most common form of primary production is through photosynthesis. Microalgae are well known for their photosynthetic capabilities and rapid growth. There are a number of variables which influence the effectiveness of photosynthesis, and these include, biomass concentration, nutrient concentration, pH, temperature, solar radiation and light penetration. Determining which of these variables are contributing most significantly to primary production of the system and how they affect production is crucial to determining the viability and effectiveness of a treatment system regarding the nutrient removal and algal biomass production.

1.4 High Rate Algal Pond

A common method employed for microalgal biomass production is HRAPs. HRAPs are open shallow raceway ponds which are utilised for microalgal production and wastewater treatment. HRAPs have been used to

treat wastewater for over half a century and were first suggested by Oswald et al. (1957). There have been many studies investigating the effects of HRAPs on water treatment. The majority of these studies have proven that HRAPs are effective in removing unwanted nutrients (Fallowfield et al., 2016, Craggs et al., 2012). HRAPs have also been extensively studied for their ability to grow microalgae. It has been demonstrated that HRAPs can be a cost-effective method to grow microalgae in large quantities, as large as 30 tonnes/ha/year with this increasing to 60 tonnes/ha/year with the addition of CO_2 as an extra carbon supply (Montemezzani et al., 2015). HRAPs are easy to operate, running costs are minimal, utilise natural light and are generally outdoor systems. The optimisation of algal growth conditions can help to reduce costs, and one of the most effective ways for optimisation is the use of a microalgal growth model. The growth model focused on in this research was based on the use of HRAPs in an outdoor environment utilising ambient consortia of algal species.

1.5 Growth Model

Microalgal growth models are used to help predict the growth of microalgae and predict the nutrient removal potential for a system. The main influencing factors considered in most microalgae growth models include light, nutrient concentration, temperature and the associated microalgae species (Jayaraman and Rhinehart, 2015). Solar radiation or light intensity is the primary energy source for most microalgae and is a variable factor. The causes of variations in light intensity among others are diurnal duration, water depth, turbidity, cloud cover and seasonal variation. Light intensity decreases with pond depth as a result of suspended particles, microalgal biomass and light absorbance by water and humic substances (Jayaraman and Rhinehart, 2015). To help minimise the effect of self-shading, caused by elevated algae biomass concentrations, HRAPs incorporate a shallow design. However, light attenuation can still occur and needs to be accounted for. All algae have an optimum light intensity range, outside of which their photosynthetic ability diminishes. Being able to model the impact light intensity will have on the algal biomass production is crucial.

Temperature is also a significant factor for microalgal growth, as it controls the rate of enzyme activation (Sutherland et al., 2015b). Each microalgal species has its own optimal temperature range at which they grow best, and this can complicate the modelling process. In a natural environment, temperature and light intensity are closely linked. Temperature fluctuates with light intensity, diurnally, day-night length, seasonally, evaporation and surface area to volume ratio of the system (Jayaraman and Rhinehart, 2015, Béchet et al., 2011). Determining the impact of temperature on the microalgae is essential to modelling the changes in the microalgal biomass accurately.

This study investigated the development of a simple microalgal growth model utilising only a few variables such as light, temperature and biomass concentration, the impact of self-shading was also investigated. The model was developed utilising HRAPs with a mixed culture of ambient microalgae. This was done so that the model could provide sufficient detail and accuracy so as to be useful in the operation and design of a microalgal wastewater treatment process in HRAPs. The model was also developed for an elevated pH environment which commonly occurs in wastewater treatment plants and algal cultures. Such microalgal growth modelling studies have not been conducted in south-eastern Australia thus far especially for Victoria. Being able to predict the microalgal biomass concentrations is crucial for the production of microalgal biofuels and other algal products.

1.6 Microalgal Products

Microalgae produce numerous compounds inside their cells such as carbohydrates, polysaccharides, fatty acids, proteins and pigments (Brennan and Owende, 2010). These compounds can be transformed into a variety of useful products such as biofuels, cosmetics, foods, animal feeds, vaccines, natural dyes, antioxidants and high value bioactive compounds (Brennan and Owende, 2010, Chisti, 2007, Hallmann, 2007, Mata et al., 2010, Pulz and Gross, 2004). The fatty acid composition of microalgae has been shown to be made up of numerous different chains which can be made into products such as; biofuels, and nutritional supplements (Brennan and Owende, 2010, Tran

et al., 2013). Carbohydrates make up the microalgal cell walls; these carbohydrates can be utilised to create ethanol and utilised as a fuel source (Chisti, 2008, Costa and De Morais, 2011). However, the production of most microalgal products is currently not economically viable, especially the main target product, biofuel. One of the significant costs involved in the microalgal production process is the harvesting process (Pragya et al., 2013).

1.7 Harvesting

Microalgae are difficult to harvest due to their small size, dilute cultures and ability to maintain a dispersed state (Pragya et al., 2013). The harvesting cost can be up to 20-50% of the total cost of production (Chisti, 2007, Leite et al., 2013, Pragya et al., 2013). Different methods for harvesting have been investigated in the past; however, none of these methods has proven to be universally feasible (Pragya et al., 2013, Barros et al., 2015). The most common of them are; centrifugation, filtration, gravity sedimentation, flotation, flocculation and electrophoresis. Each method has difficulties and limitations with cost being a predominant factor. A universally feasible method is ideal for use in HRAPs utilising wastewater as it would remove the need to determine the species of algae present. For example, harvesting large alga such as *Spirulina* sp. can be easily done by filtration or sedimentation (Pragya et al., 2013). However, harvesting small alga such as *Chlorella* sp. may require the use of a coagulant before it could be efficiently harvested (Barros et al., 2015). Furthermore, if the algal species is known a more specified harvesting technique can be utilised.

1.7.1 Filtration

Membrane filtration is a standard method for harvesting microalgae. However, most membranes foul quickly and need to be replaced regularly thus making them uneconomical. The interaction between the surface chemistry of the algae and the membranes play a significant role in the fouling of the membranes. Polymeric membranes are the most common membranes used but face severe fouling challenges. In recent times, ceramic membranes have become more common in water treatment research due to their robustness, good selectivity and their cost competitiveness (Zhang et al.,

2013). PTFE membranes have also been found to be quite effective in harvesting microalgae (Bilad et al., 2014a). There have also been trials utilising metal membranes for wastewater treatment, and these results have also proved promising, but little work has been done on the use of metal membranes regarding microalgal harvesting (Kim et al., 2007, Zhang et al., 2005). This study investigated the use of ceramic, metal and PTFE membranes to harvest algae and the results are discussed in the relevant chapter.

1.7.2 Flocculation

A method often used in conjunction with filtration is flocculation. Algal flocculation is the process of adding a compound to a culture which causes the algae to agglomerate and form into larger clumps or flocs. There are three primary forms of flocculation: chemical, physical and biological (Wan et al., 2015). Chemical flocculation involves adding a chemical to the culture which causes the microalgae to form flocs or coagulate. There are a variety of different chemicals and compounds which can cause flocculation, and metal coagulants or organic flocculants are the ones most commonly used (Vandamme et al., 2013).

The second form of flocculation is physical or mechanical flocculation which uses an outside stimulus to cause the microalgae to flocculate. The methods most commonly utilised are ultrasound, magnetic nanoparticles and electroflocculation (Barros et al., 2015, Wang et al., 2015, Vandamme et al., 2013, Hena et al., 2015, Lee et al., 2013). The main problem with these methods is the cost involved with installing the operating mechanisms and operating the system.

The third flocculation technique is bioflocculation which involves using biological compounds to induce flocculation. Certain species of microalgae can naturally self-flocculate under specific conditions (Chen et al., 2012). Studies have shown that it is possible to mix naturally bioflocculating microalgae with other species to induce flocculation (Leite et al., 2013, Vandamme et al., 2013). Other biotas that have been shown to induce bioflocculation in microalgae are fungi and bacteria (Leite et al., 2013,

Miranda et al., 2015, Muradov et al., 2015, Salim et al., 2011, Wrede et al., 2014). The use of bioflocculation is promising as the flocculating organisms could add biomass which may also contain oils and other useful compounds.

Fungal flocculation of microalgae is a promising harvesting method. Fungal flocculation is when a filamentous fungus is added to the algal culture and entraps or adheres the algae to their filaments. The flocculation is thought to be a combination of entrapped algae and the attraction of the algal cells to the fungal filaments due to the opposing surface electrical charges with algae having a negative surface charge and fungi have a positive surface charge. However, the mechanisms are still unclear, and only monocultures species of algae have been flocculated (Muradov et al., 2015). The problems with fungal flocculation are contamination, and the fungi tested so far are not able to fully flocculate all species of microalgae.

The current study investigated the relationship between the productive potential of the secondary lagoon effluent and the algal biomass production in the HRAPs. Additionally, the study focuses on the development of a predictive microalgal growth model for an elevated pH environment in south-eastern Australia. The project also investigated, the harvesting of microalgae utilising membrane filtration and fungal flocculation.

1.8 Thesis Outline

This first chapter introduces the importance of thoroughly understanding the treatment of wastewater using microalgae. It demonstrates that it is essential to understand the various factors involved and not just to focus on one aspect. This chapter also introduced why the modelling of algal growth is a critical step to consider when utilising algae for wastewater treatment. Additionally, microalgal harvesting is also of crucial importance and two different methods, membrane filtration and fungal flocculation, were introduced.

Chapter 2 provided a review of the current literature. Current and past wastewater treatment methods are discussed, as well as an examination of HRAPs for wastewater treatment and algal biomass production. This chapter also highlighted the current state of algal growth models and their various

advantages and shortcomings. Further to algal biomass production, an investigation into the use of primary productivity as a measure to determine the productivity potential of the system in assimilating raw materials into the cell biomass was examined. This chapter also explains the need for efficient harvesting techniques and gives a review on the current state of harvesting algae utilising membrane filtration and a comprehensive review on the use of fungal flocculation to remove algae from suspension and its potential uses in the production of products. Chapter 2 also highlights the current state of knowledge and highlights areas which lack sufficient research. The chapter helps to set the framework for the subsequent chapters by addressing the fundamental aspects of algal wastewater treatment and outlines the research objectives for the study.

Chapter 3 describes the experimental techniques, the pilot plant site and the quality of the wastewater effluent utilised in this study. Additionally, chapter 3 examines the production potential of the system by analysing the biosynthesis of carbon to algae in HRAPs over a 12-month period under different environmental conditions which influenced the secondary lagoon effluent at BMRWP. The production potential capacity of the secondary lagoon effluent was analysed using the light and dark bottle method to determine changes in dissolved oxygen concentration. The assimilation of carbon by the algal biomass can be determined by the amount of oxygen produced through photosynthesis. Being able to measure and predict how much biomass can be produced accurately is crucial to understanding the production potential of the system.

Chapter 4 focuses on the effectiveness of the HRAPs to remove nutrients and produce biomass. The primary nutrients investigated are nitrogen and phosphorus. This chapter also examined three different methods to enhance biomass production, the first being the addition of laboratory-grown cultures of algae during winter. Secondly, the pH of the HRAP was controlled utilising inorganic and organic acid to determine if by controlling solely the pH of the system enhanced biomass production could be achieved or if carbon was required to be added alongside pH control. Thirdly, the biomass production of

the HRAPs when filled with either primary or secondary lagoon effluent were compared.

Chapter 5 describes the development of a simple algal growth model. The model focuses on three simple factors; solar radiation, temperature and the impact biomass concentration has on self-shading. The model differs from standard models as it was developed for an outdoor environment utilising secondary lagoon effluent within an elevated pH environment, which indicates a deficiency in the availability of inorganic carbon. The model was also developed for south-eastern Australia which has not been done previously.

Chapter 6 explores the use of membrane filtration to remove algae from primary lagoon effluent samples using microfiltration. Three membranes are compared each with different properties. The membranes trialled are; a ceramic membrane in a crossflow system, a bundle of tubular metal membranes in a larger crossflow system and a bundle of hollow fibre PTFE membranes in a submerged membrane system. The change in flux and the costs of each membrane were investigated and compared.

Chapter 7 considers the use of fungal flocculation of algal cells. Fungal flocculation is a relatively new area, and the flocculation of numerous species simultaneously had not previously been investigated. The fungal-algal flocs were also tested for their ability to remove nutrients from primary lagoon effluent and compared to the algae or fungi monocultures.

Chapter 8, the final chapter, provides an opportunity to assess if the work completed met the objectives of the project. The chapter focuses on recommendations for industrial applications and future work. The limitations and drawbacks of the research are also discussed. Chapter eight also provides a summary of the outcomes, restrictions and identifies where further research is required.

Chapter 2 - Literature Review

2.1 History of Wastewater treatment

The treatment and reuse of wastewater is an environmental and public health priority. With the increase in growth and demand for potable water due to the growing population, wastewater treatment has become a major priority. The majority of the world's water resources are unfit for human consumption, with 97% being saline, almost 2% stored in polar ice caps and glaciers, and of the remaining 1%, the majority is groundwater with only 0.3% above ground and easily accessible for use (Cassardo and Jones, 2011). The primary requirement in the treatment of wastewater is the removal of pollutants, of both biological and toxicological origin (Abdel-Raouf et al., 2012). Several treatment methods are in practice to successfully remediate wastewater for recycling purposes. The majority of treatment processes include removal of settleable and suspended solids followed by biological treatment to remove any dissolved material in lagoons or other treatment processes (Lofrano and Brown, 2010, Abdel-Raouf et al., 2012). Most often this is followed by tertiary treatment wherein residual pollutants which could not be removed by the earlier methods are removed from the water, and the effluent is utilised as required (Abdel-Raouf et al., 2012).

During lagoon treatment, the initial lagoons, generally operating as anaerobic lagoons, accumulate settleable solids and most of the suspended solids, and break them down through anaerobic biological processes (Abdel-Raouf et al., 2012). Additionally, hydrogen sulphide gas which is produced by some anaerobic processes combines with heavy metals and precipitates metals as metallic sulfides. Up to 97% of the heavy metals are removed from the waste through this process (Hussainy, 1979). The anaerobic process is followed by facultative processes where in autotrophic sulfur bacteria utilise hydrogen sulfide as a hydrogen donor for carbon assimilation (Hussainy, 1979). During this process, other photosynthetic organisms occupy the system converting the facultative system into an aerobic environment. The above processes also reduce the biochemical oxygen demand (BOD) and the nitrogenous based

compounds by over 50% (Hussainy, 1979). The residual BOD and nitrogenous material are further oxidised in the aerobic lagoons downstream (Abdel-Raouf et al., 2012). However, during winter months the assimilation of nitrogen in the system is inefficient, and a high concentration of nitrogen up to 35mg/L can be discharged along with the final effluent to the receiving waters (Hussainy, 1979). Further, the lagoon system is inefficient and inconsistent in the removal of phosphorus (Bashar et al., 2018). The discharge of nutrient-rich effluent causes eutrophication in receiving waters (Christenson and Sims, 2011). Which is followed by toxic algal blooms and subsequent fish kills as experienced in the Murray-Darling, Menindee and other systems in New South Wales, Australia, in the summer of 2018-2019. The majority of the pathogenic organisms, such as *Escherichia coli,* are removed in the aerobic lagoons through a combination of pH, temperature, ultraviolet light, algal toxins and predation (Maynard et al., 1999). These treatment steps can be performed in a variety of different treatment facilities ranging from activated sludge plants, land-based treatment and lagoon-based treatment systems. One of the most common approaches to wastewater treatment is lagoon based secondary treatment with extended aeration and other advances to enhance biological nutrient removal. This study focuses on a form of lagoon-based treatment systems.

2.1.1 Lagoon Wastewater Treatment

Lagoon-based treatment plants are commonly used by both established and rural communities as they are relatively inexpensive and simple to operate (Young et al., 2017). They can also be upgraded easily as the population of the area grows. Additionally, lagoons-based systems can treat municipal and industrial wastes (Abdel-Raouf et al., 2012, Park et al., 2011). Lagoon based treatment plants are relatively simple to construct and inexpensive to operate. When operated successfully the desired results are achieved except for nutrient removal especially during winter. This study focuses on lagoon-based treatment to enhance the removal of nitrogen through the growth of algae in HRAPs. This algal biomass can be harvested and subsequently utilised for bioenergy production and feedstocks for algal bioproducts.

Lagoon based systems are first described as oxidation ponds and have been used for almost 100 years. Prior to the 1950s, lagoon based systems were thought to be a primitive and ineffective means of treatment and mainly used as a final treatment or polishing process (Parker et al., 1950). In the 1940s Caldwell (1946) investigated the use and effectiveness of oxidation ponds. They used an anaerobic settling lagoon for sedimentation and digestion and an aerobic lagoon for BOD reduction and final polishing. The study found that there was satisfactory treatment and that less area was required than the conventional land-based treatment they were using at the time. It was also determined that the unsightly microalgae probably enhanced the purification capacity of the process (Parker et al., 1950, Caldwell, 1946). Biological nutrient removal in lagoons is difficult to operate consistently and requires considerable energy. The use of HRAP treatment can help the removal of biological nutrients and reduce the amount of energy required. The use of algae as a wastewater treatment method has been widely accepted as effective. However, the process still requires some refinement (Cai et al., 2013, Abdel-Raouf et al., 2012, Park et al., 2011, Quiroz Arita et al., 2015).

2.2 High Rate Algal Ponds

HRAP have been used in wastewater treatment since the 1960s. These ponds are different from standard ponds or lagoons used for wastewater treatment as HRAPs focus on enhancing the algal biomass, and in turn, increasing the wastewater treatment efficiency. HRAPs are used all over the world, but, they are more suited to a warmer environment (Young et al., 2017). HRAPs are roughly 30 cm deep, and usually have a paddlewheel to provide mixing of the water (Chisti, 2016). HRAP can range in size from small laboratory or pilot scale devices to larger full-scale plants as seen in New Zealand, where they converted a pre-existing oxidation pond into a series of HRAPs covering 5 hectares (Craggs et al., 2012, Sutherland et al., 2018). HRAPs are seen as a cost-effective method for wastewater treatment with some sources claiming that construction costs are 30% of the cost of an activated sludge system (Young et al., 2017, Sutherland et al., 2017). Operational costs are also significantly lower than activated sludge systems as they require less energy and are simple to

operate (Young et al., 2017, Wang et al., 2017). The reduction in energy also has the added benefit of reducing the greenhouse gas emissions of the process (Acién et al., 2016). The nutrient removal capabilities of HRAP have been studied extensively (Craggs et al., 2012, Young et al., 2017, Sutherland et al., 2017). HRAPs have been shown to be able to remove large amounts of nutrients from wastewater systems with a median removal of total nitrogen and ammonium of 61.23% and 77% respectively (Young et al., 2017). The cause of this nitrogen removal is believed to be a mixture of incorporation into algal biomass and ammonia volatilisation due to elevated pH level (Young et al., 2017, Cromar and Fallowfield, 1997, García et al., 2000). Total phosphorus removal has not been as successful overall but has a broad range of removals from 10.48% to 97.2% with a median of 42.73% (Young et al., 2017). The causes of phosphorus removal are believed to be incorporation into the algal biomass as luxury uptake and pH-dependent precipitation (Young et al., 2017, Sutherland et al., 2015b).

2.3 Microalgae

Microalgal growth requires carbon, nitrogen and phosphorus, and the amount of each varies with algal species. A generally accepted ratio is the Redfield ratio of 106:16:1 of carbon, nitrogen and phosphorus respectively (Redfield, 1958). Unfortunately, this is not the nutrient ratio found in wastewaters with carbon generally found to be limiting. Algal cells contain 50% carbon by weight, and therefore carbon is the primary driver for growth (Putt et al., 2011). As carbon is the most common limiting factor in algal wastewater treatment a large number of studies have utilised various forms of carbon addition such as acetate, pure CO_2 and flue gases to increase the carbon content in the water (Sutherland et al., 2015c, Fallowfield et al., 2016, Beal et al., 2015). This addition of carbon solves two problems with microalgal production; namely carbon limitation and pH elevation. Firstly, carbon availability is increased, and the algae can utilise it for growth (Chisti, 2016). Secondly, the addition of the carbon, in the form of CO_2 or oxidised carbon species, helps to reduce the pH level and is most commonly used as a form of pH regulation (Chisti, 2016). Elevated pH levels are typical in wastewater

systems due to the photosynthetic process. When the pH level rises above 8.3, there is virtually no free/ dissolved CO_2 in the water, and this hinders the algae's growth rate (Cole and Weihe, 2015). CO_2 addition has been found to be beneficial for algal growth by numerous researchers, and its addition is standard practice in all commercial algal production HRAP systems (Craggs et al., 2012, Fallowfield et al., 2016, Park et al., 2011, Benemann, 2003, Chisti, 2016). One of the significant problems encountered with CO_2 addition is the loss to the atmosphere. Up to 70% of CO_2 sparged into a pond is lost to the atmosphere, and this reduces the overall effectiveness of the process and increases the costs (Chisti, 2016). Various techniques such as a carbon sump, increasing the bubble breakage or decreasing the bubbles sizes have been used to enhance the algal biomass production and effectiveness of the CO_2 addition (Cheng et al., 2016, Craggs et al., 2012, Park et al., 2011, Razzak et al., 2017). Additionally, products such as Oxymen, a membrane aerated biofilm reactors, could be adapted to supply CO_2 in a more energy efficient method which would reduce the loss of CO_2 to the atmosphere (OxyMem, 2018).

Microalgal cells also require a steady amount of nitrogen as it is predominately used for protein syntheses (Beuckels et al., 2015). When nitrogen becomes limiting the algal cells can continue to incorporate carbon into their cells. However, this carbon is stored as oils and lipids instead of being used for biomass production, and this reduces algal growth rates (Rawat et al., 2013). Nitrogen concentrations vary significantly between different wastewaters. Ammonia is generally the preferred nitrogen source for green algae due to the low energy requirement for its incorporation into algal cells (Decostere et al., 2016). Ammonia concentration in wastewater is linked to pH, and when there is elevated pH, ammonia volatilisation can occur causing a significant amount of ammonia to be lost to the atmosphere (Mahapatra et al., 2014, Olguin et al., 2003, Sutherland et al., 2015c). This can also limit algal growth, and it is costly to replenish the lost ammonia. Some cyanobacteria utilise nitrogen from the atmosphere, but these are generally not target organisms for use in HRAPs. Nitrogen assimilation into algal cells is believed to be linked to phosphorus assimilation (Beuckels et al., 2015). Phosphorus is mainly incorporated into the ribosomal RNA and phospholipids in algal cells (Beuckels et al., 2015). Some

algae can perform a luxury uptake of phosphorus by storing it as polyphosphate granules for use when external concentrations are low, this allows for higher than expected phosphorus removal from wastewater (Beuckels et al., 2015, Powell et al., 2009). When either nitrogen or phosphorus becomes limiting it can affect the uptake of the other, as they are both vital to protein synthesis (Beuckels et al., 2015). There are other nutrients required by algae, but these are only required in much smaller concentrations, such as calcium, silicon, magnesium, sodium, potassium and sulphur, and they are generally available in wastewaters (Cai et al., 2013).

2.4 Primary Production

Primary production is the production of organic matter utilising inorganic compounds and energy. All energy in an environmental system stems from the organic compounds created by autotrophic organisms such as algae (Cole and Weihe, 2015). Knowing the productive potential of a system is crucial, as it provides essential information regarding the growth potential of algae in a given environment. In a wastewater system, it helps to determine the algae's potential capacity to remove nutrients through nutrient assimilation into algal biomass. Primary production in HRAPs is mainly performed by algae. Algae can synthesise organic compounds, such as sugars through photosynthesis in which they utilise CO_2, water and solar radiation (Rogers et al., 2014). A by-product of photosynthesis is oxygen which can be easily measured (Yehoshua and Gophen, 2018). The algae utilise the oxygen and sugars during respiration for growth. It is worth noting that some primary production may be performed by chemotrophic bacteria that utilise the energy from chemical bonds by oxidising compounds such as sulfur and nitrogen for growth. Algal photosynthesis has been thoroughly studied, and there are numerous variables which influence its function such as; biomass concentration, solar radiation, temperature, nutrient concentration and pH level (Borowitzka and Vonshak, 2017, Béchet et al., 2015, Sutherland et al., 2015b). These factors affect the productive potential in different ways and are examined below.

2.4.1 Biomass

The first factor examined in this study was biomass. Algal biomass is the primary source of photosynthesis in HRAP, and the concentration of algae present directly influences the amount of oxygen and organic compounds which can be produced. The concentration of algal biomass affects the productive potential in three ways; firstly the higher the concentrations of algae means that there are more organisms present which can photosynthesise and in turn produce greater amounts of oxygen and organic compounds. Secondly, the algal biomass can limit the amount of solar radiation passing through the water column (Sutherland et al., 2015b). This is termed self-shading and occurs when the algal population is at a high density and is discussed in more detail below (Borowitzka and Vonshak, 2017). Thirdly, the higher the concentration of algal biomass the higher the rate of respiration which consumes the oxygen produced during photosynthesis.

2.4.2 Solar Radiation

Solar radiation provides the algal cells with the energy for photosynthesis and is the main controller of primary production (Sutherland et al., 2015b, Huesemann et al., 2016). The main difference between nutrients and solar radiation is that the photons from solar radiation need to be utilised immediately, whereas nutrients can be stored (Sutherland et al., 2015b). Solar radiation varies diurnally and seasonally, and the light reaching the bottom of the water column declines exponentially with depth as the algae scatter or absorb the light (Huesemann et al., 2016, Sutherland et al., 2015b, Yehoshua and Gophen, 2018). Algal concentrations strongly influence the penetration of solar radiation in the water column and this is termed self-shading (Borowitzka and Vonshak, 2017). High amounts of self-shading can mean that while algal cells near the top of the water column receive a sufficient amount of solar radiation or are supersaturated with solar radiation, the cells near the bottom receive little or no light and photosynthesis is negatively affected (Sutherland et al., 2015b). This self-shading effect affects the depth of the euphotic zone. The euphotic zone is the part of the water column which receives sufficient solar radiation for photosynthesis to occur. The shallow design of HRAPs optimises the amount of

algae in the euphotic zone, however, at high concentration of algae the euphotic zone may still be limited (Sutherland et al., 2014b). Outside of this zone respiration occurs freely. The larger the euphotic zone, the higher the rate of photosynthesis and therefore productivity. In addition to light penetration, different algal species can uptake solar radiation differently with some having a higher or lower photosynthetic efficiency. The capture and transformation of solar radiation is performed by pigments, such as chlorophyll, which are found in the photosystem reaction centres in the algal cells (Sutherland et al., 2015b). These pigments are affected by temperature and nutrient concentration, which in turn affects photosynthesis and productivity.

2.4.3 Temperature

The third factor examined was temperature. Temperature controls the activation of enzymes, and the majority of algae have an optimum range of 15-25℃ (Sutherland et al., 2015b). At cooler or suboptimum temperatures, growth, photosynthesis, respiration and other metabolic processes are restricted by reduced enzyme activity (Sutherland et al., 2015b). Additionally, the photosystems can become saturated at lower light intensities under cooler conditions (Borowitzka and Vonshak, 2017). Warmer temperatures increase the rate of both respiration and photorespiration causing increased consumption of oxygen (Edmundson and Huesemann, 2015). It is also important to note that temperature affects the solubility of gases in solution for example; increased temperature reduces the solubility of CO_2 in the water (Sutherland et al., 2015b, Borowitzka and Vonshak, 2017).

2.4.4 Nutrient Availability

The productive potential of a system is based on how much CO_2 can be converted into organic compounds. Therefore, the concentration of CO_2 affects primary productivity. Algae prefer to use CO_2 due to its ability to diffuse into the cell passively (Low-Décarie et al., 2011). Algae can also utilise other forms of carbon such as bicarbonate and sugars. However, this requires the use of costly energy processes such as active transport and carbon concentrating mechanisms (Sutherland et al., 2015b). Other nutrients such as nitrogen and phosphorus also affect growth. Nitrogen is utilised for proteins and enzymes

synthesis, while phosphorus is incorporated into ribosomal RNA, utilised in ATP and RuBisCO (Beuckels et al., 2015). Thus, the limitation of these nutrients diminishes growth. The combination of effects caused by nutrient limitation negatively affects the photosynthetic efficiency of the cells and leads to drastically reduced primary productivity of the algae.

2.4.5 pH

The pH level can also affect the primary production of algae cells. High pH shifts the carbon equilibrium and reduces the available CO_2. Elevated pH levels also interfere with the RuBisCO activity in the cell thus limiting the rate of photosynthesis (Sutherland et al., 2015b). Elevated pH levels cause ammonia volatilisation and phosphate sedimentation (Craggs et al., 2012). Additionally, high pH causes the dissociation of ammonium ions, and high concentrations of free ammonia have been shown to reduce photosynthetic rates drastically (Azov and Goldman, 1982). Certain species of algae are known to flocculate at high pH levels, and this may negatively affect light absorption and nutrient uptake thus affecting the photosynthetic rate (González-Fernández and Ballesteros, 2012, Ummalyma et al., 2017). Furthermore, elevated pH levels inhibit aerobic bacteria which oxidise organic matter to CO_2, thus reducing the sources of CO_2 to the system.

2.5 High Rate Algal Ponds versus Photobioreactors

Another promising method for large-scale production of algae is the use of photobioreactors. Photobioreactors are closed systems which can be used to grow algae is a controlled environment. However, there are advantages and disadvantages to both systems (Pawar, 2016, DOE., 2016). Pawar (2016) compared the two systems and the characteristics of these systems are shown in Table 2.1 below.

Table 2.1: Pros and cons associated with High Rate Algal Ponds and Photobioreactors. Adapted from Pawar (2016).

Basis	High Rate Algal Pond (HRAP)	Closed Tubular Photobioreactor (PBR)
Operating parameters		
Production capacity of a single unit	Very large	Low
Production capacity per unit land area	Moderate	Very large
Location	Outdoor	Outdoor/ Indoor
Maximum Production	0.5g/L	5g/L
Energy/ power	Low	Very high
Capital cost	Low	High
Labour cost	High	Low
Illumination	Solar light	Artificial light/ Solar light
Ease of operation	Simple	Moderate to difficult
Critical parameters		
Seasonal variation or solar intensity	Subject to high risk	Subject to low risk
Light/dark cycle	Only 12:12 hours	Can be made flexible 12:12, 16:12, 24:00 hours
Contamination risk	Very high	Low
Water loss	Very high	Low
Energy required for harvesting	Very high	Low
Cleaning and washing	Simple	Difficult

Overall, HRAPs are cheaper to run, however, they produce less biomass and are more reliant on environmental conditions (Quinn and Davis, 2015).

Photobioreactors can produce high biomass concentrations but require large amounts of energy and are difficult to operate and clean. Algae can be grown in a number of different ways and to various concentrations when controlled correctly. A proper understanding of the multiple types of algal growth and their requirements is important

2.6 Algal Growth Conditions

Algae have three different growth forms; autotrophic, heterotrophic or mixotrophic (Chen et al., 2015). Photosynthesis is the primary driver of algal growth and is reliant on available light (Sutherland et al., 2015b). Additionally, temperature affects algal growth, and each species has an optimal temperature range in which it thrives. Lastly, nutrient availability is crucial and changes how well algae grow as well as the algal cells internal composition. An effective method in which to understand and control these factors is to use a predictive growth model. There are numerous models currently in use ranging from simple models only focusing on light to more complex models involving many different factors (Huesemann et al., 2016, Jayaraman and Rhinehart, 2015, Béchet et al., 2015, Bernard and Rémond, 2012). Unfortunately, these models are usually species specific. The effects of the main factors for algal growth and some models are discussed below.

2.6.1 Light Modelling

Photosynthesis is controlled by available light which can be provided by either solar or artificial means. The growth of algal biomass in the HRAPs can be maximised by ensuring the algae receive the correct amount of solar radiation for photosynthesis (Young et al., 2017). The shallow design of the HRAP provides the algal cells with the maximum amount of solar radiation that is available (Young et al., 2017). Light is the most critical factor as it provides algae with energy for growth. Light can be provided from both natural and artificial sources; natural light is the cheaper option but is not always available. Artificial light can be continuously used, but, this uses significant amounts of energy. The effectiveness of light can vary dependant on the intensity, duration and culture conditions. Photoinhibition can occur when the light intensity is too high, causing a decrease in the rate of photosynthesis (Chisti, 2016, Undurraga

et al., 2016). Both photoinhibition and self-shading are problems for algal cultures, and cultures may experience both simultaneously (Christenson and Sims, 2011). Photoinhibition in an outdoor system will be dependent on the location of the ponds and may only occur during summer or spring where there are higher light intensities and longer illumination period. Self-shading can happen throughout the year if a culture is kept at too high of a concentration, (Sutherland et al., 2015b). Both effects can be minimised with adequate mixing and self-shading can be further controlled with regular harvesting (Chen et al., 2016, Sutherland et al., 2015b).

The main models used for predicting algal growth based on light are based on the Steele model and the Beer-Lambert law (Nagappan and Verma, 2016, Huesemann et al., 2016, Béchet et al., 2013). The Steele model is used to predict algal cell growth based on light intensity and is shown in equation (2.1) (Wu et al., 2013).

$$\mu = \mu_{max} \cdot \frac{l}{l_{opt}} \cdot e^{1\frac{l}{l_{opt}}} \quad (2.1)$$

Where μ (d^{-1}) is the specific growth rate under light intensity of I (lx); $\mu_{max(I)}$ (d^{-1}) is the maximum specific growth rate when light intensity is optimal; I (lx) is the light intensity, and I_{opt} (lx) is the optimal light intensity (Wu et al., 2013). The Steele model has some drawbacks in that it requires an optimal growth rate to be known and does not incorporate photoinhibition or self-shading effects. The majority of light models are complex and require large amounts of information some of which can be species-specific (Huesemann et al., 2016). The main problem with light models is that each algae species requires different levels of light and their photosynthetic rate can be drastically different from each other based on different light and culture conditions.

The majority of models are developed using an indoor culture under controlled conditions with artificial lighting (Huesemann et al., 2013). Some of these models use a constant light source for illumination, which is not replicable to an outdoor culture due to the diurnal variation of natural light. Constant lighting of an outdoor HRAP would require an artificial light source which would add to the overall cost of the algal production process. The wavelength of light

provided also varies due to the different light sources used with the majority having a different light spectrum to sunlight (Béchet et al., 2013). Measurements of the rates of photosynthesis are also variable due to changes in cellular composition of algal cells, thus making methods such as optical density a less accurate means of determining productivity. Techniques such as biomass concentration or oxygen production are more accurate measures of photosynthetic rate (Béchet et al., 2013, Murrell et al., 2018).

Huesemann et al. (2016) developed a predictive model for microalgal growth in outdoor ponds and validated it with three algal species. The model focused on fluctuating light and temperature conditions. The model requires predetermined values for parameters such as growth rate and biomass loss rate as a function of light and temperature, and the biomass light absorption coefficient (Huesemann et al., 2016). The research investigated how the light changed throughout the water column and highlighted the effect this would have on productivity. Beer-Lamberts law is commonly used to determine the amount of light absorption and is a function is incident light and algal concentration. This requires identifying the light intensity at different water depths at various algal concentrations. Huesemann et al. (2016) developed their model using a nutrient-replete solution which limits its use. The depletion of specific nutrients may have a negative impact on the photosynthetic rate as well as the algae's ability to transform sunlight into usable energy.

2.6.2 Temperature Modelling

Temperature is another major factor for microalgal growth. Temperature helps to regulate enzyme activity in cells, and low temperatures can lead to decreased enzymatic activity and cause reduced productivity (Eustance et al., 2016, Mehrabadi et al., 2015). All algae have a different optimal temperature production range. A significant number of algal species will have an optimum range between 15-25℃ with some slightly higher or lower (Singh and Singh, 2015, Sutherland et al., 2015b). Algae are able to acclimatise to changes in the temperature regime, but this may lead to lower productivities than their normal optimal ranges (Undurraga et al., 2016). Béchet et al. (2011) developed an in-depth temperature model for HRAPs but did not investigate the impact of

temperature on algal growth. Béchet et al. (2011) model accurately predicts water temperature, however, it requires a significant amount of data that is not commonly available, such as pond and air radiation. Bernard and Rémond (2012) used a model which required growth rates at an optimal, minimum and maximum temperature for the algae being investigated. This approach is feasible in a laboratory environment and in a study in which a single algal species is being considered, but it would not be useful in a mixed culture which contains multiple different strains and species of algae.

2.6.3 Nutrient Modelling

Nutrients are another critical factor when modelling algal growth. The majority of models considered data from carbon-rich medium, either through a high initial concentration of inorganic carbon or most commonly through the sparging of air or CO_2. The primary two nutrients for which modelling has been done are nitrogen and phosphorus. There are usually two approaches to the modelling either the Droop model or the Monod model. The Droop model considers the cells internal nutrient concentrations, whereas the Monod model considers the dissolved nutrient concentrations in the external media (Sommer, 1991). This makes it easier to gather data for the Monod model. However, the Droop model can be more accurate due to algae being able to uptake and store excess nutrients inside the cells. A combination of both models can be employed for increased accuracy but may lead to increased testing and costs (Wu et al., 2013). With nitrogen and phosphorus' assimilation into algal cells being linked, modelling of just one of the nutrients may not be sufficient. Bougaran et al. (2010) suggested that nitrogen and phosphorus should be modelled as dependant nutrients rather than biochemically independent ones, especially for nutrient-limiting conditions. In addition to these factors discussed, there are many other factors influencing algal growth such as pH, salinity, predators and interspecies competition.

There are a number of disadvantages with models. The first being that the majority of the current studies performed are in laboratory conditions and are difficult to extrapolate to outdoor situations (Huesemann et al., 2016, Béchet et al., 2013). In addition to this, the addition of CO_2 to control the pH of a culture

can be an expensive and ineffective in an outdoor culture. Being able to predict the algal growth without the addition of CO_2 to lower the pH level is poorly studied (Nagappan and Verma, 2016). Additionally, models are generally quite complex and require a large amount of data some of which can be difficult to obtain. Development of a simple model with easy to access factors is crucial for large scale operation of HRAPs (Heaven et al., 2012). Moreover, understanding what the model is required for, can alter the design. A model which is designed to maximise biomass growth may not need to consider algal growth at low nutrient concentrations. Furthermore, due to the unique optimal conditions required by each algal species and strain, developing a model for ambient cultures would be beneficial when unseeded operation of HRAPs is considered. Currently, to this researchers' knowledge, there are no models predicting the growth of algae in HRAPs in the south-eastern Australian climate.

To summarise, light is the most important factor to understand for modelling as light provides the energy for the algal cell. Second in importance to light is temperature, as temperature controls the activation of enzymes which in turn, controls the rate of growth. Nutrient modelling can also be beneficial as nutrients act as the building blocks for algal growth and understanding the requirements of the algal cells are important. Nutrients are generally supplied in excess when modelling algal growth. However, nutrient limitations are common, and generally, only one nutrient limitation is considered at a time. Monitoring the biomass concentrations and its impact on algal growth in terms of light attenuation, and self-shading is also vital as this can impact algal productivity. It is also essential to design a model which utilises data which can be readily obtained by both established HRAP systems, and water treatment plants that are investigating the feasibility of incorporating HRAPs in their systems. Considering these factors is crucial when developing an algal growth model which can be utilised to help maximise algal biomass production.

2.7 Optimisation of Algal Biomass Production

Models both predict algal biomass production and help us to understand what a system needs in order to enhance algal biomass concentrations and this is the aim of the majority of growth models. Models can also be utilised to

predict when nutrients will be limiting, or if wastewater treatment by HRAPs systems has removed a sufficient concentration of nutrients. Utilising models, it is also possible to identify optimum retention times in the HRAPs to maximise algal biomass production and nutrient removal (Wu et al., 2013). Additionally, understanding the impact of the nutrients on the algal growth and the modelling of how the nutrients are utilised can help illuminate limitations and identify if controls need to be implemented to minimise nutrient depletion from sources other than algal assimilation. For example, ammonia volatilisation due to high pH can be reduced via pH control, and this would enhance biomass production. Knowing exactly how much light or nutrients algae require is important, but if it is impossible or uneconomical to implement the optimum conditions, then modelling can be less desirable. Modelling can also provide the information needed to assess if optimisation processes are likely to be economically viable. Optimisation of algal growth can be done in several ways depending on the limiting factor. Assuming the cultures are grown in HRAPs and light is a limiting factor, there are three main ways to optimise algal production. Firstly, the construction and location of the HRAPs are important; they work best in an area with high sunlight, and long days, if the photoperiod is too short, this decreases the productive period (Chisti, 2016). Day length and shade covering should be considered when constructing a HRAP. Pond depth is also an important factor, as if the cultures are too deep light will be unable to reach the deeper water. HRAPs commonly range from 0.2m-1.0m in depth with a pond depth of 30cm considered suitable (Chisti, 2016, Park et al., 2011). Secondly, adequate mixing of the algae is required to avoid settling, limit photoinhibition, provide an optimum light/dark cycle and maximise productivity (Prussi et al., 2014). This becomes crucial the denser the algal culture becomes due to self-shading. Thirdly, regular harvesting can help to alleviate effects such as self-shading (Amini et al., 2016, Sutherland et al., 2015b). This will also enhance nutrient removal as it promotes growth. Another method that can counteract light limitation is the use of artificial lights. Unfortunately, this would add to the overall cost, power consumption and greenhouse gas emissions.

Control of the temperature can be utilised to optimise algal growth. However this is harder, and besides construction in a warm climate, there are

few other viable methods. Heaters could be used to increase the water temperature, but this would require huge amounts of energy. Building a green-house around the HRAP could help regulate the temperature but would be a substantial additional cost (Borowitzka and Vonshak, 2017). Lastly, the addition of warm flue gas could heat the water and may provide some carbon and other nutrients to the system, as well as toxic compounds such as sulfur (Barkan et al., 2018). This would also increase the overall construction cost of the system but may enhance the economics of the system in the long run (Barkan et al., 2018).

The production of algae in HRAPs is strongly linked to environmental conditions. There is excellent growth in summer with warmer temperatures, high light intensities and longer photoperiods than compared to winter which has lower temperatures and light intensities and shorter photoperiods (Sutherland et al., 2018, Mehrabadi et al., 2015). As discussed earlier, temperature and light are crucial to growth, and while control of the light and temperature are difficult and costly, another factor which helps to optimise both algal biomass production and wastewater treatment is the retention time. Varying the retention times of the effluent in the HRAPs can enhance the algal biomass production and nutrient removal. Retention times are shorter in summer due to increased growth and can be as short as a couple days whereas in winter the retention times can be 10 days or longer depending on the conditions (Mehrabadi et al., 2016, Béchet et al., 2016).

As mentioned previously nitrogen, phosphorus and carbon are the main nutrients; these nutrients can be readily found in wastewater. However, the concentrations vary significantly between different treatment plants and even within each treatment plant during different stages of treatment. Carbon is the primary nutrient required by algae and is often the first limiting nutrient as represented by high culture pH, so the addition of CO_2 is common practice (Sutherland et al., 2015b). The limitation of carbon has a variety of consequences including algal growth inhibition, ammonia volatilisation and potentially toxicity, enhanced bacterial respiration and can potentially reduce the availability of any remaining dissolved inorganic carbon. CO_2 gas can come from a variety of different sources. It is possible to use pure CO_2, but a cheaper

option is to just bubble in air or use flue gas from a power plant (de Godos et al., 2014). However, normal air does not have a high carbon content and transport of the flue gas and its potential to introduce pollutants, such as sulfur, can cause problems (Chen et al., 2015). Control of the pH of the HRAP can help to minimise the loss of CO_2 to the atmosphere and would also reduce ammonia volatilisation and decrease the amount of phosphorus sedimentation (Craggs et al., 2012, Sutherland et al., 2015b).The control of pH can be done by the addition of CO_2 or through the addition of acids and bases. The addition of CO_2 gas is believed to enhance the growth of algae through pH control, however, the added carbon would also improve growth. Both inorganic and organic acids could be utilised to help reduce the pH in HRAPs. Furthermore, organic acid would also supply additional carbon to the system which should enhance growth. Mixing of effluent types from wastewater plants may provide optimum nutrient concentrations. However, the addition of untreated effluents may have competing effects of increasing nutrient discharge and the addition of particles which may cause self-shading conditions. A carefully balanced nutrient mix and control are crucial to optimising algal biomass production.

In order to optimise biomass production and nutrient removal, it is important to build the HRAP in an area with high levels of sunlight and high temperatures to optimise growth. Additionally, having the HRAPs near a powerplant or similar facility which can provide flue gases would also be beneficial. The flue gases can add CO_2 to the system and potentially be used to heat the HRAPs if required. The use of wastewater effluent as the nutrient source is important to reduce the overall costs of algal production. However, utilising the correct effluent which has sufficient nutrients to optimise growth is crucial, if an effluent is depleted of a specific nutrient it will severely diminish growth. Furthermore, altering certain HRAP conditions can enhance the biomass and nutrient removal capacity. Altering the retention time is easy and provides good control of the algal growth and nutrient removal. Control of the pH level can also be used to promote algal growth and nutrient removal by allowing the algae to access more carbon and ammonia which are lost to the atmosphere and environment at elevated pH levels. A summary of the

influencing factors and options which can be investigated or considered for optimal algal growth are tabulated below in Table 2.2.

Table 2.2: Summary of factors which influence algal growth and methods to enhance production.

Factor	**Optimisation Options**
Light	Location (High light intensity) Long Photoperiod Shallow Depth (<30cm)
Temperature	Location (Warm climate, 20-30℃) Artificial Heating - Heaters - Hot flue gas Greenhouse
Nutrients	Carbon - CO_2 sparging - Flue gas - Bubbling Air - pH Control Nitrogen - Wastewater - pH control to reduce ammonia volatilisation Phosphorus - Wastewater - pH control to reduce sedimentation
Algal Biomass	Mixing Regular Harvesting
Other	Retention times - Short periods in summer (< four days) - Long periods in winter (> seven days)

2.8 Algal Harvesting

One of the most significant challenges of algal production is harvesting the biomass. Harvesting of algae is a significant problem due to a combination of factors including; their small size (3µm-300µm), negative surface charges (roughly -7.5~-40mV), low concentration (0.5-5g/L) and similar density to water (Ummalyma et al., 2017, Wrede et al., 2014). There are numerous methods to harvest algae each with their own advantages and disadvantages. The primary methods are centrifugation, gravity sedimentation, flotation, filtration and flocculation. Centrifugation is the most effective method with numerous studies reporting over 95% removal of algal cells (Pragya et al., 2013, Singh and Patidar, 2018). Centrifugation is also a very rapid method and works on most algal species. However, it is costly and uses a significant amount of energy. It is mainly used for laboratory studies or for the production of high-value products such as pharmaceuticals (Barros et al., 2015). Centrifugation also has the added possibility of causing cell damage due to the high shear forces exerted.

Gravity sedimentation is a relatively inexpensive and straightforward process and is commonly used in wastewater treatment processes (Barros et al., 2015). The main problems are that it is time-consuming, provides a low final algae concentration and is only useful for the larger algal species with some smaller species needing flocculation to enhance the process. Due to the extended settling period, the algae may deteriorate, and there would be a loss of product whether it be biomass or internal cellular components such as oils or proteins (Barros et al., 2015). Dissolved air floatation is another low-cost method, but it requires a small area and has short operational times. It is also feasible for large-scale production. Unfortunately, flotation usually involves the use of a chemical flocculant that contaminants the algae product (Laamanen and Scott, 2017).

2.8.1 Membrane Filtration

Membrane filtration is an established technique for particle separation with positive results regarding performance, power consumption and economically viability. In terms of algal harvesting, membranes can be used as a selective barrier to retain algae and other compounds while allowing water to

pass through thus enhancing algal concentrations and simultaneously treating the water (Bilad et al., 2014a). There are two types of membranes commonly investigated for algal filtration, microfiltration (MF) which has pore sizes ranging from 0.1-1.0μm and ultrafiltration (UF) with pore sizes ranging from 1-100nm (Sun et al., 2013). These membranes allow for almost complete retention of biomass and can remove contaminants from the supernatant potentially allowing for media to be reused (Mo et al., 2015). The harvested cells suffer minimal damage and retain their structure, mobility and properties. Additionally, membrane filtration can be operated with the use of chemicals that may reduce the cost and complexity of both the harvesting and the downstream processing (Mo et al., 2015).

In addition to the two types of membranes (MF and UF), there are several different modes of membrane operation. These modes of operation include dead-end filtration, cross-flow filtration and submerged membrane filtration (Bilad et al., 2014a, Barros et al., 2015, Shekhar et al., 2017). The composition of the membrane materials and their characteristics is also of key importance. Membranes can be made of a range of materials such as polyvinyl difluoride (PVDF), polyethersulphone (PES), polyacrylonitrile (PAN), polyvinyl chloride (PVC), PTFE, ceramic materials such as aluminium oxide and some metals (Bilad et al., 2014a, Drexler and Yeh, 2014). Membranes can be constructed as flat sheets or hollow fibres. Other factors such as flow rate, hydrophobicity and suspension characteristics can also influence filtration performance (Mo et al., 2015). The main drawback of utilising membranes is that they can be fouled or damaged and need to be replaced (Bilad et al., 2014a).

Fouling is a major impediment in filtration-based harvesting of algae. There are two sorts of fouling, surface fouling and internal fouling. Surface fouling is when a layer of organic matter such as algae or biopolymers forms a cake or gel layer on top of the membrane blocking the pores (Liao et al., 2018). Surface fouling can typically be easily removed through mechanical cleaning or a backwash (Zhang et al., 2013). Internal fouling involves smaller substances such as extracellular organic matter (EOM) and cell debris adhering to the inside of the membrane pores resulting in blocked pores (Liao et al., 2018).

Internal fouling is harder to clean and can require harsh chemical cleaning and high-pressure backwashes. Both forms of fouling can be reversible and irreversible depending on the severity. Irreversible fouling can generally be removed but requires strong chemical cleaning methods which can reduce the lifespan of the membrane leading to more frequent membrane replacement (Liao et al., 2018). As the amount of fouling increases, more energy or time is required to filter the same volume of algae, this, in turn, would increase the cost of harvesting. Understanding the membrane properties and characteristics are crucial in determining which membranes are most suited for algal harvesting.

MF is generally used to remove organic materials from suspensions such as algae, bacteria, fungi and detritus (Kumar and Ismail, 2015, Drexler and Yeh, 2014). UF is used to remove or concentrate particles, virus and large molecular weight compounds such as proteins (Xie et al., 2016, Kumar and Ismail, 2015). Both MF and UF have been proven successful in removing algae from suspension. Shekhar et al. (2017) utilised MF to harvest *Chlorella* sp. and *Chlamydomonas* sp. in a submerged system using a hollow fibre polypropylene membrane. Zhao et al. (2017) compared three hydrophilic PVDF UF membranes with different pore sizes, 0.03, 0.05 and 0.1µm, for their ability to harvest algae and determined that the largest of the three sizes tested, 0.1µm, was the most suitable for algal harvesting as it had the highest flux.

The membrane systems utilised for filtration offer different outcomes and require different operating conditions. A dead-end filtration system has a simple setup but tends to lead to high membrane fouling. However, it requires less energy than other systems such as cross-flow filtration. Dead-end filtration is commonly used to harvest larger microalgae (Elcik and Cakmakci, 2017). Cross-flow filtration is when the solution is passed parallel to the membrane surface, and a pressure gradient is applied across the membrane to remove the water. Cross-flow filtration does not tend to foul as easily as dead-end filtration, as turbulence arising from the water flowing along the membrane acts to dislodge any attached organic material (Elcik and Cakmakci, 2017). Submerged membrane filtration involves the placement of membrane modules into a culture and water is sucked through the membranes using the suction side of a pump. Bilad et al. (2012) investigated the use of submerged membranes for algal

concentration and found a low degree of fouling and claims that it is an economically viable method. Mo et al. (2015) reviewed several of the methods and claim that while cross-flow is the most common method utilised due to its low fouling tendency, other methods such as submerged membrane filtration, dynamic filtration and forward osmosis are receiving more consideration due to specific factors such as reduced fouling, energy usage and costs.

The composition and surface chemistry of membranes can play a crucial role in fouling, with most commercial membranes containing additives such as polyvinylpyrrolidone (PVP) that change the surface properties to reduce fouling (Kumar and Ismail, 2015). Standard commercial PVDF membranes have been found to foul quite readily when used for algal filtration because the algae adhere strongly to the surface of the membranes and block the pores (Kumar and Ismail, 2015). PTFE membranes may provide a better solution to fouling as commercial PTFE membranes have a strongly hydrophilic surface. Ceramic membranes can also be hydrophilic. Additionally, they are more robust than either the PTFE or the PVDF membranes, and therefore can operate under higher pressures and harsher cleaning regimes (Bilad et al., 2014a). Metal membranes are similar to ceramic membranes; they provide good filtration, are very robust and can undergo high pressures and harsh cleaning regimes. Xie et al. (2015) compared the filtration of a metal membrane against a polymeric membrane in a sludge bioreactor, and the metal membrane showed higher flux, less fouling and a slower increase in trans membrane pressure. Metal and ceramic membranes tend to require less replacement than PVDF membranes, however, they are more expensive and can be uneconomical. The majority of studies previously investigated the effect of the surface properties of common commercial membranes, but other less common membranes may provide better performance.

2.8.2 Flocculation

Flocculation is the process of adding a compound to the desired product to increase its size. Regarding algal cells, a flocculant promotes coagulation of the algal cells to form a larger floc or pellet. Flocculation is used to enhance other harvesting techniques such as gravity sedimentation, floatation and

filtration, and can be broken into chemical, physical and biological (Vandamme et al., 2013).

Chemical flocculation is rapid and straightforward; it also requires little energy beyond the mixing of the water and flocculant. Chemical flocculation is typically induced by inorganic or organic flocculants, such as chitosan, cationic starch, alum, ammonium and ferric chloride (Wan et al., 2015, Vandamme et al., 2013). The flocculants can be expensive and potentially toxic to the algae, and the use of specific flocculants, such as metal salts, can also make the algal biomass not usable for certain products without a further chemical removal step. The use of some flocculants can also alter the internal composition of algal cells. For example, residual ammonium can affect the fatty acid methyl ester (FAME) profile and composition, and may exist in the extracted lipids (Wan et al., 2015). Specific flocculants such as cationic starch and chitosan have restricted use due to their pH dependency (Wan et al., 2015). The chemical flocculant can also contaminate the culture medium and reduce recycling or reuse of the water (Vandamme et al., 2013). The addition of chemicals can also lead to pollution in the receiving waters.

Physical flocculation can be achieved by ultrasonic disruption, electro-flocculation and magnetic nanoparticles, and work on a wide variety of algal species and do not require the addition of harmful chemicals (Vandamme et al., 2013). A significant drawback is the large amount of energy required and the high equipment cost. Ultrasonic disruption is used to collapse the gas vacuoles in the algal cells and enhances the settling velocity (Zhang et al., 2006). Electro-flocculation uses cathodes, and this causes the negatively charged algae to migrate to the anode and flocculate (Lee et al., 2013). A release of bubbles at the anode rise to the surface and further entraps the microalgal flocs. Furthermore, the process adds sacrificial metals to the culture medium which can act as inorganic flocculants (aluminium or iron) and assist the flocculation of the algae. These inorganic flocculants can cause contamination of the algal cells reducing their usability as feedstocks (Chen et al., 2018). Magnetic nanoparticles can be used to adhere to the algal cell walls and act as a flocculant. Reuse of the magnetic particles is possible, and an efficient elution

process is necessary as the materials are costly and final biomass without nanoparticles is preferred (Wan et al., 2015).

Biological or bioflocculation are inexpensive methods, they can allow for culture recycling and are nontoxic (Ummalyma et al., 2017). Unfortunately, the mechanisms are poorly understood and it is currently not viable for all species of algae due to the various shapes, size, surface properties of the algae as well as the environmental conditions such as salinity, pH and temperature that can interfere with the flocculation (Ummalyma et al., 2017, Alam et al., 2016). There is also a possibility for the composition of the algae to be altered. Contamination of the biomass can occur depending on the flocculant whether it be algal, bacterial or fungal.

Certain algae self-flocculate and this can be a natural phenomenon or can be caused by changes in conditions such as elevated pH levels, dissolved oxygen concentrations and changes in the concentration of certain cations such as calcium and magnesium (Ummalyma et al., 2017). Unfortunately, these can affect growth and alter cell composition. The process does not occur for all algal species and can be slow. It is usually linked to a change in pH due to the alteration of the surface charges of the algal cells under differing pH levels (Vandamme et al., 2013). However, this is not a feasible harvesting process as altering pH levels on a large scale is uneconomical (Ummalyma et al., 2017). Increased dissolved oxygen concentration lead to more binding sites on the algal cell surface which may result in flocculation of the cells (Ummalyma et al., 2017). Certain species of algae have been shown to release specific polysaccharides that are capable of inducing flocculation in other species. Others have been shown to have cell walls enriched with phosphodiester groups and can act as flocculating organisms for other species of algae (Alam et al., 2016).

Microbial or bacterial flocculation refers to flocculation caused by compounds released by bacterial cells such as biopolymers and extracellular polymeric substances (EPS) (Ummalyma et al., 2017, Wan et al., 2015). This process has the potential to be economically viable and eliminate the need for chemicals. However, it can cause microbiological contamination to the biomass

and may reduce its usability for food and feedstocks (Vandamme et al., 2013). Flocculation efficiency of up to 97% was reported using bacterial flocculants (Zheng et al., 2012). The production of bacterial flocculants requires the cultivation and purification of the bioflocculant, and this coupled with a high required dosage leads to high operating costs (Ummalyma et al., 2017). This process may also be species specific. Ummalyma et al. (2017) have written an in-depth and comprehensive review of bioflocculation.

2.8.3 Fungal Flocculation

Fungal flocculation of algae is a natural formation and can be seen in lichens which are a symbiotic relationship between fungi and algae. Fungal flocculation of algae is an emerging area of research. Zhang and Hu (2012) were the first researchers to describe the process of fungal flocculation and showed varying levels of success with different fungal species on the flocculation of *Chlorella vulgaris*. There are two methods used for fungal flocculation of algae, firstly a fungal spore addition (FSA) flocculation and secondly, fungal pellet assisted (FPA) flocculation (Chen et al., 2018). FSA entails adding fungal spores to an algal culture, mixing the culture and allowing fungal pellets to form and flocculate the algal cells. FPA involves adding pre-made fungal pellets to an algal culture which flocculates the algal cells. Both methods have been found to be effective. However, FPA can flocculate the algae significantly faster, in a few hours, compared to FSA which can take a few days (Chen et al., 2018). Chen et al. (2018) compared FSA to FPA and found FPA to be a better method regarding time, nutrient use, overall cost and flocculating efficiency (FE%), which is commonly determined by using equation 2.2, shown below. FE% is the percentage of algae which has been removed from suspension by the fungal pellet.

$$Flocculation\ efficiency\ \% = \left[\frac{initial\ \ algal\ biomass - suspended\ biomass}{initial\ algal\ biomass}\right] \times\ 100\% \qquad (2.2)$$

FSA also requires a carbon source to be present in the algal culture which may not be feasible in wastewater treatment systems. Certain fungi are known to produce cellulase which can aid in cell wall breakdown (Xie et al., 2013,

Prajapati et al., 2016). Fungal flocculation has additionally been found to act as a pre-treatment step to enhance methane production of an algal culture by anaerobic digestion (Prajapati et al., 2016). They found that the fungus had significant cellulase production which released sugars from the algal cells. Additionally, they obtained at least a 54% increase in digestibility and up to a 50% increase in methane production during anaerobic digestion (Prajapati et al., 2016).

2.8.3.1 Mechanisms of Fungal Flocculation

Numerous fungi have been trialled against many algae with *Aspergillus* sp. and *Chlorella* sp. being the most common genera examined. Table 2.3, shows a list of fungal- algal flocculation studies and their respective FE % and has been adapted from Ummalyma et al. (2017). The mechanisms behind fungal flocculation are not fully understood and are believed to be a mixture of entrapment and adhesion of algal cells to the fungal filaments. The main cause proposed for the algal cell attachment to the fungal filaments is due to opposing surface charges with algae having a negative surface charge and the fungal filaments having a positive surface charge (Wrede et al., 2014). Algae have carboxylic, amine and phosphate groups on their cell surface that provide an overall negative charge, which is enhanced by their ability to increase the pH thus allowing more hydroxide into the system (Bhattacharya et al., 2017b). The fungal cells have aliphatic amine, aromatic compounds and carboxylic acids functional groups and due to the acidic nature of the fungal culture the surface groups are protonated (Bhattacharya et al., 2017b). This provides an overall positive charge to the fungal cells. Cell compounds such as sticky EPS or protein-carbohydrate interactions may also affect flocculation. Calcium and magnesium ions may also aid in flocculation and cell attachment by altering surface charges and acting as a bridge between proteins and carbohydrates (Zamalloa et al., 2017). A second process believed to help with the flocculation process is the entanglement of the algal cells in the fungal filaments or hyphae (Li et al., 2017). The pellet formation of the fungi creates a space in which the much smaller algal cells can be entrapped inside the pellets and fungal filaments. This may not account for all the flocculation but could enhance the process.

Table 2.3: Flocculation efficiencies (FE) of microalgae with fungus, adapted from Ummalyma et al. (2017).

Fungus	Microalgae	FE (%)	References
A. niger	*C. vulgaris*	98	(Zhang and Hu, 2012)
***Aspergillus* sp.**	*C. vulgaris*	100	(Zhou et al., 2012)
Cunninghamella echinulata	*C. vulgaris*	99	(Xie et al., 2013)
A. oryzae	*C. vulgaris*	93	(Zhou et al., 2013)
A. niger	*C. vulgaris*	90	(Gultom et al., 2014)
A. lentulus	*Chroococcus* sp.	100	(Prajapati et al., 2014, Prajapati et al., 2016)
A. nomius	*C. vulgaris*	97	(Talukder et al., 2014)
A. nomius	*Nannochloropsis* sp.	94	(Talukder et al., 2014)
A. fumigatus	*C. vulgaris*	95	(Wrede et al., 2014)
A. fumigatus	*Chlamydomonas reinhardtii*	76	(Wrede et al., 2014)
A. fumigatus	*Pseudokirchneriella subcapitata*	96	(Wrede et al., 2014)
A. fumigatus	*Scenedesmus quadricauda*	97	(Wrede et al., 2014)
A. fumigatus	*Thraustochytrid* sp.	82	(Wrede et al., 2014)
A. fumigatus	*Dunaliella tertiolecta*	81	(Wrede et al., 2014)
A. fumigatus	*D. salina*	72	(Wrede et al., 2014)
A. fumigatus	*N. oculata*	77	(Wrede et al., 2014)
A. fumigatus	*Nannochloris oculata*	77	(Wrede et al., 2014)
A. fumigatus	*Tetraselmis chuii*	71	(Wrede et al., 2014)
A. fumigatus	*Pyrocystis lunula*	38	(Wrede et al., 2014)
A. fumigatus	*Botryococcus braunii*	98	(Al-Hothaly et al., 2015)

A. fumigatus	*Synechocystis* sp.	97	(Miranda et al., 2015)
A. fumigatus	*T. suecica*	90	(Muradov et al., 2015)
A. fumigatus	*C. protothecoides*	90	(Muradov et al., 2015)
Isaria fumosorosea	*C. sorokiniana*	98	(Mackay et al., 2015)
A. niger	*C. vulgaris*	-	(Olsson, 2015)
A. niger	*Scenedesmus* sp.	-	(Olsson, 2015)
A. oryzae	*Synechocystis* sp.	99	(Choi et al., 2016)
A. fumigatus	*C. pyrenoidosa*	99	(Bhattacharya et al., 2017b)
A. fumigatus	*C. pyrenoidosa*	99	(Bhattacharya et al., 2017a)
A. niger	*C. vulgaris*	93	(Li et al., 2017)
A. niger	*C. vulgaris*	98	(Zamalloa et al., 2017)
***Penicillium* sp.**	*Chlorella* sp.	99	(Chen et al., 2018)

2.8.3.2 Fungal Pellet Formation

There are two types of pellet formation, coagulative formation wherein spores coagulate together while germinating and give rise to a net of intertwining hyphae which can be found occurring in species such as *A. niger*, and non-coagulative formation wherein one pellet is produced from one spore as found in Penicillium species and others (Zhang and Zhang, 2016, Ummalyma et al., 2017). The fungi in all the studies that investigated fungi-algae flocculation belong to the phylum Ascomycota, except for one, *C. echinulata*, which belongs to the Zygomycota phylum. The fungal part of most lichens are ascomycetes, and this may further explain their affinity to flocculation algae (Zoller and Lutzoni, 2003). Formation of fungal pellets is an important step and can be influenced by several factors such as spore inoculum, agitation, carbon content, pH, calcium content, temperature and fungal species (Zhang and Zhang, 2016). Spore inoculum was found to be

crucial in the size and formation of pellets. If the initial spore count was too high, lots of small pellets or a large clump of fungi would form. If the spore count was too low, only a small number of large pellets formed. The size of the pellets is important as it needs to be large enough to entrap algae and be easily removed by filtration while also maintaining a large surface area to volume ratio. Agitation is another factor which affects pellet size and most studies used orbital shakers to induce fungal pellet formation although other methods, such as bioreactors, can be used (Wrede et al., 2014, Espinosa-Ortiz et al., 2016, Zamalloa et al., 2017, Zhou et al., 2012). Different fungi require different speeds to induce fungal pellet formation of the desired size. Many studies for *Aspergillus* sp. grew cultures at 150rpm on an orbital shaker, and some use slower speeds such as 100 or 120rpm. Not all fungal species can naturally induce pellet formation under constant agitation, and some species require the addition of chemicals such as calcium chloride or other compounds to help induce pellet formation (Li et al., 2017). Carbon content, carbon source and pH have all also been shown to effect pellet formation. This is due to the changes in electrostatic charges at differing pH levels and the fungi's need for organic carbon for growth. Pellet formation is higher at low pH than at high pH (Zhang and Zhang, 2016).

2.8.3.3 Fungal-algal Flocculation Factors

Pellet formation is just the first part of the harvesting process, the ability of the fungal pellets to flocculate the algae can be influenced by several factors many of which are the same as the pellet formation process. Culture agitation, pH, temperature and calcium content can all affect the flocculation process (Li et al., 2017). Control of the agitation speed is essential, as if it is too slow it will allow the algae to settle too fast causing a decrease in flocculation efficiency (Bhattacharya et al., 2017a). Additionally, mixing too fast will also decrease the flocculation efficiency. This is believed to be caused by the shear forces created via the agitation overcoming the electrostatic forces attracting the algal and fungal cells to each other (Bhattacharya et al., 2017b). Changes in pH also play an essential role in the interactions of the electrostatic charges of the fungi and algae. Algal and fungal cells have an electrostatic attraction to one another as algal cells have an overall negative charge, and fungal hyphae have a positive charge thus they attract to each other (Wrede et al., 2014). Monocultures of

fungi are naturally low in pH due to their acidic functional groups, while monocultures of algae have alkali functional groups and have naturally high pH levels. At high pH levels, negative charges are found on fungal pellets and this would negatively affect flocculation (Zamalloa et al., 2017). The impact of temperature on fungal-algal flocculation is thought to influence metabolic activity. Bhattacharya et al. (2017b) investigated the flocculation of *A. fumigatus* at seven temperatures between 28 and 40℃. A higher flocculation efficiency was obtained at 38℃ than at 28℃, and they showed that there was higher metabolic activity at 38℃. This was confirmed by a comparison of the fungal-algal pellets under light microscopic that indicated a higher algal cell concentration entrapped in the pellets at higher temperatures. This optimum flocculation temperature would not be the same for all fungal strains. The calcium or ion content of the culture can help with flocculation due to ion bridging. Ion bridging occurs when the ions bind to two particles simultaneously and forms a bridge between the particles, bringing the particles together causing flocculation (Li et al., 2017). The amount of time it takes for the algae to be flocculated by the fungi can vary between species. Bhattacharya et al. (2017b) flocculated 99% of *C. pyrenoidosa* in three hours using *A. fumigatus* pellets, while Xie et al. (2013) required 48 hours to remove 99% of *C. vulgaris* using *C. echinulata*. Wrede et al. (2014) tested flocculation at 24 and 48 hours and found that in some experiments the concentration of algal cells in suspension increased after 24 hours. This could be explained by the growth of uncaptured algal cells or release of algal cells from the fungal filaments. Knowing the optimum flocculation timeframe is crucial to ensuring maximum algal harvesting and best economic value. Miranda et al. (2015) and Choi et al. (2016) both investigated the harvesting of the cyanobacterium *Synechocystis sp.* PCC 6803. Miranda et al. (2015) used *A. fumigatus* and achieved a FE% of 97% while Choi et al. (2016) used *A. oryzae* and achieved an FE% of 99%. Choi et al. (2016) also trialled *Rhizopus oryzae* but was unable to induce flocculation. *Synechocystis sp.* PCC 6803 was targeted as it was genetically modified to release fatty acids into the medium, and this ability combined with a cheap and effective harvesting method can significantly enhance the algal biofuel potential (Miranda et al., 2015). Additionally, the ability to remove

cyanobacteria is beneficial and could potentially be employed into an unwanted eutrophic bloom occurrence to remove the cyanobacteria before toxic compounds are released, or the waters become hypoxic.

2.8.3.4 Wastewater Treatment with Fungal-Algal Pellets

Zhou et al. (2012) investigated the use of the fungal-algal pellets for wastewater treatment, showing high levels of nutrient removal with 100% ammonia removal and 89.8% total phosphorus removal. Wastewater treatment with the fungal-algal pellets was also tested by Wrede et al. (2014), Muradov et al. (2015), Miranda et al. (2015) and Bhattacharya et al. (2017b). Various wastewater types were tested including swine waste, domestic water from a drain and others. The results obtained for the co-culture treatment of the different wastewater showed a higher treatment efficiency using the fungal-algal cultures than the separate monocultures. In work completed by Muradov et al. (2015) the fungal-algal cultures had a 74% and 56% removal of ammonium and phosphate respectively, compared to monoculture removal results of 36% and 25% for ammonium and phosphate by *C. prototheco*ides and 46% and 20% removal for ammonium and phosphate by *A. fumigatus*. The fungal-algal culture could be used to enhance wastewater treatments, and the fungal and algal sections of the culture could potentially be removing different unwanted nutrient components of the wastewater. Together they may improve the nutrient removal efficiency.

2.8.3.5 Oil from Fungal- Algal Pellets

Oil content in algal cells is an important topic especially as the use of biofuels is increasing. Both algae and fungi have been shown to display high internal oil concentrations. Several researchers also investigated the oil content of the fungi and algae pellets (Wrede et al., 2014, Muradov et al., 2015, Miranda et al., 2015, Mackay et al., 2015, Al-Hothaly et al., 2015, Xie et al., 2013, Zhou et al., 2013). Most of the researchers found that there was a synergistic effect and the fungal-algal culture had a higher biomass and oil content than the separate cultures. None of the studies found a negative correlation. Al-Hothaly et al. (2015) investigated using *A. fumigatus* to flocculate the high oil content algae, *B. braunii*, which have been reported to reach a hydrocarbon content of

75% of dry weight. They found that there was no significant change to the C, H, N, and bio-oil content of the harvested biomass (Al-Hothaly et al., 2015). This indicates that fungal flocculation can be used to harvest algae for biofuels. The same oil extraction method utilised for algal can be employed for fungal- algal pellets. The pellet would not need to undergo a further contamination removal step as is necessary if a chemical flocculant is used. Muradov et al. (2015) showed that the fatty acid profile of the fungal -algal pellet was conducive for use in biofuel production. It was also proposed that the fatty acid composition could be controlled through the use of different fungi and algae flocculation (Wrede et al., 2014). Muradov et al. (2015) found that the biomass of the fungi-algae pellet contained fatty acids ranging from C12 to C21, which can be directly used as a component of biodiesel.

2.8.3.6 Digestion using Fungal-Algal Pellets

The ability of fungi to produce enzymes is well known, and the production of cellulase is of crucial importance. Fungi require organic carbon for growth, and in most studies this is provided by glucose or similar sources. Algal cells have a thick cell wall containing cellulose that is difficult to break. Breakage of the cell wall is crucial to extract the various fatty acids, proteins and internal compounds and can provide fungi with a carbon source for growth. Xie et al. (2013) showed that *C. echinulata* could grow in a carbon-free media containing algae due to cellulase activity. Prajapati et al. (2016) utilised this process and combined fungal flocculation of algae with the cellulase production to enhance the digestibility of algal-fungal pellets by 54% and increased methane production by 50% during anaerobic digestion. This combination has potential uses in many algal product production areas including biomethane, biohydrogen, biodiesel and the extraction of valuable compounds from the algal cells such as pigments and proteins (Prajapati et al., 2016).

2.8.3.7 Flocculation of Different Algal Species

As shown in Table 2.3, the flocculation of several algal species has been tested. Wrede et al. (2014) used *A. fumigatus* to flocculate 11 different algal species, ranging in size from 5µm to over 300µm. Both freshwater and marine species were tested with varying results, with freshwater species overall having

a higher flocculation efficiency. This may be due to the increased ion content in marine waters interfering with the flocculation process. Autotrophic and heterotrophic species of algae were able to be flocculated, and fungal pellets have also been shown to be able to grow in an autotrophic algal culture (Xie et al., 2013). The majority of algal species trialled have been non-motile. However, some motile strains have been tested, and Wrede et al. (2014) examined *C. reinhardtii* and *T. chuii* both of which had a flocculation efficiency of over 70%.

This review showed that different types of algae can be efficiently flocculated, however, there is not a universal fungal flocculation process for algae yet. Fungal flocculation of algae is a potential low-cost harvesting option. Chen et al. (2018) claim that algal harvesting by fungal flocculation results in a cheaper cost than other methods such as filtration and floatation with a flocculant. They based this claim on laboratory studies and the small amount of time and low energy consumption required to harvest the algae using this technique, additionally, they compared the cost of harvesting the algae to other studies.

2.8.3.8 Drawbacks of Fungal Flocculation

Unfortunately, fungal flocculation is not without drawbacks. The primary and most obvious disadvantage is the biological contamination of the algal cells with fungi, which may be a drawback for the quality of the final product produced. Some of the fungi used, *Aspergillus* sp., in these studies can have detrimental effects on humans and others can be detrimental to plants and other organisms. This removes the possibility for the microalgal to be used in pharmaceutical production or food production without the biomass undergoing an extra treatment step. This drawback may be diminished with the use of a non-harmful or non-toxic fungal species such as *Penicillium sp*. Another disadvantage is the inability for one fungus to flocculate all algae. While this is not a major problem it does mean further research is required to find the optimum fungal species and conditions to flocculate the target algae.

In summary, fungal flocculation of algae is a potentially viable process and has been shown to efficiently flocculate algae from suspension. However, further research is required. Primarily, the fungal pellets ability to flocculate

numerous species of different algal species simultaneously needs investigation, and also addition work on the use of fungal-algal pellets as a wastewater treatment method is required.

2.9 Research Objectives

The aims of this study are focused on the optimisation of algal biomass production and nutrient removal in HRAPs utilising secondary lagoon effluent at the BMRWP. The specific objectives and research questions were:

1) Investigate the relationship between productive potential of secondary lagoon effluent and the production of algal biomass in HRAPs.
2) To maximise algal biomass growth and in turn nutrient removal by answering the following research questions.
    a. How does the addition of laboratory-grown algae to the HRAPs during cooler months enhance biomass production?
    b. How does the control of the pH level of the HRAPs utilising acids optimise biomass production?
    c. How does the use of an alternative nutrient source, primary lagoon effluent, to alleviate the nutrient deplete conditions observed in the warmer months, enhance biomass production?
3) To develop a simple model using minimal variables to predict algal biomass production in HRAPs under south-eastern Australian conditions at elevated pH conditions.
4) Investigation of improved algal biomass harvesting methods by:
    a. Comparing the filtration capability of three different membranes materials: PTFE, ceramic and metal to understand if material properties of the membrane can improve fouling outcomes for algae filtration.
    b. Analysing the ability of fungal flocculation to simultaneously remove numerous species of algae from suspensions of treated effluent.

Chapter 3 - Primary Productivity Potential of the High Rate Algal Ponds

3.1 Description of the Bacchus Marsh Recycled Water Plant

The Bacchus Marsh Recycled Water Plant (BMRWP) in Victoria, Australia (lat. 37°72'44.09'S, long. 144°47'61.20'E), is a land-based treatment facility treating municipal wastewater in a series of lagoons (see Figure 3.1). There are seven lagoons including an aerated lagoon at the start of the treatment process. Municipal wastewater is first pumped into the Aerated Lagoon. Air is sparged into the water column to initiate the aerobic treatment process and reduce odours, and it has a retention time of three days (Chan et al., 2009). The effluent from the Aerated Lagoon has a mean pH of 7.3; mean suspended solids of 367 mg/L and a mean ammonia concentration of 41.6 mg/L. The effluent from the aerated lagoon with the settable solids is distributed into three Primary Lagoons which are arranged and operated in parallel. These lagoons are anaerobic and are used as anaerobic settling lagoons in which the majority of the solids settle out. The anaerobic process substantially reduces the biological oxygen demand (BOD). Heavy metals are also removed in these lagoons by combining with hydrogen sulphide, produced anaerobically, to form metallic sulphides, which settle to the bottom of the lagoons as sludge along with the settleable solids. The Primary Lagoons are periodically drained and desludged; the sludge is removed for further treatment elsewhere. The effluent from the (Primary) lagoons has a low BOD with a pH of around 7.0. Additionally, most of the organic nitrogen is reduced to ammonia, which is processed in the subsequent lagoons. The primary lagoon effluent flows into the three Secondary Lagoons which are operated in series. In these lagoons, the primary lagoon effluent is further treated by aerobic processes, which are facilitated by algae and photosynthetic bacteria. The Secondary Lagoons operate as facultative systems as they contain both anaerobic and aerobic activity at different depths of the lagoons. The deeper sections of the lagoons operate anaerobically while the top layer operates aerobically. The area between these two zones is predominantly facultative, which tends to develop photosynthetic bacteria that

can utilise hydrogen sulphide for carbon assimilation as opposed to the algae that utilise water as the electron donor for carbon assimilation. The biosynthesis of carbon by algae and the reduction of sulfur by bacteria are described by the general equations shown below, Equations (3.1) and (3.2) respectively (Cole and Weihe, 2015).

$$CO_2 + 2H_2S \rightarrow (CH_2O)_n + H_2O + 2S \quad (3.1)$$

$$nCO_2 + nH_2O \rightarrow (CH_2O)_n + nO_2 \quad (3.2)$$

These equations show that the by-product of biosynthesis by phototrophic bacteria is sulfur, which is stored in their cells until needed, as opposed to oxygen, which is released to the surrounding environment, by general phototrophic organisms such as algae and cyanobacteria.

The photosynthetic process oxygenates the primary lagoon effluent while additionally converting inorganic carbon into algal and bacterial biomass. This biomass is in turn consumed by zooplankton. With the flow of effluent from one secondary lagoon to the next, the BOD is further reduced aerobically, and some or most of the ammonia is assimilated into the biomass. Since the pH of the secondary lagoon effluent from the final secondary lagoon tends to be above 8.3, there is no free CO_2 in the water, and the algae have to access carbon through metabolically expensive active transport and carbon concentrating mechanisms (Sutherland et al., 2015a). These include the dissociation of bicarbonates into mono-carbonates and CO_2. Cyanobacteria are known to be efficient in these processes, and some are known to thrive in pH environments exceeding pH levels of 10.0 such as *Spirulina* sp. (Kumar et al., 2015). Additionally, some periphyton and other phototrophs are also able to dissociate bicarbonates into mono-carbonates and CO_2. The CO_2 produced through these processes is utilised for carbon assimilation (Hutchinson, 1957). During the warmer months, the pH of the final effluent from the secondary lagoons can frequently exceed 9.0, and this allows for the potential loss of ammonia through ammonia volatilisation. The effluent from the third secondary lagoon is almost entirely treated and is discharged into the last lagoon designated as the Winter Storage lagoon, where it undergoes final polishing to help remove the remaining nutrients from the water. The effluent from the Winter Storage lagoon is

classified as ‘Class A’ water and is distributed onto the surrounding farmlands for irrigation during the warmer months. The lagoons support a large bird population which feed on the algae and zooplankton present. The birds provide an additional form of nutrient recycling through consumption of the biomass and their droppings to create a guanotrophic environment.

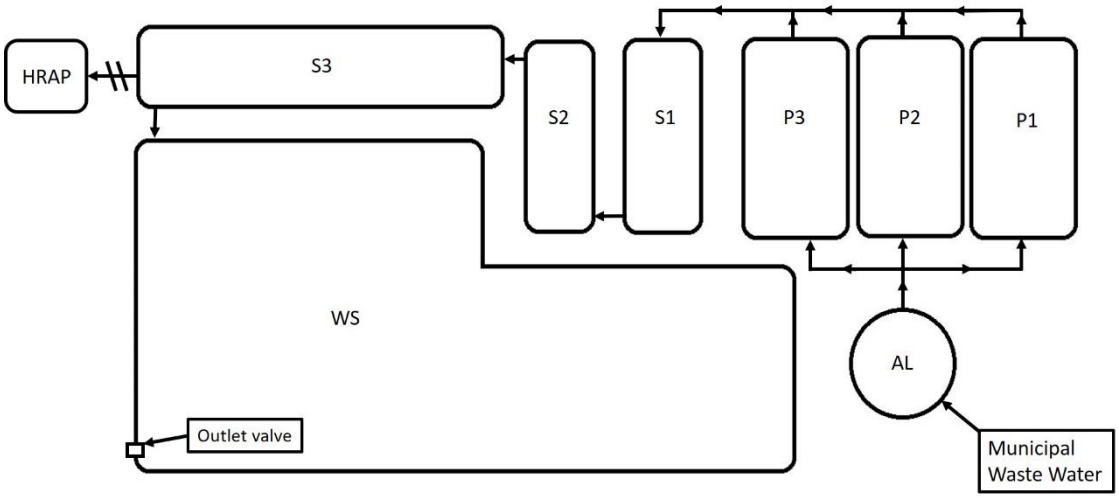

Figure 3.1: Diagrammatic flowchart and the operation of the Western Water’s Bacchus Marsh Recycled Water Plant (not to scale). Aerobic lagoon (AL), Primary lagoons (P1-P3), Secondary Lagoons (S1-3), Winter storage lagoon (WS) and HRAP.

3.2 Quality of Influent Water Researched

3.2.1 Nutrients Concentrations and pH of the HRAP Influent

The majority of water used in the HRAP experiments came from the third secondary lagoon at the BMRWP. However, four experiments which were conducted during the summer of 2017/18 utilised effluent from the first primary lagoon, and this is discussed further in sections 3.5.3 and 4.3.6. As the experiments were run all year-round, the quality of the influent to the HRAP was continually changing. The mean, maximum, minimum and standard deviation of the concentration of nutrients and biomass in the influent are tabulated in Tables 3.1 and 3.2. The methods used to analyse the samples are described in section 3.4.1. The tables are divided into overall and seasonal sections to help

explain seasonal variations, and this assists later in determining the major attributing factors.

Table 3.1: Nutrient concentration results of the influent to the HRAPs during the overall period between the 22nd of March 2016 and the 5th of February 2018 and the relevant seasonal results.

	Overall Period		Summer		Spring		Autumn		Winter	
	Mean (Std. Dev)	Minimum Maximum	Mean (Std. Dev)	Minimum Maximum	Mean (Std. Dev)	Minimum Maximum	Mean (Std. Dev)	Minimum Maximum	Mean (Std. Dev)	Minimum Maximum
Ammonia (mg/L)	14.09 (10.79)	0.41 39.25	3.74 (3.40)	0.41 10.10	22.03 (9.73)	4.48 39.25	8.40 (6.54)	0.92 19.7	21.12 (4.94)	12.95 25.50
Nitrite (mg/L)	0.15 (0.18)	0.00 0.80	0.09 (0.13)	0.01 0.47	0.17 (.015)	0.00 0.49	0.11 (0.10)	0.02 0.35	0.29 (0.36)	0.02 0.80
Nitrate and Nitrite (mg/L)	3.01 (2.13)	0.45 8.70	1.77 (0.97)	0.45 4.00	3.79 (2.00)	0.90 6.80	3.15 (2.30)	1.20 8.40	3.34 (3.24)	1.10 8.70
Total Nitrogen (mg/L)	13.97 (9.27)	2.89 29.54	4.10 (1.63)	2.89 6.93	17.81 (8.50)	7.05 26.67	10.39 (6.36)	3.80 23.64	24.18 (5.46)	15.27 29.54
Orthophosphate (mg/L)	8.69 (3.55)	1.99 14.30	10.44 (2.09)	7.70 14.50	8.25 (3.25)	3.43 12.00	7.89 (4.57)	2.32 13.00	7.48 (4.87)	1.99 12.00
Total Phosphorus (mg/L)	8.53 (2.26)	4.28 12.31	6.49 (1.69)	4.28 8.71	11.10 (1.22)	9.23 12.31	8.05 (1.96)	5.79 11.47	7.75 (0.97)	6.26 8.73
pH	8.53 (4.96)	7.85 10.04	8.97 (0.6)	8.07 10.04	8.18 (0.27)	7.86 8.96	8.69 (0.55)	7.85 9.63	8.25 (0.25)	7.86 8.67

Table 3.2: Biomass concentration results of the influent to the HRAPs during the overall period between the 22nd of March 2016 and the 5th of February 2018 and the relevant seasonal results.

	Overall		**Summer**		**Spring**		**Autumn**		**Winter**	
	Mean (Std. Dev)	**Minimum Maximum**	**Mean (Std. Dev)**	**Minimum Maximum**	**Mean (Std. Dev)**	**Minimum Maximum**	**Mean (Std. Dev)**	**Minimum Maximum**	**Mean (Std. Dev)**	**Minimum Maximum**
750nm	0.051 (0.05)	0.005 0.200	0.093 (0.054)	0.019 0.201	0.03 (0.021)	0.005 0.072	0.059 (0.049)	0.017 0.127	0.021 (0.013)	0.005 0.036
680nm	0.065 (0.06)	0.006 0.220	0.108 (0.06)	0.023 0.222	0.036 (0.025)	0.006 0.086	0.08 (0.063)	0.021 0.153	0.026 (0.014)	0.010 0.043
440nm	0.082 (0.07)	0.009 0.290	0.138 (0.08)	0.035 0.295	0.049 (0.03)	0.009 0.110	0.110 (0.08)	0.028 0.201	0.042 (0.02)	0.015 0.066
Dried Biomass (mg/L)	37.9 (30.2)	5.0 129.0	60.9 (37.7)	12.0 129.0	26.7 (16.8)	5.0 50.0	44.0 (31.8)	13.5 89.0	18.1 (7.4)	8.5 29.0

The majority of experiments were conducted and analysed between the 22nd of March 2016 and the 5th of February 2018. The total dissolved inorganic nitrogen (TIN) and total dissolved inorganic phosphorus (TIP) results were analysed from the 22nd of March 2106 to the 4th of July 2017 only. The TIN and TIP analyses were ceased as the results obtained provided data for over a year and were suitable to provide an estimate of the variation in these parameters on an annual basis. Furthermore, there was only minor phosphorus removal in the HRAPs, and the orthophosphate concentration of the feed was sufficient to specify this parameter. Additionally, as ammonia was the primary nitrogen source utilised by algae, variations in its concentrations, as well as the nitrite and nitrate concentrations, were sufficient to determine the changes in the nitrogen cycle in the HRAPs.

Examining the overall results for nutrient concentrations, it is clear that the concentration of ammonia exhibits the widest variation with a mean concentration of 14.09± 10.79 mg/L and a range of 38.84 mg/L. Spring had the highest concentrations of ammonia with 39.25 mg/L and summer the lowest with 0.41 mg/L. The influent to the HRAPs in summer had the lowest mean ammonia concentration with 3.74 mg/L, whereas the highest mean concentration was in spring with 22.03 mg/L with winter closely behind with 21.12 mg/L. This broad range of ammonia concentrations and significant variations between seasons are due to a combination of biomass production in the previous lagoons and the coinciding changes in pH which cause ammonia volatilisation. Consequently, these results coincide with the pH results which are on average highest in summer, pH = 8.97, which lead to high losses of ammonia to the atmosphere through volatilisation, and lowest, in spring, pH = 8.18.

In addition to pH affecting the concentration of ammonia, it was also strongly influenced by the biomass concentration of the lagoon. The biomass results are tabulated below in Table 3.2. The biomass results were recorded in two different ways: firstly, dried biomass and secondly the optical density of water samples measured using a spectrophotometer at three different wavelengths. Both methods were used to confirm the results. Additionally, the use of optical density can provide a quick and easy method for estimating algal

biomass concentrations in waters. However, a strong correlation needs to be developed for the particular water body to allow accurate algal biomass estimations from optical density values. A graph displaying the correlations between the dried biomass concentration and the three optical densities values are displayed in a later chapter (see figure 4.8). However, dried biomass concentration is the standard method used to describe algal concentration in wastewater research and will be the primary result used in this text to describe algal concentration. Dried biomass concentration results do not only contain algae but also contains other particulates, primarily secondary produced biomass, which are commonly found in water samples. The composition of the biomass can include bacteria, zooplankton, detritus and other particulate matter. While this is not an ideal measure for algal concentration, it is a universally adopted method, and hence the results are easily comparable with other findings in the literature (APHA/AWWA/WEF, 1998).

Overall, the dried biomass concentration in the HRAP influent ranged from 5.0 to 129.0 mg/L with a mean of 37.9± 30.2 mg/L. Summer had the highest mean dried biomass concentration and winter the lowest with 60.9± 37.7 and 18.1± 7.4 mg/L respectively. Spring had a similar mean dried biomass concentration to winter with 26.7± 16.8 mg/L. Autumn was in the middle with a mean dried biomass concentration of 44.0± 31.8 mg/L. These means highlight the significance of the biomass concentration variations throughout the year. These variations are due to the fluctuations in nutrient availability and environmental conditions such as temperature, solar radiation, wind and rainfall. Additionally, there are biological factors such as the seasonal variations in species composition and the interactions between different types of algae, the contamination by bacteria, fungi, protozoans, viruses and predation by zooplankton and other organisms.

Summer experienced the most significant variations in dried biomass with a minimum of 12.0 mg/L and a maximum of 129.0 mg/L. Fluctuations in predation most likely caused the low biomass concentrations, as blooms of zooplankton have been known to consume the majority of algae in a water body. For example, rotifers can consume up to 200 algal cells per min and can double their density daily (Carney et al., 2016). Additionally, experiences at the

lagoons at Melbourne Water's Werribee Treatment Plant have shown that swarms of Cladocera can appear in the final lagoon reproducing asexually and consuming algae causing it to be free of algae (Hussainy, 2018). Proper control of zooplankton is vital for the successful production of algal biomass and the treatment of wastewater (Montemezzani et al., 2015). High biomass concentrations can be caused by high temperatures, high solar radiation intensity and high nutrient concentrations, which would have led to increased periods of high biomass productivity in the secondary lagoons and significantly increased the biomass concentrations of the HRAP influent.

3.2.2 Capacity of Algae to Sequester Heavy Metal

Algae can also sequester heavy metals in their biomass and remove the metals from the surrounding medium (Mani and Kumar, 2014, Abdel-Raouf et al., 2012, He and Chen, 2014). The heavy metal concentrations of the raw sewage and the effluent from Secondary Lagoon 3 were analysed at a NATA (National Association of Testing Authorities) registered laboratory (ALS Water) using NATA approved methods at the start of the experimental period. The results are tabulated in Table 3.3. It can be seen from the results in Table 3.3, that the lagoon treatment system at BMRWP was capable of removing heavy metals from the wastewater during the process.

As the heavy metals were in such low concentrations, it is unlikely they would have any harmful impact on the treatment process or the biota associated with it. Therefore, it was considered unnecessary to investigate the fate of the heavy metals in the system further. An earlier publication by Hussainy (1979) corroborates the proposition that low concentrations of metals have no effect on algal processes.

Table 3.3: Heavy metal concentrations in the Raw Sewage and Secondary Lagoon 3 effluent at the BMRWP.

	Raw Sewage	**Secondary Lagoon 3 Effluent**
Arsenic (mg/L)	<0.01	<0.01
Cadmium (mg/L)	<0.002	<0.002
Chromium (mg/L)	<0.01	<0.01
Copper (mg/L)	0.41	<0.01
Iron (mg/L)	0.80	<0.20
Lead (mg/L)	<0.01	<0.01
Mercury (mg/L)	<0.001	<0.001
Nickel (mg/L)	<0.01	<0.01
Zinc (mg/L)	0.12	<0.01

3.2.3 Biota of the HRAP Influent

The biomass in the influent to the HRAP contained numerous species of algae, zooplankton and other microorganisms. The photosynthetic organisms of the influent included green algae (Chlorophyta) such as *Chlorella* sp., *Dictyosphaerium* sp. and *Scenedesmus* sp., as well as cyanobacteria such as *Golenkinia* sp., Microcystis sp. *Arthrospira* sp. A list of algal and cyanobacterial genus observed in the HRAP influent is given below. This list is not comprehensive of the total algal population in the lagoons.

- Chlorophyceae
    - *Chlorella* sp.
    - *Scenedesmus* ps.
    - *Dictyosphaerium* sp.
    - *Chlamydomonas* sp.
    - *Chlorococcum* sp.
    - *Ankistrodesmus* sp.
    - *Actinastrum* sp.
    - *Pediastrum* sp.
    - *Coelastrum sp.*
    - Volvox sp.
    - Ourococcus sp.
    - *Micractinium* sp.
    - *Golenkinia* sp.
- Charophyceae
    - *Closterium* sp.
    - *Spirogyra* sp.
- Cyanophyceae/ Cyanobacteria
    - *Arthrospira* sp.
    - *Oscillatoria* sp.
    - *Microcystis sp.*
- Euglenophyceae
    - *Euglena* sp.
    - *Phacus* sp.

Chlorella sp., *Scenedesmus* sp., *Dictyosphaerium* sp. and *Micractinium* sp. were the most dominant species throughout the year. Summer and had the highest biomass concentrations, the maximum being 275mg/L. *Chlorella* sp. and *Scenedesmus* sp. were the most dominant species in spring. In addition to these species, spring also had blooms of *Micractinium* sp. and *Microcystis* sp. These blooms resulted in relatively high biomass concentrations reaching over 140mg/L for the majority of these runs and a maximum of 212mg/L. Autumn also had high concentration of *Micractinium* sp. and *Microcystis* sp. with these being the dominant species, while autumn didn't have biomass concentration as high as spring it still had several runs with biomass concentrations of over 100mg/L. Winter had low biomass concentrations, with some runs having less than 10mg/L, and the biomass was dominated by *Chlamydomonas* sp.

In addition to the primary producers, the photosynthetic organisms, there are many secondary producers such as zooplankton that were seen in the secondary lagoons in significant numbers. However, the zooplankton were rarely observed in significant numbers in the HRAPs. This is due to the zooplankton being killed and destroyed by the pump used to fill the HRAPs. The high hydrodynamic shear forces exerted on the water by the pump killed the zooplankton. Additionally, zooplankton can crash into pipe walls and get destroyed (Montemezzani et al., 2015). The zooplankton observed in the lagoons included but are not limited to, *Brachionus* sp., *Moina* sp., *Bosmina* sp., *Daphnia* sp. and cyclopoid copepods.

3.3 Description of the High Rate Algal Ponds (HRAP)

Two HRAPs were utilised during this research. They were built and operated at the BMRWP adjacent to Secondary Lagoon 3. The effluent from Secondary Lagoon 3 forms the influent for experimentation in the HRAPs. The two HRAPs were single loop raceway ponds with a central baffle, with an operational depth of 0.3m, surface areas of 2.8m^2 and a total volume of 850L (see Figure 3.2) (Wrede et al., 2018). The influent in the HRAPs was continuously mixed with paddle wheels. The influent was pumped into the HRAPs via pipework and float valves. The HRAPs were scrubbed cleaned and filled at the start of each experimental run, and the water level was kept

constant with the aid of float valves. The HRAPs were operated in batch mode with a retention time of seven days during most of the year. However, a shorter retention time of four days was utilised during four of the summer runs because of the biomass high growth rates under these conditions.

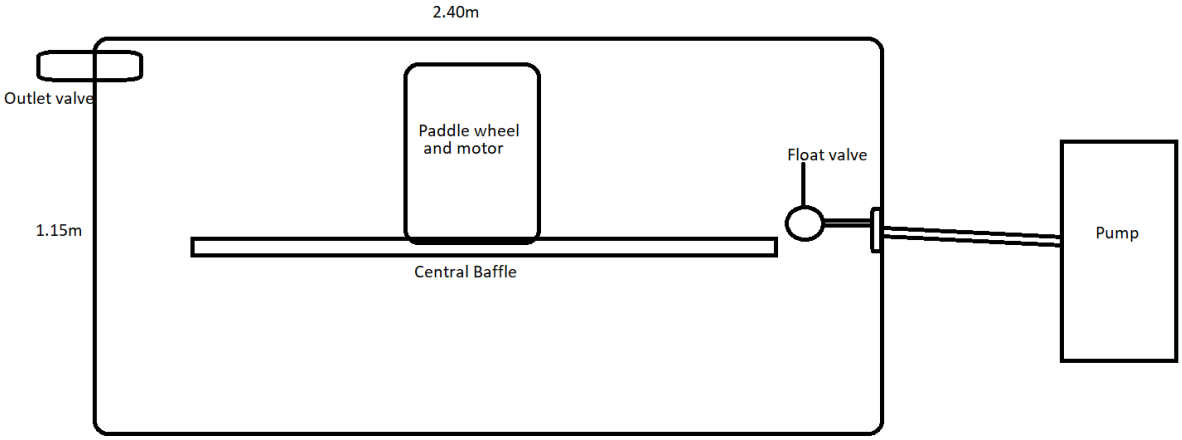

Figure 3.2: Schematic of HRAP and pump (not to scale)(Wrede et al., 2018).

3.4 Production Potential of the Influent

The availability of primary resources and their utilisation determines the concentration of biomass and the productive potential (PP) of a body of water. Seasonal variation in the availability of resources and their effect on the PP of an aquatic system has been the subject of study by Reynolds (1984), Vanni and Temte (1990), Rosemond et al. (2000), Sutherland et al. (2014a), Béchet et al. (2016) and Yehoshua and Gophen (2018). In eutrophic waters such as the effluent from the BMRWP, there can be significant fluctuations in the algal biomass and its species diversity. Vanni and Temte (1990) found that changes in the algal biomass in eutrophic waters were caused by various factors changing from season to season. However, nutrient limitation was relatively more pronounced in summer than in other seasons.

This study investigated the ability of HRAPs to utilise and integrate nutrients into the algal biomass. An effective method to determine the potential of feedwater to support algal growth is to measure the PP of the system. The PP of a water body is determined by its ability to convert inorganic carbon into

organic compounds through carbon assimilation (Sutherland et al., 2015b). In HRAPs the main method in which PP occurs is through photosynthesis, in which the biota, mainly algae, utilise CO_2 and water to biosynthesise simple sugars. Furthermore, the algae utilise these simple sugars and assimilate other nutrients such as ammonia and phosphates to create algal biomass. The higher the PP of the HRAP, the greater the potential to remove nutrients during the treatment process. Other factors which influence a system's PP include; biomass concentration, solar radiation, temperature, nutrient content, pH level and mixing, either naturally (wind) or artificially (paddlewheel) (Sutherland et al., 2015b). According to Liebig's law of the minimum, if any of the influencing factors fall below the required level, the entire process would be affected (Chen et al., 2009).

The PP experiments were conducted between February 2017 to March 2018. During these experiments, data was collected from the HRAPs and the surrounding environment to examine the influence of various factors on PP including, biomass concentration, optical density, depth of the euphotic zone, nutrient concentration, water temperature, solar radiation and pH levels. Each of these factors affects the PP differently. Water temperature controls the enzyme activity in the algae, affecting both photosynthesis and respiration. Solar radiation provides the energy for photosynthesis, and conversely, high levels of solar radiation can also damage the cells (Sutherland et al., 2015b). The concentration of available nutrients, primarily carbon, nitrogen and phosphorus in the system provide the building blocks for algae to grow. The biomass concentration in the system relates to how many photosynthetic cells there are available for the process. In contrast to this, a higher biomass concentration also has a higher respiration rate. Furthermore, high biomass concentrations can affect production due to limiting the amount of solar radiation reaching the cells through self-shading (Sutherland et al., 2015b, Verspagen et al., 2014). The pH of the system affects the consumption and the availability of carbon and other nutrients in water (Cole and Weihe, 2015). High pH can also interfere with the activity of enzymes such as RuBisCO (Sutherland et al., 2015b). Additionally, high pH levels inhibit the activity of aerobic bacteria which oxidise organic matter to CO_2 (Sutherland et al., 2015b).

3.4.1 Method for Measuring Production Potential

The production potential of the secondary lagoon effluent was measured using the light and dark bottle method analysing the changes in dissolved oxygen concentration which were then converted into the amount of carbon biosynthesised (APHA/AWWA/WEF, 1998). Dissolved oxygen is the by-product produced during photosynthesis. CO_2 and the hydrogen electron from water combine to form a carbohydrate molecule with oxygen released into the surrounding waters. The oxygen is dissolved in the water up to the saturation point. The light bottle was a clear bottle (300 ml) through which light could pass while the dark bottle was covered in aluminium foil to prevent light penetration into the bottle. The light bottle was used to measure the photosynthetic rate while the dark bottle was used to measure the rate of respiration. Light and dark BOD bottles were filled with HRAP effluent and placed at the bottom of the HRAPs at a depth of 30cm. The dissolved oxygen (DO) in the bottles was measured with a HACH HQ 40d meter with an attached LDO probe. Measurements were taken at the start of each run and after two hours at the end of the run. Using these results, net and gross photosynthesis, as well as respiration, was determined as shown in equations 3.3- 3.5 (APHA/AWWA/WEF, 1998).

$$\text{Net photosynthesis} = DO_{LB} - DO_i \quad (3.3)$$

$$\text{Respiration} = DO_i - DO_{DB} \quad (3.4)$$

$$\text{Gross photosynthesis} = DO_{LB} - DO_{DB} \quad (3.5)$$

Where DO_i is the initial dissolved oxygen concentration of the water sample used in both the light and dark bottles, DO_{LB} is the dissolved oxygen concentration of the light bottle at the end of the test and DO_{DB} is the dissolved oxygen concentration of the dark bottle at the end of the test.

Primary production is the synthesis of organic matter from inorganic compounds. This is most commonly performed during carbon assimilation by algae in HRAPs (Sutherland et al., 2015b). The photosynthetic equation states that for every one molecule of oxygen produced as a by-product, one molecule of CO_2 is assimilated. Therefore, it is possible to determine the amount of

carbon assimilated into organic matter (algal biomass) from the amount of oxygen produced during photosynthesis. Utilising stoichiometry, it is possible to calculate the amount of carbon assimilated per oxygen produced. Utilising the molar ratio of 12g of carbon in CO_2 and 32g per mole of oxygen as a gas, it can be determined that for every 1g of oxygen (O_2) produced there is 0.375g of carbon fixed in biomass. This calculation assumes an ideal situation where there are no losses in energy or inefficient processes. However, this is not normally the case, and a photosynthetic quotient (PQ) is utilised to correct the results. A mean PQ of 1.2 was used in this study as stated by Wielgat-Rychert et al. (2017). Equation 3.6/3.6a was used to calculate the amount of carbon fixed (APHA/AWWA/WEF, 1998).

$$mg\ carbon\ fixed/m^3 = mg\ oxygen\ released/L \times \frac{12}{32} \times 1000L/m^3 \times PQ \qquad (3.6)$$

$$C(mg/m^3) = O_2(mg/L) \times 450 \qquad (3.6a)$$

3.4.2 Analytical Methods for the Factors Affecting Primary Production

The PP or amount of carbon fixation is affected by biomass concentration and the availability of resources such as ammonia, pH, temperature, solar radiation and the depth of the euphotic zone. Waters samples were taken at the start of each experiment, placed on ice and returned to the laboratory for analysis.

3.4.2.1 Ammonia

Ammonia was determined by filtering the sample through a glass fibre grade C Whatman filter and tested for Ammonia (NH_3–N) concentration using a Hach test kit (Method 8038) and analysed on a bench top Hach spectrophotometer (Hach, 2008).

3.4.2.2 Biomass

Dry weight was used to measure algal biomass. Dried biomass was determined using the method described in Standard Methods for the Examination of Water and Wastewater 20th edn (1998). This involved filtering a sample through a previously, washed, dried and weighed, glass fibre grade C

Whatman filter and placing the retentate in an oven at 105℃ for 24 hours and weighing the retentate on the filter paper.

3.4.2.3 pH Level

The pH level was measured with a YSI Quatro probe. Only the pH of the initial sample was measured.

3.4.2.4 Temperature and Solar Radiation

The YSI Quatro probe also measured the temperature of the HRAP in which the light and dark bottles were suspended, and the mean temperature is reported. The solar radiation was provided by Western Water from an on-site weather station. The results were recorded on a half hourly basis, and the mean solar radiation over the experimental period was used in this study.

3.4.2.5 Depth of the Euphotic zone

To determine the depth of the euphotic zone, a Secchi disk was utilised. The depth of the euphotic zone was measured by lowering the Secchi disk, a black and white disk 30cm in diameter, into the water column and measuring the point at which it could not be seen (Preisendorfer, 1986). In this study, a result of 30+ cm indicated that the Secchi disk was on the bottom of the HRAP and that the whole HRAP was in the euphotic zone. Shallower Secchi disk depths suggest that some form of light attenuation or self-shading was occurring.

3.4.2.6 Presentation of results

The results from the experiments are tabulated on a seasonal basis (see Tables 3.4- 3.7). Dividing the results into seasons allows for grouping of similar results such as higher temperature or high solar radiation, and this allows for easier understanding and analysis of the influencing factors.

3.4.3 Statistical Analysis

All statistical analyses were performed in Microsoft Excel, including calculating mean, minimum, maximum and standard deviation values. The t-test was utilised to determine differences between populations and a confidence

interval of 95% was utilised (p= 0.05). Multiple regression analyses were performed to determine influencing factors. Correlation tables were created in Microsoft Excel and used to identify which factors were influencing each other to remove the bias of multicollinearity and redundancy.

3.5 Results and Discussion

3.5.1 Description of the Variation of the Influencing Factors and their Effects

3.5.1.1 Net Carbon Production

The net carbon production varied significantly throughout the year, and the results are tabulated in Tables 3.4-3.7. Examining net productivity, which was determined based on the oxygen produced in the light bottles over the two-hour incubation period, the mean amount of net carbon fixed was 457 $mg/m^3/h$. The two highest results were recorded in spring and summer with 1946, 1888 $mg/m^3/h$ of carbon fixed respectively. These two results had similar temperatures and solar radiations, however, biomass, ammonia and pH were considerably different. In January 2018, a negative reading of 199 $mg/m^3/h$ was recorded indicating that respiration was greater than production. This was due to the high biomass concentration and the consequence of self-shading impairing photosynthesis. On average, autumn had the highest productivity of 651 $mg/m^3/h$ of carbon fixed, while winter had the lowest productivity with 105 $mg/m^3/h$ of carbon fixed. Summer and spring had mean productivities of 482 and 590 $mg/m^3/h$ of carbon fixed respectively. The maximum concentration of carbon fixed in this study was comparable to that of Berner et al. (1986) which displayed a maximum of 2140 $mg/m^3/h$ of carbon fixed in high rate oxidation ponds. A similar study by Harding (1997) on the productivity of shallow lakes (<0.5m) displayed a maximum productivity of 1524$mg/m^3/h$ of carbon fixed. The similarities of the studies suggest that the results are reproducible and that HRAPs are able to achieve a higher primary productivity than that of shallow lakes.

3.5.1.2 Amount of Respiration

Respiration was calculated based on the amount of oxygen consumed in the dark bottles during the two-hour incubation period. Respiration was highest in summer and lowest in winter with 392, and 11 mg/m^3/h of C consumed respectively. Spring and autumn had mean respiration concentrations of 237, and 127 mg/m^3/h of C consumed respectively.

3.5.1.3 Biomass Concentration

Dried biomass was utilised to represent the algal population in the HRAPs. The results in Tables 3.4- 3.7 list the initial biomass concentrations of the light and dark bottles, and the biomass concentration was assumed not to change significantly over the two-hour incubation period. Summer had the highest mean biomass concentration of 126mg/L and winter had the lowest biomass concentration with 11mg/L. Autumn and spring had similar mean biomass concentrations with 75 and 72 mg/L respectively. However, the range of biomass concentrations for both seasons was significant, with maximum variations of 123 and 177 mg/L respectively. These broad ranges are due to the overlapping of seasons with autumn having high concentrations at the start of the season due to summer-like conditions and lower concentrations at the end of the season due to winter-like conditions and vice versa for spring.

Table 3.4: Autumn results from the light and dark bottle experiments for oxygen production utilising effluent from the HRAPs filled with secondary lagoon effluent.

Autumn							
	13/03/17	**15/03/17**	**17/03/17**	**11/04/17**	**12/04/17**	**3/05/17**	**5/05/17**
Net C Production ($mg/m^3/h$)	989	1096	1098	323	399	251	407
Respiration (C $mg/m^3/h$)	20	243	200	69	113	106	105
Dried Biomass (mg/L)	120	155	123	32	40	25	32
Secchi Disk (cm)	13.5	11.0	14.5*	30.0+	30.0+	30.0+	30.0+
Solar radiation (W/m_2)	364	631	689	279	489	399	371
Temperature (℃)	23.4	23.5	18.4	15.3	16.7	14.1	13.1
Ammonia (mg/L)	0.82	0.22	0.25	3.22	2.70	6.20	5.25
pH	9.63	9.97	9.79	8.63	8.68	8.57	8.52

* Secchi disk reading was predicted utilising biomass concentration (see figure 3.4)

Table 3.5: Winter results from the light and dark bottle experiments for oxygen production utilising effluent from the HRAPs filled with secondary lagoon effluent.

Winter							
	1/06/17	**5/06/17**	**4/07/17**	**7/07/17**	**10/07/17**	**10/08/17**	**14/08/17**
Net C Production ($mg/m^3/h$)	21	80	144	177	488	2	106
Respiration (C $mg/m^3/h$)	7	26	-11	46	-14	16	8
Dried Biomass (mg/L)	7	8	16	14	19	8	8
Secchi Disk (cm)	30.0+	30.0+	30.0+	30.0+	30.0+	30.0+	30.0+
Solar radiation (W/m_2)	254	218	216	361	335	345	504
Temperature (℃)	9.8	10.9	10.3	9.6	9.4	11.0	14.8
Ammonia (mg/L)	11.50	9.80	25.50	21.25	19.00	19.25	13.25
pH	8.54	8.47	8.24	8.78	8.62	8.58	8.64

Table 3.6: Spring results from the light and dark bottle experiments for oxygen production utilising effluent from the HRAPs filled with secondary lagoon effluent.

Spring						
	14/9/17	**18/9/17**	**4/10/17**	**30/10/17**	**17/11/17**	**20/11/17**
Net C Production ($mg/m^3/h$)	38	260	26	396	1946	225
Respiration (C $mg/m^3/h$)	10	137	57	290	389	536
Dried Biomass (mg/L)	17	13	7	144	68	240
Secchi Disk (cm)	30.0+	30.0+	30.0+	16.5	20.0	10.0
Solar radiation (W/m_2)	387	586	795	306	700	771
Temperature (℃)	12.5	12.5	18.1	12.9	21.6	21.9
Ammonia (mg/L)	20.50	14.00	34.13	0.22	7.33	0.15
pH	8.57	8.51	8.57	9.66	9.05	10.31

Table 3.7: Summer results from the light and dark bottle experiments for oxygen production utilising effluent from the HRAPs filled with secondary lagoon effluent.

Summer					
	15/12/17	**12/01/18**	**15/01/18**	**24/01/18**	**9/02/18**
Net C Production ($mg/m^3/h$)	1888	286	199	1010	-34
Respiration (C $mg/m^3/h$)	379	261	770	255	294
Dried Biomass (mg/L)	111	82	124	133	180
Secchi Disk (cm)	13.0	13.0	16.0	14.0	7.0
Solar radiation (W/m_2)	766	123	486	464	201
Temperature (℃)	21.7	21.3	18.7	19.1	20.3
Ammonia (mg/L)	0.12	0.24	0.18	0.02	0.26
pH	10.52	9.23	10.14	10.08	9.89

3.5.1.4 Solar Radiation

Mean solar radiation over the two-hour incubation period is displayed in Tables 3.4- 3.7. The highest mean solar radiation was recorded during spring followed by autumn, then summer and winter last and the means were 590, 460, 408 and 319 W/m^2 respectively. These values are not an accurate representation of the seasons but of when the PP experiments were performed. Summer would have had a higher level of solar radiation, but due to safety concerns, experiments were not performed on very hot days when the fire risk was extreme (>35℃).

3.5.1.5 Secchi Disk Depth

The Secchi disk depth represents the euphotic zone of the HRAP. Winter was the only season during which the whole water column (30cm) of the HRAP was constantly euphotic. In contrast, summer was the only season in which all the experiments showed a reduction in the depth of the euphotic zone, and on average the depth of the euphotic zone was only 12.5cm. Spring and autumn had a mixture of both reduced and complete euphotic zone and had means of 23cm for the euphotic zone.

3.5.1.6 Water Temperature

The mean water temperature over the two-hour period was highest in summer and lowest in winter with 22.2℃ and 10.8℃, respectively. Autumn and spring had similar mean temperatures with 17.8℃ and 16.6℃ respectively. As with solar radiation, safety concerns with regard to fire risk prevented experiments being conducted when temperatures exceeded 35℃. Thus, higher temperatures were likely to occur in the HRAPs.

3.5.1.7 Ammonia Concentration

Tables 3.4- 3.7 display the initial concentration of ammonia in the light and dark bottles, and it was assumed to not change significantly over the two-hour period. Summer had the lowest concentrations of ammonia with a mean of 0.16 mg/L, and algal growth was most likely nitrogen limited. Winter and spring both had substantial concentrations of ammonia with means of 17.08 and 12.72 mg/L. However, spring had two runs which were similar to nitrogen-limited summer ammonia concentrations. Autumn had a low concentration of ammonia with a mean of 2.67 mg/L and had three runs which had very low ammonia concentrations (<1.0mg/L). Runs that were warmer generally had lower ammonia concentrations (see Figure 3.3), this was most likely due to enhanced algal growth causing assimilation of the ammonia into the biomass and an increase in pH causing ammonia volatilisation.

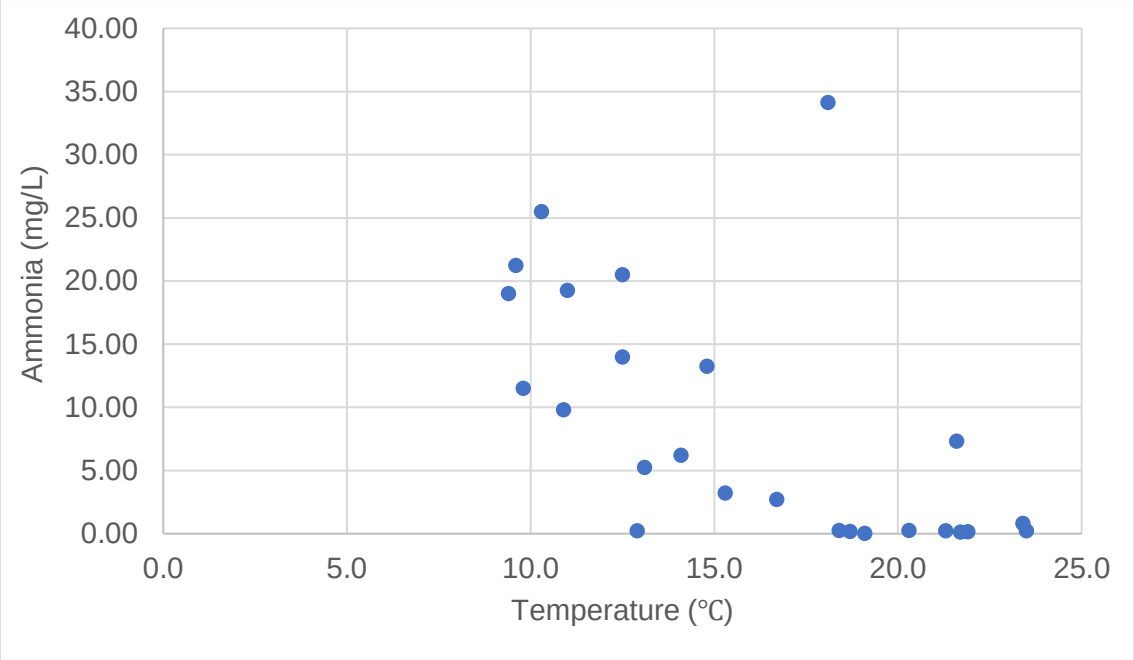

Figure 3.3: Ammonia concentration versus water temperature.

3.5.1.8 pH Level

The initial pH levels of the light and dark bottles used in PP experiments are tabulated in Tables 3.4 - 3.7. All runs except one winter-run were over 8.3, which suggests that the availability of free CO_2 might have been a limiting factor. Summer had the highest mean pH level with 9.97 and winter had the lowest mean pH level of 8.55. Autumn and spring had the same mean pH level of 9.11.

3.5.2 Correlation and Regression Analysis

To determine which factors were significantly impacting on the productivity a multiple linear regression analysis was performed. The linear regression analysis gave a correlation coefficient. The correlation coefficient determines the correlation between the predicted values and the actual values of the dependent variable. Using this it is possible to determine how strong the relationship between numerous variables is, and correlation coefficient values closer to 1 indicate a stronger relationship with less error. To avoid redundancy and multicollinearity, a correlation analysis was performed and is tabulated in Table 3.8. The correlation analysis can determine which variables are interrelated to each other, multicollinearity, and can also potentially highlight

variables that may be redundant. Redundancy is when two variables display the same information, and multicollinearity is when the variables correlate strongly with each other. Both redundancy and multi-collinearity should be avoided so as not to confuse the results and make them statistically insignificant. Examining the correlation analysis showed that Net C production had the strongest relationship with temperature closely followed by solar radiation with 0.54 and 0.50 respectively, both of which were not strong relationships. These results suggest that it was not just environmental factors affecting productivity. The work done by Sutherland et al. (2015b) supports this assertion. Additionally, after examining the correlation analysis results, it was clear that a couple of variables needed to be omitted from the regression analysis so as not to provide an inaccurate regression result. The two variables omitted were, Secchi disk readings and pH level. Secchi disk readings had strong correlations with dried biomass, temperature, and pH of with correlation values of -0.96, -0.81 and 0.93 respectively. These variables showed high levels of multicollinearity, a correlation value of 1.0 would indicate that the two variables tested are displaying the same information. These high correlation values suggested that the dry biomass and Secchi disk readings were redundant results (i.e. the higher the biomass concentration, the lower the Secchi disk reading). The other variable omitted was pH, in addition to Secchi disk depth, it had strong correlations with dried biomass, temperature and ammonia with correlations values of 0.93, 0.75 and - 0.70 respectively. These values indicate that pH is strongly influenced by these variables and the inclusion of pH into the regression analysis would cause significant amounts of multicollinearity. Respiration was also not included in the multiple regression analysis as Net C production was already included the effects of respiration, and it would be redundant. Utilising the remaining variables, dried biomass, solar radiation, temperature and ammonia, a multiple regression analysis for Net C production was performed. This regression analysis returned a correlation coefficient statistic of 0.66, and this low R-value suggests that while these variables do impact the Net production of C, other variables are also influencing productivity.

Table 3.8: Correlation analysis results for all results from primary productivity experiments utilising secondary lagoon effluent.

	Net C (mg/m^3)	Respiration (mg/m^3)	Dried Biomass (mg/L)	Secchi Disk (cm)	Solar radiation (W/m^2)	Temperature (℃)	Ammonia (mg/L)	pH
Respiration (mg/m^3)	-0.22	1.00						
Dried Biomass (mg/L)	0.35	-0.71	1.00					
Secchi Disk Depth (cm)	-0.42	0.69	-0.96	1.00				
Solar radiation (W/m^2)	0.50	-0.39	0.24	-0.22	1.00			
Temperature (℃)	0.54	-0.59	0.74	-0.81	0.48	1.00		
Ammonia (mg/L)	-0.39	0.59	-0.74	0.71	-0.02	-0.60	1.00	
pH	0.45	-0.76	0.93	-0.93	0.39	0.75	-0.70	1.00

One such variable could be the amount of light the algae are exposed too. One of the limitations in utilising solar radiation is that it is not an accurate representation of the amount of light the algal cells are exposed to throughout the water column due to self-shading and light attenuation. In a clear and uniform water body, solar radiation would be sufficient to provide information on whether light is a significant variable affecting productivity. However, this is not the case in most water bodies, especially in a HRAP used for algae production and nutrient removal. High biomass concentrations do not allow the light to fully penetrate the water column and reach the bottom of the HRAP (Sutherland et al., 2014b). This diminishes the amount of light reaching the algal cell and reduces the photosynthetic rate thus reducing the productivity of the system. In order to determine if light penetration, or the lack thereof, is causing significant changes in the productivity of the system, another set of regression analyses were performed. These regression analyses used the same set of data as the previous analyses. However, the results were separated into two sections, light replete and light limited. The first regression analysis only used data from experiments in which the Secchi disk depth was 30cm or higher, indicating that the entire depth of the HRAP was in the euphotic zone. This dataset would not be light limited, and variation in solar radiation would affect the whole water column equally. This analysis returned a multiple regression value of 0.88 indicating a much stronger relationship. The second regression analysis only used data from experiments in which the Secchi disk depth was below 30cm, indicating that some form of light attenuation or self-shading was occurring in the water column thus reducing the euphotic zone. This data set is light limited, and therefore variations in solar radiation are not affecting the whole water column equally, and some parts may not receive any light. The regression analyses returned a strong relationship of 0.80. These stronger relationships indicate light penetration is a significant variable and that the loss of light by self-shading in the HRAP is a substantial variable that cannot be accounted for by utilising other variables such as biomass and solar radiation.

3.5.3 Effect of Biomass and Light Limitation

In addition to the above results, the results of the primary productivity experiments that utilised primary lagoon effluent were included in the dataset

(see Table 3.9), and a multiple regression analysis was performed. Overall a multiple regression value of 0.70 was returned. In addition to this, another regression analysis was performed utilising only the results with Secchi readings below 30cm, as none of the primary lagoon effluent Secchi disk readings were 30cm or above. This multiple regression analysis returned a value of 0.87. This result is similar to the previous light limiting results and strengthens the statement that light loss by self-shading was a significant variable.

Table 3.9: Results from the light and dark bottle experiments for oxygen production utilising effluent from the HRAPs filled with primary lagoon effluent.

	15/12/2017	12/01/2018	15/01/2018	24/01/2018	9/02/2018
Net C Production ($mg/m^3/h$)	-333	294	-318	-61	-239
Respiration ($mg/m^3/h$)	543	114	495	369	347
Dried Biomass (mg/L)	452	224	280	250	213
Secchi Disk (cm)	6.0	9.0	8.0	10.0	7.0
Solar radiation (W/m_2)	766	123	486	464	201
Temperature (℃)	22.2	22.1	18.1	19.5	20.5
Ammonia (mg/L)	0.24	8.60	0.30	0.29	0.34
pH	9.86	8.41	9.07	9.50	8.68

When comparing the two data sets, one light limiting and the other not, it was important to determine which of the other variables were statistically significant. To do this, multiple t-tests were performed on the data. The t-tests determined that dried biomass, Secchi disk depth and temperature were

statistically different between the populations. As stated previously, the amount of biomass attributes to the productivity of the system in two ways. Firstly, the higher the concentration of biomass the higher the number of photosynthesising organisms, which would enhance the PP. Secondly, the biomass inhibits light penetration through self-shading which can be confirmed by the difference in Secchi disk depths, and this light limitation would reduce the PP.

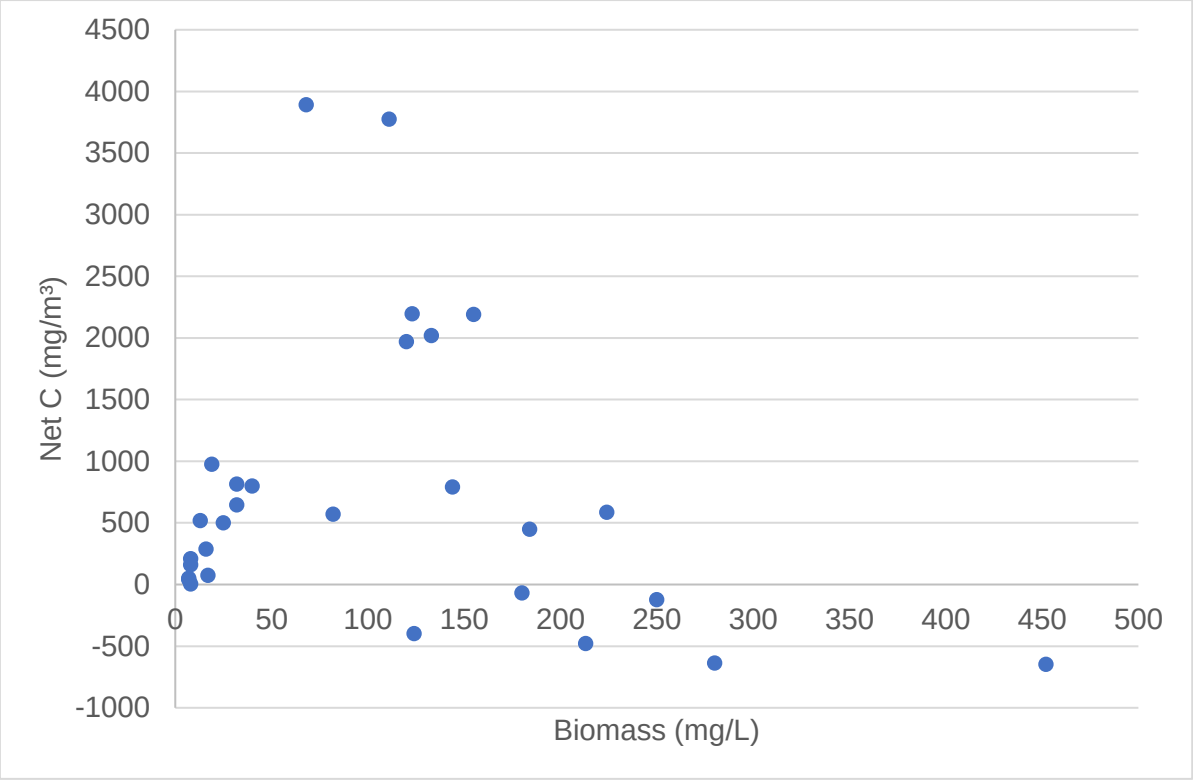

Figure 3.4: Biomass concentration against the concentration of net C fixed during the light and dark bottle experiments.

The effect of biomass on net C fixed can be seen in Figure 3.4. As the biomass concentration increases so does the amount of net C fixed. However, once a biomass concentration of roughly 120 mg/L is reached the PP starts to diminish, and at biomass concentrations around 200 mg/L, there were losses in net C fixation. This indicates that elevated biomass concentration caused significant self-shading and that the respiration rate of the biota in the system was higher than the photosynthetic rate leading to a loss in carbon. To confirm that these biomass concentrations could inhibit the penetration of light into the water column, the biomass concentrations were plotted against the Secchi disk depths (see Figure 3.5). The results follow a logarithmic curve and display an

R^2 value of 0.88. Figure 3.5 indicates that light limitation may occur at a biomass concentration of 100 mg/L. At a biomass concentration of 50 mg/L a third of the HRAP is experiencing self-shading, and for 110 mg/L biomass concentration almost half of the HRAP is shaded according to Secchi disk depth. However, some runs still had a positive production potential as seen in Figure 3.4 (above). For example, one run had a biomass concentration of 224 mg/L also had a net C production of 587 mg/m^3. This would again support the idea that it is a combination of factors affecting the PP of the system, and that the system cannot be correlated to a single uniform component. A limitation of this work was that the light and dark bottle were placed on the bottom of the HRAPs. This means that the bottles were only representing what is occurring in the bottom third of the HRAP. In this case, it is not possible to determine the level of PP for the whole water column when the HRAP is significantly shaded. In the run mentioned above where the biomass concentration was 224 mg/L, the Secchi disk depth was 9cm implying that over two-thirds of the water column were shaded, and this would mean that the light and dark bottles were shaded and would have received very little light. The respiration of that run was -114mg/m^3/h which would suggest that there was another form of PP occurring such as bacterial chemosynthesis or heterotrophic growth.

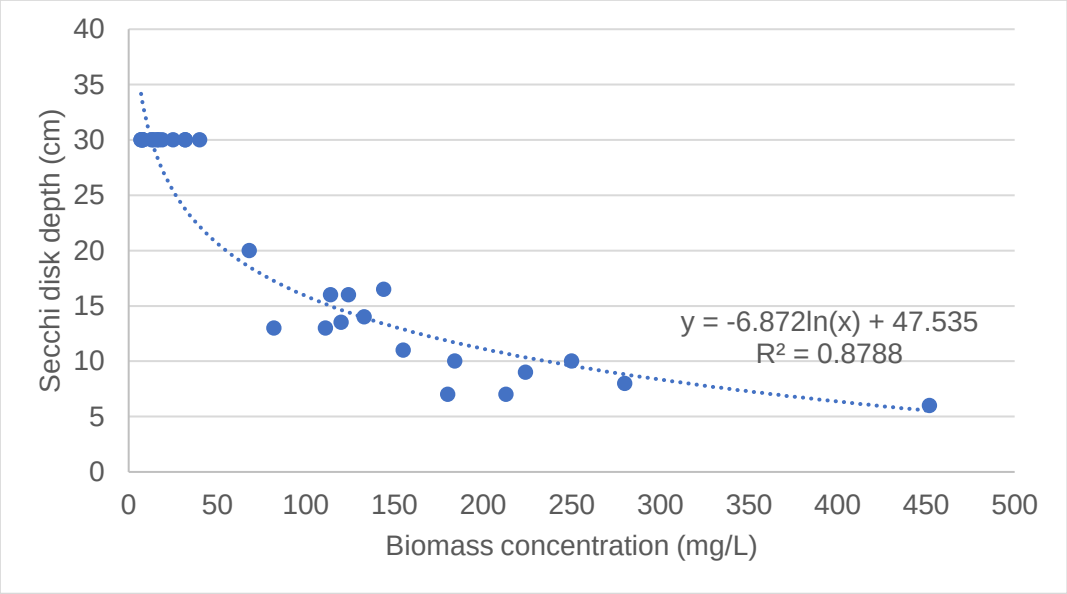

Figure 3.5: Correlation between the biomass concentration and the Secchi disk readings.

The temperature of the water controls the rate of algal enzyme activity which controls both photosynthesis and respiration and consequently controls the growth rate and productivity of the algae (Sutherland et al., 2015b). As shown earlier in Table 3.8, biomass and temperature have a strong correlation with a value of 0.74 indicating that there is an established relationship between these variables. In this case, as the temperature increases so do the algal biomass concentration. Increases in temperature have also been shown to enhance metabolic activity and hence increase the rate of respiration (Pulz, 2001).

To further explore the effect that biomass concentration has on PP and for the prediction of the PP of the HRAPs, a comparison of the results from the experiments in which the primary production of the primary and secondary lagoon effluent samples were tested in parallel was performed. These experiments were performed simultaneously and experienced the same solar radiation, similar temperatures, had similar limiting ammonia concentrations, (except one primary lagoon effluent run), both populations had elevated pH levels, and both systems had some level of self-shading. This enables comparison of only one variable, biomass concentration. Several t-tests were performed, and there was a significant difference between the biomass concentrations of the two populations. However, the PP of the two populations could not be shown to be different. This is explained by the combination of effects namely, an increase in the number of organisms photosynthesising and respiring and the self-shading effect by these organisms. These processes are counteracting each other in the HRAPs resulting in biomass concentration having no significant effect on PP.

3.5.4 Other Processes Affecting Production in HRAP

In addition to PP, it is important to note the other biological and chemical processes occurring in the HRAP. Firstly, photosynthesis utilising CO_2 and water is not the only electron donor for the biological production process. Certain bacteria can perform photosynthesis utilising H_2S and CO_2 to synthesise organic molecules and can perform more effectively in dim light than algal

photosynthesis (Cole and Weihe, 2015). This could contribute significantly, as much as 25%, to the primary production of the system (Takahashi and Ichimura, 1968). Secondly, certain algae do not require sunlight for growth and some species can grow by utilising simple sugars, i.e. heterotrophic growth (Razzak et al., 2017). This would allow for increased PP and biomass concentrations even in low light conditions. Thirdly, mixing of the water either by natural methods such as wind or mechanical methods such as paddlewheels can supplement oxygen and CO_2 into the water for the metabolic activities. However, the reverse of this is also possible depending on the oxygen gradient between the air and the water. Additionally, mixing moves the photosynthesising cells in and out of the euphotic zone in highly concentrated solutions. These short light intervals may interfere with the cells ability to photosynthesise optimally. Fourthly, respiration and decomposition play an essential part in the utilisation of oxygen in the water system. Oxygen is recycled into organic matter and CO_2 that can potentially be used for production (Sutherland et al., 2015b). Respiration and decomposition are both strongly linked to the majority of the factors discussed above which enhance productivity, for example, increased temperatures enhance the respiration rate (Pulz, 2001).

3.5.4 Photosynthesis to Respiration (P/R) Ratio

The ratio of photosynthesis to respiration (P/R) was examined and plotted as shown in Figure 3.6 and Figure 3.7. A P/R ratio of 1.0 proposes that the system is in equilibrium, with the daily photosynthetic rate equalling the daily respiration rate (Cole and Weihe, 2015). A P/R ratio above 1.0 indicates that the system is autotrophic and produces more organic matter than it consumes through respiration (Cole and Weihe, 2015). A P/R ratio below 1.0 suggests that the organic matter in the system is an allochthonous addition and the organic matter did not come from photosynthetic organisms within the ecosystem and implies that in said system the rate of respiration is higher than the rate of photosynthesis (Cole and Weihe, 2015). The P/R ratio in this study was determined over a two-hour incubation period, around midday, and will not accurately represent the activity of the whole day (see Figure 3.6). The results implied that during this incubation period, the HRAPs were generally autotrophic

and would produce more organic matter then they consume. The majority of the PP runs were performed during the late morning; when solar radiation may be reaching its peak resulting in an enhanced photosynthetic rate compared to the average rate for the day.

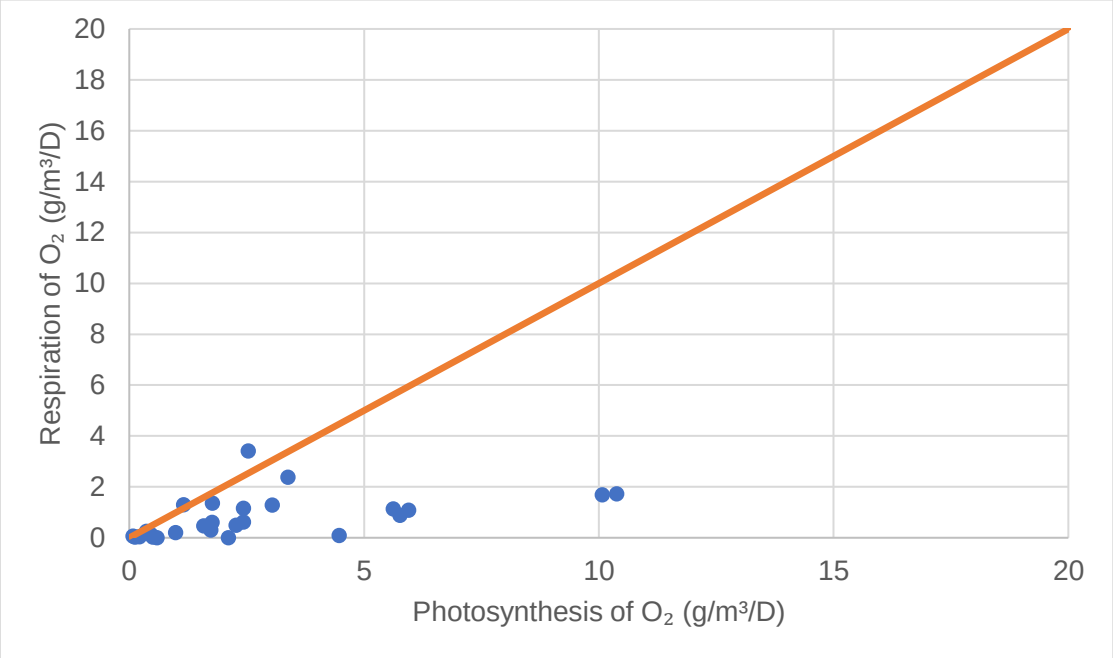

Figure 3.6: Log plot of the photosynthesis versus respiration from the PP experiments. The diagonal line represents a P/R of 1.0.

While it is useful to know the P/R ratio during the short two-hour experimental period, it is important to be able to determine the P/R ratio over a full day. Knowing the P/R ratio over a full day provides the necessary information to determine how effective a system will be in producing algal biomass and in turn reducing nutrient concentration. A series of assumptions were required to determine the daily photosynthetic rate and daily the respiration rate. Firstly, to determine the daily photosynthetic rate one must assume that the photosynthetic rate in the two-hour experimental period is constant throughout the day and then multiple this rate to 12 hours to represent the daily illuminated period. Secondly, to determine the daily respiration rate it can be assumed that the respiration rate is the same throughout the day and it is, therefore, possible to extrapolate the respiration rate over the two-hour experimental period to a 24-hour period. Making these assumptions, it is possible to get a rough estimate of the daily P/R ratio. The majority of data

points still fall under the trendline indicating an autotrophic environment, see Figure 3.7. However, these assumptions may be overestimating both the photosynthetic rate and the respiration rate of the system as the environmental conditions such as the light intensity and temperature along with the nutrient concentrations are not the same throughout the day. The importance of utilising HRAPs which have autotrophic growth conditions is that autotrophic growth does not require the addition of sugars and other sources of carbon and can utilise nitrogen and phosphorus. Autotrophic growth is also capable of bioassimilation of a large amount of CO_2 from the atmosphere thus offsetting greenhouse gas emissions of the wastewater treatment plant while removing nutrients. By installing HRAPs and harvesting the biomass, there is a potential to remove the nutrients from already treated 'Class A' water and to reduce its potential to eutrophicate the receiving waters, and thereby reducing the risk of algal blooms which cause anoxic conditions leading to fish mortality.

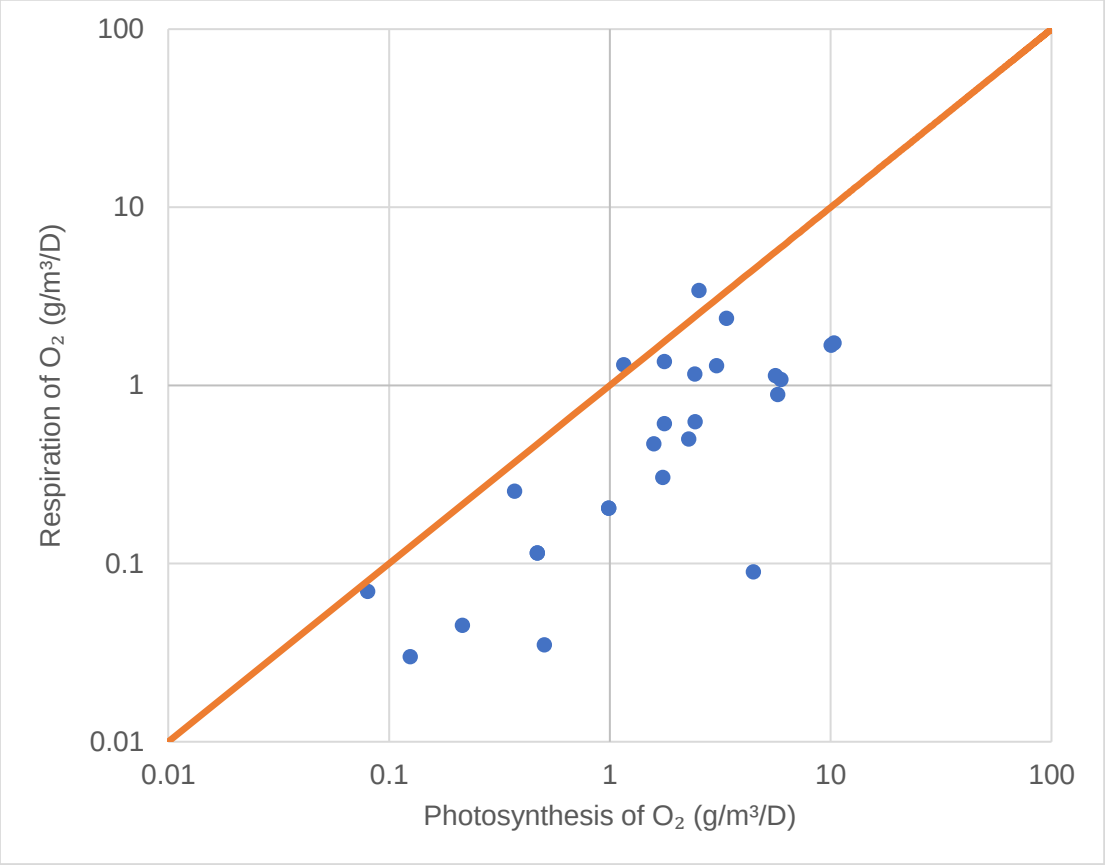

Figure 3.7: Log plot of the photosynthesis versus respiration, extrapolated over a 24-hour period, from PP tests. The diagonal line represents a P/R of 1.0.

3.6 Summary and Recommendations

The influent to the HRAPs experienced large fluctuations in nutrient concentrations with ammonia varying the most with a minimum of 0.41mg/L and a maximum of 39.25mg/L. These variations in nutrient concentration are caused by the natural variation in environmental conditions, such as solar radiation and temperature affecting the growth of algae and other organic biomass in the preceding lagoons. It was found that nutrients concentrations were lower in summer and autumn and higher in spring and winter. The varying nutrient concentrations of the HRAP influent can affect the PP of the HRAPs with deficiencies in nutrients such as ammonia limiting algal growth. Furthermore, this chapter investigated the production potential of the HRAPs in order to determine how effectively the HRAPs can utilise the primary resources in the secondary lagoon effluent to grow algal biomass and determine which are the strongest influencing variables.

The results of this chapter conclude that there is not one single factor which influences the productivity of the system. It is clear that there are numerous factors influencing productivity and these include but are not limited to; solar radiation, temperature, biomass concentration, nutrient concentrations and pH. Limitations in the factors mentioned were the main influencers of PP. The algal biomass was strongly influenced by the other factors and also by itself. High levels of solar radiation, temperature and nutrients all enhance algal productivity. This enhanced algal productivity leads to an increased algal biomass concentration which causes self-shading limiting the amount of light available to the algal cells in the lower parts of the water column. This can cause a decline in the productivity of the HRAP. The P/R ratio determined in this chapter showed that the HRAPs are autotrophic which is ideal for an algal production system which utilises secondary lagoon effluent. Moreover, understanding the natural operation of the HRAPs in terms of the nutrient concentration and the environmental conditions is crucial to being able to promote the highest algal biomass production and in turn treat the secondary lagoon effluent in a quick and efficient manner. By understanding the natural operation of the HRAP, it is possible to identify aspects which need to be altered to optimise algal biomass production and nutrient removal.

With these factors in mind, an optimal system would need to keep biomass at an intermediate concentration to enhance the primary production while ensuring all the algal cells can receive sufficient solar radiation for photosynthesis. Mixing the water can assist in alleviating self-shading issues by circulating the algae throughout the water column and ensuring that all cells are exposed to solar radiation frequently. Additionally, the inclusion of an algal harvesting step would be beneficial in helping to maintain an optimum biomass concentration. A harvesting method that removed excessive algal but keeps the water and nutrients in the system would be ideal, and possible techniques include membrane filtration. Furthermore, nutrient and pH levels in the system contribute considerably to the growth of biomass and would need to be controlled adequately. All the runs in this study were considered to be carbon deficient due to the elevated pH levels. Reducing the pH levels would enhance the amount of free CO_2 available which may enhance the PP, and it would also increase the amount of available ammonia in the water as less of it would be lost through ammonia volatilisation. The availability of higher carbon and ammonia concentrations would also enhance the PP of the system by providing more substrates to be converted into biomass and additionally would increase the amount of photosynthetic enzymes and pigments in the algal cells (Juneja et al., 2013).

Chapter 4 – Utilising High Rate Algal Ponds to Enhance Algal Biomass and Reduce Nutrient Concentrations

4.1 Introduction

The various physiochemical factors which influence algal growth and the productive potential of the effluent were examined in chapter 3. Chapter 4 examines the changes in biomass and nutrient concentrations in the HRAPs during the experimental period. This chapter will also examine methods to enhance biomass productivity and therefore nutrient reduction. The natural operation of the HRAPs utilising secondary lagoon effluent as the media source was investigated for almost two years. From this data, a simple predictive algal growth model was developed and is explained in detail in Chapter 5. The methods employed to enhance the biomass productivity included; the addition of laboratory cultured algae during periods of low algal concentration, the control of the pH in the HRAPs using two different acids, and the use of primary lagoon effluent to increase the nutrient concentrations in the HRAPs during periods in which some nutrients were normally limited (<1mg/L) in the secondary lagoon effluent thus limiting algal growth. The data collected in this chapter provides an understanding of some of the benefits and limitations of HRAPs prior to developing the simple predictive algal growth model. The questions answered in this chapter are as follows, how does the addition of laboratory-grown algae to the HRAPs during cooler months enhance biomass production? How does the control of the pH level of the HRAPs utilising acids optimise biomass production? How does the use of an alternative nutrient source, primary lagoon effluent, to alleviate the nutrient deplete conditions observed in the warmer months, enhance biomass production?

4.2 Methods

4.2.1 Analytical Methods

4.2.1.1 Sensors

The two HRAPs were fitted with “YSI Quatro” probes to monitor and record water temperature (℃), conductivity (µS/cm), pH, ammonia concentration (mg/L) and dissolved oxygen concentrations (mg/L) at a frequency of half an hour. The probes were calibrated on day 0, days 2/3 and days 4/5. The data was retrieved at the end of each run. Additional, dissolved oxygen measurements were also conducted in different spots of the HRAPs to check that they were sufficiently mixed and also to confirm the uniformity of the compounds in the HRAPs.

4.2.1.2 Chemical Analysis

In addition to the parameters measured by the sensors listed above, water samples for analysis were collected at the start and the end of each experiment. Further samples were taken during each of the runs to monitor the changes in the nutrient concentrations throughout the experiments. An initial sample was taken and filtered through a 0.45µm syringe filter, and the filtrate was analysed immediately for orthophosphate (P-PO_4) concentration using a “Hach kit” (method 8048) and results were measured on a handheld “Hach DR890 colourimeter” (Hach, 2008). A second sample was collected and placed on ice and taken to the laboratory. It was filtered through a glass fibre grade C Whatman filter and tested for ammonia (NH_3–N), nitrate and nitrite (NO_3–N, NO_2-N), TIN (N), and TIP (P) concentrations using Hach test kits and analysed on a bench top Hach spectrophotometer (Hach, 2008). The change in nutrient concentrations are displayed as absolute values and percentage values, these are utilised to help convey the changes in nutrient concentrations. The percentage values were calculated using equation 4.1 below.

$$\%\ change = \frac{N_i - N_f}{N_i} \cdot 100 \qquad (4.1)$$

In equation 4.1, above, N_i is the initial nutrient concentration, and N_f is the final nutrient concentration.

4.2.1.3 Biomass Analysis

Dry weight and optical density (OD) results were used to measure algal biomass. The biomass dry weight was determined using the method described earlier in chapter 3. The OD of the sample was determined by measuring the absorbance of the sample at three different wavelengths: 440nm, 680nm and 750nm using a Biochrom Libra S22 spectrophotometer (Standard Methods for the Examination of Water and Wastewater 20th edn, 1998, Wang et al., 2010, Huesemann et al., 2013). A graphical relationship between the three OD values and dried biomass is shown in the results section (see figure 4.8).

The biomass growth rates were calculated using the dried biomass data, see equation 4.2 below.

$$G = \frac{fb - ib}{t} \tag{4.2}$$

In equation 4.2, above, G is growth rate in (mg/L/D), *fb* is the final biomass in (mg/L), *ib* is the initial biomass (mg/L), and *t* is time (D (days).

4.2.1.4 Weather Data

The weather data was provided by Western Water from their on-site weather station. The data included; solar radiation (W/m^2), air temperature (℃), wind direction, wind speed (m/s), daily rainfall (mm), daily evapotranspiration (mm), barometric pressure (hPa) and humidity (%). The data was recorded at half-hour intervals.

4.2.2 Experimental Design

The HRAPs were used for several different experiments over the course of two years from May 2016 to February 2018. The operational parameters, such as the speed of mixing by the paddlewheel and the depth of the water column in the HRAPs, were kept constant in all the experiments. Several experiments were conducted to assess the impact of different factors on the ability of the system to utilise nutrients for biosynthesis of algal biomass. Each set of experiments is described in the following section, and the results are presented.

4.2.2.1 HRAP Natural Run

The two HRAPs were run in parallel during the first 12 months from May 2016 to May 2017. During this period there were minimal changes to the operation of the HRAPs and minimal non-natural changes to the influent unless otherwise stated. This was done partially to observe the natural variations occurring in the HRAPs and the surrounding environmental conditions. The HRAPs were compared with each other to determine if there were any differences between their biomass production and the nutrient reduction capabilities of the two HRAPS and to gain an estimate of experimental reproducibility. The variations in environmental conditions such as solar radiation and temperatures and their impact on the algal productivity and biomass in the HRAPS during the different seasons were also examined. Additionally, the results from the unaltered HRAP runs are also utilised in chapter 5, in which a simple predictive algal growth model was developed utilising easily accessible variables.

4.2.2.2 Addition of Algae to HRAP

During the cooler months of the year such as winter and early spring (June to October), the production of biomass and thereby the nutrient reductions were observed to be low. A set of experiments to enhance the algal productivity and in turn, the nutrient reduction of the HRAPs were performed. These experiments aimed to determine whether the addition of laboratory-cultured ambient strains of algae, isolated earlier from the HRAPs, were able to enhance the biomass production and thereby improve the nutrient reduction of the HRAPs. On three separate occasions, 20L of laboratory-cultured algae were added to one of the 850L HRAPs, the other HRAP was maintained as a control HRAP. The details of these cultures are as follows: the first batch of algae added was 20L of *Dictyosphaerium* sp. on the 5th of October 2016, resulting in an increase in biomass concentration of the HRAP by 6mg/L (35%). The second batch was 20L of *S. acuminatus* added on the 4th of July 2017 resulting in an increase in biomass concentration of the HRAP by 2mg/L (12%). The third batch was 20L of *S. quadricauda* added on the 11th of September 2017 and resulted in an increase in biomass concentration of the HRAP by 5mg/L (56%).

The impact of such additions on the biomass and nutrient concentrations in the HRAPs were monitored during the experimental period.

4.2.2.3 pH Regulation in HRAP utilising the addition of Acid

The pH in HRAP has a significant impact on the availability of carbon and thereby on the overall productivity of the algal biomass. The depletion of dissolved CO_2 through algal utilisation shifts the carbonate/ bicarbonate equilibrium and when the pH exceeds 8.3, the amount of free/ dissolved CO_2 reduces to near zero (Cole and Weihe, 2015). Although algae are still able to grow in these elevated pH conditions, their growth rates are diminished by the algae's need to break down bicarbonate to access carbon. Additionally, high pH values (>9) also cause ammonia volatilisation, and this would further hinder the growth of algae due to the possibility of nitrogen becoming a limiting factor (Cai et al., 2013). The impacts of elevated pH on algal growth were discussed in Chapter 2 and Chapter 3. Many researchers have utilised the addition of CO_2 gas into a system in order to control pH and enhance biomass production (Craggs et al., 2012, Sutherland et al., 2015b, Sutherland et al., 2015c, Fallowfield et al., 2016). This method can be very costly, and a large proportion of the supplemented CO_2 is lost to the atmosphere, and this could also be environmentally unfriendly (de Godos et al., 2014). This series of experiments is aimed to determine, whether it is possible to meet the CO_2 demand for algal biomass production by lowering the pH to 8.3 or less. To test this hypothesis, the pH in one of the HRAP was controlled between 7.3 and 8.3 by utilising an acid dosing pump connected to a pH meter and control box (TPS miniChem). The pH controlled HRAP, henceforth known as the pH HRAP, was compared to the control HRAP for changes in algal biomass, pH and changes in nutrient concentrations. Two different types of acids were utilised during this experiment. In one set of experiments an inorganic acid, 1M hydrochloric acid (HCl), was utilised to control the pH and in the second set, an organic acid, 1M acetic acid, was utilised. The use of organic acid also aimed to determine if the addition of carbon in the organic acid as well as the pH control would significantly enhance biomass production.

4.2.2.4 Comparison of utilising Primary Lagoon Effluent and Secondary Lagoon Effluent in HRAPs

It was found during the warmer summer period that the nutrient concentrations of the HRAPs were low and in some cases deficient. The primary lagoon effluent at BMRWP contains higher nutrient concentrations compared to the secondary lagoon effluent, which was generally nutrient depleted in the warmer months. This set of experiments aimed to test the hypothesis that the effluent from the Primary Lagoon would promote a higher growth rate in the HRAPs compared to the effluent from Secondary Lagoon 3. The use of primary lagoon effluent and secondary lagoon effluent in the HRAP was compared in a series of experiments. The primary lagoon effluent was collected from the end of Primary Lagoon 1 using an eductor truck and transferred to the one of the HRAPs; the other HRAP was filled with effluent from the Secondary Lagoon 3 as per normal. These experiments were run in batch mode and did not receive water from Secondary Lagoon 3 to compensate for evaporation. Each experiment was conducted over seven days, and the results compared.

4.2.2.5 Algae Identification and Isolation

During the experimental period, numerous algae samples were collected and identified to their respective taxa using the taxonomical key in Standard Methods (APHA/AWWA/WEF, 1998). Additionally, several algal species were isolated and were utilised for laboratory experiments. Isolation of various species was performed by a combination of serial dilution and plate streaking. All algal samples were grown in Bold's Basal Medium (BBM) containing the following chemicals: sodium nitrate (0.25g/L), calcium chloride dihydrate (0.025g/L), magnesium sulphate heptahydrate (0.075g/L), potassium phosphate dibasic (0.075g/L), potassium phosphate monobasic (0.175g/L), sodium chloride (0.025g/L), EDTA (0.05g/L), potassium hydroxide (0.031g/L), iron sulphate heptahydrate (0.00498g/L), sulphuric acid (0.0001ml/L), boric acid (0.01142g/L) and 1ml/L of microelements mix consisting of zinc sulphate heptahydrate (8.82g/L), manganese chloride tetrahydrate (1.44g/L), molybdenum oxide (0.71g/L), copper sulphate pentahydrate (1.57g/L), and

cobalt nitrate hexahydrate (0.49g/L) (CSIRO Marine Research). Bolds Basal agar (BBA) was prepared using BBM with 1.5% agar. Solutions were autoclaved at 121℃ for 15 minutes. Algal samples were grown in both liquid and plate cultures. The cultures were grown under fluorescent lights providing 2.95 lux with a photoperiod of 16 hours on and 8 hours off at room temperature (~20℃).

4.2.2.6 Statistical Analysis

The mean, minimum, maximum, standard deviation and standard error values for the water quality data were calculated. Percentage values were used to show the changes in nutrient concentrations and biomass concentrations during HRAP trials. Correlations, t-tests and ANOVA analyses were utilised to compare data sets, and a confidence interval of 95% was used. Analyses of the data and the creation of the graphs and trendlines was done in Microsoft Excel 365.

4.3 Results and Discussion

4.3.1 Comparison of the Performance of the Two HRAPs

During the first 12 months of the experimental period, May 2016 to May 2017, the two HRAPs were operated in parallel and their performance measured over the period. A correlation analysis was performed, on the similarity of the changes occurring between the two HRAPS based on several variables such as ammonia, nitrate and nitrite, orthophosphate, TIN, TIP, dried biomass and OD at 750nm. A correlation analysis is a statistical evaluation which studies the strength of the relationship between variables. Sixteen sets of runs were compared, and each run had multiple sets of data taken at various time points: day 0, day 2/3, day 4/5 and day 7. The results of the correlation analyse are tabulated in Table 4.1, a perfect correlation between the two HRAPs has a result of 1.00 and would indicate the changes were identical in each HRAP (i.e. HRAP1 = HRAP2).

Table 4.1: Correlation coefficients of HRAP 1 and 2 when operated in parallel.

	P-PO_4	**NO_2-N and NO_3-N**	**NH_3-N**	**TIN**	**TIP**	**Dried Biomass**	**750nm**
Day 0	0.99	0.95	1.00	0.98	0.98	0.99	1.00
Day 2/3	0.98	0.98	1.00	0.98	0.99	1.00	1.00
Day 4/5	1.00	0.86	0.93	0.97	0.98	1.00	1.00
Day 7	0.98	0.73	0.99	0.96	0.90	0.99	1.00

Overall, it was evident that the correlation results indicated a strong relationship between the two HRAPs. The mean value for the correlation coefficients was 0.97. All the results had strong relationships, and there was little variation over time. The only exception being, nitrate and nitrite whose correlation value declines with experimental time; this may be more due to the natural changes between the HRAPs rather than human error. To clarify the difference in the data between the two HRAPs, the nitrate and nitrite data was reviewed. On average the nitrate and nitrite concentrations displayed a 20% difference between the two HRAPs. This difference was relatively small and roughly equates to 1.5 mg/L of nitrate and nitrite in the HRAPs. This difference does not fall into the experimental error range. This would suggest that one of HRAPs was better at promoting either denitrification or nitrification, this may be due to the location of the HRAPs with one potentially having more shading from the nearby shed. Alternatively, the HRAPs were constructed with different materials with one being metal and the other plastic and this may have influenced the processes.

Apart from the nitrate and nitrite concentrations, the two HRAPs had strong correlations confirming that the two HRAPs performed similarly and that the natural variations had a similar effect on biomass production and nutrient reduction in both HRAPs. Overall, the data indicates that the performance of the HRAPs is reproducible and that the operation of one HRAP would be able to provide reliable results regarding the operation, nutrient reduction and biomass

production of both of the HRAPs. This facilitated one HRAP to be used as a control for the other, which was utilised for experimentation in which the conditions were altered. The dissolved oxygen concentration was also measured during the runs and the HRAPs were found to be near or above saturation point majority of the time. Spot checks were performed to ensure the mixing from the paddlewheel was sufficient and to confirm the uniformity of the HRAPs. There was no difference in the dissolved oxygen concentrations in different spots of the HRAPs.

4.3.2 Natural Fluctuations in Water Quality

The efficiency of the HRAPs varied throughout the year both for nutrient reduction and algal biomass productivity. This was caused by the changes in the nutrient concentration of the influent to the HRAPs and the changes in environmental conditions. Both sets of factors affect biomass productivity as discussed in chapter 3. The nutrient concentration of the HRAP influent varied throughout the year as discussed earlier in chapter 3.2.1. This section will investigate the changes in the nutrient concentrations of the HRAPs from May 2016 to February 2018. The data from May 2016 to March 2017 as well as May 2017 and August 2017 are mean values from runs in which both HRAPs were operated similarly. The data from March 2017 to February 2018, except May 2017 and August 2017, are from the results of one HRAP, which was used as a control, as the other HRAP was altered for experimental work which is discussed later in this chapter. There was a high level of reproducibility between the HRAPs when operated under the same conditions, as discussed in the above section, so differences in performance between the two HRAPs could confidently be attributed to the different operating conditions.

4.2.2.1 Natural Variations in Temperature and Solar Radiation

The mean water temperature and the mean solar radiation during each of the unaltered HRAP runs are displayed in Figure 4.1. Water temperature and solar radiation follow similar trends, and as solar radiation increases so does the water temperature (Edwards et al., 2016). The peak season for both temperature and solar radiation was summer (December to February) with a maximum mean solar radiation of 314±30 W/m^2 (12/12/17) and a maximum

water temperature of 23.0±0.3℃ (22/01/18). The lowest mean solar radiation was recorded near the start of winter, 52±7 W/m^2 (03/06/16). The lowest recorded mean water temperature was in the middle of winter, 7.4±0.2℃ (04/07/17). There were large variations in temperatures between the seasons. Summer had the highest mean temperature of 19.1±0.3℃ and highest mean solar radiation of 245±26 W/m². The mean temperature of each summer run ranged from 15.9-23.0℃. Spring (September- November) had the next highest with a mean temperature of 14.0±0.2℃ and a mean solar radiation of 192±19 W/m². The mean temperature of each spring run ranged from 10.3-19.6℃. Autumn (March-May) had a slightly lower mean temperature, 12.9±0.2℃ and the mean solar radiation,103±14W/m² was just over half the spring mean. The mean temperature of each autumn run ranged 8.0- 19.8℃. Winter (June-August) had the lowest mean temperature, 9.7±0.2℃ and lowest mean solar radiation 80±9W/m². The mean temperature of each winter run ranged from 7.4-11.9℃.

Temperature and solar radiation are both strong influencers of algal growth, with temperature helping to control enzyme activation and solar radiation providing the energy required for growth. Warmer temperature and higher levels of solar radiations such as the conditions in summer promote high algal biomass production. Conversely, colder temperatures and lower levels of solar radiation, such as the conditions in winter, support algal growth less and algal biomass productivity diminishes.

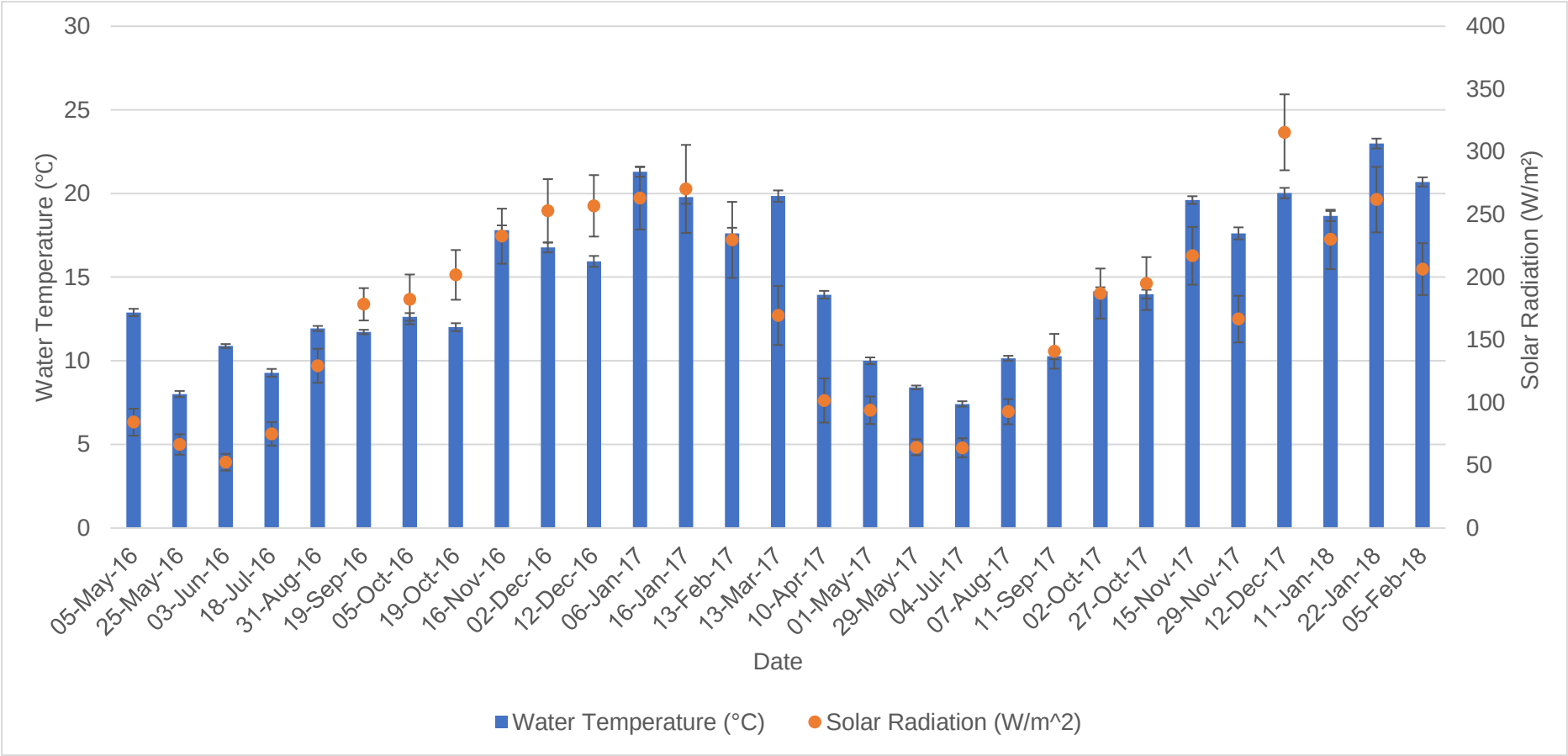

Figure 4.1: Mean water temperature and mean solar radiation for each run between May 2016 and February 2018.

4.2.2.1 Natural Variation in Algal Biomass

Figure 4.2 displays the dried biomass concentrations during the course of each run and the overall percentage change for each run from May 2016 to February 2018. Upon examination of the data on algal biomass, it was evident that there was a significant variation throughout the year. The most significant changes in biomass were in the spring and summer periods, in which the biomass concentration in most runs increased by at least 100%. The highest percentage increase in biomass concentration was in December 2017 with an increase of 1433% (172 mg/L). The second highest was in December 2016 with an increase of 500% (143 mg/L). Overall, summer had the highest mean percentage increase of 316% (108 mg/L) closely followed by spring with a mean increase of 300% (88 mg/L). Autumn had just over half the productivity of summer with a mean increase of 174% (40 mg/L). Winter had the lowest increase in algal biomass with a mean increase of 60% (12 mg/L). It is evident when comparing Figure 4.1 to Figure 4.2, that the periods of increased biomass production and concentration coincide with the periods of warmer water temperatures and higher levels of solar radiation such as summer. This is due to the higher temperature and solar radiation enhancing the algal photosynthetic ability and thus increasing the algal productivity. The lower biomass production in winter coincides with low temperatures and low solar radiations both of which are not conducive for biomass production. When examining each season in more detail, a better understanding of the operation of the HRAPs can be achieved.

Autumn had relatively low initial biomass concentrations, but it was moderately productive. It had a mean biomass increase of 174% (40 mg/L) and a minimum and maximum increase in biomass of 18% (6 mg/L) (10/04/17) and 351% (83 mg/L) (05/05/16) respectively. The initial concentrations of the HRAPs ranged from 13.5 to 92 mg/L, and the final algal biomass concentrations of the run during autumn ranged from 40 to 155 mg/L. Additionally, these results indicate that autumn is overall a productive season. However, there was a substantial variation in the operation of the HRAPs during this time. This variation was most likely due to the large fluctuations in the mean temperatures (8.0-19.8℃) of each run during this period.

The biomass results obtained during winter were lower than the autumnal results. The biomass concentrations had a mean increase of 60% (12 mg/L) and a maximum increase of 183% (53mg/L) (03/06/16). In two runs, 18/07/16 and 07/08/17, the final biomass in the HRAPs was lower than the initial biomass by 29% (6 mg/L) and 45% (7 mg/L) indicating that there was a loss in algal biomass. This may be attributed to predation, metabolic breakdown or both. The run in which there was an increase of 183% (53mg/L), occurred shortly after the start of winter and conditions were similar to those of autumn, as the temperature was slightly warmer during that period than that which commonly occurs in winter. Overall, the results obtained during winter indicate that the HRAPs and natural environmental conditions do not support a productive algal culture during the cooler parts of winter and only support a moderate productive potential during the rest of the season which was slightly warmer.

The results obtained during spring exhibited a broader variation in algal production. Spring had a mean increase in biomass of 300% (88 mg/L) and a minimum and maximum biomass increase of 88% (8 mg/l) (19/09/16) and 471% (198 mg/L) (15/11/17) respectively. The lowest increase in production occurred at the start of spring in September 2016, and this would most likely occur due to colder temperatures during the first few weeks of spring. In contrast to this, the highest increase in production occurred towards the end of spring, with warmer temperatures similar to that of summer influencing algal growth. These results indicate that spring is a productive period and that there can be some seasonal overlap with temperatures ranging from 10.3-19.6℃. The fluctuations in spring are also influenced by the algal species composition and the tendency of certain algal species to bloom during the spring season.

Summer was the most productive season with a mean increase of 316% (108 mg/L) and a minimum increase of 50% (64 mg/L) and a maximum increase 1433% (172 mg/L). This was due to summers long photo-illumination periods, greater solar radiation intensities as well as higher temperatures, all of which promote the photosynthetic rate. Summer had a vast range of productivity due to both the optimum environmental conditions and the fluctuations in the nutrient concentrations. Although the optimum environmental conditions support

the higher algal growth, the variations in nutrient concentrations may severely limit the growth rate. Summer periods which are nutrient replete have high productivity and periods which are nutrient deplete have lower productivity. During the run in which there an increase of 1433% (172 mg/L), the initial biomass concentration was very low, most likely due to predation in the previous lagoons. However, the algae were able to grow rapidly in the HRAP and achieved a concentration of 184 mg/L. Furthermore, it should be noted that this run did not have the highest final biomass concentration, which was achieved in an earlier summer run with a concentration of 275mg/L which only had a percentage increase of 113% (146mg/L). In addition to this, the run which only had a 50% (64 mg/L) increase in biomass had a final biomass concentration of 192 mg/L. This was caused by the algal growth plateauing after a few days of rapid algal growth in the HRAPs, due to the nutrients in the HRAP becoming deplete as a result of the rapid algal growth. To overcome this, some of the summer runs were performed over four days instead of seven. These results indicate that summer has high productivity and high biomass concentration, which is most likely caused by its higher mean temperatures, 15.9-23.0℃, than the other seasons.

The variations in algal biomass concentrations observed throughout the experimental period are due to environmental changes, such as temperature and solar radiation accompanied by changes in nutrient concentrations. The dried biomass concentration results had an error range of 4.4mg/L. The other significant factor that influences algal biomass production is the amount of available nutrients, mainly phosphorus and nitrogen.

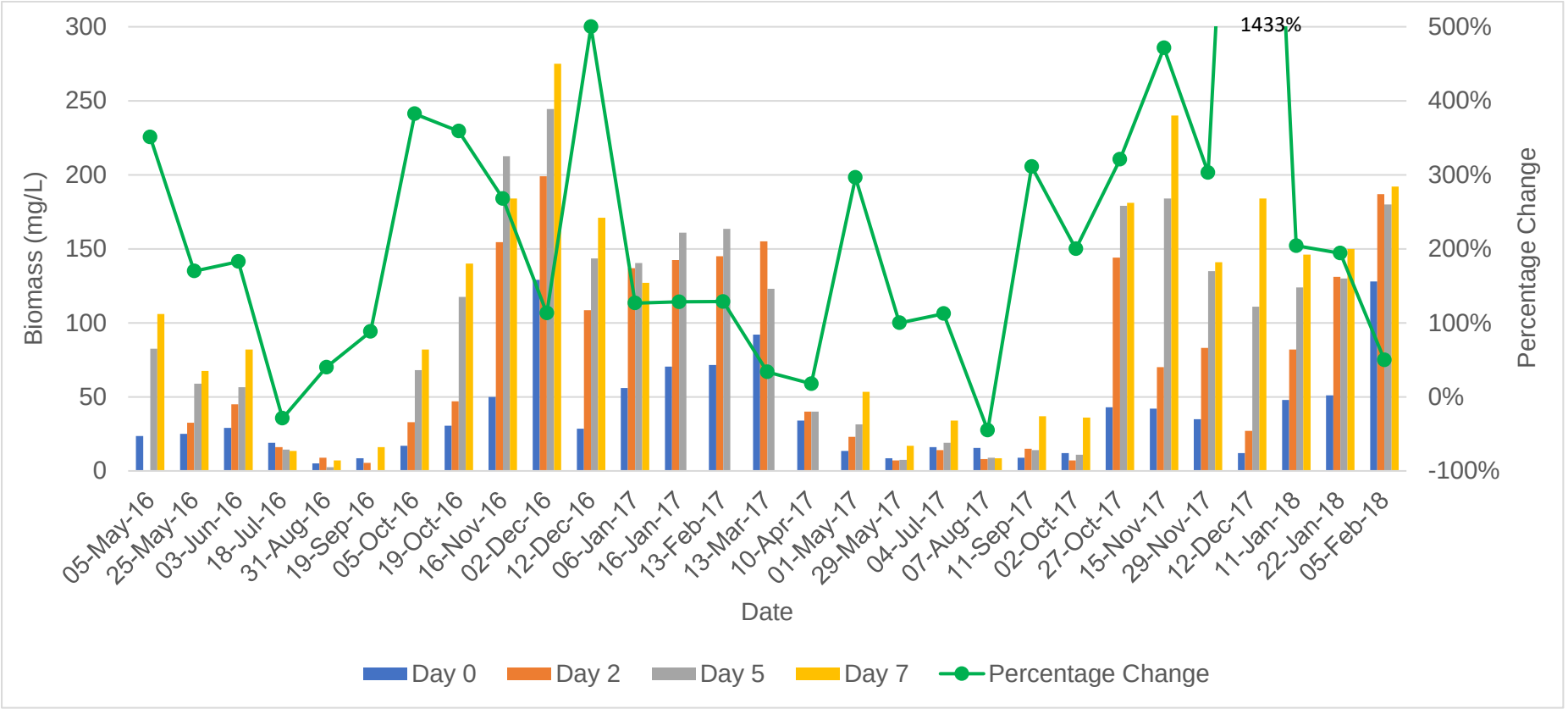

Figure 4.2: Dried biomass concentrations and overall percentage change for each run between May 2016 and February 2018.

4.3.2.2 Natural Variation in Phosphorus Concentrations

Phosphorus is incorporated into ribosomal RNA and phospholipids in algal cells, and can also be stored and sequestered in excess in the cells for future use; this is also referred to as luxury uptake in the literature (Powell et al., 2009, Beuckels et al., 2015). Orthophosphate is the most common form of phosphorus utilised by algae, and its concentrations and reduction percentages are displayed below in Figure 4.3. The results for TIP are also displayed below in Figure 4.4.

The orthophosphate concentrations changed dramatically throughout the year both in the initial concentrations and the amount of orthophosphate reduction in the HRAPs, see Figure 4.3. The mean initial concentration was 8.97 ± 3.88 mg/L, and the minimum and maximum concentrations were 1.99 mg/L (18th July 16) and 14.50 mg/L (12th December 16) respectively. The highest percentage reductions of orthophosphate were recorded in November 2017 with a percentage reduction of 81% (8.2 mg/L) and 78% (8 mg/l). In contrast, September 2016 recorded a slight increase of 13% (0.5 mg/L) in orthophosphate concentration and increases in orthophosphate concentration were also recorded in July and August 2016. The initial concentrations fluctuated throughout the year and from year to year with 2017 and 2018 having higher initial concentrations compared to 2016. The percentage reductions of orthophosphate by the HRAP were highest during late spring and summer. This would imply that the higher bioactivity of algae is enhancing orthophosphate reduction either through assimilation into the algal biomass or settling out as salts due to the elevated pH level during high photo-bioassimilation of carbon compounds. This is also a reflection of the microbial activity in the HRAPs.

HRAP water samples were analysed for TIP and the results recorded for 14 months (May 2016- July 2017). The initial concentration of TIP varied significantly throughout the year with a range of 8.0mg/L, see Figure 4.4. The initial mean TIP concentration was 8.0± 2.3 mg/L, and the minimum and maximum concentrations were 4.3 mg/L (2nd December 2016) and 12.3 mg/L (19th October 2016) respectively. The highest percentage reduction was in November 2016 in which 90% (8.3 mg/L) of the TIP was removed. In contrast,

there was almost no change in TIP concentrations in May and September 2016 as well as April, May and July 2017. This was most likely caused by the low biomass concentrations of these runs. Additionally, the concentration of TIP increased during July 2016 by 16% (0.9 mg/L). Overall, it is evident that the reduction of TIP is highest in spring and summer and lowest in winter.

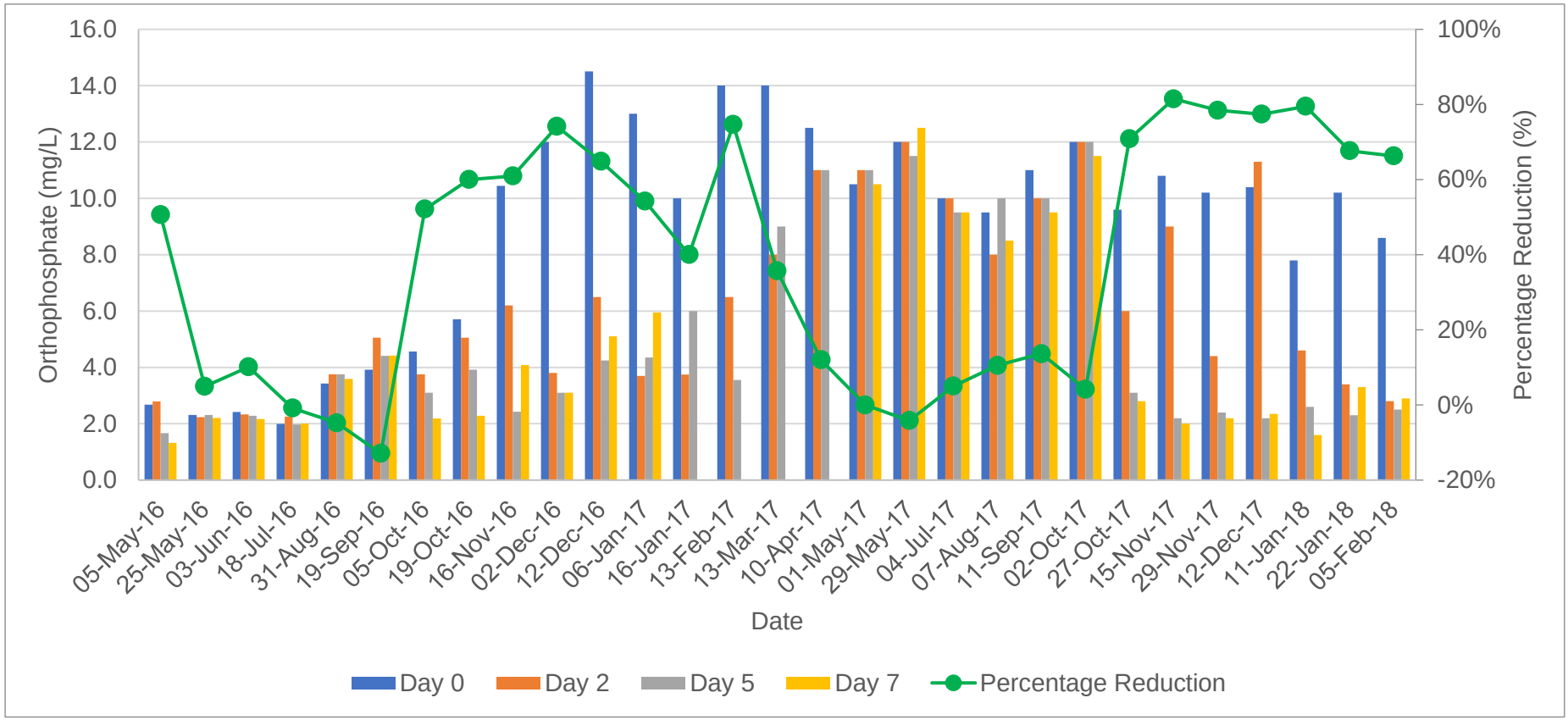

Figure 4.3: Orthophosphate concentrations over time and final percentage reduction.

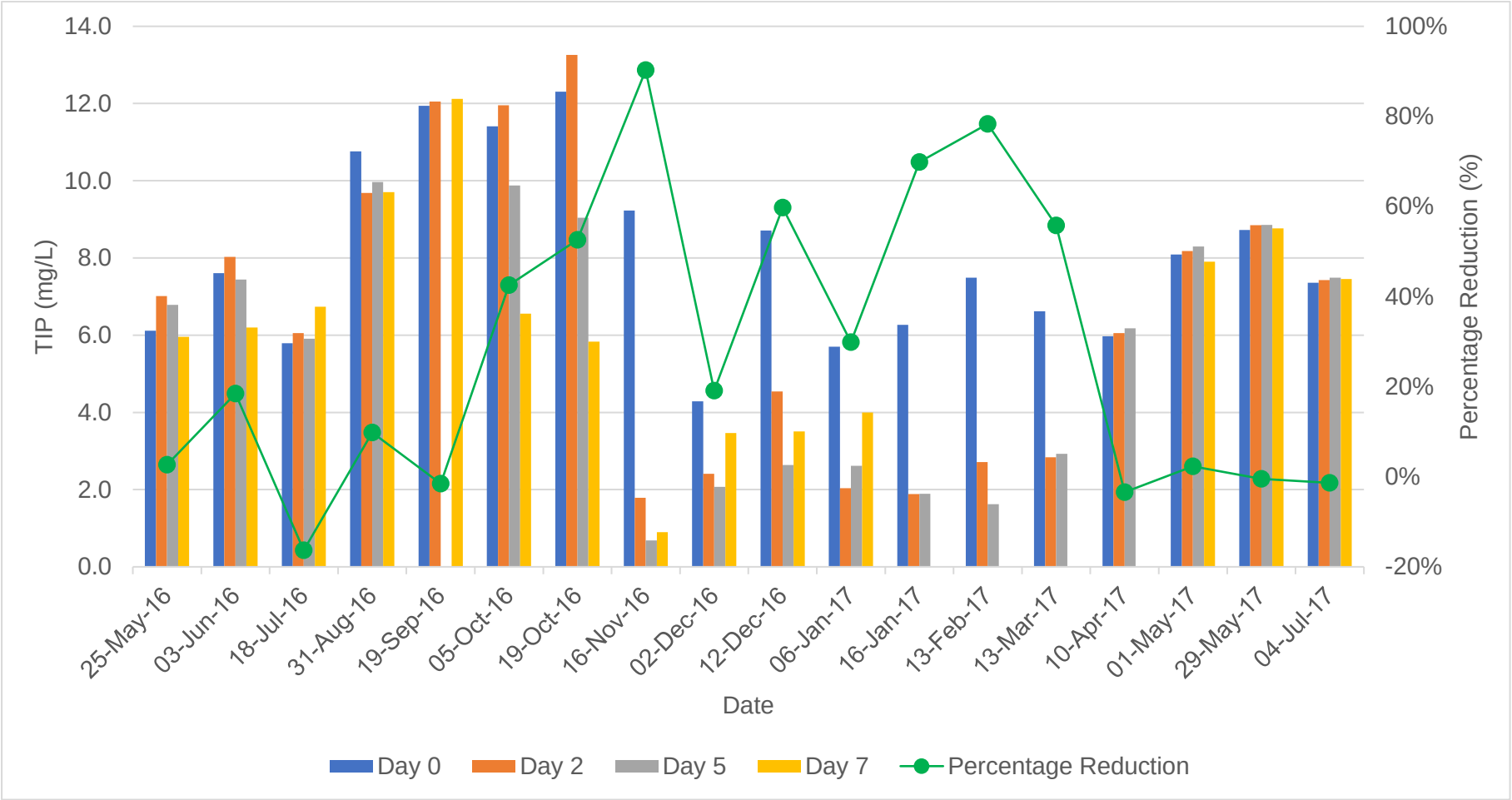

Figure 4.4: TIP concentrations over time and final percentage reduction.

4.3.2.3 Natural Variations in Nitrogen Concentrations

Nitrogen is essential for protein synthesis, and therefore its concentration can influence the growth and productivity of algal systems (Beuckels et al., 2015). Nitrogen in wastewater is generally available to algae in three different forms, ammonia, nitrite and nitrate. The concentration of nitrate and nitrite are displayed together in Figure 4.5, and ammonia is displayed in Figure 4.6. Samples were analysed for TIN, and these are shown in Figure 4.7.

The initial concentrations of nitrate and nitrite in the HRAPs had a mean of 2.74± 2.08 mg/L and ranged from 0.10 mg/L (12/12/17) to 8.70mg/L (18/07/16). The highest percentage reduction of nitrate and nitrite was in October 2017 with a reduction of 98% (3.1 mg/L). However, in August 2017 there was an increase in the nitrate and nitrite concentration by 395% (4 mg/L). The changes in nitrate and nitrite could be a reflection of the microbial bioactivity in the HRAP and the preceding lagoons. The increase in nitrate and nitrite may be caused by the presence of nitrifying bacteria such as *Nitrosomonas* sp. and *Nitrobacter* sp. The photosynthetic bacteria, *Nitrosomonas* sp., can oxidise ammonia into nitrite and this can be further oxidised to nitrate by *Nitrobacter* sp. thus increasing the nitrite and nitrate concentration. This process reduces the ammonia concentrations of the HRAPs while increasing the nitrate and nitrite concentrations. The overall mean percentage change of nitrate and nitrite was an increase in the concentration by 33% (0.1 mg/L).

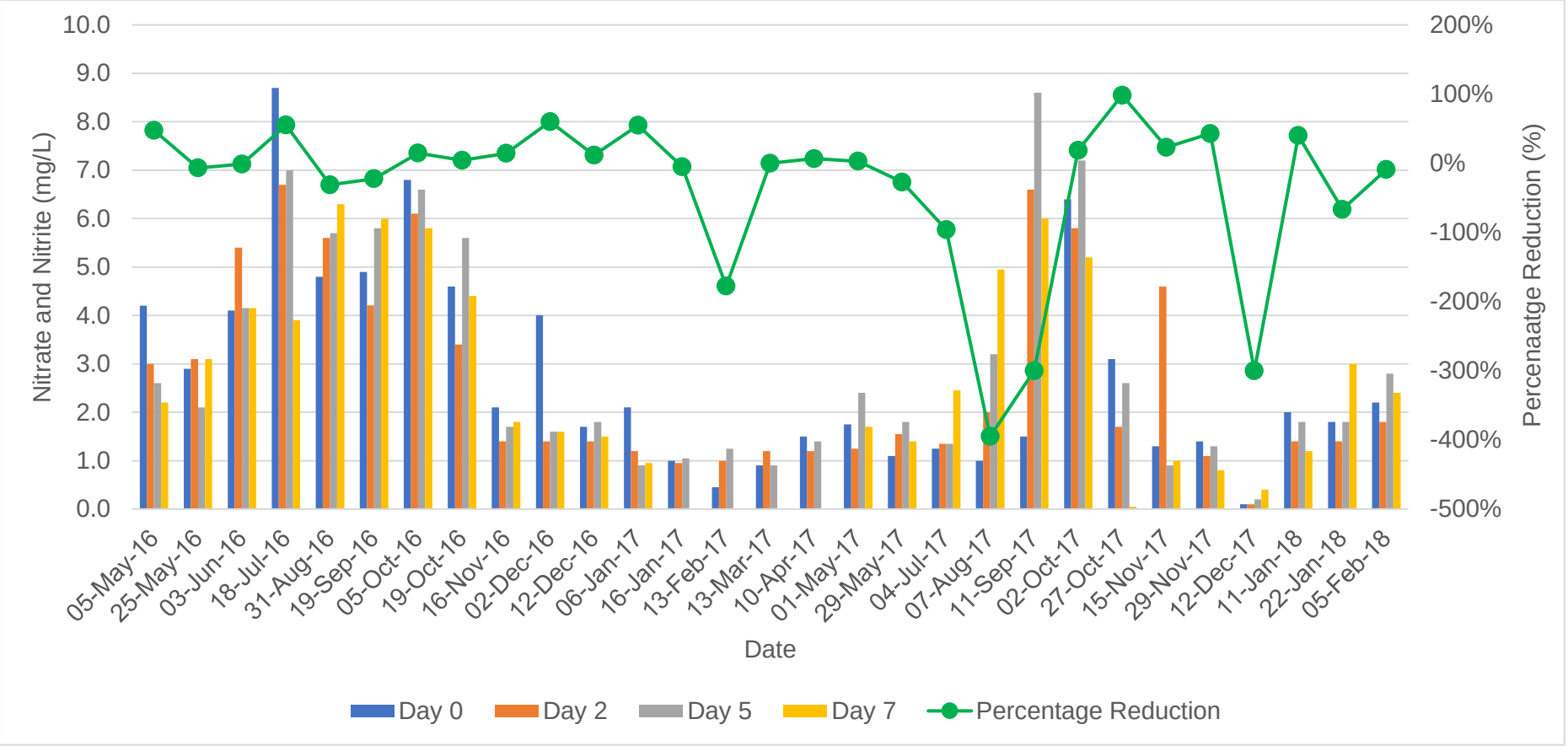

Figure 4.5: Nitrate and Nitrite concentrations over time and final percentage reduction.

As seen in Figure 4.6, the concentrations of ammonia varied significantly throughout the year. The mean concentration of ammonia in the HRAP influent was 13.03± 10.72 mg/L with a maximum of 39.25 mg/L (02/10/17) and a minimum of 0.41mg/L (02/12/17). The highest percentage reductions of ammonia were recorded during October and November 2017 with reductions ranging from 98% (23 mg/L) to 99% (19 mg/L). The lowest percentage reduction was in December 2016 with a reduction of 26% (0.1 mg/L). The initial concentration of ammonia in the run mentioned above was already reduced to 0.41mg/L, and approximately 0.30mg/L appeared to be the minimum ammonia concentration achievable in the HRAPs, as this was approximately the lowest ammonia concentration recorded during numerous runs. It was hypothesised that due to the warmer conditions in December, most of the ammonia would have either been biosynthesised into algal biomass in the earlier lagoons, reduced by other organisms such as *Nitrosomonas* sp. or was volatilised due to the high pH (>9) experienced as a result of the high photosynthetic activity. The second lowest percentage reduction was 43% (8.8 mg/L) in August 2016. Overall, the percentage reductions were highest in spring and summer due to the high productivity in the HRAPs and elevated pH levels facilitating ammonia volatilisation. During spring and summer as explained earlier, the majority of the runs had very low ammonia concentrations by the end of the run. Additionally, winter still obtained a 57% (12.9 mg/L) reduction of ammonia concentration. However, it is hypothesised that this winter ammonia reduction was more likely due to ammonia volatilisation than assimilation into the algal biomass as the biomass production during winter was low, and this may not have facilitated efficient biosynthesis of ammonia.

HRAP water samples were analysed for TIN and the results recorded for 14 months (May 2016- July 2017). Figure 4.7 shows the variation in the TIN. TIN had a mean initial concentration of 13.9± 10.2 mg/L and an initial minimum and maximum of 2.9 mg/L (02/12/17) and 29.5 mg/L (04/07/17) respectively. The highest reduction of TIN was in October 2016 with 96% (12 mg/L). In contrast, during March 2017, there was a slight increase of 26% (1 mg/). Overall, TIN reduction was relatively stable throughout the year with the highest reduction occurring during spring and summer.

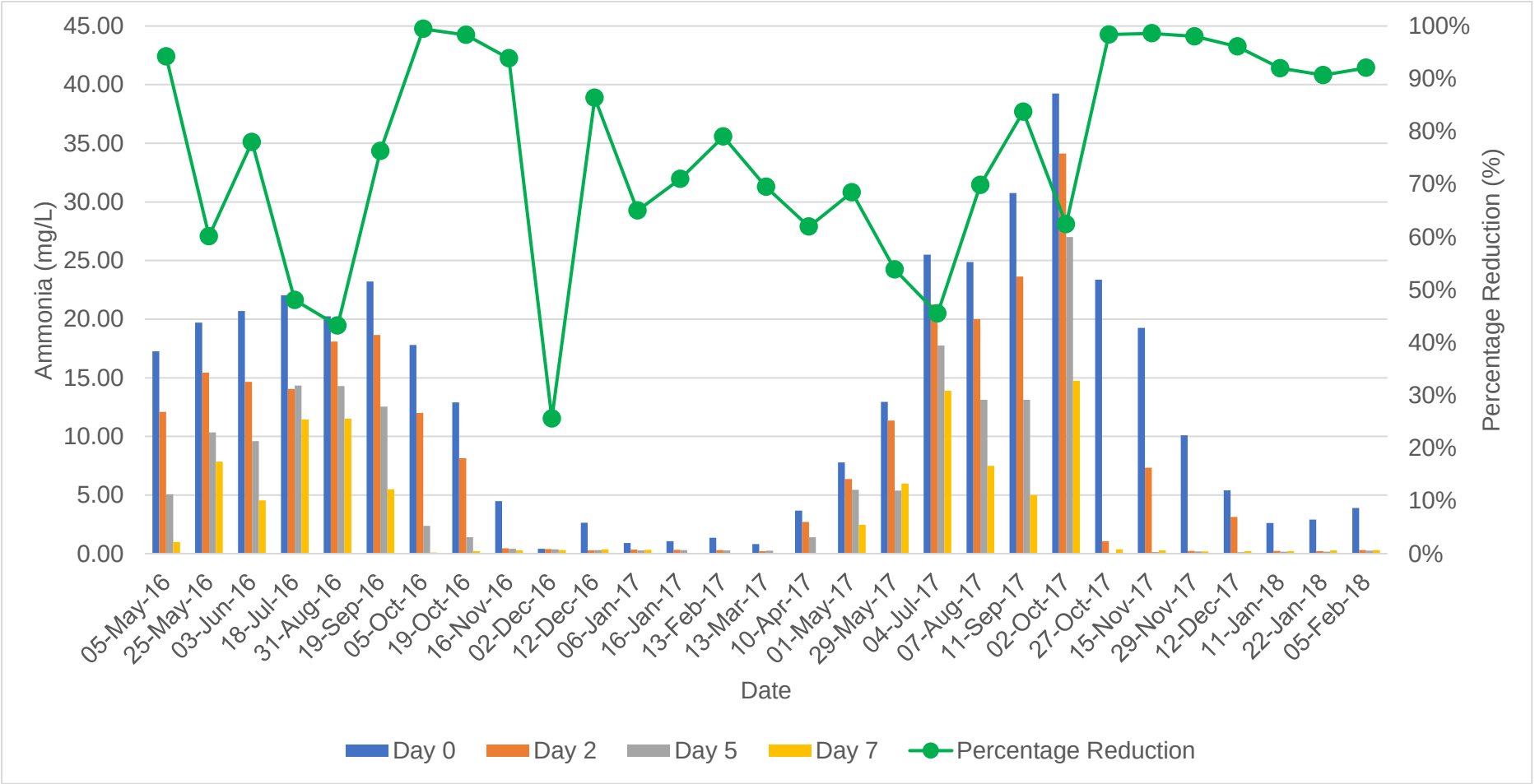

Figure 4.6: Ammonia concentrations over time and final percentage reduction.

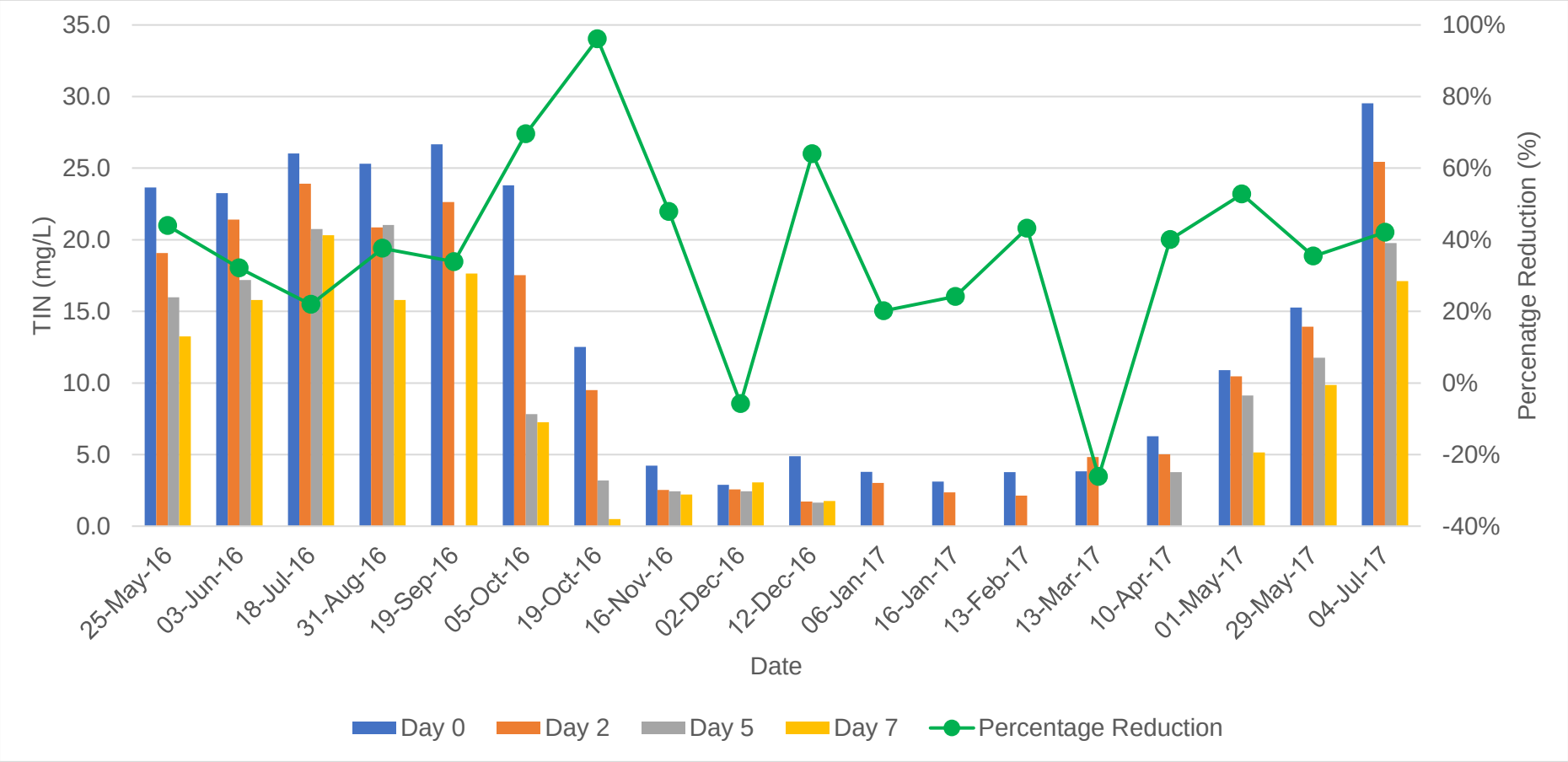

Figure 4.7: TIN concentrations over time and final percentage reduction.

The assimilation of nitrogen and phosphorus into algal cells are believed to be linked with one another as discussed in chapter 2. A limitation in one causes a decrease in the reduction of the other. Comparing the above graphs, Figures 4.3-4.7, to each other, it is evident that there is a reduction in nutrient concentrations in the warmer conditions during the summer and spring months especially from October to February. This period of lower nutrients overlaps with the period of peak biomass production which is also from October to February. This is due to the biosynthesis of the nutrients into the algal biomass. This period is also a warmer and more illuminated time of the year, see Figure 4.1, enabling higher algal productivity.

4.3.3 Comparisons of the Analytical Methods for Biomass Quantification

Dried biomass is the primary method utilised to represent the concentration of algae (see chapter 3 for detailed procedure) present in the HRAPs. However, OD is another method which has also been used. The OD method is quicker, easier, less cumbersome and gives an immediate result compared to the gravimetric method. In this study, three different wavelengths to measure the OD of samples were used, and they are 750nm, 680nm and 440nm. While using OD values can provide a reasonable estimate of the algal population it requires a good reference base and dataset to be able to provide accurate algal concentration values. Figure 4.8 compares the three OD values to the respective dried biomass concentration of various samples. From the results tabulated in Figure 4.8, it is evident that the OD values for the three wavelengths provide an accurate representation of the dried biomass results and could be used to as a quick method to determine the biomass concentrations in the water samples. The equations in Figure 4.8 can be utilised to calculate the dried biomass concentration from an OD value.

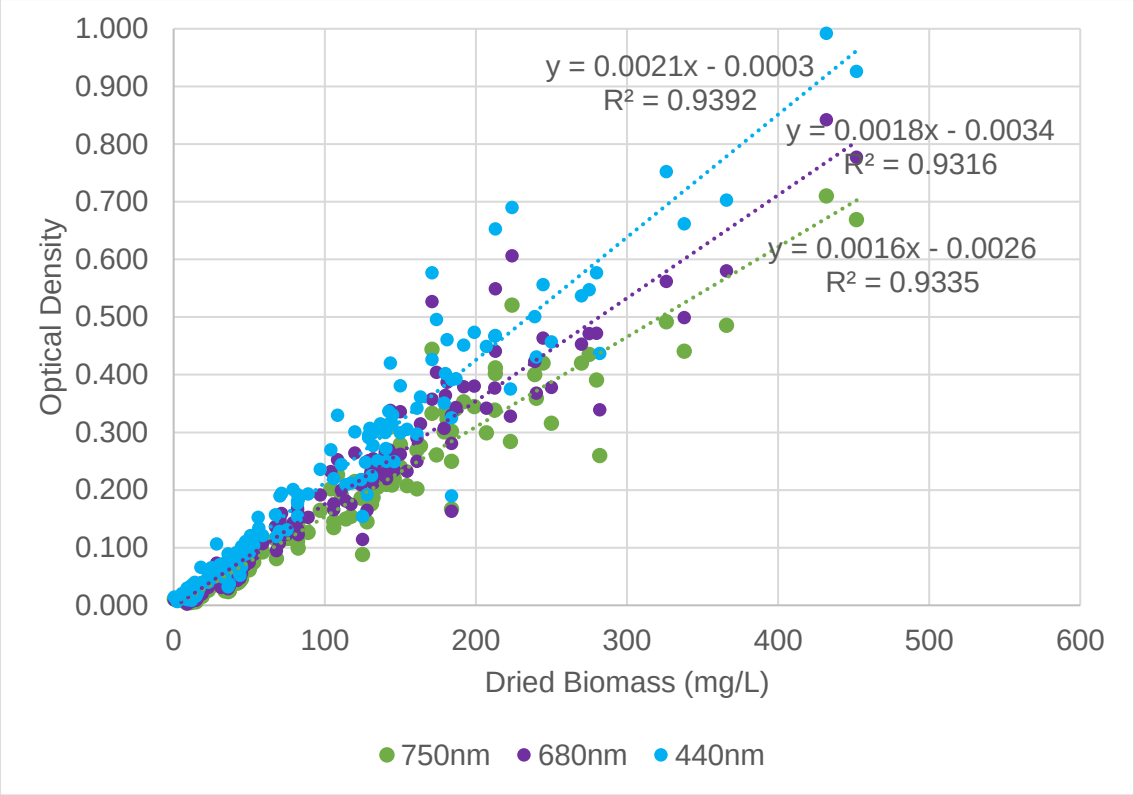

Figure 4.8: Optical Density versus Dried Biomass Concentration.

4.3.4 Effects of the Addition of Algae to HRAPs

During the colder winter months, the biomass of algae in the effluent from the secondary lagoons was low, and in turn, the algal biomass in the HRAPs was also low. This was believed to be caused by a combination of slow growth conditions and an algal population depletion caused by zooplankton grazing on algae in the secondary lagoon effluent and the preceding lagoons. Such low algal population in the HRAPs would diminish biomass productivity and in turn, diminish the nutrient reduction potential of the HRAPs. To overcome this situation, laboratory-cultured batches of ambient species of algae were added as a seed inoculum to the HRAPs with the aim to enhance algal biomass productivity and thus improve the nutrient reduction. Three separate 20L monocultures of algae which had been previously isolated, and laboratory cultured from the HRAPs namely; *Dictyosphaerium* sp., *S. acuminatus* and *S. quadricauda* were added to one of the HRAPs during different runs, and the other HRAP was run as a control without the addition of algae.

Dictyosphaerium sp. is a fast-growing algal species and was prevalent in the HRAP influent throughout the year. It was added to a HRAP on the 5th of October 2016 and increased the dried biomass concentration by 6 mg/L (35%). Both *Scenedesmus* species were common in the HRAP influent throughout the year. They were added separately to determine which species performed better in colder temperatures. *S. acuminatus* was added to a HRAP on the 4th of July 2017 and increased the dried biomass concentration by 2 mg/L (12%), and *S. quadricauda* was added on the 11th of September 2017 and increased the dried biomass concentration by 5 mg/L (56%). The mean water temperature during each of these runs was 12.61℃ for the *Dictyosphaerium* sp. run, 7.42℃ during the *S. acuminatus* run and 10.27℃ during the *S. quadricauda* run. There was no statistical difference between the pH of the experimental and control HRAPs during each of these experiments with a mean pH value of 9.03 and 8.96 for the *Dictyosphaerium* sp. run, 8.63 and 8.64 for the *S. acuminatus* experiment and 8.50 and 8.66 for the *S. quadricauda* experiment. The percentage nutrient reduction results are tabulated in Table 4.2, and the biomass results are tabulated in Table 4.3

Table 4.2: Percentage reduction of nutrients results from the two HRAP experiments in which laboratory-grown cultures of algae were added.

	$P\text{-}PO_4$ (%)	**TIP (%)**	**NH_3-N (%)**	**NO_2-N and NO_3-N (%)**	**TIN (%)**
***Dictyosphaerium* sp.**	58	50	99	30	66
Control 1	52	43	99	15	70
S. acuminatus	5	-1	45	-96	42
Control 2	0	1	55	-6	50
S. quadricauda	14	-	84	-300	-
Control 3	0	-	71	-507	-

Examining the results from the experiment utilising *Dictyosphaerium* sp., it was evident that the majority of the nutrients such as orthophosphate, ammonia, TIN and TIP, did not vary significantly between the control and experimental HRAP, see Table 4.2. The reduction of nitrite and nitrate appeared different, with the experimental HRAP removing double the nitrate and nitrite as the control HRAP, with reductions of 30% (1.6 mg/L) and 15% (1.0 mg/L) respectively, however, both of which were within the experimental error.

The results from the experiment utilising *S. acuminatus* demonstrated that the reductions of most of the nutrients did not vary significantly between the experimental and control HRAP, see Table 4.2. The only result that was different between the two HRAPs was again the nitrite and nitrate concentration, with increased concentrations of 96% in the experimental HRAP and 7% in the control HRAP. These absolute changes in concentration are small and equate to 0.9mg/L in the *S. acuminatus* HRAP and 0.1mg/L in the control HRAP. The difference between the HRAPs does not fall within the experimental error suggesting that the added *S. acuminatus* had an impact on the nitrate and nitrite concentration.

The results from the experiment utilising *S. quadricauda* had a large amount of variation between the experimental HRAP and the control HRAP. The difference between the reduction of the orthophosphate in the two HRAP was 13.6%, and the difference in ammonia reduction in the two HRAP was 12.3%. In both cases, the experimental HRAP had a higher reduction. Nitrite and nitrate were again different between the experimental HRAP and the control HRAP with increases in the concentration of 506% (7.6 mg/L) and 300% (4.5 mg/L) respectively. The difference in the concentration of nitrite and nitrate between the experimental and control HRAPs was 40% at the end of the experiment. To provide a better understanding of how the addition of the three algal species was affecting the algal population in the two HRAP, the biomass increases over the run, the daily biomass productivity and the final dried biomass concentrations were examined and are tabulated in Table 4.3.

Table 4.3: Daily biomass productivity results and final biomass concentrations results from the two HRAP experiments in which laboratory-grown cultures of algae were added.

	Biomass increase over run (mg/L) *	**Daily biomass productivity (mg/L/D)**	**Final biomass concentration (mg/L) ***
***Dictyosphaerium* sp.**	81	11.6	104
Control 1	65	9.3	82
S. acuminatus	35	5.0	53
Control 2	18	2.6	34
S. quadricauda	16	1.6	30
Control 3	28	2.8	37

* Error range of 4 mg/L

After perusing the results for the biomass increases over the runs, the daily biomass productivity and final dried biomass, see Table 4.3, it is evident that there were differences between the experimental and control HRAPs. The addition of algae in the *Dictyosphaerium* sp. and the *S. acuminatus* experiments both showed higher results for the biomass increase over the run, the daily biomass productivity and final biomass concentration compared to their control HRAPs. The *Dictyosphaerium* sp. experiment had the highest productivity of the three experiments, this, in turn, produced the highest biomass production and the highest final biomass concentration. This high productivity is likely due to the higher mean temperature recorded during this run of 12.61℃ compared to the other two runs. The experimental HRAP in the *S. acuminatus* run also had higher biomass increase, daily biomass productivity and final biomass concentration than the control HRAP. The only experiment in which the experimental HRAP performed worse than the control HRAP was when *S. quadricauda* was used. The experimental HRAP in the *S. quadricauda* experiment had lower biomass increase, daily productivity and a lower final

biomass concentration than the control HRAP. The change in biomass and the daily productivity of the *S. quadricauda* experimental HRAP are almost half that of the control HRAP. This could be due to the experimental HRAP requiring an extended acclimatisation period or lag phase. During the lag phase, the added laboratory-cultured algae need to expend energy and resources to change their physiological structure to acclimatise to the condition in the experimental HRAP (Mathur et al., 2017). Whereas, the native algae does not need to expend the energy and resources on acclimatisation and can focus these on growth. However, by adding a large concentration of laboratory-cultured algae the majority of the energy and resource in the HRAP to may have been utilised by the laboratory-cultured algae to acclimatise rather than for the algae to grow. The added culture of *S. quadricauda* accounted for 36% of the algal biomass in the experimental HRAP. In the other two experiments, the laboratory-cultured algae only accounted for 26% of the algal biomass in the *Dictyosphaerium* sp. experiment and 11% for the *S. acuminatus* experiment. These smaller proportions of laboratory-cultured algae in the HRAPs would have required less energy and resources to acclimatise. Additionally, the mixed cultures in the HRAP would require less time to acclimatise as there is a greater amount of native algal already acclimatised to the environment. Furthermore, each species of algae acclimatises to changes in conditions differently and can take varying amounts of time depending on the conditions (Mennaa et al., 2015, Lynch et al., 2015). Furthermore, the *Dictyosphaerium* sp. experiment also had a higher mean temperature, 12.61℃ compared to 7.42℃ for the *S. acuminatus* and 10.27℃ *S. quadricauda* experiments. This higher temperature was to closer to the temperature the laboratory-cultured algae were grown in (~20℃) thus reducing the acclimatisation period. Alternatively, the *S. quadricauda* may not have been able to grow in the cold conditions and may have died, this may have negatively impacted the remaining algae in the HRAP thus slowing their growth.

While it was hypothesised that the addition of algae would enhance biomass productivity, this was only found to be the case in two of the three experiments. The experimental HRAP in the *Dictyosphaerium* sp. run and the *S. acuminatus* run performed better than the control HRAP with differences

between the changes in biomass concentration of the two HRAPs of 16 and 17 mg/L respectively. The experimental HRAP in the *S. quadricauda* run performed worse than the control HRAP and had a biomass concentration 12mg/L less than the control HRAP. These results demonstrate the need to select appropriate algae for inoculation. Additionally, laboratory-cultured batches of algae will require an acclimatisation period, which is commonly seen as a lag phase in growth, before they are able to grow efficiently. The amount of algae added as an inoculum needs to be carefully controlled to help minimise this acclimatisation period. If the acclimatisation period is too long the native species of algae may out-compete the laboratory-cultured algae, or the laboratory-cultured algae may die off.

Overall, the results from this experiment show that it is possible to increase the daily biomass productivity of the HRAP and the final biomass concentrations by adding algae to the HRAPs. However, it cannot be stated currently that the addition of algae significantly alters the nutrient reduction ability of the HRAPs. The results demonstrate the need for the appropriate algae to be utilised to optimise growth. Bioprospecting for cold temperature algae would be beneficial, along with growing the algae in similar conditions in the laboratory to those the algae would be exposed to in the HRAPs. This would help reduce the lag phase in the laboratory-cultured algae's growth cycle and ensure the algae added are able to survive and prosper.

4.3.5 Effects of pH Regulation in HRAPs utilising Acid

The pH level of the HRAP can affect algal productivity. Elevated pH levels can negatively affect the algal growth, due to the absence of free/dissolved CO_2 in water with a pH above 8.3. At these high pH levels, the algae need to dissociate the bicarbonate in the water to access the CO_2 which they utilise for growth. Control of the pH level to below 8.3 was investigated to enhance productivity. Two acids were utilised to regulate the pH in the pH HRAP. An inorganic acid, hydrochloric acid (HCl) (1M), was used for five runs and an organic acid, acetic acid (1M), was used for one run. The inorganic acid was utilised to test the hypothesis that it is only the adjustment of pH which is required to enhance algal biomass production by reducing the pH to facilitate

the presence of free CO_2 for biosynthesis by algae. A further experiment utilising organic acid was performed to test the hypothesis that it is the addition of carbon in conjunction with the pH adjustment which enhances algal biomass growth. The experiments included a control HRAP to compare the results of the experimental HRAP (pH HRAP) with the natural growth in the HRAPs. There were two runs which utilised inorganic acid to regulate pH in which either a power failure or the dosing pump tripped and caused the experiment to stop prematurely, and these results are not included. The results from the six runs utilising acid are tabulated in Table 4.4. The results are displayed as reductions in concentration for orthophosphate and ammonia, and increases in concentration for dried biomass data. Additionally, the mean for the pH data for the HRAPs is also included in Table 4.4.

Table 4.4: Results from the HRAP runs utilising acid to regulate the pH level.

		Orthophosphate (mg/L)	Ammonia (mg/L)	Biomass (mg/L)	pH
13/03/2017	Control	5.0	0.6	31	9.95
	pH HRAP	-1.0	0.6	37	7.63
10/04/2017	Control	1.5	2.3	6	8.57
	pH HRAP	3.0	1.7	9	8.27
2/10/2017	Control	0.5	24.5	24	8.48
	pH HRAP	0.5	9.1	33	7.92
27/10/2017	Control	6.8	23.0	138	9.58
	pH HRAP	-0.1	8.8	105	7.47
15/11/2017	Control	8.8	19.0	198	9.71
	pH HRAP	0.3	16.1	63	7.37
Mean for Control HRAP	Control	4.5	13.9	79	9.26
Mean for Inorganic acid	pH HRAP	0.5	7.3	49	7.73
29/11/2017	Control	8.0	9.9	106	9.99
	pH HRAP*	4.3	9.6	230	8.19

* Organic acid was utilised instead of an inorganic acid

The reduction of orthophosphate in the five hydrochloric acid runs varied significantly with the three of the five runs displaying a higher orthophosphate reduction in the control HRAPs than the pH-regulated HRAP and a mean reduction of 4.5mg/L in the control HRAPs compared to 0.5mg/L in the pH-regulated HRAPs. This may be due to the elevated pH, 9.26, in the control HRAP compared to 7.73 in the pH-regulated HRAP, which can cause phosphorus to form salts, such as the insoluble calcium phosphate, which

would settle out of suspension (Hu et al., 2012, Ruiz-Marin et al., 2010). The acetic acid run also displayed an increased orthophosphate reduction in the control HRAP with a reduction of 8.0mg/L compared to the pH-regulated HRAP with a reduction of 4.3mg/L. The reduction of orthophosphate in the pH-regulated HRAP could be explained by incorporation into the algal biomass due to the significant biomass production and by luxury uptake.

The ammonia reductions in all six runs were higher in the control HRAPs than compared to the pH-regulated HRAPs. The inorganic runs had a mean reduction of 13.9mg/L for the control HRAP compared to 7.3mg/L for the pH-regulated HRAPs. The cause of this is likely to be due to ammonia volatilisation which occurs at elevated pH levels (>9.0). Both HRAPs in the organic acid run had similar ammonia reductions with 9.9 mg/L of ammonia removed in the control HRAP, and 9.6mg/L removed in the pH-regulated HRAP; both runs had depleted the ammonia in the HRAPs within a few days. The ammonia reduction in the HRAPs could also be due to the ammonia being incorporated into the algal biomass.

The production of biomass varied throughout the experiments. The first three runs, 13/03/17, 10/04/17 and 2/10/17 and the organic acid run, 29/11/17 had a higher biomass increase in the pH-regulated HRAP compared to the control HRAP, see Table 4.4. However, during the fourth and fifth runs, 27/10/17 and 15/11/17, the pH HRAP displayed a lower biomass increase than the control HRAP. The biomass increase in the control HRAP (198mg/L) in the 5th run was three times higher than the pH HRAP (63mg/L), and such a significant variation was not seen elsewhere. The mean increase in biomass was 79mg/L in the control HRAP and 49mg/L in the pH-regulated HRAP. To investigate this further, the initial, final and change in the biomass concentrations are displayed in Table 4.5, as well as, the daily biomass productivity.

Table 4.5: The initial, final and change in dried biomass concentrations and daily biomass productivity of each run.

		Biomass (mg/L) *			Daily Biomass Productivity (mg/L/D)
		Start	**End**	**Change**	
13/03/2017	Control	92	123	31	7.8
	pH HRAP	95	132	37	9.3
10/04/2017	Control	34	40	6	1.5
	pH HRAP	34	43	9	2.3
2/10/2017	Control	12	36	24	3.4
	pH HRAP	11	44	33	4.7
27/10/2017	Control	43	181	138	19.7
	pH HRAP	45	150	105	15.0
15/11/2017	Control	42	240	198	28.3
	pH HRAP	43	106	63	9.0
Mean for inorganic acid	Control	45	124	79	12.1
	pH HRAP	46	95	49	8.0
29/11/2017**	Control	35	141	106	15.1
	pH HRAP	40	270	230	32.9

* Error range of 4mg/L **Acetic acid utilised to regulate pH instead of HCl acid.

After examining Table 4.5, it is evident that even between the inorganic acid runs there are significant differences. The inorganic runs had a mean increase in biomass concentration of 79mg/L in the control HRAP and 49mg/L in the pH HRAP. Moreover, the mean daily biomass productivity rates were different with the control HRAP having a rate of 12.1 mg/L/D and the pH HRAP

having a rate of 8.0mg/L/D. The 4th and 5th runs, 27/10/2017 and 15/11/2017, are significantly different from runs 1-3, 13/03/2017, 10/04/2017 and 2/10/2017. The pH HRAPs in runs 1-3 had a higher change in biomass and greater daily biomass productivity than their respective control HRAPs. Whereas runs 4 and 5 had much lower changes in biomass and lower daily biomass productivities than their respective control HRAPs. An explanation of these varying results could be due to the amount of inorganic acid required by the HRAP to reduce the pH in each experiment. The first three experiments conducted on, 13/03/17, 10/04/17 and 2/10/17, had lower increases in the algal biomass and relatively small daily biomass productivities, and they were 9.3, 2.3 and 4.7 mg/L/D in the control HRAP respectively. These low daily biomass productivities would indicate that less biosynthesis occurred and as less carbon was assimilated, there was less need to reduce the pH level as frequently or as drastically. The daily biomass productivities in the fourth (27/10/17) and fifth (15/11/17) runs were significantly higher in the control HRAP than the previous runs and the pH-regulated HRAP; they were 19.7 and 28.3 mg/L/D respectively. These higher productivities resulted in greater biomass production in the HRAP, and this would, in turn, increase the photosynthetic rate of the HRAP which would cause more carbon to be assimilated into the algal biomass increasing the pH level of the HRAPs, thus requiring more acid to control the pH level. The pH-regulated HRAP should be enhancing the biomass production by keeping the pH below 8.3 at which pH there is still free CO_2 in the water. However, by reducing the pH level of the water, there may be a reduction in the amount of soluble CO_2. This is because the solubility of CO_2 increases with pH, therefore by reducing the pH there is a reduction in the amount of dissolved CO_2. This could consequently cause the pH-regulated HRAP to have less available carbon in the water than the control HRAP which would cause a decline in biomass production. This reduction in available carbon in the water and the negative impact on biomass production suggests that the hypothesis that it is only a reduction in pH which enhances the algal biomass production should be rejected.

The inorganic acid may have had a destructive and damaging impact on the algal cells which was not observed in the first three runs due to the small amount of acid required to control the pH level. However, in the fourth and fifth

runs, as more acid was required to control the pH, the inorganic acid may have had a negative or harmful impact on the algal biomass production. Overall, the addition of inorganic acid was able to enhance the biomass production in experimental HRAPs in which the productivity was low. However, as the productivity of the system increased the addition of more inorganic acid caused the biomass production in the HRAPs to drop below that which was occurring in their respective control HRAP.

The sixth experimental run was completed using organic acid (acetic acid) instead of an inorganic acid (hydrochloric acid). There were two reasons for this, firstly to test the addition of carbon in conjunction with the pH regulation, and secondly to test the impact of a weaker acid on the growth of the algae. The utilisation of organic acid to regulate the pH had a significant, positive effect on the production of algae in the HRAP. The control HRAP had a biomass increase of 106 mg/L with daily biomass productivity of 15.1 mg/L/D, while the pH-regulated HRAP had a biomass increase of 230 mg/L with daily biomass productivity of 32.9 mg/L/D. These results are supported by Zhu et al. (2014) in which they utilised acetic acid to regulate pH of photobioreactors in winter, they also found higher biomass productivity when using acetic acid to control pH. The results in the current study suggest that there were no visible adverse effects on the algae due to the addition of an organic acid. However, while under similar environmental conditions with an inorganic acid in the earlier runs, 27/10/2017, 15/11/2017, there appeared to be a reduction in the biomass productivity which could have been a result of the damaging effects of the inorganic acid.

In summary, this research supports the hypothesis that it is not solely the regulation of pH that enhances the algal biomass production in HRAP but the addition of carbon in conjunction with the pH control that significantly promotes the process. This assists the understanding of why processes such as CO_2 addition to control pH, as performed by other researchers, have such a significant impact on algal biomass production. In addition to this, it demonstrates that the control the pH of HRAPs by utilising an inorganic acid is not productive and should not be performed. Conversely, it suggests that the use of an organic acid not only reduces the pH but also adds organic carbon

which can be utilised by algae thus enabling a significant enhancement in biomass production. Further work on the use of acetic acid to regulate pH and the economic feasibility of the process is required before this can be implemented on a larger scale.

4.3.6 Comparison of utilising Primary Lagoon Effluent and Secondary Lagoon Effluent in HRAPs

The secondary lagoon effluent was utilised as the influent to the HRAP for the majority of the experiments. However, secondary lagoon effluent can be low in nutrients, such as carbon and ammonia, which are required for algal growth. The low concentration of the nutrients in secondary lagoon effluent can be rapidly consumed in the HRAPs, thus causing algal growth to plateau. The rapid depletion of nutrients, mainly nitrogen, is shown in figures 4.5 and 4.6 where during summer most of the ammonia and nitrogen in the HRAPs was removed by day two. Section 4.2.2.1 demonstrated the plateauing of algal growth, as the increases in algal biomass concentrations were not consistent and the runs which had the highest percentage increases did not have the highest algal biomass concentrations. The use of primary lagoon effluent as the influent for the HRAP was investigated in an attempt to alleviate nutrient limitation in the HRAPs and increase biomass growth. The primary lagoon effluent results were compared against the results from a HRAP filled with secondary lagoon effluent.

Table 4.6 provides the results for the experimental runs in which secondary and primary lagoon effluent were utilised in the HRAPs. Examining the results between the two HRAPs, the most significant difference is the biomass concentration, with the secondary lagoon effluent HRAP displaying a mean increase of 108mg/L and the primary lagoon effluent HRAP displaying a mean increase of 2mg/L. The ammonia reduction was higher in the primary effluent HRAP with a reduction of 10.5mg/L in the primary effluent HRAP and 3.5mg/L in the secondary lagoon effluent HRAP. However, both HRAP had percentage reductions over 90%, except one run utilising primary lagoon effluent which had a removal of 74%, suggesting that the majority of the ammonia may have been utilised in each run. This reduction of ammonia may

be likely due to a combination of bioassimilation and volatilisation due to the elevated pH levels of the HRAPs. The mean orthophosphate reductions were higher in the secondary lagoon effluent HRAP with 6.7mg/L removed compared to the primary effluent HRAPs with only 4.5mg/L removed. The difference in orthophosphate reduction may be caused by the differences in pH, with an elevated pH in the secondary lagoon effluent HRAP causing orthophosphate to form salts and settle out of suspension as discussed earlier. Both HRAPs displayed an increase in mean pH with the secondary lagoon effluent HRAP increasing slightly more than the primary lagoon effluent HRAP with an increase of 1.4 and 0.8 respectively.

The initial biomass concentrations of the two HRAPs were very different in each run. The most substantial difference was in the first run, 12/12/17, the secondary lagoon effluent HRAP had an initial biomass concentration of 12 mg/L while the primary lagoon effluent HRAP had a dried biomass concentration of 338mg/L. The following runs also displayed differences in the dried biomass concentrations between the two HRAPs, see Figure 4.9. In each run, the primary lagoon effluent HRAP had a significantly higher initial dried biomass concentration, and it is important to note that both the primary lagoon effluent and the secondary lagoon effluent contained live algae and also bacteria and other colloidal particles including dead algal cells. The primary lagoon effluent would have a higher amount of dead or inactive suspended particles as it is earlier in the treatment process. It can be seen in Table 4.6 and Figure 4.9 that the biomass concentration in the primary lagoon effluent HRAPs does not change as drastically compared to the secondary lagoon effluent HRAPs. In two of the runs, 11/01/18 and 22/01/18, the biomass concentration in the primary lagoon effluent HRAP was lower after the experimental period with a reduction of 48mg/L and 49mg/L respectively. Meanwhile, the biomass concentration in the secondary lagoon effluent HRAP of both runs increased by 98 mg/L and 99mg/L respectively. The other two runs, 12/12/17 and 05/02/18, had increases in biomass for both the secondary lagoon effluent and primary lagoon effluent HRAPs. The secondary lagoon effluent HRAP always had a more substantial increase than the primary lagoon effluent. The first run, 12/12/17, had an increase of 172mg/L in the secondary lagoon effluent HRAP

compared to the primary lagoon effluents increase of 94mg/L. The last run, 05/02/18, had an increase of 64mg/L in the secondary lagoon effluent HRAP and 10mg/L in the primary lagoon effluent HRAP.

The reduced productivity of the primary lagoon effluent is most likely due to a few factors. The first reason being the high biomass concentrations causing self-shading in the HRAPs. As discussed earlier in Chapter 3, the depth of the euphotic zone is related to the concentration of biomass. Table 3.9 displays the Secchi disk readings for the primary lagoon effluent HRAPs, the Secchi disk readings for each productivity test, light and dark bottle tests, were 10cm or below the surface, which indicates that there was reduced light penetration. Figure 3.4 corroborates this when a biomass concentration of 110mg/L was achieved more than half of the HRAP did not receive sufficient light. Each of the primary lagoon effluent HRAP had biomass concentrations exceeding 110mg/L, with the lowest initial biomass concentration in the primary lagoon effluent HRAP being 174mg/L and the highest being 414mg/L. These high biomass concentrations would limit the productivity of the HRAPs. The second factor that influences productivity is an elevated respiration rate due to a high amount of dead and decaying organic matter in the primary lagoon effluent compared to the secondary lagoon effluent. Table 3.9 again corroborates this. During three of the productivity experiments utilising primary lagoon effluent, the respiration rate was higher than that of the production rate, which caused a decline in the net production of carbon. Table 3.9 also displays the ammonia concentrations, in four of the five runs the ammonia concentrations were very low (<0.4mg/L). This low concentration of ammonia also limits production as discussed in Chapter 3.

The experiments utilising secondary lagoon effluent exhibited a higher biomass production in comparison to the ones in which primary lagoon effluent was utilised. These experiments were conducted under the same conditions to eliminate the environmental impact, if any, on the experiment. Thus, it illustrates that the main influences would have been the solutes in the media. The experiments were run in the warmer summer months which should promote algal growth. The mean water temperature of each run is displayed in Figure 4.9. The mean temperature ranged from 18.7 to 23.0℃.

Table 4.6: Changes in algal biomass and nutrient concentration results of the experiments comparing the primary and secondary lagoon effluent. Initial and final concentration in brackets.

	12/12/17		**11/01/18**		**22/01/18**		**05/02/18**		**Mean**	
	Secondary effluent	Primary effluent	Secondary effluent	Primary effluent	Secondary effluent	Primary effluent	Secondary effluent	Primary effluent	Secondary effluent	Primary effluent
Dried Biomass (mg/L)	172 (12- 184)	94 (338- 432)	98 (48- 146)	-48 (414- 366)	99 (51- 150)	-49 (174- 125)	64 (128- 192)	10 (213- 223)	108	2
Ammonia ($N\text{-}NH_3$)	-5.2 (5.4- 0.2)	-13.6 (13.9- 0.3)	-2.4 (2.6- 0.2)	-18.7 (19.1- 0.4)	-2.6 (2.9- 0.3)	-3.0 (4.1- 1.1)	-3.6 (3.9- 0.3)	-6.7 (7.0- 0.3)	-3.5	-10.5
Phosphorus ($P\text{-}PO_4$)	-8.0 (10.4- 2.4)	-7.3 (8.3- 1.0)	-6.2 (7.8- 1.6)	-5.0 (9.2- 4.2)	-6.9 (10.2- 3.3)	-2.1 (9.0- 6.9)	-5.7 (8.6- 2.9)	-3.7 (8.4- 4.7)	-6.7	-4.5
pH -Change (Initial-Final)	2.4 (8.3- 10.7)	1.9 (8.6- 10.5)	1.3 (8.9- 10.2)	0.5 (8.7- 9.2)	0.7 (8.9- 9.6)	0.1 (8.5- 8.6)	1.0 (9.0- 10.0)	0.7 (8.6- 9.3)	1.4	0.8

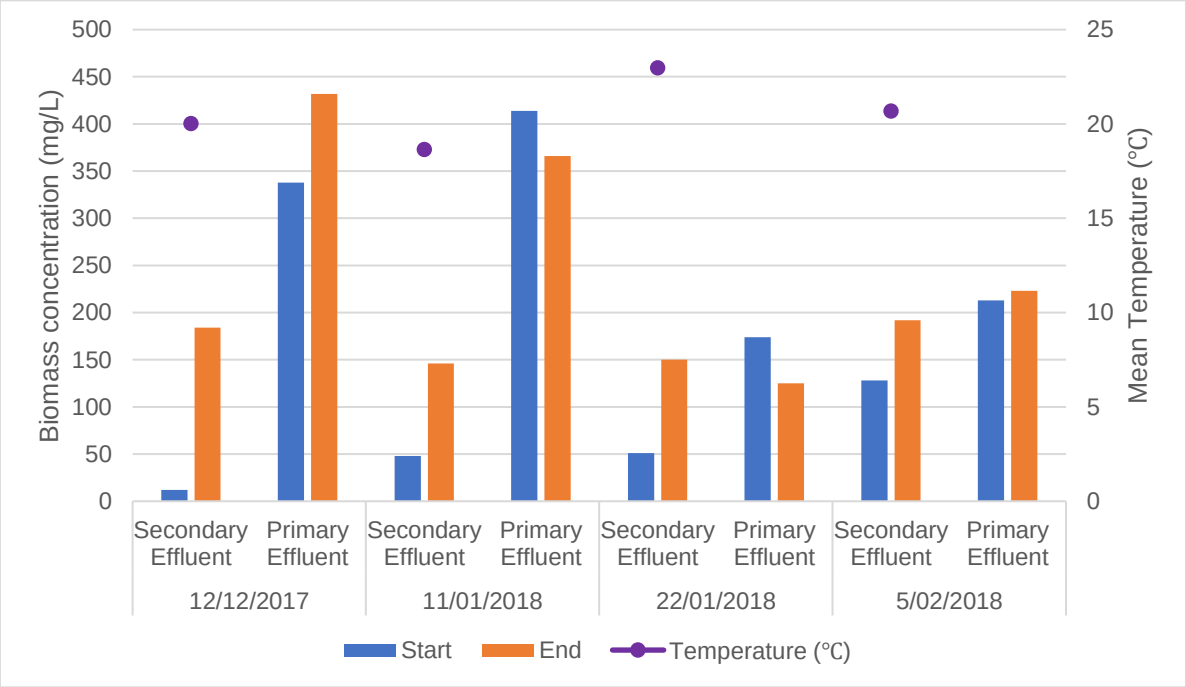

Figure 4.9: The biomass concentrations at the start and end of each run comparing the secondary lagoon effluent to the primary lagoon effluent and the mean temperature of the HRAPs.

The use of primary lagoon effluent did not benefit algal production in the HRAPs. This was due to the already higher concentration of colloidal biomass in the primary lagoon effluent compared to the secondary lagoon effluent causing the waters to become turbid. The primary lagoon effluent in the HRAPs was not able to support its algal population due to this colloidal turbidity as well as a high demand for oxidative metabolism of the media and ammonia becoming deficient during the experimental period. The primary lagoon effluent could be beneficial if the majority of algal and other colloidal biomass were removed from suspension before use in the HRAPs. A mixture of filtered primary lagoon effluent and natural secondary lagoon effluent could potentially be beneficial as the addition of nutrients to the secondary lagoon effluent would significantly promote algal biomass production.

4.4 Summary

The natural growth of algae in HRAPs utilising secondary lagoon effluent as the influent media was investigated. It was found that the two HRAPs operated in the same manner with a mean correlation coefficient value of 0.97

and that either one could be used as a control while the other was used for experimentation. Throughout the experimental period, there were numerous limitations and problems with the functioning of the HRAPs.

During the colder winter months, the algal biomass was low which impacted on algal productivity and in turn on nutrient reduction. The addition of laboratory-cultured algae was investigated. It was found that the addition of algae could potentially enhance biomass production. However, there was no significant reduction in nutrient reduction due to the additional algae. The difference in the change in biomass concentrations between the control HRAP and the experimental HRAP ranged from the experimentally HRAP having 17mg/L more biomass than the control HRAP to the experiment HRAP having 12mg/L less biomass than the control HRAP depending on the algal species utilised. It was determined that the addition of suitable species of algae would be required as some species were unable to compete with the ambient species in the HRAPs due to the cold weather. Bioprospecting would be required to determine the optimum algae to add to the HRAPs.

Another limitation commonly found in HRAPs is the amount of available carbon which is impacted by the higher pH, as this influences bicarbonate chemistry. A series of experiments were performed to determine if, by only controlling the pH, whether it was possible to increase the algal production. The majority of other studies controlled the pH in HRAPs by sparging CO_2. This study investigated if it was the addition of carbon to the system or the lowering of the pH which impacted algal productivity. Two different acids were utilised, an inorganic acid and an organic acid. It was determined that the inorganic acid was able to slightly enhance algal growth when algal productivity was low, but when algal productivity increased the addition of the inorganic acid was found to reduce biomass production. The organic acid was used during warmer conditions, and it was found to enhance algal productivity significantly. From these results, it was determined that it was not solely the control of the pH which promoted algal productivity but also the addition of carbon.

Another method to promote algal productivity was investigated, utilising nutrient-rich primary lagoon effluent as the media source in the HRAPs. The

biomass productivity of the HRAPs filled with either primary or secondary lagoon effluent as the influent were compared. It was found that the HRAPs filled with primary lagoon effluent had a higher initial algal biomass concentration whereas the HRAPs filled with secondary lagoon effluent had higher algal productivity with mean increases of 108mg/L in the secondary lagoon effluent HRAP and a mean increase of 2mg/L in the primary lagoon effluent HRAPs. This was due to a combination of self-shading, higher oxidative metabolic rates and potential ammonia limitations in the HRAP filled with primary lagoon effluent. A mixture of filtered primary lagoon effluent and secondary lagoon effluent may provide a better media for improving algal productivity.

Optimising the operation of the HRAPs is required to enhance the algal biomass productivity and in turn the nutrient reduction. A combination of the methods tested above could prove beneficial. Additionally, an understanding as to when the factors become limiting and adjusting the conditions according to the situation could also enhance algal productivity. In order to do this, a simple predictive growth model could be employed. The model would need to focus on environmental factors such as solar radiation and temperature as well as the vital limiting nutrients.

Chapter 5 - Modelling

5.1 Introduction

In order to optimise algal biomass production, it is important to understand how the system operates. In chapters 3 and 4, the productivity and operation of the HRAPs at the BMRWP was investigated. The experiments in chapter 4 provided an abundance of information regarding the growth of algae, the environmental conditions and the nutrient profile of the waters in the HRAPs. Utilising this data, it was possible to develop a simple predictive algal growth model. The model developed in this chapter focuses on a few simple variables which all algal growth systems likely have data for; solar radiation, water temperature and biomass concentration. The majority of predictive algal growth models in literature are complex and species-specific (Huesemann et al., 2016). Furthermore, this model is different from most as it was developed for an elevated pH environment. The majority of studies which examined or develop algal growth models control the pH of the media either through chemical pH adjustments or by sparging CO_2 gas (Nagappan and Verma, 2016, Huesemann et al., 2016, Béchet et al., 2016, Decostere et al., 2016). The model in this chapter was developed to be simple and to work with uncontrolled variables, including environmental and biotic changes. Moreover, a predictive algal growth model has not previously been developed for the Australian south-eastern environment.

5.2 Microalgae Biomass Predictive Growth Model

5.2.1 Development of Model

The microalgal growth model presented in this chapter was designed to facilitate the prediction of algal biomass concentration in HRAP utilising wastewater as its feed. The model was validated utilising three different sets of water of samples, the use of unaltered secondary lagoon effluent, the use of primary lagoon effluent and the use of a pH controlled (<8.3) secondary lagoon effluent. The model assumes that light and temperature are the primary determinants for algal growth and productivity. Furthermore, ammonia limited

samples (<1.0mg/L) were removed from the dataset to not interfere with the results, and it was assumed that no other factors other than an elevated pH environment were limiting the algal growth.

The microalgae growth model presented in this chapter is based on a pre-existing model, the Steele model (Steele, 1962). The Steele model has been used to predict algal growth based on light intensity (Wu et al., 2013). This chapter will discuss several modifications which were made to the Steele model to enhance its ability to predict algal growth. The Steele model is shown in Equation 5.1.

$$\mu = \mu_{max} \cdot \frac{l}{l_{opt}} \cdot e^{1\left(\frac{l}{l_{opt}}\right)} \quad (5.1)$$

Where μ (d^{-1}) is the specific growth rate under light intensity of l (lx); μ_{max} (d^{-1}) is the maximum specific growth rate when light intensity is optimal; l(lx) is the light intensity; and l_{opt} (lx) is the optimum light intensity (Wu et al., 2013).

The first modification to the Steele model was to eliminate the exponential function of the Steele equation. This was done as the algal growth in the HRAPs was determined to be linear and not exponential, and this is demonstrated in section 5.3.1. Furthermore, the model was modified to incorporate both light intensity, in the form of mean daily incident solar radiation (W/m^2), and mean daily water temperature (℃). The light intensity variable of the Steele equation was replaced with a mean incident solar radiation variable. The Steele model assumes constant temperature, and this was not the case in this research. The mean daily water temperature was included in the model to account for variations in temperature. It was assumed that the effect of temperature on growth was linear and that the maximum temperature, after which algal growth is diminished, was not achieved. The two variables were placed together due to their interrelatedness, as light intensity increases so does water temperature (Edwards et al., 2016). The incorporation of the two variables into the equation enhanced the correlation between algal growth and the joint light intensity and water temperature value compared to when the two variables were examined separately. This is also demonstrated in section 5.3.1. The modified Steele model is shown in equation 5.2.

$$\mu = \mu_{\max(tl)} \cdot \frac{tl}{tl_{opt}} \quad (5.2)$$

Where μ (mg/L/D) is the specific growth rate under conditions of tl (℃·W·m^{-2}); $\mu_{max(tl)}$ (g/L/D) is the maximum growth rate when temperature and solar radiation were optimal; tl (℃·W·m^{-2}) is the mean daily water temperature (℃) multiplied by the mean daily solar radiation (Wm^{-2}). The modified version of the Steele model incorporates the effects that both solar radiation and temperature have on algal growth. Solar radiation provides the energy algae require for growth while temperature helps to regulate the enzyme activation kinetics of algae.

Further modifications were required for accurate prediction of the algal biomass growth. The solar radiation value used in the above equation (5.2) was the daily mean solar radiation taken at a nearby weather station and provided data on the surface solar radiation of the HRAPs. It was demonstrated in chapter 3 that self-shading caused by the algal biomass had a drastic effect on the productive potential of the HRAP system. In order to account for the impact self-shading can have on the growth of algae a new equation was incorporated into the modelling. The equation in question, equation 5.3a, was the equation for the slope of the trend line in Figure 3.5. Figure 3.5 is a graph comparing the concentration of algal biomass in the HRAP to the depth at which the Secchi disk was visible. This equation helps to identify the depth of the HRAPs water column which was receiving sufficient light and was thus in the euphotic zone. Equation 5.3a provides a result in centimetres.

$$\delta = -6.872 * LN(\frac{1}{2}\mu + \beta_i) + 47.535 \quad (5.3a)$$

Where δ is the depth of the euphotic zone in the HRAP (cm), μ is the growth rate predicted by the Steele model, (mg/L/D), β_i is the initial biomass concentration (mg/L) of the HRAP. In order to account for the change in biomass concentration over the course of the prediction period, half the of the growth rate predicted by the Steele model was added to the initial biomass concentration. This provides a mean result between the initial biomass and what would be the final biomass concentration of the HRAPs. The algae in the

HRAPs was assumed to be photoautotrophic and thus would not grow outside of the euphotic zone.

The depth of the euphotic zone as calculated in 5.3a was then divided by the total depth of the HRAP (30cm). This value represents the proportion of algae in the HRAP that was in the euphotic zone, see equation 5.3b.

$$\gamma = \frac{\delta}{30} \qquad (5.3b)$$

Where γ is the value representing how much of the water column is in the euphotic zone. The combined Equations 3.5a and Equation 3.5b will be referred to as the Secchi equation. The Secchi equation could be substituted with other equations, such as the Beer-Lambert's Law, which can accurately determine the depth at which the light can penetrate the HRAP water column. Nevertheless, due to the fundamental nature of the Secchi equation and the ease of use and accessibility of a Secchi disk, equations 5.3a and 5.3b were developed and utilised for this research. Additionally, this equation accounts for light attenuation caused by; self-shading by the algal biomass, and the natural absorbance of the light by water. It also accounts for any light scattering caused by the algal cells and other particles in the HRAPs. Equation 5.3a contains constants which are site and potentially algal species specific and may need to be determined at each new location tested, which can be quickly and easily done with a Secchi disk. This data was collected from wastewater samples containing a mixed culture of algae and so may prove accurate over a broad range of wastewater samples.

Using the modified Steele equation in conjunction with the Secchi equation gave an algal growth rate which was based on the mean solar radiation and mean temperature of the system, as well as accounting for the light attenuation caused by the algal biomass and other particles in the HRAP. The final model, henceforth referred to as the Steele-Secchi model is shown in equation 5.4.

$$\mu_f = \mu_{\max(tl)} \cdot \frac{tl}{tl_{opt}} \cdot \gamma \qquad (5.4)$$

Where μ_f is the specific algal growth rate (mg/L/D) under tl ($°C \cdot W \cdot m^{-2}$) while accounting for the light attenuation effects (γ) of the algal biomass.

The Steele- Secchi model was validated against the growth results obtained in chapter 4 including experiments in which the parameters were altered. These three different sets of data are as follows. Firstly, experiments in which the HRAP utilised secondary lagoon effluent as the media source; secondly, experiments that utilised primary lagoon effluent as the media source in the HRAPs; and lastly runs which utilised acids to control the pH of the HRAPs to below 8.3 utilising secondary lagoon effluent. Validating the model against these differing parameters demonstrated its strength and accuracy. The results were validated by adding the predicted and experimentally obtained growth rates to the initial biomass concentration of the HRAP and plotting the results against each other and determining a linear R^2 value. HRAPs are operated over differing periods based on the time of the year and the environmental conditions. During summer the HRAP will be able to treat the influent more efficiently due to the high temperatures and high light intensities, and may only require a retention time of two or three days. Conversely, in winter the HRAPs may require retention times of seven days or longer to fully treat the influent.

5.2.2 Statistics

Mean solar radiation and water temperature values were determined in Microsoft Excel 365. R^2 values for exponential and linear algal growth rates from each HRAP run were compared using a t-test assuming equal variance, utilising a confidence interval of 95 % (p-value of 0.05). R^2 values were also utilised to indicate the accuracy of the correlation between the recorded and predicted algal growth rates. Additionally, root mean square error (RMSE) was utilised to calculate the amount of error between the recorded and predicted algal biomass concentration data sets. The RMSE was also utilised to help identify if the data points from the pH altered runs were accurately predicted by the model. T-tests assuming unequal variance, utilising a confidence interval of 95% (p-value0.05) were also utilised to compare to predicted and experimentally obtained biomass concentrations after 1 day, 2/3 days and 4/5

days. Graphs, trendlines and statistical testing were compiled and performed using Microsoft Excel 365.

5.3 Model Development, Justification and Validation

5.3.1 Modifications to the Steele Model

The above statements and modification to the Steele equations are discussed and justified in this section. The first modification to the Steele Model discussed will be the use of a linear relationship in the predictive growth model instead of an exponential relationship. The changes in biomass concentration versus time (i.e. growth rate) for each of the unaltered HRAP runs were plotted, and the R^2 values for the linear and exponential relationships were determined, see Table 5.1. The growth rates utilised in this comparison and throughout the model validation section were selected based on the ammonia concentrations of each run. When the ammonia concentration was below 1.0mg/L, algal growth was considered to be limited by low ammonia availability, and these data points were not utilised. If the ammonia concentration dropped below 1.0mg/L during a run, the growth rate was then determined based on the biomass concentration and time passed at the next sampling time point. For example, if the initial ammonia concentration of a run was 5.0mg/L and on day three it was <1.0mg/L, the growth rate would be determined based on three days of growth. This was done as it was not possible to determine precisely when the ammonia concentration dropped below 1.0mg/L. Furthermore, the ammonia concentration of 1.0mg/L and not the previously mentioned minimum concentration of 0.3mg/L was used as it was not possible to know precisely when the ammonia concentration reached 0.3mg/L, and use of a 1.0 mg/L cut-off was conservative.

Table 5.1: R2 values based on time versus biomass concentration for each separate HRAP run.

	Linear R^2	Exponential R^2
25/05/2016	0.9767	0.9757
3/06/2016	0.9483	0.9934
18/07/2016	0.9786	0.9900
31/08/2016	0.0112	0.0206
19/09/2016	0.5595	0.1317
5/10/2016	0.9917	0.9629
19/10/2016	0.9713	0.9713
16/11/2016	0.7068	0.6712
2/12/2016	0.9924	0.9676
12/12/2016	0.8846	0.7394
6/01/2017	0.5967	0.6318
16/01/2017	0.8957	0.8581
13/02/2017	0.8935	0.8563
6/03/2017	0.8820	0.9083
13/03/2017	0.7923	0.7835
10/04/2017	0.8313	0.8193
1/05/2017	0.9820	0.9875
29/05/2017	0.5578	0.5152
4/07/2017	0.7054	0.7181
7/08/2017	0.5040	0.4741
11/09/2017	0.7065	0.7851
2/10/2017	0.6544	0.5767
27/10/2017	0.8750	0.8130
15/11/2017	0.9098	0.9492
29/11/2017	0.9479	0.9220
12/12/2017	0.9832	0.8956
11/01/2018	0.9324	0.8449
22/01/2018	0.7039	0.6414
5/02/2018	0.6000	0.5889
Mean	0.7922	0.7584

The mean R^2 values for the linear algal growth of 0.79 and exponential algal growth of 0.76 were similar. An ANOVA and t-test analyses were performed on the two sets of R^2 value, and this showed no difference between the two datasets. As there was no significant difference between the two datasets, the linear relationship was selected for further research. The use of a linear relationship removed some of the complexity of the original model and did not decrease the accuracy. It is well known that algae grow exponentially under optimum conditions (Brennan and Owende, 2010, Chisti, 2007). However, as stated in the earlier chapters, the conditions in the HRAPs utilising secondary lagoon effluent were not optimal, and the water temperature and solar radiation fluctuated and were not kept within an ideal range. The suboptimal and non-steady state conditions limited the algal growth. This limited algal growth would result in a decline in growth rate and cause the algal growth to significantly slow. If the algal growth is sufficiently slowed and limited the exponential growth rate of the algae would approach a value of 1. This would indicate that the growth of algae is occurring in a linear manner and not occurring exponentially. This allows for a linear growth rate to be utilised which is simpler and easier to use.

The second modification to the Steele model was to incorporate daily mean light intensity in the model and the addition of daily mean temperature to account for the changes in temperature throughout the day. This was done as it was previously shown that both temperature and light intensity have a significant impact on algal growth (Huesemann et al., 2016, Béchet et al., 2015). It was essential to understand how strongly the light and temperature variables were related to the algal growth rate, and this was assessed by determining the R^2 values of light and temperature plotted against the observed experimental algal growth rates. Firstly, the effect of water temperature was investigated. The mean water temperature and observed experimental growth rate were plotted against each other. This returned an R^2 value of 0.62 which does not indicate that water temperature had a particularly strong effect on algal growth rate. However, it suggests that temperature does affect algal growth, and this assumption is well supported in published literature and by experimental data in the earlier chapters (Talbot et al., 1991, Singh and Singh,

2015, Huesemann et al., 2016). Secondly, the effect of light intensity or solar radiation on the algal growth rate was investigated. The mean solar radiation was plotted against the observed experimental growth rate. This returned an R^2 value of 0.65. Again this does not indicate that solar radiation had a particularly strong effect on algal growth rate but suggests that there was a relationship between solar radiation and algal growth which was also supported by previous literature (Steele (1962), Béchet et al. (2013), Singh and Singh (2015)) and the earlier chapters. Furthermore, the effects of differing photoperiods of light and dark were investigated and provided the same R^2 value as the solar radiation relationship; this was because the dark and light periods were already accounted for, as the mean daily solar radiation values were utilised. In addition to these relationships, it is well known that water temperature and solar radiation are linked to each other, and as solar radiation increases so does water temperature (Edwards et al., 2016). By incorporating both the mean water temperature and mean solar radiation, it was possible to observe the effect both variables had on algal growth simultaneously. A simple multiplication of daily mean temperature and daily mean solar radiation was plotted against the observed experimental algal growth rate, see Figure 5.1.

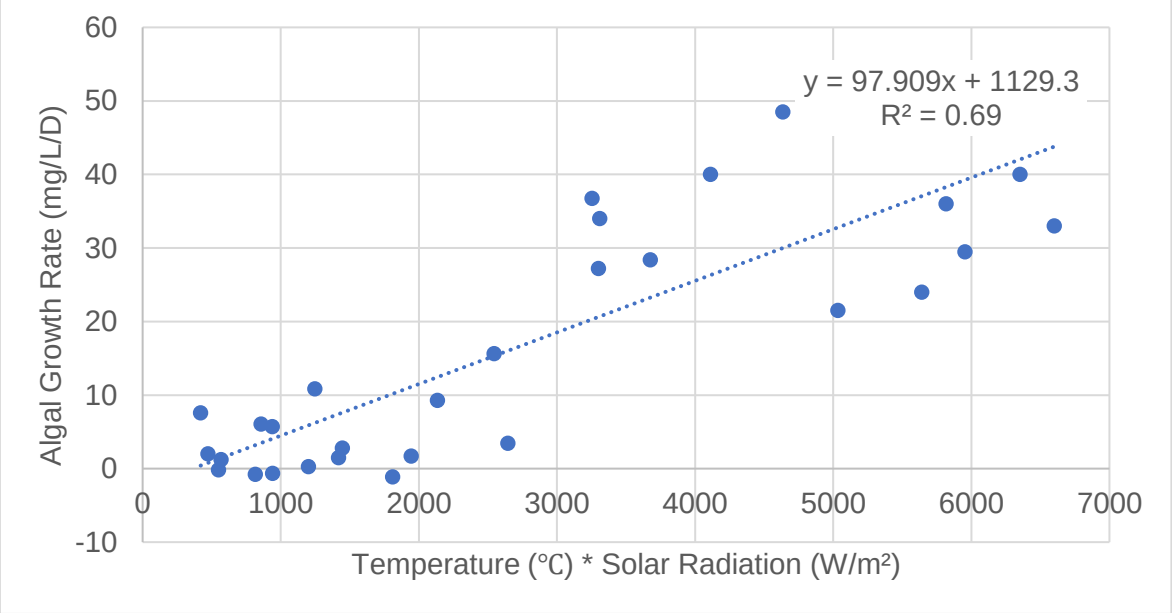

Figure 5.1: Experimentally determined algal growth rate (mg/L/D) versus mean temperature (°C) multiplied by mean solar radiation (W/m²).

This combination of temperature and solar radiation was determined to have a stronger relationship to algal growth rate than the two factors separately as shown by the increase in the R^2 value, 0.69. Therefore, this combination was utilised in the model development and the light variable in the Steele model was replaced by light and temperature variables. The biomass growth rate predicted by the modified Steele model, equation 5.1, was plotted against the growth rates determined from the experimental work and an R^2 value of 0.69 was achieved, see Figure 5.2.

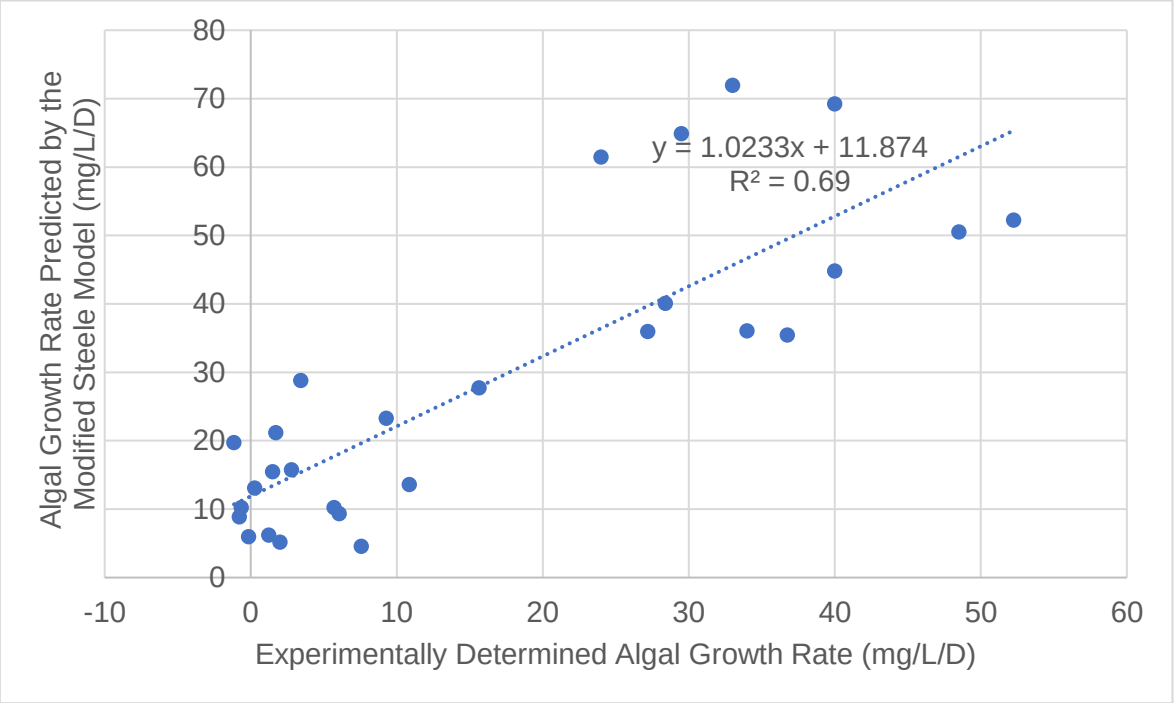

Figure 5.2: The algal growth rate predicted by the modified Steel model (mg/L/D) versus the experimentally determined algal growth rate (mg/L/D).

An R^2 value of 0.69 is not a strong enough relationship to support using this modified Steele model by itself, and some further modifications were necessary. As can be seen, in Figure 5.2 there was some variation between the predicted and experimentally obtained growth rates, notably as the growth rates increased. The variation suggests that there are some factors limiting growth, and these limitations could be in the secondary lagoon effluent or caused by the surrounding environment. One of the main limitations determined in previous chapters was light attenuation or self-shading. To account for this limitation, further modifications to the model were performed. In order to adjust the model to incorporate self-shading, it was imperative to know how much self-shading

was caused by the biomass concentration. To do so the Secchi disk versus biomass concentration relationship determined in Chapter 3, Figure 3.5, was utilised. The equation from Figure 3.5 was utilised to determine the depth (cm) of the euphotic zone in the HRAP's water column. This depth (cm) was then divided by the total depth (30cm) of the HRAP to give a value representing how much of the HRAP water column received sufficient light for algal growth, see equation 5.3a and 5.3b.

The final model, Steele-Secchi, was the modified Steele equation multiplied by the Secchi equation to provide a growth rate (mg/L/D) which was determined by the solar radiation, temperature and the self-shading in the HRAPs.

5.3.2 Model Validation

In order to validate the Steele-Secchi model, the predicted growth rate and the experimentally observed growth rate were added to the initial biomass in the HRAPs (see equation 5.5a and 5.5b). By doing so, it was possible to compare the predicted biomass concentrations to the biomass concentrations obtained during the experimental HRAP runs.

$$\omega = \mu_f + \beta_i \quad (5.5a)$$

$$\omega_e = \mu_e + \beta_i \quad (5.5b)$$

Where ω is the predicted biomass concentration of the HRAP (mg/L), μ_f is growth rate predicted by the Steele-Secchi model, β_i is the initial biomass of the HRAP, ω_e is the experimentally observed biomass concentration of the HRAP (mg/L), and μ_e is the experimentally observed biomass growth rate (mg/L/D). These equations were plotted against each other, and the results are shown in Figure 5.3.

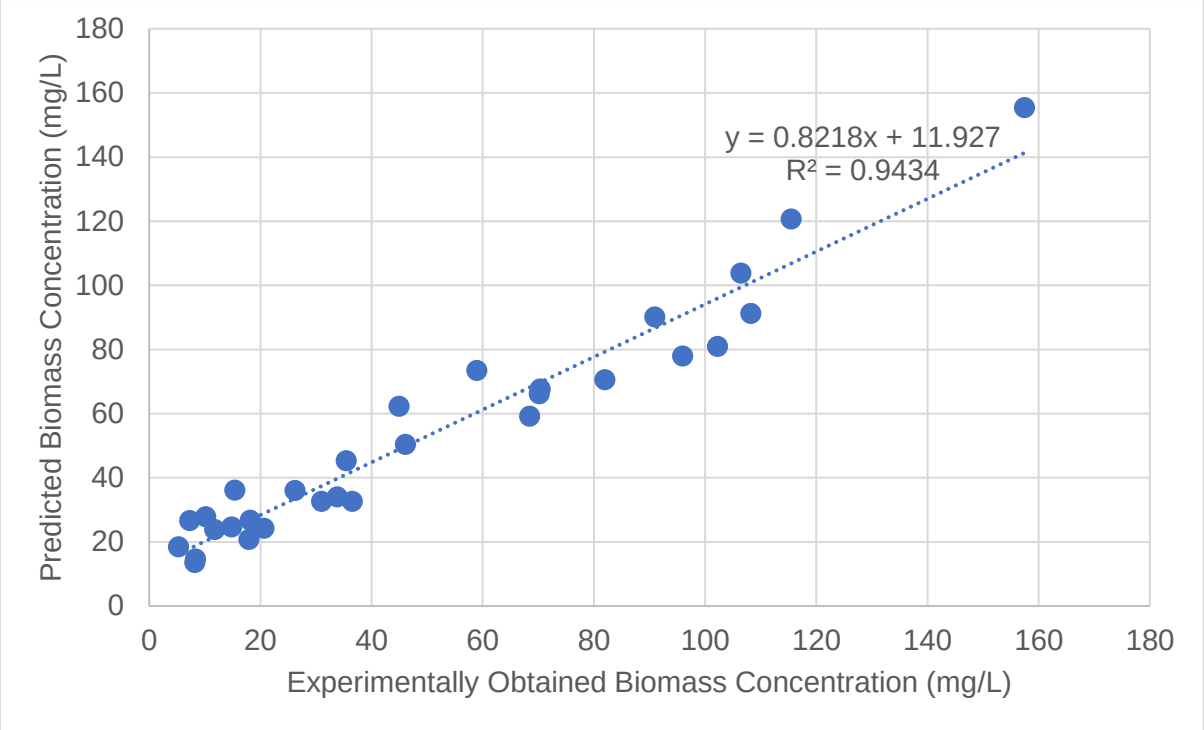

Figure 5.3: Predicted biomass concentration versus the experimentally obtained biomass concentration of the unaltered secondary lagoon effluent HRAP runs for one day of growth.

As seen in Figure 5.3, this comparison of values had an R^2 value of 0.94 which indicates a very strong relationship. This strong relationship implies that the developed Steele-Secchi model can be employed to accurately predict the biomass concentration in a HRAP utilising secondary lagoon effluent as its growth media. To further validate the model, the results from runs which were performed under altered conditions were added to the dataset, and the validation was repeated. In order to determine if the new data points from the altered HRAP runs accurately fit the model, an RMSE analysis of the difference between the predicted and experimentally obtained biomass concentrations was performed. If the data point was larger than the RMSE, it implies that the model did not accurately predict the biomass concentration. The RMSE for the difference between the unaltered runs was 11.2 mg/L.

5.3.3 Model Validation under Altered Conditions

5.3.3.1 Primary Lagoon Effluent

The results from the runs which utilised primary lagoon effluent instead of secondary lagoon effluent as the nutrient source were the first to be added to

the dataset. The Steele-Secchi model was able to predict new data points from these results, and this new dataset was validated utilising the same methods previously described in section 5.3.2. The two new sets of biomass concentrations were plotted against each other, and the graph is shown in Figure 5.4.

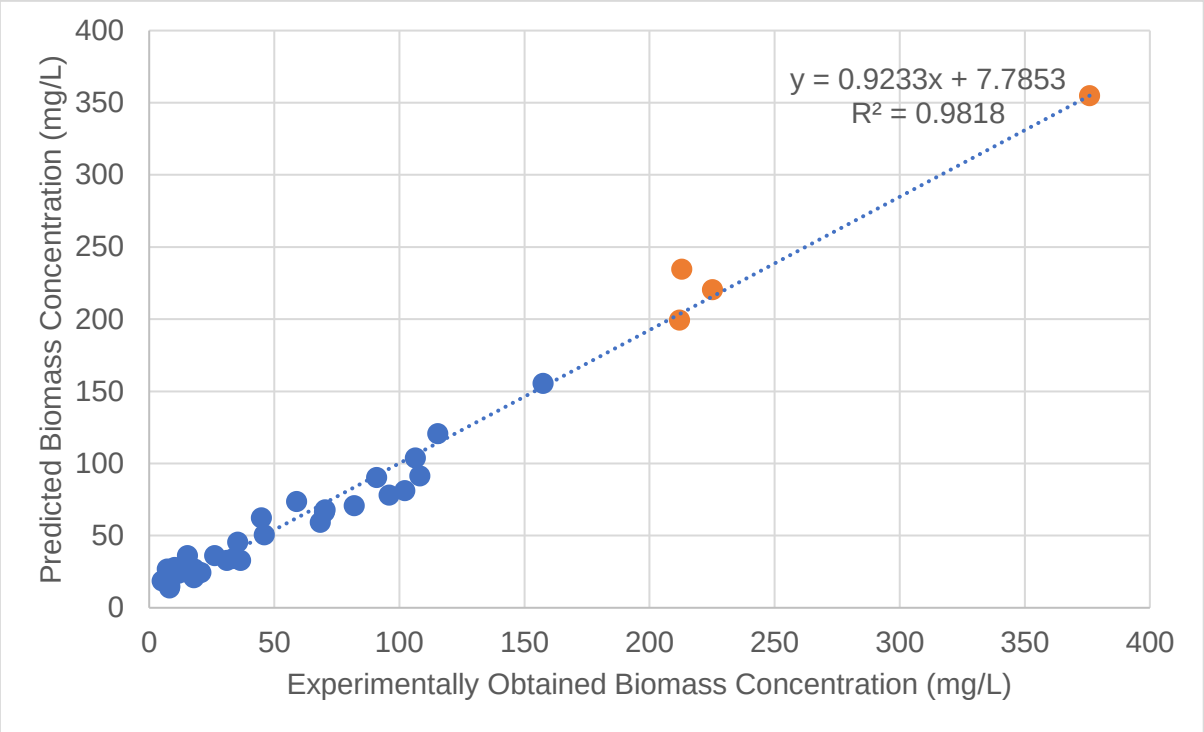

Figure 5.4: Predicted biomass concentration versus the experimentally obtained biomass concentration of the unaltered secondary lagoon effluent HRAP runs (blue) and the primary lagoon effluent runs (orange) for one day of growth.

Figure 5.4 has an improved R^2 value indicating that the results from the runs in which the primary lagoon effluent was utilised can be accurately predicted by the Steele-Secchi model. This demonstrates that the growth rates for runs which had high biomass concentrations were able to be accurately predicted, which was not determinable with the previous dataset. The improvement of the R^2 value from 0.94 to 0.98 suggests that the new dataset further supports the use of the Steele-Secchi model. The improvement of the R^2 value after the inclusion of the high biomass concentration results may imply that the model is less accurate at low growth rates. The lower accuracy at low growth rates may be due to some of the experimentally observed growth rates

being zero or negative at low temperatures, which would indicate a higher respiration rate than photosynthetic rate.

The mean difference between the predicted and experimentally obtained biomass concentrations of the primary lagoon effluent runs was an underprediction of the biomass concentration by 4.3 mg/L. Thus, as it was smaller than the RMSE, it can be implied that the model was able to predict the biomass concentration accurately. The runs which utilised primary lagoon effluent in the HRAPs were initially expected to provide a nutrient-rich media which would enhance the algal growth rate. However, due to the high biomass concentration, there was significant self-shading. The potential for an enhanced growth rate caused by the elevated nutrient concentration was reduced as the majority of the algae in the HRAP were not able to receive sufficient light for photosynthesis. The two factors, elevated nutrient concentrations and self-shading balanced each other, and the growth rate was able to be predicted by the Steele-Secchi model.

5.3.3.2 pH-controlled Runs

The second set of results added to the dataset were the results from the runs in which the pH was controlled. The Steel-Secchi model was used to predict new data points, and they were added to the dataset. The new dataset was validated utilising the same method used previously in section 5.3.2. The new sets of biomass concentrations were plotted against each other, and the result is shown in Figure 5.5.

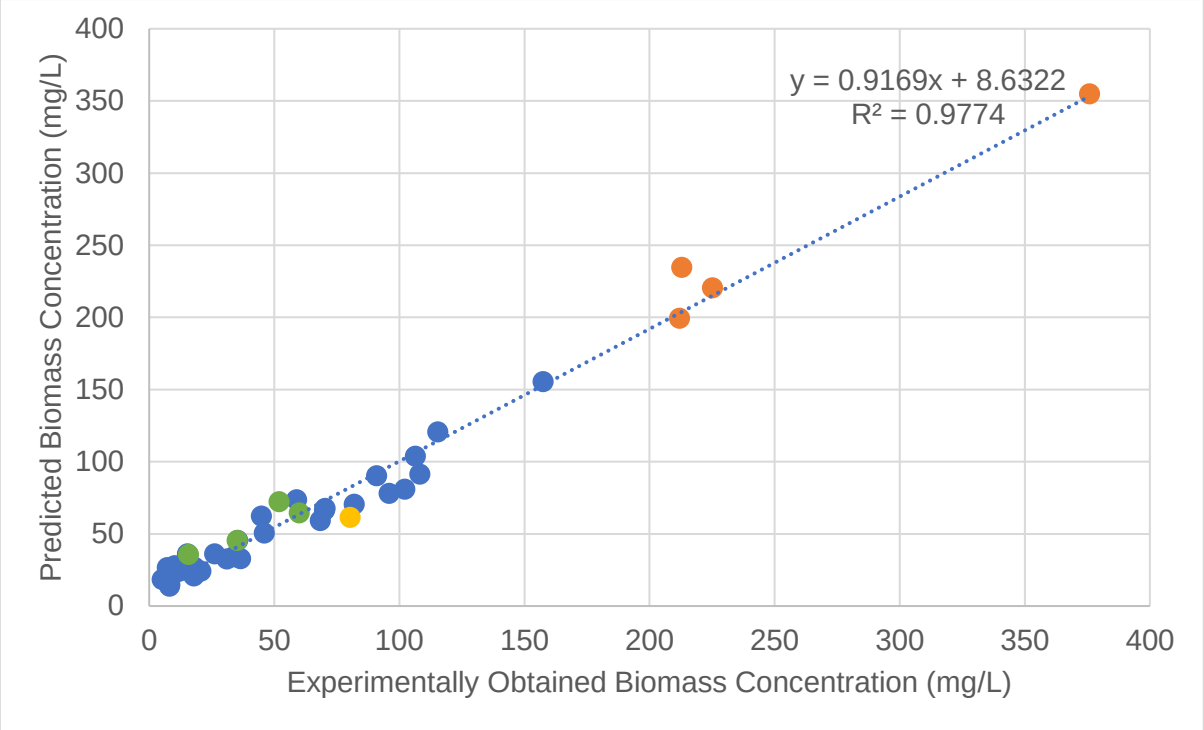

Figure 5.5: Predicted biomass concentration versus the experimentally obtained biomass concentration of the secondary lagoon effluent HRAP runs (blue), primary lagoon effluent runs (orange) and the pH-controlled runs (HCl- green) (acetic acid- yellow) for one day of growth.

Figure 5.5 has a negligible decrease in the R^2 value from the previous validation (0.9818 to 0.9774). As there is a marginal difference, the R^2 value cannot be utilised to suggest if the model was able to predict the biomass concentration accurately. The RMSE was used to determine the accuracy. The mean difference between the predicted and experimentally obtained biomass concentrations for the experiments which utilised inorganic acid to control the pH indicated an over-predicted by the model by 13.6mg/L. This was larger than the RMSE for the unaltered runs and implies that the model was not able to accurately predict the biomass concentration for these runs. However, as this result was very similar to the RMSE for the unaltered runs and due to the small number, four, of pH-controlled runs which utilised inorganic acid that were utilised for validation, further work is required to confirm this result.

The runs in which the pH was controlled were expected to enhance the biomass concentration. However, as explained in chapter 4, the control of the pH utilising inorganic acid did not significantly enhance the biomass concentration during all the runs, and several runs were adversely affected as a

result of the addition of inorganic acid. This caused the model to overpredict the biomass concentration for these inorganic acid runs. In contrast to this, the run in which an organic acid was utilised to control the pH of the HRAPs was underpredicted by the Steele-Secchi model by 19mg/L. As this was higher than the RMSE, it can also be implied that the model was not able to accurately predict the biomass concentration when organic acid was utilised to enhance biomass production. However, there was only one run in which the pH was controlled utilising organic acid and more runs are required to confirm this result. Both these results suggest that the model was not able to accurately predict the biomass concentration when the pH was controlled utilising acid. This outcome supports the use of the Steele-Secchi model as a method to predict algal biomass concentration in an elevated pH environment.

The Steele-Secchi model was shown to accurately predict the biomass concentration of the HRAPs under both natural and some altered conditions. It is conceivable to suggest that the model can be used to predict the biomass concentration of HRAPs utilising a number of different media sources when algae are grown in an elevated pH environment.

5.3.4 Validation over Time

The previous validations were done utilising the predicted and experimentally obtained growth rates for one day. This section investigates the accuracy of the model over extended periods of time, namely, 2/3 days and 4/5 days. The predicted biomass concentration was obtained by recalculating the growth rate determined by the Steele-Secchi model and adding this to the previous day's biomass concentration. This was done as the light attenuation factor of the model was required to be recalculated for each day to ensure that the model accurately represented the growth rate based on the current biomass concentration of the HRAP for that day. The experimentally obtained biomass concentrations for days two and three were plotted together and days four and five were also plotted together. Figures 5.6 and 5.7 display the biomass concentrations predicted by the Steele-Secchi model versus the biomass concentration experimentally observed data after two/three days and four/five days. The results for two days are plotted together on the same graph as there

were insufficient data points for only one day at a time. The results from the unaltered experiments and the primary lagoon effluent experiments were utilised for the validations of days two/three biomass concentrations as the model was shown to be unable to accurately predict the biomass concentration of the pH-controlled experiments. The validation of days four/five biomass concentration only utilised data from the unaltered HRAP experiments as the ammonia in the primary lagoon effluent experiments was depleted after three days.

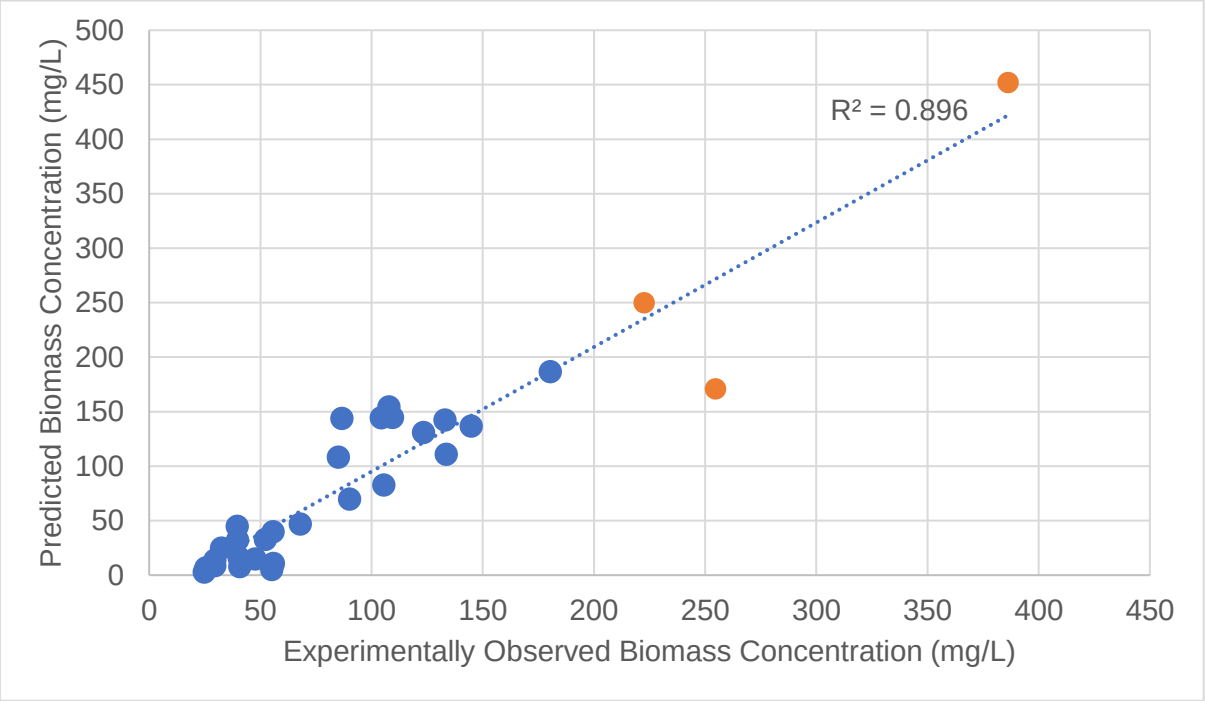

Figure 5.6: Biomass concentrations of the HRAPs after two/three days utilising the predicted and experimentally obtained biomass concentrations. Unaltered secondary lagoon effluent HRAP runs (blue) and primary lagoon effluent runs (orange).

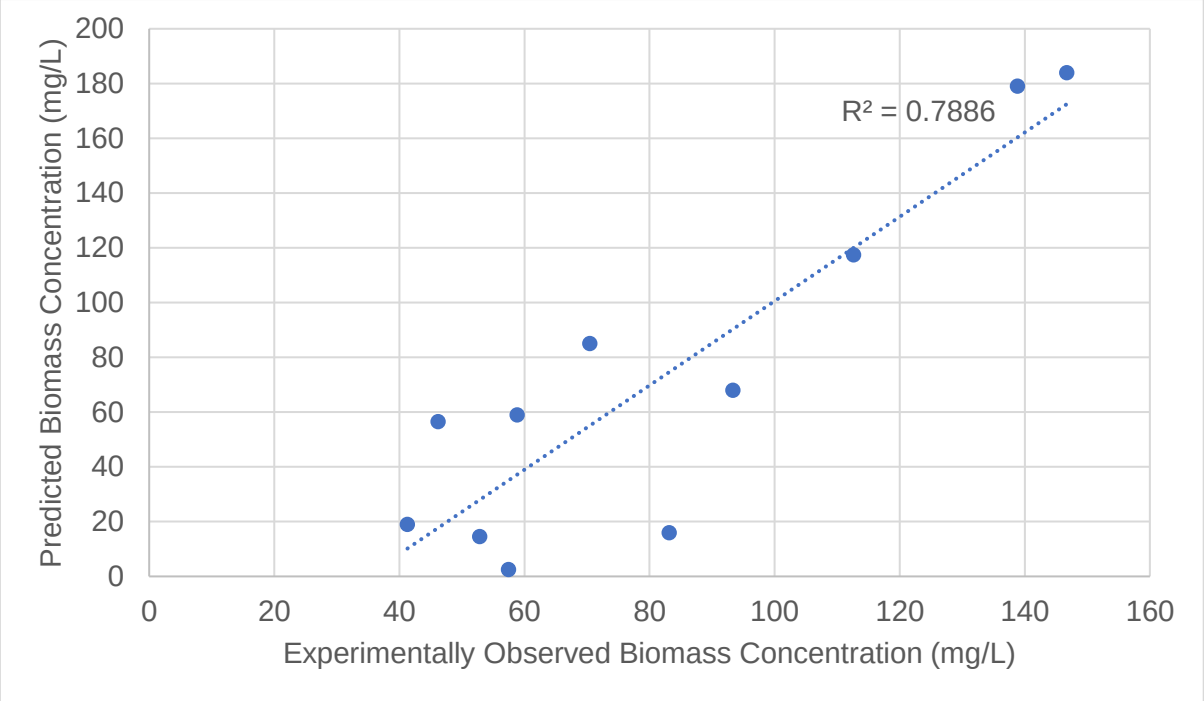

Figure 5.7: Biomass Concentrations of all the HRAPs after 4/5 days utilising the predicted and experimentally obtained growth rates, unaltered secondary lagoon effluent HRAP runs (blue).

The correlation between the biomass concentrations predicted by the Steele-Secchi model and the experimentally obtained biomass concentrations varied with time. The longer the prediction period, the less accurate the model predictions were. The R^2 value decreased with time starting at 0.98 after 1 day, 0.90 after 2/3 days and reduced to 0.79 after 4/5 days. An R^2 value of 0.79 is still a moderately strong prediction of correlation and still implies that the model is accurately predicting the algal biomass concentration. The decrease in accuracy may be due to the large variation of different algal species being present in the HRAPs, each having their own unique optimum conditions. The decrease in the R^2 value could also be due to the reduced number of data points. Additionally, the experimental errors are higher and the model less accurate at low growth rates due to the possibility of zero or negative growth at low temperatures. As the high growth rates experiments concluded after three days, the data for growth on days four/five contained a higher proportion of low growth rate experimental data and had fewer data points that could act to reduce the fit between data and model predictions. Furthermore, over time nutrient deficiencies can affect the growth rates and this may be present on

days four/five. Several t-tests were performed to compare the biomass concentrations predicted by the Steele-Secchi model and the experimentally observed biomass concentration after one day, two/three days, and four/five days. All the t-tests found there to be no statistical difference between the two datasets, suggesting that the Steele-Secchi model was able to accurately predict the biomass concentration of the HRAPs over these timeframes.

5.4 Discussion

The above validations verify that the Steele-Secchi model can accurately predict the concentration of algal biomass in HRAPs which utilise various types of wastewater as their influent. The Steele-Secchi model was able to account for high biomass concentrations which cause self-shading and increased respiration. All the HRAP runs utilised to validate the Steele-Secchi model had an elevated pH level which indicates a carbon deficiency and the Steele-Secchi model was able to predict the biomass concentration of those runs accurately which has not been done previously. The model was validated over a growth rate range of 3-50mg/L/D and the initial biomass concentrations of the experiments ranged from 5- 338mg/L. The highest biomass concentration obtained in these experiments, 452mg/L, was less than the typical peak biomass concentrations from other research of 500mg/L. This may be due to the large number of different algae present in the HRAPs at one time.

It is important to note that the Steele-Secchi model does not include the impact of nutrient limitation other than assuming low carbon concentration as a result of the elevated pH of the system. The impact of nitrogen limitation was reduced by only utilising data from runs and time points which had sufficient ammonia concentrations to support growth. The reduction in nutrients as the biomass increases would need to be addressed if the prediction of algal growth over extended periods of time is required.

The model described in this chapter is different from the previously published work compiled from this research, Wrede et al. (2018). Wrede et al. (2018) was published utilising the first 12 months of data from the experimental period and was not validated against the second 12 months of control data and

the data from the altered runs. The first part of the model proposed by Wrede et al. (2018) is the same as the modified Steele model proposed in this chapter, with the exponential function was removed, and the light intensity variable replaced by a daily mean solar radiation and the incorporation of mean daily temperature. However, the second part of the two models differ. The model proposed in Wrede et al. (2018) included a secondary model, the Monod model, which accounted for low ammonia concentrations which occurred during some of the runs. The Monod model was utilised to adjust for overpredicted biomass growth rates caused by high solar radiation and temperature values when the ammonia concentrations were low, which happened most commonly in summer and spring. A similar overprediction occurred in this chapter but was alternatively accounted for by including the Secchi equation which accounted for the effect of self-shading. The data utilised in this chapter utilised growth rates from points in each run which had a sufficient ammonia concentration to support growth (>1.0mg/L). Therefore, the impact of ammonia limitation was not deemed to have a significant impact on the prediction of the algal biomass concentration in the HRAP for the results examined and was thus not included in the Steele-Secchi model. Furthermore, the addition of the Monod model into the Steele-Secchi model was trialled, however, this caused a large number of results to be underpredicted. Therefore, it was not included in this work. The addition of two equations with the purpose of reducing the overprediction of the model would be counterproductive and the model would underpredict the algal growth rate. The Steele-Secchi model proposed in this chapter provided a more accurate prediction than that of the model previously described by Wrede et al. (2018).

A few disadvantages which are commonly addressed in algal growth modelling are the complexity of algae's optimum growth requirements, the vast number of algal species each requiring their own model parameters, the inter and intra-species interactions between algae, light photoinhibition and limitation, nutrient depletion/ limitations and the application of the proposed model. The Steele-Secchi model addressed some of these disadvantages. Firstly, the Steele-Secchi model was validated by utilising data from outdoor HRAP cultures containing numerous naturally occurring algal species. This

demonstrates that the model can account for variations in the algal community as well as how the algal interact with each other. However, the highest biomass concentrations obtained in this research were below the typical peak biomass concentration of 500mg/L (Chisti, 2016). This lower biomass may be due to the large number of different algal species present in the HRAPs. Secondly, the effects of photoinhibition and photo limitations or self-shading have been accounted for in the model and the design of HRAPs. The use of a simple paddlewheel moves the algae on the surface of the HRAPs water column, which under extreme conditions may be affected by high solar radiation and exhibit the effects of photoinhibition, to lower in the water column. This process is continuously occurring, and this allows for the algae to reach a maximum saturation point for light after which it is then removed from the light and can repair damage caused by photoinhibition. Thirdly, nutrient depletion, the model was validated utilising both primary and secondary lagoon effluent samples which were carbon limited as indicated by an elevated pH, and the model was able to predict the biomass concentration under these conditions accurately. As the HRAPs were mixed with a large paddlewheel relative to their volume they were assumed to be uniformly mixed. This would include the concentrations of dissolved oxygen and dissolved carbon. The dissolved oxygen concentrations, which were near or above saturation point for the majority of the time, were spot checked in a number of different spots in the HRAPs to confirm the uniformity of the compounds in the HRAPs. This uniformity may not be present on larger scale systems and this may influence production.

The Steele-Secchi model can be utilised to determine the biomass concentration of a HRAP system based on naturally occurring environmental data and the algal biomass concentrations. Utilising this data, water treatment plants can investigate if it would be feasible to implement a HRAP system to treat their effluent and produce algal biomass. The predicted biomass concentrations obtained from the Steele-Secchi model could be used to determine if the HRAPs would produce a sufficient quantity of algal biomass under natural conditions, i.e. under elevated pH conditions, or if methods to enhance the biomass production should be utilised. Additionally, the model can be utilised to determine the maximum algal biomass concentration which would

support the highest growth rate possible in an already established system. The data could be utilised to develop a harvesting regime which would promote the most effective biomass growth rate based on the impact of self-shading and biomass concentration.

5.5 Summary

A simple predictive algal growth model was developed and validated utilising secondary lagoon effluent in the south-eastern Australian climate. The Steele-Secchi model utilised the daily mean incident solar radiation, daily mean water temperature and the impact of self-shading, as a result of biomass concentration, to predict algal growth. The validation of the Steele-Secchi model demonstrated that it was accurate (R^2=0.98) in predicting the growth of algae in an elevated pH environment utilising both primary and secondary lagoon effluent. The model could be utilised to optimise algal growth in already establish HRAP treatment plants and could help to determine if a treatment plant could support the introduction of a HRAP system for algal biomass production. The Steele-Secchi model utilises simple readily available data and can be easily adapted to a variety of different circumstances if required. The model does not account for substantial changes in nutrient concentration, such as ammonia depletion over time and this may need to be investigated. However, a system which continually supplies fresh nutrients or regularly removes nutrient deplete effluent and replenishes it with nutrient replete effluent samples may not need to account for nutrient depletion. Furthermore, it may prove beneficial to test the validity of the model when utilising continuous operation rather than batch mode operation that was used in this research.

This chapter and the previous chapters have demonstrated and highlighted the critical factors required for algal growth as well as methods to enhance algal growth and additionally provided a simple and accurate algal growth model. Following on from this, the crucial next step is to harvest the algae from the water. The two harvesting methods examined in this research were the use of membrane filtration and the use of fungal flocculation.

Chapter 6 - Membrane Filtration

6.1 Introduction

Harvesting algae is a significant challenge for both algal production and algal wastewater treatment. Algal harvesting is notoriously difficult due to the algae's small size and disperse nature in suspension (Bilad et al., 2014a). There are several techniques utilised to harvest algae such as centrifugation, filtration and flocculation (Bilad et al., 2014a, Christenson and Sims, 2011, Singh and Patidar, 2018). The majority of these techniques are not feasible on a large scale due to the costs involved in running and purchasing the harvesting facilities (Milledge and Heaven, 2013, Sathe and Durand, 2015). In addition to this, some of the techniques used such as chemical flocculation can contaminate the harvested algal product making it unusable for feedstocks and requiring further refining for biofuels (Ummalyma et al., 2017). Membrane filtration is a common algal harvesting method and is commonly used in the wastewater industry to remove particles and other compounds (Bilad et al., 2014a). It is an effective method and can harvest both algal and algal organic compounds such as pigments and toxins (Liu et al., 2017). The benefit of utilising membrane filtration is that it is possible to screen for specific products while allowing others to pass through. For example, it is possible to remove algae from suspension and reuse the permeate as the growth medium for the following batch of algae (Bilad et al., 2014b). Single step membrane filtration has been shown to have a volumetric concentration factor (VCF) as high as 150 - 200 potentially resulting in a reduction of 99.5% water (Bilad et al., 2014a). This could also be achieved in a multiple step process utilising membranes and/or additional harvesting techniques such as flocculation and centrifugation (Bilad et al., 2014a). There are numerous different filtration membranes that have been utilised for algal harvesting or the removal of organic particles, including membranes comprising of PTFE, PVDF, ceramic and metal (Drexler and Yeh, 2014, Johir et al., 2013, Bilad et al., 2014a). In addition to the composition of membranes, there are several different membrane systems that can be utilised for algal harvesting including, dead-end filtration, cross-flow

filtration and submerged membrane filtration (Bilad et al., 2014a, Shekhar et al., 2017, Barros et al., 2015). There are commonly two ranges of pore sizes which been investigated for algal harvesting and are classified as microfiltration (0.1-1 μm) and ultrafiltration (0.01- 0.1 μm) (Sun et al., 2013).

In order to compare different membranes and operating systems to each other, it is essential to know the operating flux of the membrane. Understanding the flux and the variations between membranes and filtration systems is important when selecting the most appropriate membrane filtration system. The flux is the rate at which the filtered sample can be passed through the membrane and is generally recorded as litre per square meter of membrane per hour (Mathur et al., 2017). The pore size, number of pores and the membranes physical structure can influence flux. In addition to this, one of the main factors which limits flux is fouling of the membrane (Zhao et al., 2017).

There are three types of fouling; reversible, irreversible and irrecoverable. Reversible fouling refers to fouling that can be removed through physical methods such as backwashing or relaxation of the membrane. Irreversible fouling is when the foulant requires intensive chemical cleaning and maintenance to be removed. Irrecoverable fouling is permanent fouling which cannot be removed by any means. Fouling occurs when particles block the membrane pores and this can occur in two ways. The first type of pore blocking is external and occurs when cells, cell debris, and other rejected particulate matter form a layer or cake on the membrane surface and this impedes the flow of water through the pores thus reducing the flux. The second type of pore blocking is internal and occurs when particles, if small enough, such as extracellular biopolymers, adhere to the inside of the pores through intermolecular interactions, namely, hydrophobic interaction, hydrogen bonding, and interactions between the foulant and membrane surfaces. These interactions reduce the size of the pore or completely block the opening (Liao et al., 2018). There are ways to reduce fouling such as backwashing, chemical cleaning and the use of different membrane systems. Backwashing and back pulsing are useful as they can be performed during the filtration process. Backwashing can be performed with either air, water or the filtrate. A backwash or back pulse applies pressure to the sample on the permeate side of the

membrane which dislodges organic particles that have settled on the retentate side of the membrane. Backwashing can be used preventatively and as a method to remove significant reversible fouling. Backwashing can be effective at removing or reducing external pore blocking but is generally not effective at removing internal pore blocking. Chemical cleaning is normally required to effectively remove internal pore blocking. Chemical cleaning can remove a significant amount of both internal and external fouling and can include the use of acidic and caustic washes (Kumar and Ismail, 2015, Bhave et al., 2012). Continual use of strong cleaning chemicals can damage polymeric membranes and reduce their lifespan. Chemical cleaning is a time-consuming process and requires the shut-down of the filtration process. Naturally, this is not desired, and other methods are preferred. Once the flux of a membrane cannot be recovered or if there is irrecoverable fouling the membrane may need to be replaced (Liao et al., 2018).

The fouling rate of membranes can be reduced through selection of appropriate membrane materials and systems. A cross-flow system reduces fouling by keeping particles in suspension (Ahmad et al., 2012). This is done by creating a high velocity of flowing water parallel to the membrane surface, thus the water acts to lift particles from the membrane surface. In line backwashing can also be utilised to minimise fouling during the filtration process. The structure and surface chemistry of the membrane also helps to reduce fouling and should be selected carefully. An example of this is the difference between a hydrophilic membrane and a hydrophobic membrane. Sun et al. (2013) demonstrated that a PVDF membrane with a hydrophilic modified surface showed very little fouling when filtering algal samples compared to a hydrophobic PVDF membrane. Additionally, the strength of a membrane is also important, as stronger membranes would allow for higher pressures to be used in both filtration and cleaning processes. More robust membranes such as the ceramic and metal membranes can be used with more stringent cleaning processes that utilise harsher chemicals and higher pressure backwashes (Xie et al., 2015). These higher pressures and harsher cleaning processes reduce the fouling of the membrane and therefore would reduce the need to replace it. There is a considerable amount of research on the use of PVDF membranes for

algal harvesting but there is only a small amount on ceramic and PTFE membranes, and none utilising metal membranes to harvest algae. There are several studies investigating the use of metal membranes to treat wastewaters and remove organic matter, but to the researcher's knowledge none specifically on the removal of algae. The cost of the membrane is crucial as metal membranes and ceramic membranes are significantly more expensive to make than the PTFE or other polymeric membranes. However, the higher flux of these membranes can make this extra cost a viable option. As irreversible fouling is a common problem, a membrane which fouls less but is more expensive can be the economical option in the long run.

This study investigated the use of three different membranes each of which was used in a different membrane system. The aim was to determine which membrane and membrane system performed the most efficiently. Firstly, a ceramic membrane was tested in a cross-flow filtration system. Secondly, a bundle of PTFE hollow fibre membranes was tested in a submerged membrane system. Lastly, a bundle of metal tubular membranes was tested in a larger cross flow system. All the membranes used are classified as microfiltration membranes and have a hydrophilic surface. The capital cost of the membranes and sustainable operating flux was used to identify which membrane was attractive for further investigation use.

6.2 Material and methods

6.2.1 Ceramic Membrane System

The characteristics of the cross-flow ceramic membrane system utilised in this study are listed in Table 6.1. The membrane system was supplied by Metawater Co., Ltd. The ceramic membrane was housed inside a cross-flow module. A pump was utilised to push the effluent sample across the ceramic membrane and through the system. The pump was controlled by a frequency inverter at 25 Hz and had an initial pressure of 3 bar. The experimental set up for the ceramic membrane system is displayed in Figure 6.1, and the operating conditions are tabulated in Table 6.2.

Table 6.1: Characteristics of the Metawater ceramic membrane.

Characteristics	**100nm Ceramic Membrane**
Length (cm)	10
Outer diameter (cm)	18
Channel diameter (cm)	2
Number of channels	55
Support material	α-Al_2O_3
Membrane surface area (m^2)	0.04

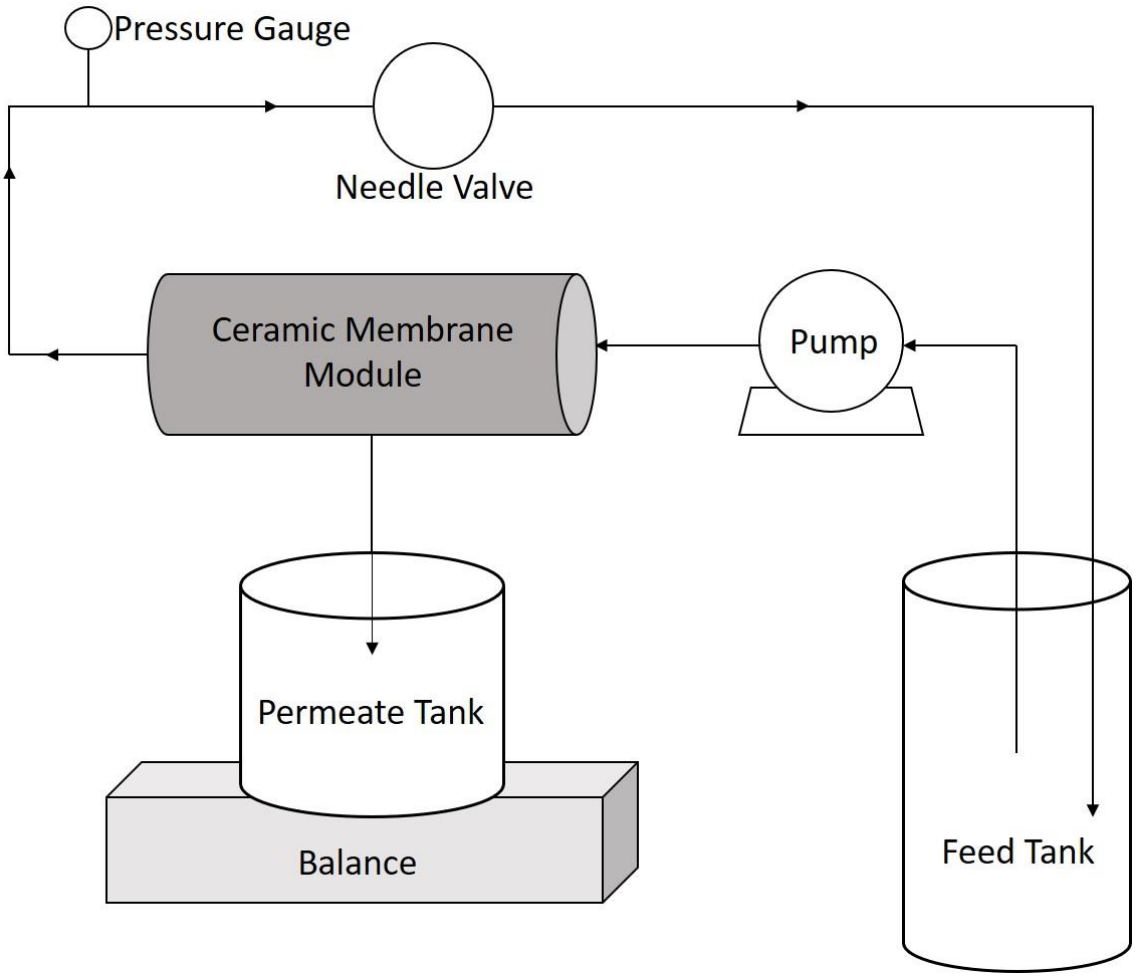

Figure 6.1: Experimental set up for the ceramic membrane system.

Table 6.2: Experimental operating conditions of the ceramic cross-flow filtration system.

Parameter	**Setting**
Pump Frequency Setting	25 Hz
Needle valve setting	Fully open
Pressure	3 Bar
Feed Flow rate (g/min)	4057
Total Volume (mL, reservoir + pump and lines)	1500
Temperature (°C)	20 (RT)

Two sets of experiments were performed each consisting of filtering three batches of primary lagoon effluent containing algae. Each experiment followed the plan outlined in Table 6.3.

Table 6.3: Plan for ceramic membrane flirtation experiment.

Stage	**Description**
1	Clean water flux test on the clean membrane for 5 minutes
2	Primary lagoon effluent filtration performed until 1000mL of permeate was achieved
3	Backwash performed utilised distilled water
4	Clean water flux test on the fouled membrane for 10 minutes
5	Repeat stages 2-4
6	Repeat stages 2-4

It should be noted that each backwash led to a loss of water due to a release by the pumps pressure valve. The backwashes were started at a lower pressure than the filtration experiments and slowly increased, starting at 12.5Hz up to 25Hz. A connection on the rig was also prone to leaking during the backwashes. The leak was between the membrane and the connection for the pressure gauge. The connection was checked and tighten, but the leaking

continued. The system did not leak or release the pumps pressure valve during normal filtration operation.

Each ceramic membrane experiment used a balance connected to a computer to record the weight of the filtrate during primary lagoon effluent filtration and to determine the clean water flux. The filtrate weight was recorded at 5-second intervals. Optical density readings of the primary lagoon effluent sample were taken before and after each relative stage as previously described in Chapter 3. The membrane was cleaned with a 1000mg/L solution of sodium hypochlorite after each experiment.

Clean water flux tests are performed throughout the experiments in this chapter. They are done at the start of each experiment and provide an initial baseline of flux for the membrane system. Further clean water flux tests were performed at constant pressure, before each membrane filtration run and at the end of the experiment to measure the change in flux as a result of any irreversible or irrecoverable fouling that occurred during the previous run.

6.2.2 PTFE Membrane System

The second membrane tested was a bundle of PTFE membranes. The PTFE membranes were supplied by Sumitomo Electric Industries Ltd. The PTFE membranes are hollow fibre and were used in a submerged filtration system. The membrane bundle was completely submerged in the sample, and the filtrate was sucked through the hollow fibres by pumping from the filtrate side of the membrane (outside-in mode). The pore size of the membrane was 0.1 µm, and the module had a surface area of 0.1m². The system utilised air to create bubbles at the base of the module and the bubbles scoured the membrane surface to minimise fouling. The system was operated in a cycle of 9 minutes on and 1 minute off. This was done to help minimise fouling. The 1 minute off or ‘rest/relaxation’ period could also be utilised for a backwash period if required. No backwashing was performed during this experiment. The experimental operating conditions are tabulated in Table 6.4.

Table 6.4: Experimental operating conditions of the PTFE membrane system.

Parameter	**Setting**
TMP	10-30 kPa
Pump flow rate (g/min)	42
Bubbling air flow (L/min)	6-7
Temperature (℃)	20
Total Volume (mL, reservoir + pump and lines + top up)	3000ml

The PTFE system was initially filled with 2000ml of primary lagoon effluent and was topped up periodically once the volume dropped below 1600ml, to a total volume of 3000ml. This was done as the feed reservoir used in the system could not contain the total volume, and due to the length of the membrane module. The total filtrate for each run was between 1100ml and 1400ml. The transmembrane pressure was measured during this experiment using a TPI 665 digital differential manometer connected to a computer recording the pressure (kPa) with 5-second intervals. The permeate weight was recorded using the method previously described in section 6.2.1. Optical density readings of the primary lagoon effluent sample were taken before and after each relative stage as previously described in Chapter 3. The experimental set up for the submerged PTFE module is displayed in Figure 6.2.

The PTFE membrane was utilised in three experimental runs each with three operating cycles of 9 minutes each. Clean water flux tests were performed before each run. The change in pressure and weight were recorded during each cycle and compared. After each run, the module was submerged in a mixture of 1000mg/L sodium hypochlorite and 1000mg/L sodium hydroxide for cleaning. The mixture was circulated through the membrane system for an hour. This was followed by thorough rinsing with distilled water to remove any traces of the cleaning mixture. Prior to use, the PTFE membrane had been unused for a significant period of time. To ensure the PTFE membrane was functioning correctly it was submerged in distilled water and sparged with air for two hours.

This allowed for the membrane to acclimatise to the system and removed any particulates such as dust which may have settled on the membrane surface. Additionally, it also removed any air trapped inside the membranes fibres and ensured the fibres were wetted.

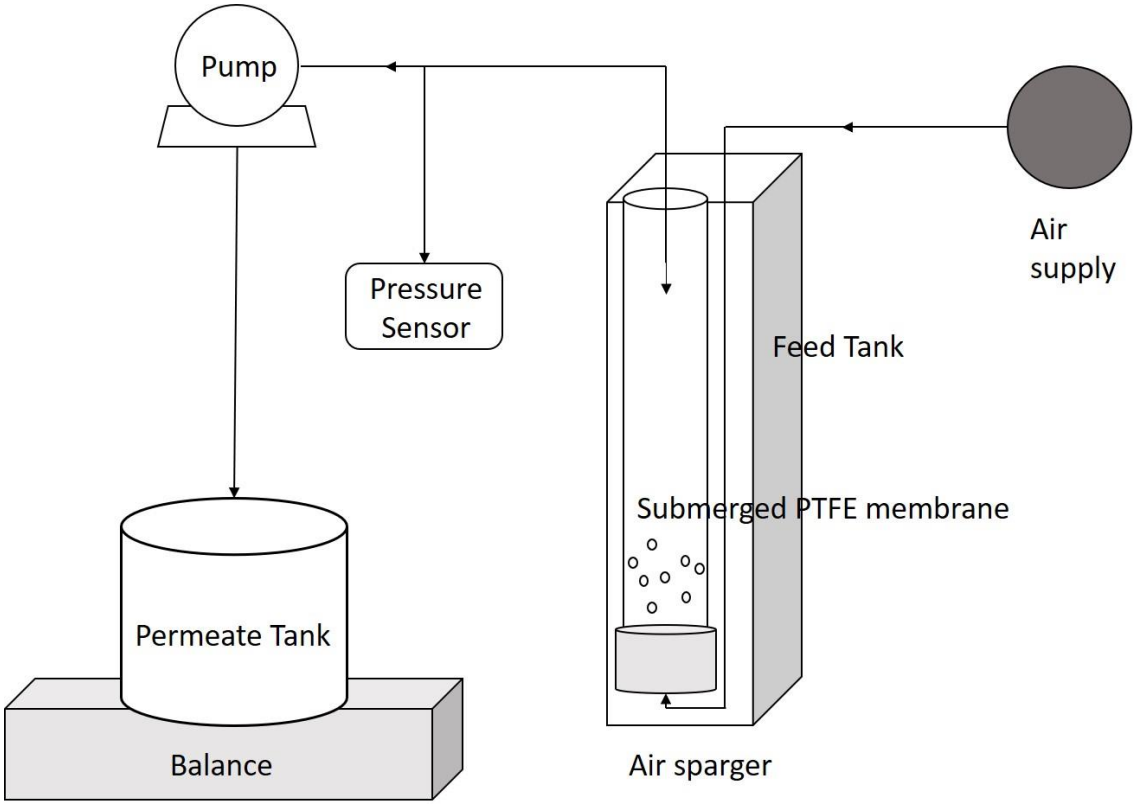

Figure 6.2: Experimental setup for the PTFE membrane system.

6.2.3 Metal Membrane system

The third membrane tested was a bundle of metal membranes. The metal membrane rig and bundle were supplied by Advanced Metallurgical Solutions (AMS), Adelaide. The bundle was 450mm long and consisted of 58 tubes with a pore size of 0.1µm and a total surface area of 0.49m². The membrane was a stainless-steel microfiltration membrane. A pump was utilised to push the primary lagoon effluent sample across the metal membrane and through the system. Each run consisted of a 10L sample and was operated in batch mode. Each run was split into two parts, in the first part roughly 3000ml of filtrate was removed, and in the second part, a further 2000ml was removed. This was done as the weighing balance used had a maximum measured weight of 3000g, and after 5000ml there was insufficient sample in the feed tank to

continue. The permeate weight was recorded using the method previously described in section 6.2.1. Optical density readings of the primary lagoon effluent sample were taken before and after each batch as described in Chapter 3. Back pulsing was used in each run to help reduce fouling of the membrane. The back pulsing was an automated feature of the rig, which supplied a burst of compressed air pressure of approximately 1.0 bar into the filtrate every 6 seconds. This provided a small backflow of filtrate to remove solids which had accumulated on the feed side of the membrane surface. Clean water flux tests were performed prior to the initial experiment, between batches 2 and 3, and after the last batch. The experimental setup is displayed in Figure 6.3. The metal membrane was cleaned before and after the experiment with a 2% sodium hydroxide solution which was circulated for 30 minutes and the system was thoroughly flushed with clean water afterwards.

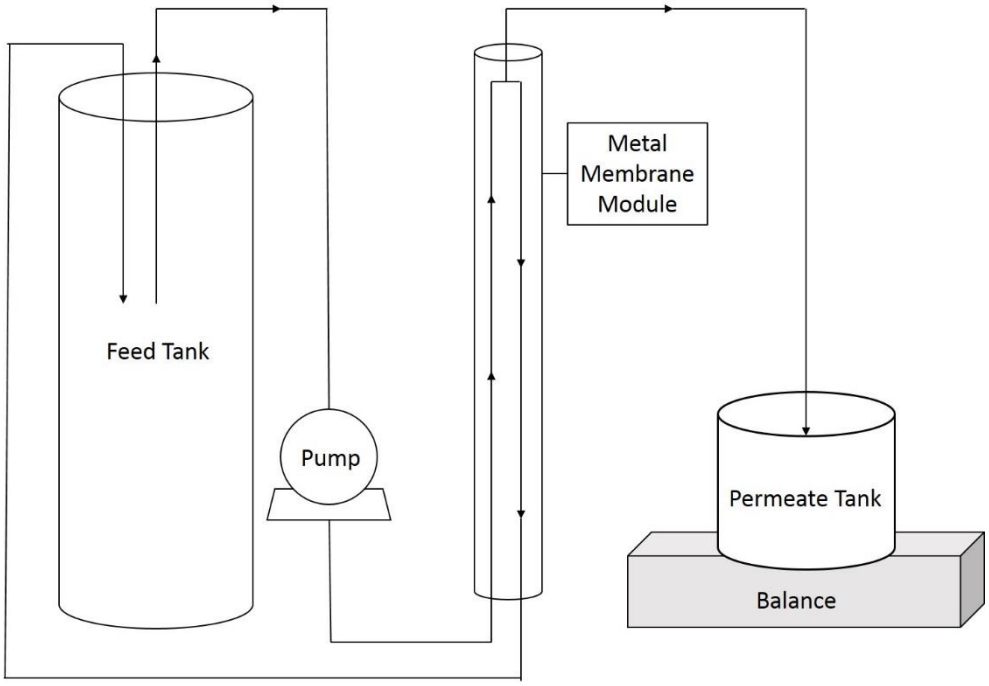

Figure 6.3: Experimental set up for the metal membrane system.

6.2.4 Description of sample

An effluent sample was collected from the first primary lagoon located at BMRWP. The primary lagoon effluent sample had a dried biomass concentration of 40mg/L. Primary lagoon effluent was chosen as filtered primary lagoon effluent was proposed in chapter 4 as a potential nutrient-rich media to

grow algae in. The sample was stored in a 20L carboy and stored at 4℃ until required.

6.2.5 Membrane Cost

Comparison of the costs of the three membranes was performed. The equation utilised to determine the capital cost of the membrane per cubic metre filtered is below, see Equation 6.1.

$$\frac{Cost}{Flux} = \frac{\$m^2}{L/m^2/h} = \frac{\$}{L/h} = \frac{\$1000}{m^3/h} \quad (6.1)$$

Where *Cost* is the price 1m^{2}of the membranes in USD $, and *Flux* was the sustained operating flux from the experimental data. The results are the capital cost of membrane capacity expressed as dollars per cubic metre of sample filtered per hour at the sustainable flux of the system.

6.3 Results and Discussion

6.3.1 Cross-flow Ceramic Membrane

The ceramic membrane was operated in crossflow configuration and the results are displayed in Tables 6.4 and 6.5. The tables show the initial and final percentage change in flux and the duration of each run. The results for the first set of runs are displayed in Table 6.4 and Figure 6.4. After perusing Table 6.4, it is evident that each clean water run acted as a cleaning step as the flux increased during the course of them. In contrast to this, the first run filtering primary lagoon effluent showed a significant flux loss of 61%. The subsequent runs displayed minor increases in flux. However, the overall flux declined by 88% over the course of the experiment. This was due to decreases in flux occurring between runs, as seen most dramatically between primary lagoon effluent run 1 and clean water run 2 in which the flux dropped from 56.22 L/m^2/h at the end of the primary lagoon effluent run 1 to 22.80 L/m^2/h at the start of clean water run 2, see Figure 6.4. This is a prime example of irreversible fouling and this could be due to the backwash damaging algal cells and causing a release of EOM which may cause some internal blocking of the membrane

pores. The amount of time required to filter 1L of permeate increased for each run due to the significant fouling of the membrane.

The rapid initial decline followed by a small gradual decline indicates that there was significant initial fouling, most likely pore blocking and potentially followed by an accumulation of a cake layer, which was not removed through backwashing and after which there was a further accumulation of fouling either through additional accumulation of the cake layer. The change in flux from the initial clean water run to the sustainable flux of the final run (approx. 20 L/m^2/h) was 156.08 L/m^2/h equalling to an 88% decline in flux.

Table 6.5: Initial and final flux of the first set of results for the filtration of primary lagoon effluent utilising ceramic membranes.

2-May-18				
	Initial flux (L/m²/h)	**Final Flux (L/m²/h)**	**Decline in Flux (%)**	**Time (min: sec)**
Clean Water 1	167.27	175.08	-5%	08:25
Primary Effluent 1	143.08	56.22	61%	15:25
Clean Water 2	22.80	28.92	-27%	16:00
Primary Effluent 2	25.97	29.10	-12%	34:35
Clean Water 3	13.98	19.34	-38%	30:00
Primary Effluent 3	17.25	21.39	-24%	38:05
Clean Water 4	19.34	20.03	-4%	12:20
Overall			88%	154:50

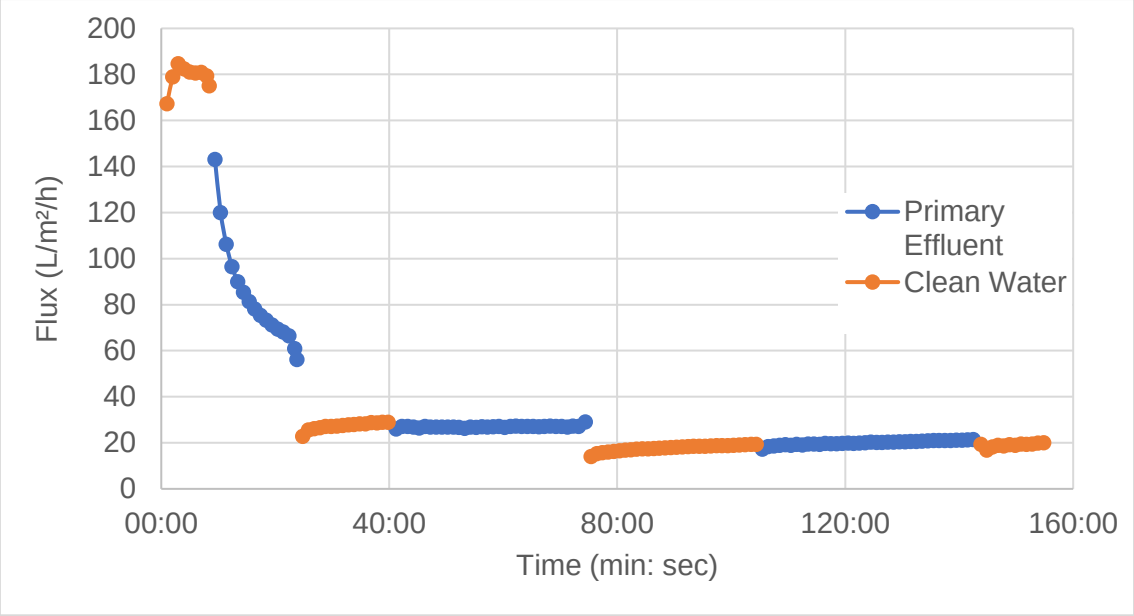

Figure 6.4: The flux over time for the first set of crossflow ceramic membrane runs. Displaying changes in clean water and primary lagoon effluent.

The results for the second set of runs are shown in Table 6.5 and Figure 6.5. This second set of runs was performed after the membrane had undergone a caustic clean in order to try and remove the irreversible fouling. The cleaned membrane had a higher initial clean water flux, indicating that the membrane may have been fouled by organic compounds prior to the first set of runs. The two sets of runs displayed similar patterns in flux behaviour with a rapid decline in flux followed by a plateau phase. During the second set of runs, the plateau phases consisted of slight decreases in flux after filtration of the primary lagoon effluent and after each backwash followed by slight increases in flux through the clean water runs. The second set of runs performed better than the first set of runs with higher fluxes and a slight recovery in flux after each clean water flux test. However, after examining Table 6.5 it was evident that there were still large amounts of fouling occurring with an overall decline of 81%. The initial run of this second set of runs behaved almost identically to the initial run in the first set with a flux decline of 60%. Due to the higher starting flux and higher fluxes overall the second set of runs was conducted much faster than the first set of runs.

The problem of low recovery of flux after the backwashes caused by rapid irreversible fouling is a major concern. A membrane system which

requires a chemical clean after every run is not feasible and the operation of the membrane system needs to be altered. Reducing the flux of the system may lead to less fouling and there is less pressure causing the algae to adhere to the membrane surface. Additionally, the backwashing method utilised in this system may not have been effective due to the leaks which occurred during the backwashing. The leaking may have reduced the pressure of the backwash thus reducing its effectiveness in dislodging the cake layer and any other particles which may have settled on the membrane surface. Alternatively, the backwash may not have been strong enough to dislodge the fouling, and this would lead to a build-up of pressure in other areas of the membrane system causing the leak. The reduction in the backwashes effectiveness would cause a reduction in the flux of the system as there would be less open pores available to filter the sample through. The addition of more frequent short backwashes or the addition of short back pulses may reduce the build-up of a cake layer on the membrane surface.

Table 6.6: Initial and final flux of the second set of results for the filtration of primary lagoon effluent utilising ceramic membranes.

8-May-18				
	Initial flux (L/m²/h)	**Final Flux (L/m²/h)**	**Decline in Flux (%)**	**Time (min: sec)**
Clean Water 1	320.04	343.38	-7	05:20
Primary Effluent 1	385.63	154.82	60	09:20
Clean Water 2	116.82	164.93	-41	10:50
Primary Effluent 2	211.41	111.38	47	15:55
Clean Water 3	76.92	104.78	-36	11:35
Primary Effluent 3	127.78	88.54	31	21:25
Clean Water 4	56.34	66.60	-18	13:00
Overall			81	87:25

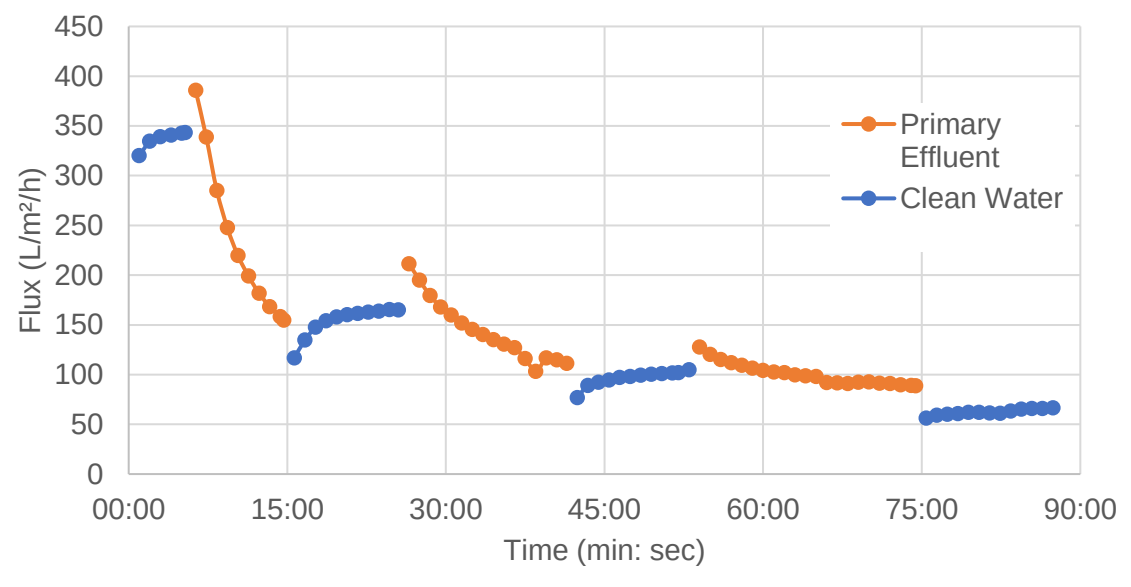

Figure 6.5: The flux over time for the second set of crossflow ceramic membrane runs. Displaying changes in clean water and primary effluent.

Both runs display a similar pattern for decline in flux and the recovery after a backwash, with a swift decline in flux and minimal recovery from the backwash and a small amount flux recovery occurring during the clean water flux tests. Overall the ceramic membrane experienced rapid and significant irreversible fouling which was not recoverable by the backwash method utilised and a sustainable operating flux of 80L/m^2/h was estimated for the second run.

6.3.2 Submerged PTFE Membrane

The second membrane system tested was the PTFE membrane which was operated in a submerged rig and the primary lagoon effluent was filtered through the membrane in 9-minute intervals. The results for the PTFE membrane experiments are in Table 6.7 which displays the initial and final flux, the percentage flux decline and the time elapsed for each run. Table 6.7 shows that there was minimal fouling and that the majority of any fouling that occurred was recovered after a rest period. It should be noted that the initial flux was lower than the peak flux due to the pump powering up and the effluent passing through the system. The initial run (18-May-18) had the largest amount of fouling with changes in flux of 1.4 L/m^2/h between the peak flux (26.1 L/m^2/h) and final flux (24.7 L/m^2/h). This decline in flux was almost fully recovered after a rest period. The same fouling pattern and recovery was observed in the two following runs. Overall there was a 5.5% decline in flux over the 3 runs.

The second and third sets of runs had minimal fouling throughout the experiments. The second set of runs had an overall decline flux of 10% and the third set of runs had a decline of 0.5%. The difference between the sets of runs could potentially be due to fouling prior to the start of the experiments or the fact that the membrane had not been used for a significant period of time prior to this experiment and may have required a longer period submerged in the distilled water to acclimatise the system. The recovery in flux between rest periods as shown by similar initial and peak fluxes demonstrated that the 1-minute rest period between each run allowed for adequate removal of fouling on the membrane surface. Figure 6.6 demonstrates the fouling and recovery of the flux during each run.

Table 6.7: Initial and final flux of the second set of results for the filtration of primary lagoon effluent utilising PTFE membranes.

	Initial Flux (L/m²/h)	**Final Flux (L/m²/h)**	**Decline in Flux (%)**	**Time (min: sec)**
Run 1				
Clean Water 1	24.80	25.90	-4	09:00
Primary Effluent 1	21.90	24.70	-13	09:00
Primary Effluent 2	24.70	24.50	1	09:00
Primary Effluent 3	23.40	24.40	-4	09:00
Run Total			6	39:00
Run 2				
Clean Water 1	25.98	26.15	-1	09:00
Primary Effluent 1	24.20	26.14	-8	09:00
Primary Effluent 2	24.20	26.14	-8	09:00
Primary Effluent 3	24.08	23.42	3	09:00
Run Total			10	39:00
Run 3				
Clean Water 1	24.97	26.23	-5	09:00
Primary Effluent 1	25.30	26.24	-4	09:00
Primary Effluent 2	25.42	26.13	-3	09:00
Primary Effluent 3	24.79	26.28	-6	09:00
Primary Effluent 4	26.26	26.14	0	09:00
Run Total			0.3	49:00
Mean Total			5.5	

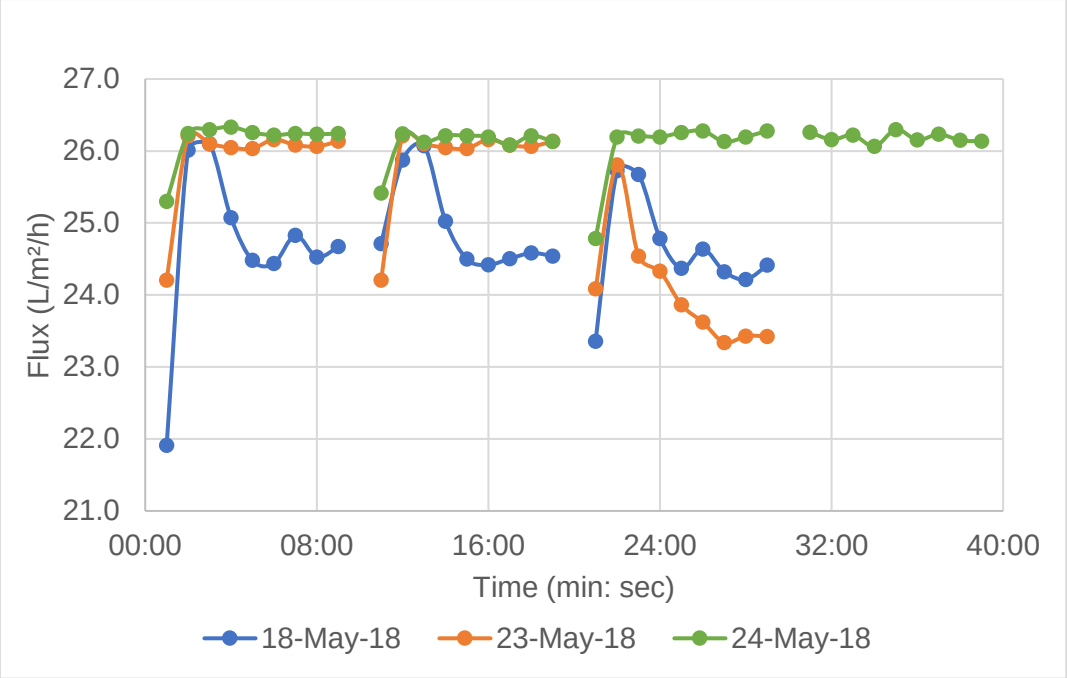

Figure 6.6: Submerged PTFE membrane flux when filtering algae from a sample of primary lagoon effluent. Rest periods of one minute between each run.

A clean water flux test was performed at the start of each experiment. Figure 6.7 displays the results of the clean water flux tests. There were minimal changes between the fluxes during each of the tests. This provides a good baseline of results and confirms that the membrane was functioning similarly to a clean membrane after cleaning with 1000mg/L sodium hypochlorite. The clean water flux tests also show minimal variation over the course of each run after the results had been stabilised.

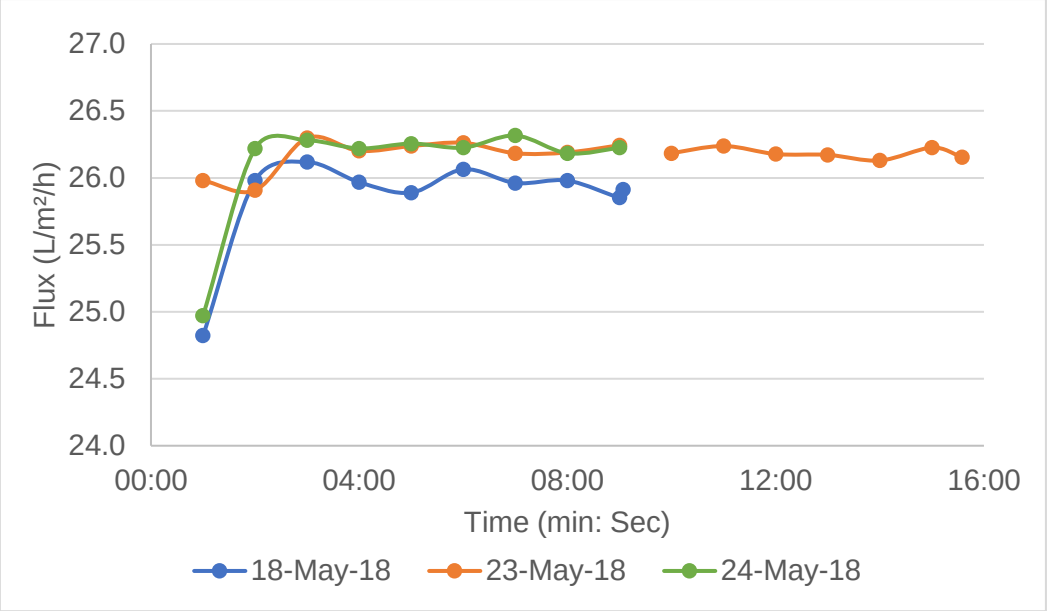

Figure 6.7: Submerged PTFE membrane clean water flux test.

The increase in filtrate weight over time is displayed graphically in Figure 6.8. It can be clearly seen that there is minimal variation between the three sets of experiments which would indicate very little long-term fouling and that the operating conditions were the same. Additionally, Figure 6.9 displays the increase in filtrate weight during the clean water flux tests. Once more, it can be clearly seen that there is little to no variation between the runs.

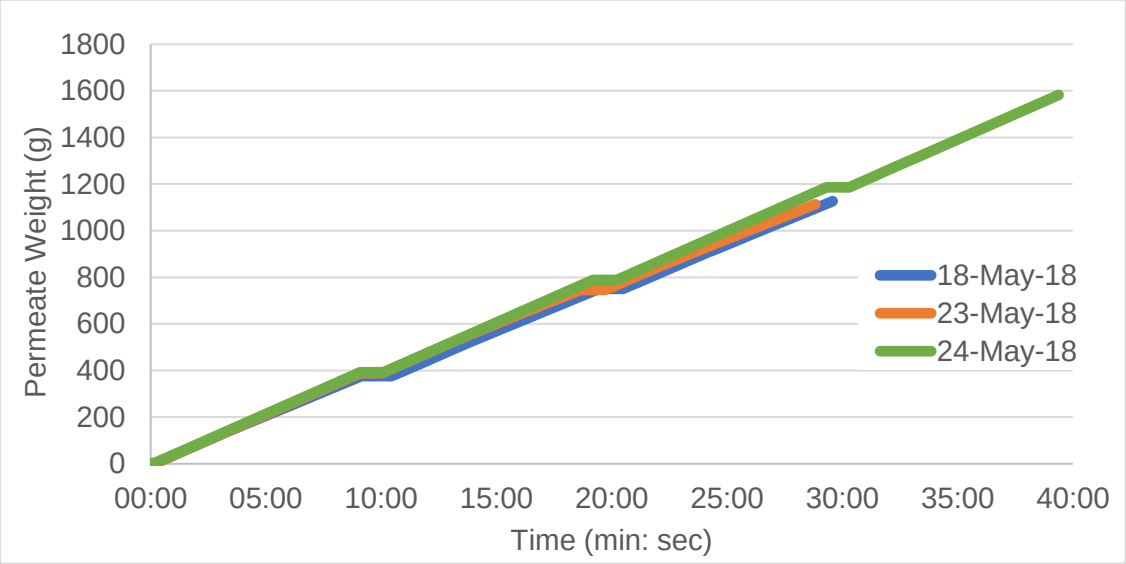

Figure 6.8: Permeate weight increase over time for each experiment utilising the submerged PTFE membrane when filtering primary lagoon effluent.

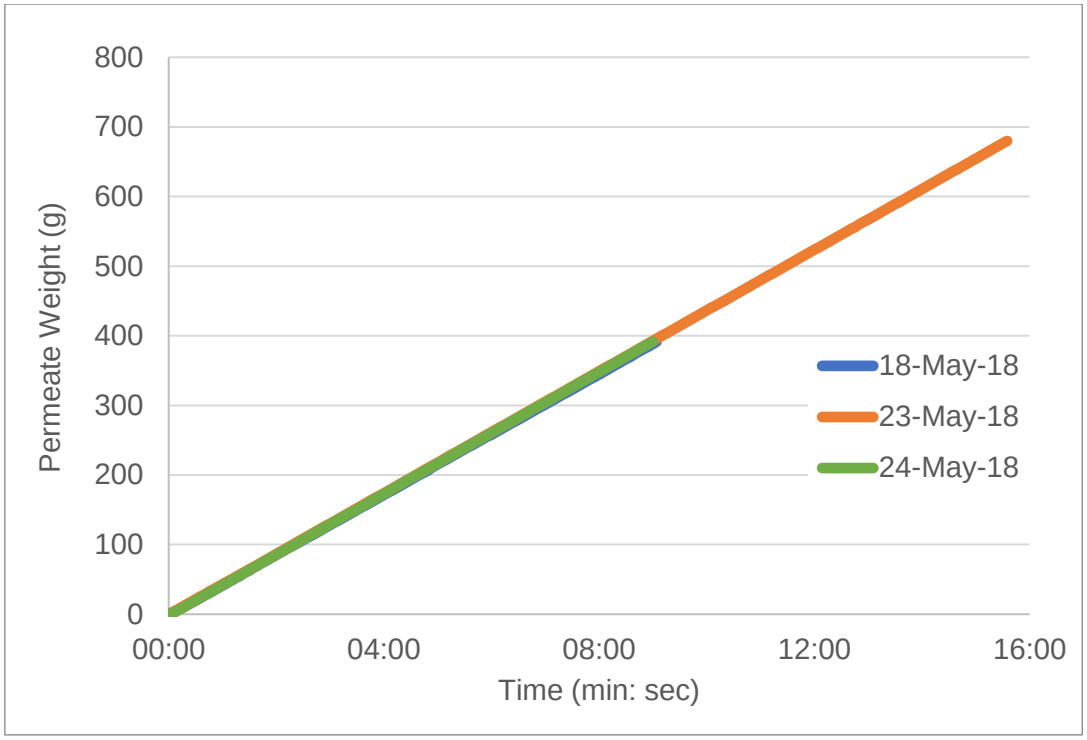

Figure 6.9: Permeate weight increase over time for each experiment utilising the submerged PTFE membrane when filtering clean water.

The submerged membrane showed very little fouling over the course of the experiments and when fouling occurred such as during the first experiment the rest period was able to recover the membrane back to the initial flux. These results can be attributed to the hydrophilic surface of the PTFE membrane which reduces fouling by repelling algae and reducing attachment to the surface. Additionally, the air bubbles sparged into the system at the base of the membrane helped to dislodge any particles that had settled on the membrane surface. The rest period also enhanced the fouling recovery by allowing for any particles which were attaching to the surface through vacuum pressure to become dislodged by the air bubbles. The low operating flux may have also minimised fouling. The majority of fouling which occurred during this set of experiments was reversible fouling and the rest period and bubble sparging were sufficient to dislodge any particles which had settled on the membrane surface. There were no signs of major irreversible fouling occurring during this set of experiments.

6.3.3 Crossflow Metal Membrane

The third membrane tested was the metal membrane. The metal membrane rig had two sets of runs each comprising of a 3000ml and 2000ml step. Table 6.7 displays the initial flux, final flux, the percentage decline in flux and the time elapsed in each run. After inspection of Table 6.7, it is evident that fouling occurred during the course of the experiments with a 51% decline in flux. The two sets of runs performed similarly with comparable initial fluxes and similar declines in the flux of 16.11 and 14.50 L/m²/h which equate to a 22% and a 21% decline in flux. The flux declined in a comparable manner in each run with R-squared values ranging from 0.94-0.99. The performance of the runs can be seen in Figure 6.10.

Between the two sets, the feed tank was drained and flushed with water and a clean water flux test was performed. This process was found to act as a cleaning step, and the flux of the membrane was almost fully recovered. The draining of the feed tank had two effects on the fouling of the membrane. Firstly, the draining would have acted as a relaxation step and allowed for the membrane to release some particles which were adhered to the membrane by the force of the vacuum created by the pump. Secondly, the flushing of the system with clean water would have also dislodged the majority of the cake layer and removed the particles fouling the membrane.

Table 6.8: Initial flux and final flux of the metal crossflow membranes.

	Initial flux (L/m²/h)	Final Flux (L/m²/h)	Decline in Flux (%)	Time (min: sec)
Clean Water 1	98.59	106.13	-8	03:30
Primary Effluent 1	72.12	61.69	14	05:40
Primary Effluent 2	61.29	56.01	9	04:15
Clean Water 2	66.47	62.68	6	05:45
Primary Effluent 3	68.86	59.25	14	05:50
Primary Effluent 4	61.51	54.36	12	04:00
Clean Water 3	49.94	52.45	-5	07:25
Overall			51	36:25

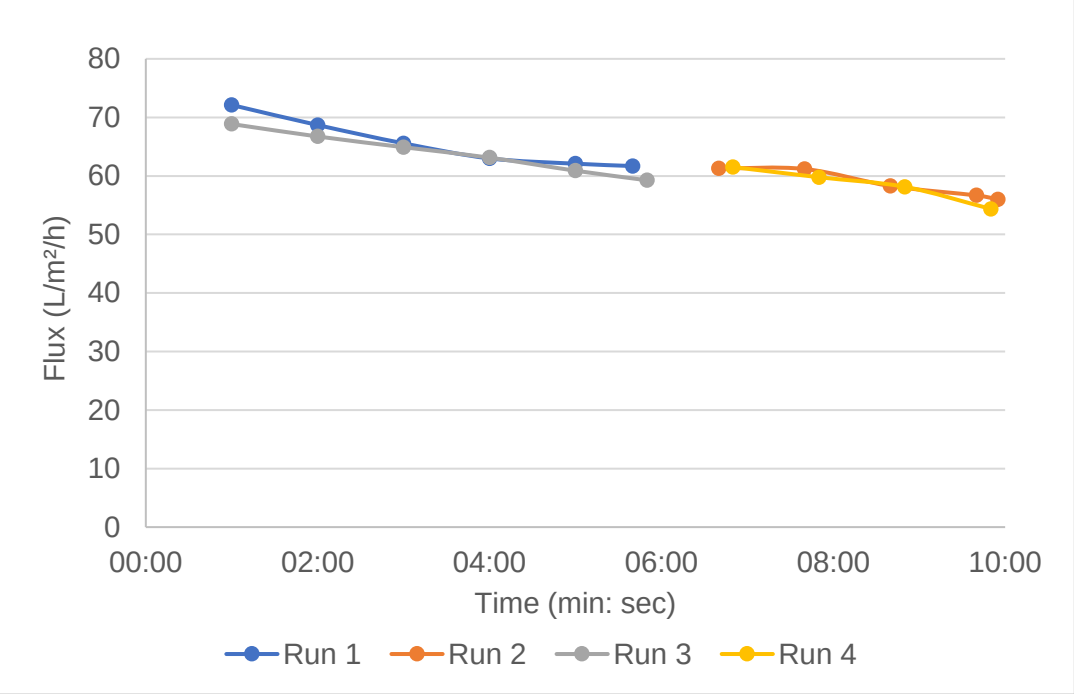

Figure 6.10: Flux (L/m²/h) versus time (minutes: seconds) in each of the experimental filtration runs utilising the crossflow metal membrane system.

Figure 6.11 displays the changes in permeate weight over time for the two batches. This graph shows that the two runs were very similar in operation and results.

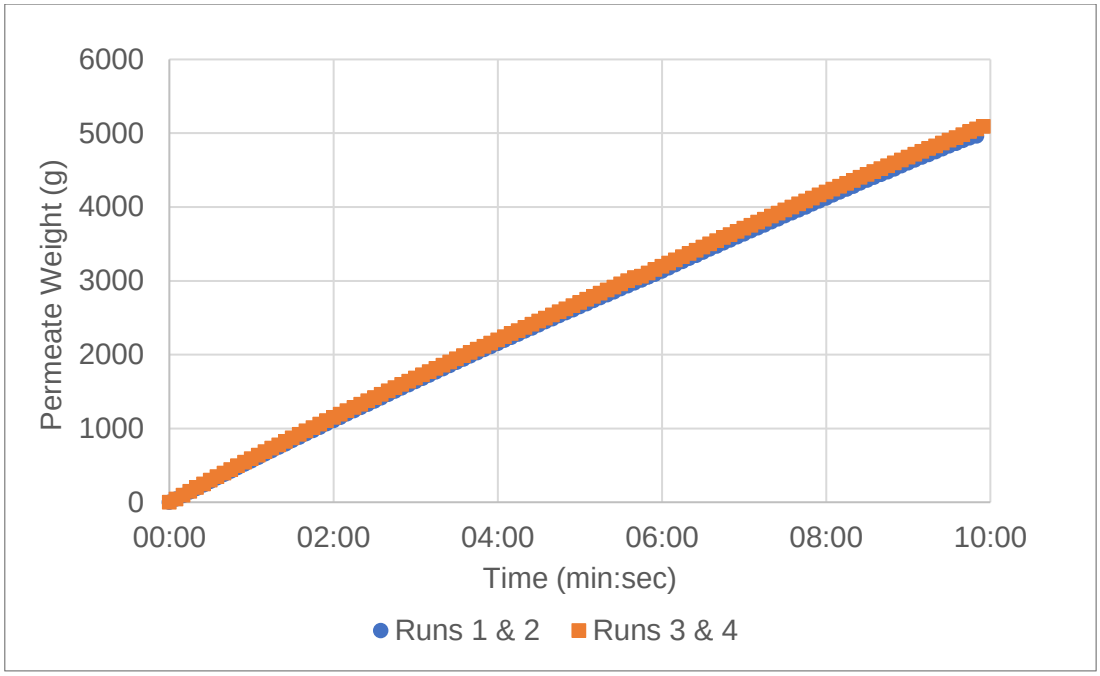

Figure 6.11: Change in permeate weight (g) over time (minutes: seconds) of the two sets of runs, runs 1-2 and runs 3-4.

In addition, clean water flux tests were performed throughout the experiment and prior to the start of the experiment. The initial test had a maximum flux of 109.48 L/m²/h. The other clean water flux tests were performed between runs 2 and 3 and at the end of the experiment (run 4). These clean water flux tests had significantly lower flux results due to fouling by the primary lagoon effluent with 68 and 53 L/m²/h respectively. It is important to note that there was a significant difference, 34 L/m²/h, in the final flux of the first clean water test and the initial flux of the first primary lagoon effluent run. This would indicate that the membrane was substantially fouled within the first minute of operation. It may be possible to reduce this rapid fouling by reducing the flux of the system, however, this would reduce the economic competitiveness of the metal membrane and make it an uneconomical option compared to other systems such as the PTFE membrane.

To help overcome and reduce fouling the system had continual back pulsing every six seconds. However, the pressure used in this back pulse may not have been strong enough to dislodge a significant amount of particles which had settled on the membrane surface. The system did not undergo any additional deliberate flux recovery such as backwashing. Overall, the membrane

performance was constant and predictable. There was modest fouling during each run, which was somewhat recoverable after a clean water flush of the system. However, by the end of the two sets of runs there was a 51% reduction in flux. This indicates a significant amount of irreversible fouling occurred. The recovery of the membrane fouling through the clean water flush of the system was promising and suggests that a more effective and optimised backwashing/ back pulsing procedure would enhance the economics of the membrane, its algal harvesting efficiency and reduce the amount of irreversible fouling.

6.3.4 Comparison

The flux results for the three membranes tested are tabulated in table 6.9. Table 6.9 contains the results for the maximum and minimum flux obtained during the experiments as well as the mean decline in flux (%) for each set of experiments, for example, the two batches of the metal membrane filtration experiments had declines in flux of 23% and 26% resulting in a mean of 24.5%. The ceramic membrane fouled easily and showed poor flux recovery after backwashing. There was a decline in flux of over 80% for both sets of runs indicating significant irreversible fouling. The PTFE membrane showed very little fouling, and any fouling that occurred was easily removed by a relaxation period and bubbling air. The mean decline in flux for the PTFE membrane was 5.5% over the course of the three runs indicating minimal irreversible fouling. The metal membrane showed a small amount of fouling, roughly 20% per batch, and between the batches there was a recovery of the flux as a by-product of preparation for the clean water flux test. However, the overall result showed a reduction in flux of 51% over the course of the experiment indicating significant irreversible fouling. This high amount of fouling would mean that twice the membrane area would be required to provide the same amount of filtered water.

The ceramic membrane had the highest initial flux overall but also the lowest final flux indicating the largest reduction in flux. In comparison, the PTFE membrane had the lowest initial flux but displayed the smallest decline in flux. The metal and ceramic membranes are able to withstand harsher chemical cleaning and stronger physical cleaning methods to recover fouling than the

PTFE membrane. Furthermore, they were also able to and are required to be operated at a higher flux to be run economically. Even though the metal and ceramic membrane are able to withstand harsher cleaning methods this may not be beneficial as they require more cleaning than the PTFE membrane. This would increase the operating cost, due to the cost of the chemicals required and the time needed to shut down the system for cleaning. The PTFE membrane had the lowest rate of fouling out of the membranes tested but was operated at a much lower flux than the other two membranes.

Table 6.9: Flux results for the three tested membranes.

	Ceramic Membrane	**PTFE Membrane**	**Metal Membrane**
Highest Flux ($L/m^2/h$)	257.1	26.3	106.1
Lowest Flux ($L/m^2/h$)	14.0	21.9	49.9
Mean Decline in Flux (%)	84.5	5.5	24.5

6.3.5 Economic Analysis

The price of each membrane and the operating price for filtration of a cubic metre of sample are displayed in Table 6.10. The sustainable flux of each membrane was utilised, 80 $L/m^2/h$ for the ceramic membrane, 25 $L/m^2/h$ for the PTFE membrane and 65 $L/m^2/h$ for the metal membrane.

Table 6.10: Membrane capital cost.

Membrane	**Price per m^2 (USD$)**	**Sustainable Flux ($L/m^2/h$)**	**Price per m^3/hr (USD$)**
Ceramic	~450	80	5,625
PTFE	43	25	1,720
Metal	~870	65	13,385

When examining the results from Table 6.10 and combining these with the information from the previous section some observations can be made. The ceramic membrane was expensive and fouled quite readily. The PTFE membrane while much cheaper than the other two membranes showed very little fouling. The metal membrane was the most expensive and had moderate fouling. Examining these results, it is clear that the PTFE membrane is the most feasible of the three membranes to be considered for use due to its lower cost and fouling. In the future, this may change as ceramic and metal membranes become more affordable and the properties are further enhanced to repel algae more effectively. The long-term fouling, recovery effects and cleaning cost of the membranes were not examined in these experiments and these may affect the long-term cost.

6.4 Summary

The performance of three different membranes and membrane rigs were assessed and compared. Overall the PTFE membrane showed the least amount of fouling and had a relatively constant flux. The ceramic membrane fouled most readily and severely (80%). and required caustic cleaning to recover the membrane rather than mechanical cleaning (backwash). The metal membrane fouled significantly with an overall flux reduction of 51%. However, each metal membrane run showed significant recovery through the clean water flux test. The costs of the three membranes were examined and PTFE was found to be the most economical when comparing the membranes in this study. The current operational processes for the membrane rigs utilised in this study were not optimised and this would impact the effectiveness and overall

economics of the systems. Due to the PTFE low cost, constant filtration and minimal fouling it was determined to be the most cost-effective filtration membrane of the three tested and is the most suitable for further large scale, longer term testing. The PTFE system utilised in this study is commercially available, can be upscaled and so is easily implemented into wastewater treatment plants. In theory, the PTFE system could be placed into an existing wastewater treatment lagoon and filtered sample could be easily removed as the system removes filtered water rather than trying to push unfiltered water through or across a membrane. This would be easier to implement than a cross flow system and requires less energy as it does not need to move as much water, so it would have a lower operating cost. The low fouling of the PTFE means it would require less maintenance and can be run for extended periods of time without the need for chemical cleaning, further reducing operating costs. This form of membrane filtration could also be used downstream after algae have been grown in a HRAP and are ready for harvesting. The PTFE system would be able to remove unwanted water from HRAP and leave behind a highly concentrated slurry of algae which can be removed and treated further. The algal slurry could be utilised as is or it could be dried and stored for future use. The Zobi Harvester which is utilised by the by Global Algae Innovation is also a submerged algal harvesting system and utilises bundles of hollow fibre membranes to harvest algae, similar to that of the PTFE membrane system used in this study. It has been shown to concentrate algae into a 15-20% slurry and has been proven to be effective on a large scale. It also requires very little energy and has been demonstrated to be effective on numerous different algal species (Hazlebeck, 2018). However, the membrane type, design and construction has not been divulged to the public, even though the Zobi Harvester is commercially available. With further testing and optimisation, the PTFE membrane has the potential to produce similar or better results.

The filtration of algae and removal of the filtrate could potentially be enhanced with the use of a flocculant. An ideal flocculant would not cause fouling of the membrane and would not need to be removed for downstream processing of the algal biomass. Fungal flocculation of algae was tested and is discussed in the following chapter.

Chapter 7 - Fungal Flocculation of Microalgae

7.1 Introduction

In the search to efficiently and economically harvest algal cells, fungal flocculation stands out as a major competitor. However, there are still some questions which need to be answered. Numerous fungi have been shown to be able to form pellets and flocculate monocultures of microalgae (see table 2.3). However, there are no previous studies published which investigated the flocculation of multiple algal species simultaneously. While testing the flocculation of monoculture of algae will help in understanding the mechanisms behind fungal flocculation of algae, it leaves a significant gap in knowledge regarding the practical applications of this procedure. This piece of information is vital, especially as more research is performed on the use of HRAP utilising a natural consortium of algae for wastewater treatment.

A common problem in wastewater treatment lagoons is the presence of algae in the effluents, while these algae help remove the nutrients and polish the lagoons they can also create anoxic conditions and kill fish in the receiving waters. As experienced in the Murray-Daring river system and the Menindee Lakes during the summer of 2018-2019. Alternatively, if and when the effluents are discharged to farmland, there is a requirement that the algae need to be removed first. The removal of algae is an expensive and complicated process, and some methods can do more harm than good to the remaining water. Currently, fungal flocculation is not considered as it has only been proven to work on monocultures of algae. However, if it can be shown to flocculate a mixed culture of algae, it could be employed before the discharge of the effluent as a viable method of algal harvesting. Additionally, the use of fungi-algae pellets has been shown to remove more nutrients from a few different types of wastewater then fungi or algae separately (Zhou et al., 2012, Wrede et al., 2014). While it may not be possible to completely remove all the algae, as some species have proven to be more difficult to remove than other, it would still offer

a significant reduction in the algal concentration and could also significantly reduce the cost of harvesting the algae.

In this chapter, several fungal species were trialled for their ability to flocculate monocultures of algae. The fungal species which showed the highest proficiency to harvest monocultures of algae was tested for its flocculation proficiency against numerous effluent samples containing a consortium of different algal species. Three batches of algae- fungi pellets were also investigated for their ability to remove nutrients from primary lagoon effluent and compared against monocultures of algae and fungi.

7.2 Methods

7.2.1 Experimental Design

The experiments were performed in four phases. In the first phase, the fungal samples were isolated and screened for pellet formation on Potato dextrose broth (PDB). In the second phase, the efficiency of harvesting monocultures of algal cells by flocculation by fungal pellets was investigated. The third phase examined the use of *A. oryzae* pellets to harvest many algae species simultaneously by flocculation. In the fourth phase, fungal-algae pellets were collected and reused as immobilised cells to treat primary lagoon effluent samples and determine the nutrient removal efficiency.

7.2.2 Fungal Isolation and Screening for Pellet Formation

Majority of fungal samples were collected from the ground soil around the laboratory at Victoria University, Werribee campus (Werribee, Australia, 37°53'22.4"S 144°42'00.2"E). Two additional fungi samples were isolated as contaminants from laboratory microalgal cultures. They were grown on Potato Dextrose Agar (PDA) (Sigma- Aldrich) at 30℃ for 72 hours. Fungal isolates were obtained from the collected samples via selection with inoculum loop or the back of a 1ml pipette, depending on size and formation, the isolates were added to 100ml conical flasks containing 50ml of potato dextrose broth (PDB) (Sigma- Aldrich). The flasks were placed on to an orbital shaker (Thermo Fisher MaxQ 4450) at 150rpm for three days at room temperature. Observation of pellet formation was performed, and only isolates which could form pellets were

selected for further testing. Lawn cultures of these species were made on PDA. Samples were stored at 4℃ until required for further investigation.

7.2.3 Preparation of Seed Fungal Spores

To generate fungal spores, fungal samples were grown on PDA at 30℃ for three days. Sterile water was added to collect the spores; the spore solution was utilised as the inoculum for the development of fungal pellets in PDB.

7.2.4 Formation of Fungal Pellets

Fungal pelletisation was achieved by adding the spore inoculum to flasks containing PDB and cultivated at 28±2℃ on an orbital shaker at 150rpm for three days. After pellet formation, the fungal pellets were washed thrice with sterile water to remove any PDB which could interfere with results.

7.2.5 Preparation of Algal Cultures

Six algal cultures were utilised to test for algal flocculation. The first five species; *Dictyosphaerium* sp., *Scenedesmus* sp., *S. quadricauda, S. acuminatus* and an unknown filamentous algal sp. were isolated from secondary lagoon effluent samples collected from the HRAP at BMRWP. Isolation was performed by a combination of serial dilution and plate streaking. They were identified using Standard Methods (APHA/AWWA/WEF, 1998). The sixth species used was *Chlorella vulgaris* (strain CCAP 211/11) which was purchased from the Australian National Algal Culture Collection. All algal samples were cultured in BBM. Cultures were grown in 2L flasks containing 1.5L BBM and mixing was done by sparging filtered air into the culture. Cultures were exposed to 2.95 lux fluorescent lights and a photoperiod of 16 hours on and 8 hours off at room temperature. Algal cultures were stored in either liquid stocks of BBM or on agar plates BBM containing 1.5% agar. The culture media were autoclaved at 121℃ for 15 minutes.

7.2.6 Screening for Algal Flocculation

The fungi which could form pellets in solution were inoculated with three different algal samples. The first batch of screening tests examined the flocculation of *Dictyosphaerium* sp. *Scenedesmus* sp. and an unidentified

filamentous algal species. The next three batches of fungi were screened for their flocculating capabilities utilising *C. vulgaris, S. quadricauda* and a *Dictyosphaerium* sp. Twenty-one fungal samples were tested for their efficiency to flocculate algae. Mixtures of fungi and algae were placed on to an orbital shaker at 100rpm at room temperature for 24, 48 or 72 hours. Flocculation efficiency was determined by optical density measurements made using a BioRad iMark™ Microplate reader. Cultures were prepared in triplicate or duplicate with triplicate measurements performed to confirm results. Flocculation efficiency (FE) was calculated based on changes in optical density at 750nm (equation 7.1).

$$FE\% = \left[\frac{A-B}{A}\right] \times 100 \qquad (7.1)$$

Where A is initial optical density at time zero, B is the optical density after 24, 48 or 72 hours. Percentage flocculation was used to compare results as starting algal concentration varied between experiments. A single fungal species was chosen for further study and identification. The morphology of fungal- algal pellets was observed under bright field microscopy using a Motic BA310 compound microscope with an attached camera.

7.2.7 Fungal Species Identification

The selected fungus species was identified as *A. oryzae* based on morphology using bright field microscopy. A taxonomic key was utilised to help identify the fungal species (Samson et al., 1981).

7.2.8 Flocculation of Monocultures of Algae

A. oryzae flocculation efficiency was tested on a number of different monocultures of algae. The monoculture of algal tested were *C. vulgaris, S, quadricauda, Dictyosphaerium* sp. and *S. acuminatus.* Different concentrations of fungal pellets were tested to determine if an optimum ratio was necessary for efficient flocculation. Experiments were performed over 24, 48 and 72 hour periods. FE measurements were performed using the same method as the screening experiments.

7.2.9 Flocculation of Multiple Species of Algae in Effluent Samples

The efficiency of *A. oryzae* to flocculate numerous species of algae simultaneously was tested using various effluent samples. These samples were collected from the BMRWP. Several samples of differing algal concentrations and species composition were collected from the primary lagoon, secondary lagoon and the HRAPs. Sampling was performed at different periods to ensure a greater species composition and diversity, sampling dates are tabulated in table 7.3. The fungal-effluent mixture was placed on an orbital shaker at 100 or 150rpm at room temperature for 72 hours to test their FE. FE measurements were performed using the same method as the screening experiments.

7.2.10 Treatment of Primary Lagoon Effluent Utilising Fungal-Algal Pellets

Primary lagoon effluent collected from Pond 1 at BMRWP was used to test the efficiency of fungal-algal pellets to remove nutrients. The sample was filtered through a Whatman glass fibre filter paper grade C filter to remove any algae and larger particulates (henceforth referred to as filtered primary effluent) (fPE). Three effluent samples, two primary lagoon effluent samples (primary lagoon effluent 23/02/18 and primary lagoon effluent 17/04/18) and one HRAP effluent sample (HRAP 03/11/17), containing various naturally occurring algae were added to *A. oryzae* pellets and placed on an orbital shaker for two days to induce flocculation. After two days the samples were filtered using Whatman glass fibre paper grade C and rinsed three times with deionised water to remove any traces of the previous effluent sample. The fungal-algal co-culture pellets were placed into a flask containing fPE and placed on the shaker at 100rpm at RT for 48 hours. Monocultures of *A. oryzae* pellets and the natural algae consortiums from the relative effluent samples were added to fPE for comparison and placed under the same conditions as the co-culture. The cultures of the natural algae consortium were centrifuged at 4000rpm for 10 minutes and rinsed with water before reconcentrating by centrifugation; the algal pellets were resuspended in fPE. A control of fPE was kept on the bench top next to the orbital shaker to monitor any chemical changes over the 48 hours. After 48 hours the samples were filtered through Whatman glass fibre paper grade C to remove the pellets and loose algal cells. Filtered samples

were correctly diluted and analysed for Ammonia (NH_3-N), Nitrate (NO_3-N) and orthophosphate (P-PO_4) concentrations using Hach kits and measured on a bench top Hach DR 5000 Spectrophotometer (Hach, 2008). The pH levels were measured using a benchtop pH meter. Results are tabulated as percentage change, see equation 7.2 and table 7.5.

$$\text{Change in nutrient concentration } \% = \left[\frac{N_0 - N_1}{N_0}\right] \times 100 \qquad (7.2)$$

Where N_0 is the initial nutrient concentration (mg/L) and N_1 is the final nutrient concentration (mg/L) after 48 hours. Samples were prepared in triplicate, and nutrient removal tests were performed in triplicate to confirm results.

7.2.11 Statistics of Analysis

Flocculation screening experiments were conducted in replicates of two or three. All experiments utilising *A. oryzae* were performed in triplicate, including the effluent treatment experiment. All OD measurements were performed in triplicate. Nutrient concentrations were performed in triplicate. Mean values and percentage removal of algae and nutrients results were analysed. Graphs and tables were compiled using Microsoft Excel 365.

7.3 Results and Discussion

7.3.1 Fungal Isolation and Screening for Pellet Formation

Thirty-five fungal samples were isolated and tested for pellet formation. Twenty-one samples were found to form pellets of varying shapes and sizes through mixing at 150rpm, and these were isolated to test their ability to flocculate single species of algae.

7.3.2 Screening for Algal Flocculation

Twenty-one species were shown to form pellets. Four sets of experiments were conducted investigating the efficiency of the different fungi to flocculate some of the algal species. Table 7.1 shows the percentage removal results for the four sets of experiments over 24, 48 and 72 hours. After the first set of data was analysed, running the experiment for 72 hours was deemed unnecessary as 48 hours provided sufficient data regarding flocculation. The

data was obtained using OD at 750nm. Percentage removal results are used to help compare different runs and species of algae as the initial OD values were different for each set of experiments. The unknown filamentous species was not used after the first experiment as the results varied significantly. The use of OD to measure the algal concentration proved a poor method when using filamentous algae as the algae does not disperse uniformly throughout the medium. *C. vulgaris* was used as it is a 'model' species and has been utilised thoroughly by other researchers examining fungal flocculation and the results provide a reliable comparison between studies.

The fungal sample referred to as 'SQ' showed the highest flocculation efficiency with over 95% removal of all the algal species tested. Sample 'SQ' was identified and utilised for further study. Table 7.2 contains some negative values which indicate that the fungi were not able to remove the algal cells from suspension and additionally the algal culture grew over the testing period.

Table 7.1: Screening experiments, percentage removal of algae from suspension by fungal flocculation after 24 and 48 hours.

16/08/2016	**24 hours**			**48 Hours**		
	Dictyosphaerium sp.	**Scenedesmus sp.**	**Filamentous sp.**	**Dictyosphaerium sp.**	**Scenedesmus sp.**	**Filamentous sp.**
AL Black 1	-48%	3%	-20%	-51%	15%	2%
9 White	-31%	12%	62%	-31%	19%	31%
7	-15%	37%	48%	-23%	23%	-29%
10	14%	14%	45%	-26%	13%	66%
3	14%	43%	65%	-22%	-11%	43%
1a	-54%	-8%	16%	12%	72%	81%
9 Pink	-15%	1%	-3%	-41%	-28%	71%
CF	-72%	23%	-163%	-18%	0%	36%
21/06/2017	**24 Hours**			**48 Hours**		
	C. vulgaris	***S. quadricauda***	***Dictyosphaerium sp.***	***C. vulgaris***	***S. quadricauda***	***Dictyosphaerium sp.***
CF	-323%	-85%	-71%	-314%	-70%	-54%
SQ	95%	99%	99%	96%	96%	95%
26	-173%	-65%	-102%	-215%	-75%	-67%
7	90%	-21%	20%	87%	13%	65%

7B	-58%	-22%	-23%	-11%	-2%	33%
14/08/2017	**24 Hours**			**48 Hours**		
	C. vulgaris	***S. quadricauda***	***Dictyosphaerium sp.***	***C. vulgaris***	***S. quadricauda***	***Dictyosphaerium sp.***
7f	91%	94%	68%	85%	90%	82%
26	-3%	7%	-69%	-36%	29%	-76%
26grey	46%	-12%	-24%	37%	-1%	-2%
30	11%	-1%	-2%	-25%	-30%	-9%
31	59%	-5%	12%	58%	-14%	-12%
SQ	98%	99%	99%	95%	98%	95%
21/08/2017	**24 Hours**			**48 Hours**		
	C. vulgaris	***S. quadricauda***	***Dictyosphaerium sp.***	***C. vulgaris***	***S. quadricauda***	***Dictyosphaerium sp.***
1a	36%	3%	-1%	51%	12%	8%
5green	72%	96%	0%	69%	93%	0%
8	57%	84%	7%	81%	96%	21%
9w	41%	-2%	3%	52%	14%	0%
34	84%	86%	12%	87%	89%	5%
35	94%	100%	96%	93%	96%	28%

ALbw2	59%	98%	9%	61%	95%	12%

Table 7.2: First screening experiment, percentage removal of algae from suspension by fungal flocculation after 72 hours.

16/08/16	**72 Hours**		
	Dictyosphaerium sp.	***Scenedesmus sp.***	***Filamentous sp.***
AL Black 1	-3%	-14%	59%
9 White	-31%	-107%	77%
7	-73%	5%	51%
10	19%	33%	82%
3	-17%	-56%	69%
1a	-25%	45%	26%
9 Pink	-61%	-139%	62%
CF	19%	89%	91%

7.3.3 Identification of Fungal Species

Fungal identification was carried out using Samson et al. (1981), the sample 'SQ' was identified as *A. oryzae* based on its morphological details. It was decided that gene sequencing was not required as *Aspergillus* has been the common fungal genus used in fungal flocculation. The pellets formed by *A. oryzae* were white and were uniform in size and 3-5mm in diameter. However, the fungal sample grown on PDA was green in colour.

7.3.4 Flocculation of Monocultures of Algal using *A. oryzae*

The flocculation of single species of algae was confirmed in a set of experiments following the initial screening experiments. Results are displayed as percentage removal of algae in figure 7.1. Three of the algae species, namely, *S. quadricauda, C. vulgaris and S. acuminatus,* trialled had over 85% of algae removed from suspension in all experiments. *S. quadricauda* was found to be the most readily flocculated algae closely followed by *C. vulgaris* with a mean percentage removal of 99% and 94% after 24 hours respectively. The flocculation of the algae varied marginally over time. *S. quadricauda* showed very little change in efficiency within the duration of the experiments, with a change in the removal of only 3%. *C. vulgaris* followed a similar trend with a change of 6%. *S. acuminatus* did not show any difference between removal after 24 hours or 48 hours. Additionally, *S. quadricauda, C. vulgaris and S. acuminatus* displayed a change of 1%, 1% and no difference after 72 hours respectively. The *A. oryzae* effectively flocculated *Dictyosphaerium* sp. during these experiments, with the highest removal recorded being 48%. This was most likely due to the shape and a mucilaginous envelope encasing the *Dictyosphaerium* sp. algal cells. The cells are arranged tetrachotomously and attached with a thin stalk. The structure of the algal colony could prevent flocculation by causing the algae to interact differently with the fungal hyphae compared to other species, especially regarding surface charge neutralisation. The mucilaginous envelope may interfere with the binding capacity of the fungi. The sheath may prevent the surface charges of the fungi and algae from interacting with each other. The increase in *Dictyosphaerium* sp. indicated by the negative removal value may be due to a combination of algal cell growth

and the cells were not being flocculated by the *A. oryzae*. The fungi-algae pellets were examined under a light microscope, and it was observed that a combination of flocculation techniques was employed. The algae, *C. vulgaris*, *S. acuminatus* and *S. quadricauda,* were both entrapped by the fungal hyphae in the pellets and were adhered to them. *Dictyosphaerium* sp. cells, however, were not observed to adhere to any fungal hyphae however some entrapment may have occurred, see figure 7.2.

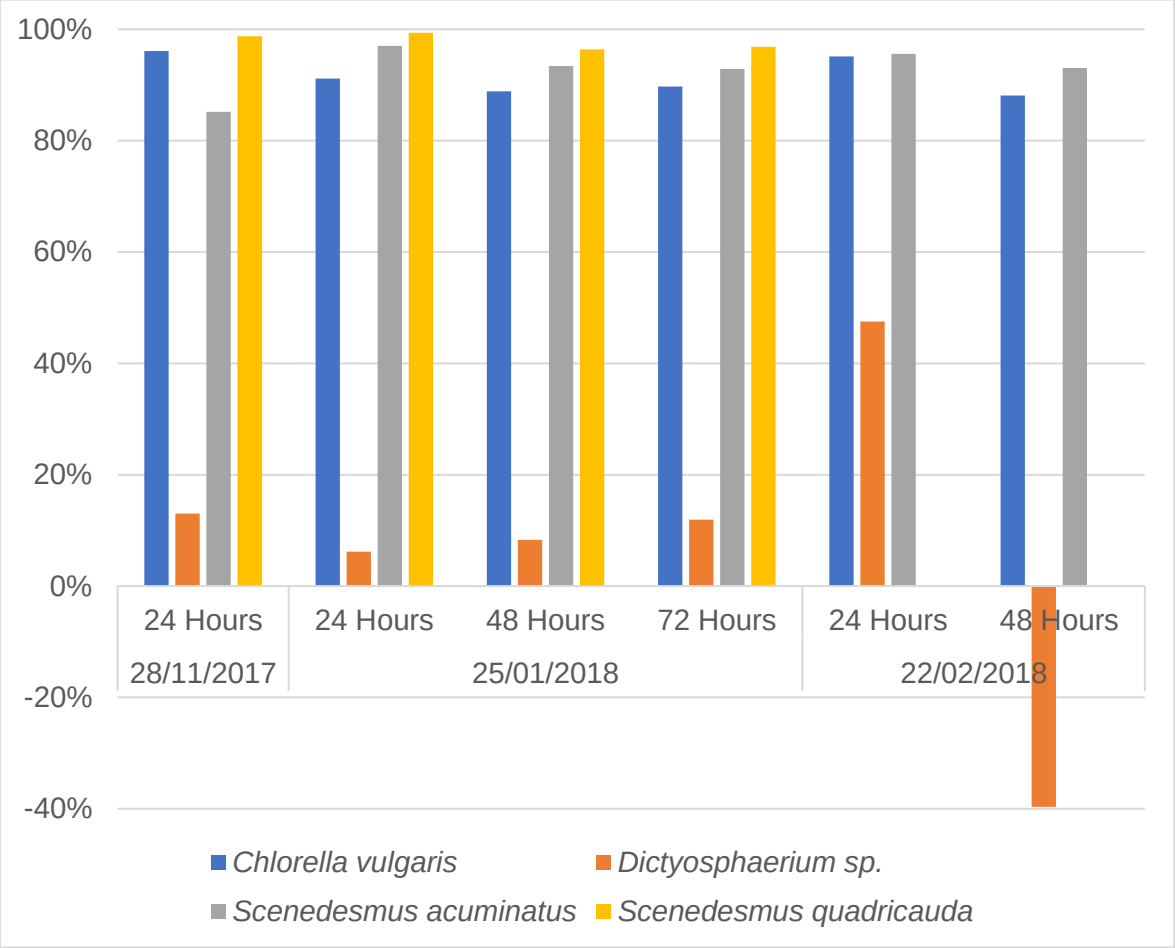

Figure 7.1: Percentage removal of algae from the suspension of monocultures of four different algae using A. oryzae as the fungal flocculation.

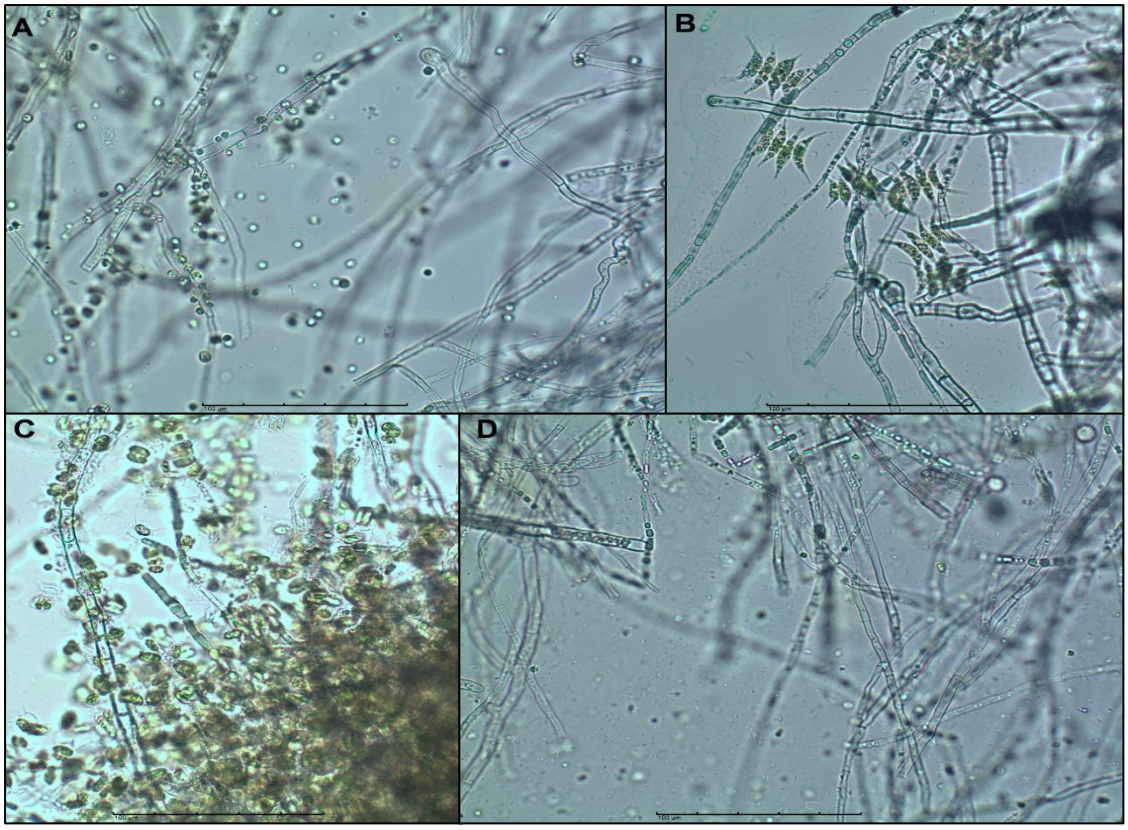

Figure 7.2: Microscopic analysis of A. oryzae flocculation of monocultures of algae. A) C. vulgaris; B) S. acuminatus; C) S. quadricauda; D) Dictyosphaerium sp. Scale = 100µm.

A. oryzae could flocculate varying concentrations of algae when used in the correct ratio of fungi to algae. The initial optical density readings for the above experiments ranged from 0.34-1.3 for *C. vulgaris,* 0.23-0.88 for *S. quadricauda*, 0.22- 1.24 for *S. acuminatus* and 0.28-1.33 for *Dictyosphaerium* sp. Although this was not the aim of the experiments upon examination of the data, this phenomenon was highlighted. Figure 7.3 illustrates a comparison between two batches of algal flocculation with varying amounts of fungal pellets. The first batch grown in 100ml flasks contained 50ml of algae and had a very low concentration of fungal pellets and the second batch which was performed in 6 well plates containing 5ml of algae and had three times more fungal pellets relative to the volume than the flasks did. This comparison shows that the ratio of fungi to algae is crucial and too low of a concentration of fungi would not provide sufficient flocculation. This is supported by the research conducted by Al-Hothaly et al. (2015) who also state that too high a concentration of fungal pellets can hinder the flocculation efficiency. These results indicate that with the

correct ratio of fungi to algae almost all the *C. vulgaris*, *S. quadricauda* and *S. acuminatus* cells could be flocculated out of the medium.

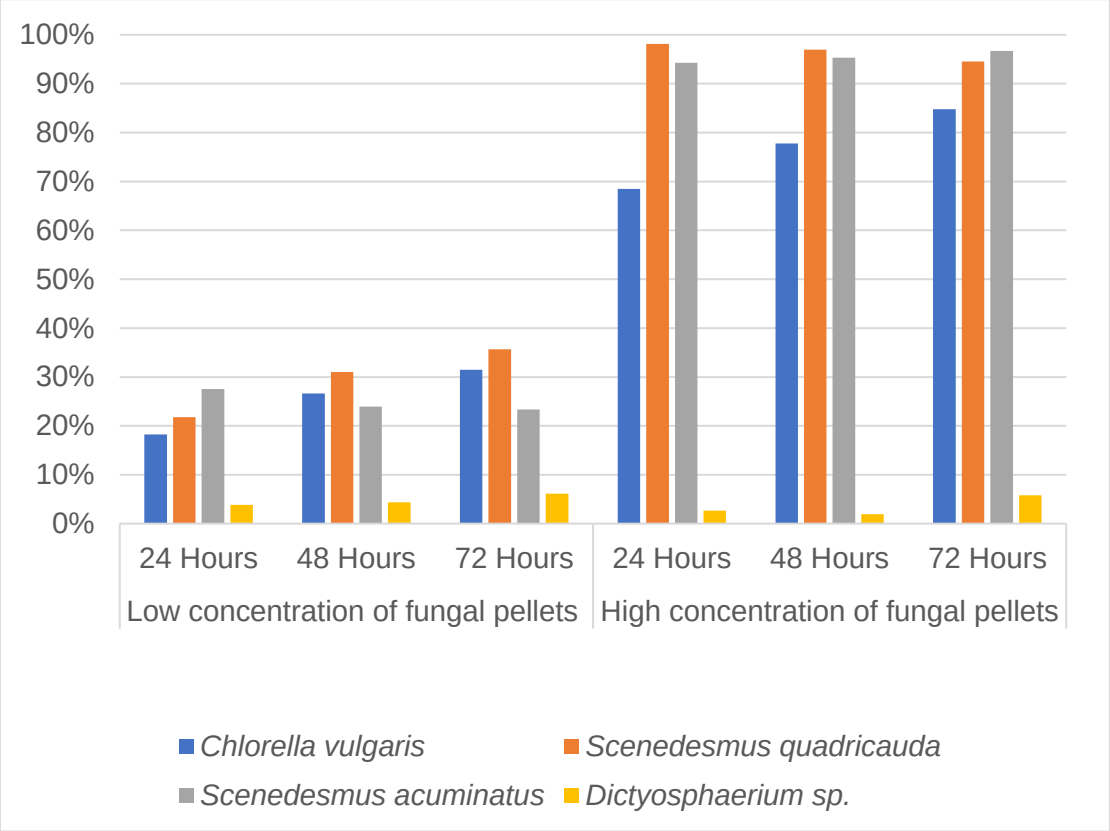

Figure 7.3: Percentage removal of algae using different concentrations of A. oryzae.

7.3.5 Flocculation of Numerous Species of Algae in Effluent Samples

The flocculation of multiple species of algae simultaneously was performed by using 14 different effluent samples. The samples were tested at various concentrations of algal biomass and different species compositions. Six of the effluent samples contained very low concentrations of algae, and an OD minimum of 0.05 was chosen to ensure the percentage results were not skewed by low starting values. For example, an initial starting OD value for a secondary lagoon effluent sample taken on the 22nd of November 2017 was 0.006, and after 24 hours it had increased to 0.009, while this was a small change in concentration it equated to a percentage increase of 50% in algal concentration. It is worth noting that the mean percentage removal over all the effluent samples was 71%, 70% and 71% over 24, 48 and 72 hours respectively. The effluent samples with only the initial OD values over 0.05 had percentage

removal results of 73%, 79% and 71% over 24, 48 and 72 hours respectively. This indicates that there was the same high percentage of flocculated algae at low algae concentrations and as there was when higher concentration of algae were flocculated, this would suggest that the fungal pellets did not reach a saturation of algal cells in any of the experiments. Figure 7.4 shows the mean percentage removal of algae in the eight effluent samples. Table 7.3 displayed the mean minimum and maximum percentage removal values for the eight effluent samples. The minimum and maximum values highlight the variation possible between runs and effluent samples.

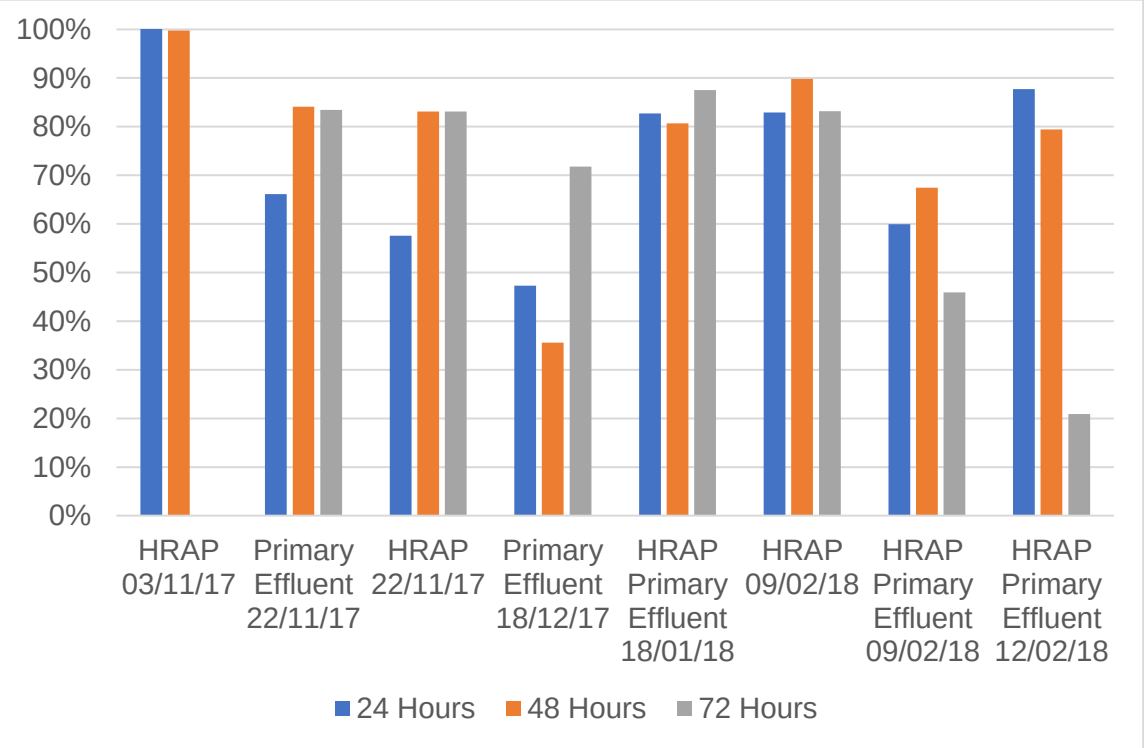

Figure 7.4: The mean percentage removal of algae from eight different effluent samples.

Table 7.3: Mean percentage removal over 24, 48 and 72 hours and minimum and maximum removal for each effluent sample.

	24 Hours	48 Hours	72 Hours	Minimum	Maximum
HRAP (03/11/17)	101%	100%		96%	104%
Primary Effluent (22/11/17)	66%	84%	83%	31%	98%
HRAP (22/11/17)	58%	83%	83%	26%	100%
Primary Effluent (18/12/17)	47%	36%	72%	10%	73%
HRAP Primary Effluent (18/01/18)	83%	81%	88%	50%	103%
HRAP (09/02/18)	83%	90%	83%	40%	99%
HRAP Primary Effluent (09/02/18)	60%	67%	46%	32%	81%
HRAP Primary Effluent (12/02/18)	88%	79%	21%	11%	104%

The percentage removal of algae from suspension ranged in each run with a maximum mean removal of 100% and a minimum mean removal of 36%. Four of the runs had removal of algae over 80% after 24 hours and two of the remaining runs had removal over 80% after 48 hours. The variations between runs could be due to a few factors; concentration of fungal pellets, the age of the algal culture and mixing speed. Mixing speed has been investigated previously, and it was shown that a lower speed could promote flocculation over a higher speed. This effect was examined by Bhattacharya et al. (2017b) who hypothesise that a lower RPM provides enough energy for the algal cells to overcome the electrostatic repulsion and adhere to the fungal hyphae. Additionally, a high RPM is believed to disrupt the adhesive forces between the

algal and fungal hyphae (Bhattacharya et al., 2017b). The results were investigated further to examine this phenomenon.

7.3.6 Effect on Mixing Speed on Flocculation Efficiency

Two different mixing speeds were used and compared to confirm if slower mixing speeds induce higher flocculation. Samples were mixed at 100rpm and 150rpm, and flocculation percentage removal values were compared. The results are shown in table 7.4. These results indicate that initially there was a small difference between the two speeds with 150rpm showing slightly better flocculation. However, after 72 hours, 100rpm had a better flocculation efficiency. Over time the interactions between the fungi and algae may weaken. The bond between the fungal filaments and the algal cells may be overcome by the mixing shear forces produced by the higher mixing speed which would dislodge algal cells and redisperse them into the culture medium. These results contradict Bhattacharya et al. (2017b) results; this could be caused by a few factors, firstly they used a single species of algae, a different fungal species and lastly they tested the flocculation efficiency over a short period (4 hours). The fungal species, *A. fumigatus* and the algal species, *C. pyrenoidosa* used by Bhattacharya et al. (2017b) may require different conditions to flocculate and could be more likely to flocculate readily. The consortium of algal species in the effluent may need higher energy to overcome the electrostatic charges and could need less time to adhere to the *A. oryzae* filaments. Algal species such as *Chlorella* sp. are small and spherical with no spines or flagella, whereas the consortium of natural algae may contain species such as *Scenedesmus, Euglena* and *Microcystis* which have protrusions which may affect flocculation. This effect may slow or even hinder flocculation.

Table 7.4: The mean flocculation efficiency between the two rotation mixing speeds, 150rpm and 100rpm after 24, 48 and 72 hours flocculation.

	24 Hours	**48 Hours**	**72 Hours**
150rpm	80%	81%	65%
100rpm	74%	85%	80%

These experiments show that *A. oryzae* can flocculate mixtures and concentrations of algae from effluent samples. The results also demonstrate that the flocculation mixing speed does affect flocculation efficiency in mixed consortiums of algae. However, this effect was marginal and more pronounced over an extended period.

7.3.7 Treatment of Primary Lagoon Effluent Utilising Fungal-Algal Pellets

Three co-cultures of fungi and algae were tested for their ability to remove nutrients from primary lagoon effluent in comparison to fungi and algae cultures. The results are tabulated in table 7.5.

Table 7.5: The percentage change of nutrients when treated utilising fungi and algae co-cultures and monocultures.

	Ammonia ($N\text{-}NH_3$)	**Orthophosphate ($P\text{-}PO_4$)**	**Nitrate (NO_2)**	**pH**
Control	-2%	-1%	11%	-1%
Fungi	61%	-45%	272%	0%
Primary effluent 23/02/18	-46%	-1%	192%	2%
Primary effluent 17/04/18	-36%	-18%	163%	3%
HRAP 03/11/17	-96%	-33%	100%	-10%
Fungi and Primary Effluent 23/02/18	-41%	-83%	33%	-4%
Fungi and Primary Effluent 17/04/18	141%	-27%	25%	0%
Fungi and HRAP 03/11/17	-39%	-27%	25%	-3%

When examining the changes in ammonia concentrations, it was observed that the algal cultures from all the effluent samples could remove ammonia. The fungi, however, showed an increase in ammonia levels in both the monoculture and one of the co-cultures. *A. oryzae* has been known to produce cellulase, chitinase and some other enzymes which would enable the fungi to break down the algal cells walls and potentially release the cellular compounds, which may potentially contain ammonia (Pandey et al., 1999, Xia et al., 2001). Ammonification may have also occurred by the breaking down of the organic nitrogen into ammonia. The co-cultures which did not show an increase in ammonia concentration may have contained different algal species which were harder for the fungi to digest or the remaining algae were able to remove the ammonia or a combination of both.

Investigating the changes in orthophosphate shows that all the cultures could remove some level of orthophosphate. The highest removal was seen in a co-culture sample with 83% removal, and the lowest observed was by an effluent algae sample with only a 1% removal. The fungi monoculture could remove 45% of the orthophosphate.

Nitrate concentrations in all samples showed no removal after 48 hours, and all the monoculture showed significant increases with the fungi monoculture increasing by 272% and all the algal cultures rising by 100% or more. The co-culture of fungi and algae showed significantly less change in nitrate concentration, although there was an increase. The effluent cultures may have contained nitrifying bacteria which were able to convert other forms of nitrogen into nitrate. The combined fungi and algae cultures may have assimilated nitrogen into the biomass instead of being converted into nitrate.

The pH in the cultures did not vary significantly during the experiment with the most substantial change being a reduction of 10%. This would be due to fungi preferring a lower pH environment and can reduce the pH through respiration, while algae tend to increase the pH through the consumption of carbon via photosynthesis. The mixture of the two processes may have neutralised the effects of each other.

Overall, this mixture of fungi and algae may not be applicable for nutrient removal from primary lagoon effluent in this investigation. Previous research has demonstrated that a combination of fungi and algae can reduce the nutrient concentration more efficiently then monoculture of the fungi or algae (Wrede et al., 2014). It would be beneficial to trail fungal species which are found at a wastewater treatment plant and potentially a higher concentration of algae. The effect of enzyme production of the fungi and algae co-culture needs to be investigated further especially regarding wastewater treatment.

7.4 Summary

The use of fungal flocculation of algae is an effective and economical method. This research has demonstrated that both single species of algae and mixtures of algae from effluent samples can be successfully harvested. From

the fungal species screened, *A. oryzae* was determined to be the most effective at flocculating single species of algae with flocculation of 99% achieved for certain algae. *A. oryzae* was also able to flocculate over 80% of the algae in the majority of the wastewater samples containing multiple species of algae after 48 hours. While the harvesting of mixed cultures was not perfect, this could be preferred as it would leave a seed culture of algae behind which would repopulate the effluent without the need for a new inoculum. Alternatively, the fungi may be flocculating only certain species of algae and leaving other species in suspension. These undesirable species, such as cyanobacteria, which would create problems due to higher concentrations in the receiving waters.

The mixing speed of the fungi-algal culture was also investigated for its impact on flocculation efficiency and it was determined that over 48 hours there was minimal difference in the removal of algae from suspension between the two speeds, 100 and 150 rpm. However, after 72 hours the slower speed had a higher percentage removal of algae than the faster speed. The ability of the fungal-algae culture to remove nutrients from wastewater samples was also investigated. The co-culture of fungi and algae tested were not able to enhance the nutrient removal of the system compared to the monocultures of algae and fungi.

Further research is required to identify the limitations of fungal flocculation of multiple species. High importance should be focused on the flocculation of toxic algae such as cyanobacterial species belonging to *Microcystis* and *Anabaena*, commonly found in wastewater and effluent samples. Additionally, the ability of fungi- algae co-cultures to sufficiently remove nutrients from wastewater should also be further investigated.

Chapter 8 - Conclusions and Recommendations

8.1 Summary

Successful treatment of wastewater can be challenging and costly. The use of microalgae in HRAPs is a well-established method for efficiently treating wastewater and has been shown to be cost-effective (Sutherland et al., 2015b, Craggs et al., 2014). This study investigated the treatment of secondary lagoon effluent utilising HRAP at Western Waters' Bacchus Marsh Recycled Water Plant Victoria, Australia. The aim of this study was to determine the nutrient removal capacity of the HRAPs, identify the productive potential of the HRAPs to grow algae and to test a number of algal harvesting techniques. The research objective and questions were as follows

- Investigate the relationship between productive potential of secondary lagoon effluent and the production of algal biomass in HRAPs.
- Maximise algal biomass growth and in turn nutrient removal by answering the following research questions.
    - How does the addition of laboratory-grown algae to the HRAPs during cooler months enhance biomass production?
    - How does the control of the pH level of the HRAPs utilising acids optimise biomass production?
    - How does the use of an alternative nutrient source, primary lagoon effluent, to alleviate the nutrient deplete conditions observed in the warmer months, enhance biomass production?
- To develop a simple model using minimal variables to predict algal biomass production in HRAPs under south-eastern Australian conditions at elevated pH conditions.
- Investigation of improved algal biomass harvesting methods by:
    - Comparing the filtration capability of three different membranes materials: PTFE, ceramic and metal to understand if material

properties of the membrane can improve fouling outcomes for algae filtration.

- Analysing the ability of fungal flocculation be utilised to simultaneously remove numerous species of algae from suspensions of treated effluent.

The outcomes of this research have led to (a) determining the productive potential of the HRAPs throughout the year, (b) determined which methods to enhance biomass production were effective, (c) the development of a simple and accurate predictive algal growth model, (d) determined that PTFE membranes were more effective than the other tested for harvesting algal biomass and (e) determined that *A. oryzae* could be utilised to flocculate several single species of algae as well as effectively flocculating a mixed algal community.

8.1.1 Summary of New Knowledge

The new knowledge produced during this research will help with the future use of HRAPs and potentially with their design and operation. The determination of productive potential in HRAPs utilising the light and dark bottle method had not been performed in HRAPs previously. Moreover, there are minimal other studies investigating the productive potential of HRAPs using alternative methods. The use of the light and dark bottle method provided easy to understand results and can be conducted without the need for complicated equipment. This method could be used to assess the potential for biomass production in different water bodies especially various wastewaters.

The biomass enhancement experiments each shed valuable insight into the growth of algae in the HRAPs. Firstly, the addition of laboratory-grown algae demonstrated the need for bioprospecting. Secondly, the control of the pH with acids provide a new insight into the impact of pH control on the algal growth in HRAPs. The addition of the two acids demonstrated that it is not solely the control of pH which enhances biomass production but also the addition of carbon. Previously, the addition of CO_2 gas was always inferred to enhance biomass production by the control of the pH of the system, but this research suggests that this is not the case. Thirdly, the comparison of the growth and

nutrient removal capacity of algae utilising secondary and primary lagoon effluent in HRAPs had not been done previously. These experiments provided valuable knowledge on why the primary lagoon effluent was ineffective in enhancing the algal biomass concentration and reinforced the significance of turbidity.

The model developed during this research is novel in that it was designed with the intention of utilising it for HRAPs which had elevated pH levels indicating a severely limited carbon supply. In addition to this, as far as this researcher knows there are no other predictive growth models developed and validated in the south-eastern Australian climate.

The use of metal membranes had not previously been trialled for their ability to harvest algae and there is also very little work on the algal filtration capabilities of PTFE membranes. While there is more research on ceramic membranes ability to harvest algae compared to the other two membranes, there still isn't an abundant amount and the results from this research would be beneficial. This research identified a membrane, PTFE, which displayed a very low fouling tendency and would be suitable for implementation into an already established HRAP or wastewater treatment plant.

The flocculation of algae utilising fungal pellets is a relatively new area and the ability to flocculate numerous species of algae simultaneously had not been done previously. Additionally, the fungal flocculation of *Dictyosphaerium* sp. and *S. acuminatus* had not previously been performed either.

The large amount of new knowledge produced from this research should provide valuable insight and will be of great benefit to the wider scientific community.

8.1.2 Summary of the Results

The PP of the HRAP was found to be highest in summer and spring with a maximum of 1946mg/m^3/h of carbon fixed while winter had the lowest PP of 105mg/m^3/h of carbon fixed. The maximum carbon fixed in this study was similar to that reported by Berner et al. (1986) which had a maximum of 2140mg/m^3/h of carbon fixed in high rate oxidation ponds. The addition of

laboratory-grown algal cultures during winter months was found to enhance the biomass production in the HRAPs but not the nutrient removal capacity. The use of inorganic acid to control the pH of the HRAPs was found to have a negative impact on algal production under conditions which would normally promote higher production. The largest difference observed was a decrease in biomass productivity of 19.3mg/L/D compared to the control HRAP. Additionally, the mean final biomass concentration for the inorganic pH HRAP was 30mg/L lower than the control HRAP which was outside of the error range of 4mg/L implying a statistical difference. Conversely, the use of organic acid was found to have a positive impact on algal productivity and increased biomass productivity by 17.3mg/L/D compared to the control HRAP. Furthermore, the final biomass concentration of the organic pH HRAP was 124mg/L higher than the control HRAP implying a significant statistical difference. The use of primary lagoon effluent instead of secondary lagoon effluent was not found to be effective in enhancing the biomass production in the HRAPs due to a strong light limitation effect caused by the turbidity of the primary lagoon effluent.

A simple predictive algal growth model was developed for HRAPs which utilised effluent samples in the south-eastern Australian climate. The model was simple and utilised data which can be easily obtained. The model was validated utilising both primary and secondary lagoon effluent that had elevated pH levels. When control of the pH level was trialled, the model was found to decrease in accuracy. The model overpredicted the biomass concentration when inorganic acid was utilised by 13.6mg/L and underpredicted when organic acid was utilised by 19.0mg/L and were both outside the established RMSE for the unaltered HRAPs of 11.2mg/L.

Algal harvesting can account for up to 20-30% of production costs and is notoriously difficult due to algae’s small size and disperse nature. An economic and effective method to harvest algae is required to enhance the feasibility of both algal wastewater treatment and algal biomass production. Three different membranes and membrane systems were tested for the ability to remove algal biomass from primary lagoon effluent. The PTFE submerged membrane rig was found to be the most effective as it had very little fouling and it was likely to be the most economical. The other membranes trialled, ceramic crossflow

membrane and the metal crossflow membrane were found to foul quickly and irreversibly, and the costs were significantly higher than the PTFE membrane.

The fungal species *A. oryzae* was able to flocculate three of the four single species of algae tested in monocultures. *A. oryzae* was also able to flocculate the algae in various wastewater samples with the flocculation efficiency ranging from 73% -100% for the removal of algal biomass from suspension.

8.2 Implication of the Results

8.2.1 Productive Potential of the HRAPs

The HRAPs were tested for their ability to treat secondary lagoon effluent and demonstrated they were able to efficiently remove ammonia, with removals ranging from 43% in winter to 99% in spring. Phosphorus was also removed to a lesser extent, with orthophosphate having slight increases in concentration during some winter and early spring runs and up to 81% removal in late spring. These removals were a combination of assimilation of nutrients into the algal biomass as well as ammonia volatilisation and phosphate precipitation. The PP results obtained also demonstrate that during spring, summer and autumn the production of carbon by the HRAPs was sufficient to support high levels of algal biomass and that in winter it did not assimilate a sufficient amount of carbon to support algal biomass production adequately. Summer and spring each had a mean biomass increase of over 300% (88mg/L) and autumn had a mean biomass increase of 174% (40mg/L) whereas winter only had a mean biomass increase of 60% (12mg/L). Furthermore, during winter there were a number of runs in which the biomass concentration decreased. The low mean biomass increase and the runs with negative biomass production during winter suggest that based on these results winter would be unable to adequately support algal biomass production.

The PP of the HRAPs when utilising primary lagoon effluent as the influent source was lower than when utilising secondary lagoon effluent due to the higher biomass concentration of the primary lagoon effluent resulting in a significant limitation in useable light. The results of this study suggest that

during spring, summer and autumn the algal biomass produced in the HRAPs was able to treat the wastewater effectively and could be harvested for either bioenergy production or as a feedstock for algae-based products. Additionally, controlled harvesting could enhance the productive potential of the HRAPs by ensuring the biomass concentration does not reach a level which diminishes the productive potential of the HRAPs due to self-shading. The Secchi disk readings collected during this research implies that almost half the HRAP was shaded once the biomass concentration reached 110mg/L. Further research would be required to ascertain the precise biomass concentration required to optimise production.

8.2.2 Algal Biomass Enhancement

8.2.2.1 Addition of Algae

During winter the algal concentration in the HRAPs was low due to the cold temperatures hindering algal growth and in turn, the low productive potential of the system. Three different algal species were grown in the laboratory in 20L cultures and added to the HRAPs with the aim to enhance biomass production and nutrient removal. The addition of *Dictyosphaerium* sp. *S. acuminatus* and *S. quadricauda* increased the initial biomass concentration of the HRAP by 35%, 11% and 56% respectively. The runs in which 20L of *Dictyosphaerium* sp. and *S. acuminatus* were added to the HRAPs both had final biomass concentration higher than the control HRAP. In contrast, the run in which *S. quadricauda* was added to the HRAP had a final biomass concentration lower than the control HRAP. This demonstrated the necessity for bioprospecting, an algal species which grows well in cold temperatures would be ideal to enhance biomass production. It also highlighted the need to carefully control the amount of algal biomass added to the HRAPs as this may influence the acclimatisation period and overall growth rate. The addition of laboratory-grown algal cultures to the HRAPs did not have a significant impact on the removal of nutrients such as ammonia and phosphorus as the results were comparable to that of the control HRAPs.

8.2.2.2 Control of the pH of the HRAPs utilising Acids

The pH and carbon limitation of HRAPs and algal cultures can impact algal growth, and these are commonly controlled by sparging CO_2 gas into the media. This technique is well established in the literature and is utilised in a number of large-scale facilities (Craggs et al., 2012, Chisti, 2016, Lundquist et al., 2018). During this research, the pH of the HRAPs was controlled to below 8.3 utilising either an inorganic or organic acid to discern whether it was solely the control of pH which enhanced algal growth or if it was also the addition of a carbon source. When inorganic acid was utilised to control the pH of the culture, there was a small increase in biomass production under low growth rate conditions. However, when the conditions promoted higher growth rates, the addition of inorganic acid negatively impacted algal growth. In contrast to this, the addition of organic acid enhanced the biomass production of the HRAPs in conditions which would naturally have a high growth rate. This research implies that it was not only the control of pH which enhanced algal growth but also the addition of a carbon source in conjunction with the pH control which significantly promoted algal biomass production. Further work on the use of organic acid to regulate pH and the economic feasibility of the process is required before this could be implemented on a larger scale.

8.2.2.3 Comparison between the use of Primary and Secondary Lagoon Effluent in the HRAPs

The nutrient concentration of secondary lagoon effluent was occasionally low in summer and spring and this negatively impacted algal biomass production. The use of primary lagoon effluent was investigated in an attempt to enhance the biomass production of the HRAPs. This research found that the use of only primary lagoon effluent in the HRAPs did not benefit the productivity of algal biomass. This was due to the turbidity of the primary lagoon effluent causing severe light limitations as well as a high oxidative metabolic rate which could not be supported. The use of a filtered primary lagoon effluent to supplement secondary lagoon effluent with the required nutrients could prove to be beneficial. However, further research is required to determine if this would be

an economically viable option as the filtration of the primary lagoon effluent and transport of the filtered effluent could add significant costs to the process.

8.2.3 Development and Validation of the Algal Growth Model

Algal growth models are often complex and species-specific. This research aimed to develop a model that is simple and utilised data which was commonly available to all HRAP systems. The developed model employed a modified version of the Steele model and the use of a Secchi disk to calculate the amount of light attenuation. The model utilised solar radiation, water temperature and biomass concentration. The Steele-Secchi model was validated and shown to be very accurate when utilising both secondary and primary lagoon effluent (R^2 = 0.98). The Steele-Secchi model was designed to be utilised in an elevated pH environment in south-eastern Australian and for effluent samples with an ammonia concentration higher than 1.0mg/L. The use of the model in an elevated pH environment is beneficial as HRAPs without pH control would rapidly reach an elevated pH level. Moreover, pH control can be costly and having the ability to predict algal growth without controlling the pH and focusing on other factors which are inexpensive to control could reduce overall costs. The model was not applicable when the pH of the HRAPs was controlled, and this was in accordance with the aim of the model. The Steele-Secchi model does not account for substantial changes in nutrient concentration and would be best utilised in a system which constantly ensures that the nutrient concentrations are maintained at a sufficient concentration. The use of Steele- Secchi model to predict biomass production in a continuous system was not validated, and this would be required if the model is to be implemented on an industrial scale.

8.2.4 Algal Harvesting via Membrane Filtration

Algal harvesting is a difficult and costly procedure for both algal biomass production and wastewater treatment due to algae's small size, disperse nature in suspension and ability to readily foul filters. Three different membranes each with a different filtration system and membrane composition were trialled to ascertain which was the most effective at removing algae from suspension and resisting fouling from algae. The membranes utilised were; a ceramic

membrane in a crossflow system, a PTFE membrane in a submerged membrane system and a metal membrane also in a crossflow system. The membranes in the crossflow arrangements were backwashed between filtration cycles and the submerged PTFE membranes had a relaxation period between filtration cycles. The PTFE membrane was found to be the most effective due to its low fouling rate and had a constant flux. The ceramic membrane fouled the most readily and severely (80%), and the metal membrane was also fouled significantly (51%). Furthermore, the cost of the three membranes was examined, and the PTFE membrane was proposed to be the most economical of the three membranes in this study. The systems utilised in this study were not optimised, and this would impact the effectiveness and economics of the systems. Due to the PTFE membranes low capital cost per treated volume of effluent, and low fouling rate it was determined to be the most suitable for larger scale, long term testing. The PTFE membrane could be utilised in both a wastewater system as well as an algal biomass production plant. The operation of the system would need to be optimised and trialled on a number of different wastewater samples as well as different biomass concentrations before it could be implemented with confidence in an industrial system.

8.2.5 Fungal Flocculation of Algae

Fungal flocculation is a relatively new technique utilised to harvest algae. Fungi are added to algal cultures, in this research as a pellet, and the algal cells attach or are entangled in the fungal pellet which can be easily removed by filtration. This research investigated if fungal pellets could flocculate a number of single species of algae as well as a mixed consortium of algae. The fungi *A. oryzae* was isolated and tested alongside numerous other isolates to identify its flocculation capacity, and it was found to be the most effective fungi tested. *A. oryzae* was able to flocculate three of the four single species of algae tested and had previously been shown to flocculate others. Fungal flocculation had not previously been performed on a mixed consortium of algae and based on the findings of this research it was determined that *A. oryzae* was successful in flocculating over 70% of the algae cells in all samples tested with 100% removal of algae cells in one of the tests. Further research is required to identify the limitations of fungal flocculation especially regarding the flocculation of multiple

species, and more research on which algal species can be successfully flocculated by various fungi is required. A large amount of focus should be on the ability of fungi to flocculate toxic cyanobacteria such as *Microcystis* sp. and *Anabaena* sp. both of which are commonly found in wastewater samples. The co-culture of flocculated fungi and algae were also tested for the ability to enhance nutrient removal; however, they did not sufficiently remove nutrients compared to monocultures of fungi and algae.

8.3 Limitations of the Research

As shown above, the objectives of this research were successfully met. However, it is important to understand that the outcomes were influenced by a number of limitations. The research attempted to answer the questions proposed fully, but limitations, such as scope, method and practicality influenced the outcomes.

8.3.1 Environmental Conditions

The outdoor location of the HRAPs introduced some limitations in terms of environmental conditions, natural hazards and constantly changing variables. Extreme conditions such as elevated temperatures (>35℃) hindered the collection of data and on-site testing as access to the site was prohibited because of the risk of bushfires. Natural hazards such as fire danger or native animals such as venomous snakes and spiders, were also present in the testing area and careful monitoring and considerations were needed to avoid these hazards. The constantly changing quality of the secondary lagoon effluent into the HRAPs in terms of nutrient concentrations influenced the growth of the algal biomass, and an artificial media would have provided a more controlled growth environment. However, this would not have been practical given the size of the HRAPs.

Additionally, biotic contaminants such as fungi, bacteria, virus and unwanted algal species introduced into the HRAPS from the environment and animals, were unavoidable and blooms of any of these may severely impact algal growth. Furthermore, zooplankton, such as Cladocera, rotifers and predatory insects would have had access to the open HRAPs and could have

consumed the algal biomass and decimated the algal biomass, although pumping the secondary lagoon effluent into the HRAPs appeared to control the presence of these species.

8.3.2 HRAP

The size and design of the HRAPs were not ideal and may have influenced the results. The size of the HRAPs was only pilot scale with a capacity of ~850L. Furthermore, the rectangular design of the HRAPs would cause eddies and dead spots in the flow of the effluent inside the HRAPs during operation. These dead spots while observed in this research did not influence the uniformity of the HRAPs as confirmed by the dissolved oxygen spot-check results. However, larger HRAPs of the same or similar design may have larger dead spots which could influence the uniformity and production of the system.

8.3.3 Algal Growth Modelling

In order to maintain the simplicity of the model, the impact of nutrient concentrations was excluded from the modelling. Major nutrients other than carbon were assumed to be non-limiting during the algal growth. As discussed in chapter 5 the use of the linear model does reduce the complexity of the algal growth model but implies that there are limiting factors which are unaccounted for. Furthermore, the model utilised daily means for temperature and solar radiation which may not have provided a complete representation of what was occurring in the HRAPs throughout the day. The accuracy of the model was reduced at low temperatures due to a number of runs experiencing decreases in biomass concentration. The impact of ammonia limitation was not fully investigated in this research and has been previously shown to strongly influence the PP of the HRAPs. In addition to this, the impact of very high ammonia concentrations and high temperatures can also negatively impact algal growth and these elevated conditions were not considered in this study. As the model was only validated in the south-eastern Australian environment this may reduce its application and the model may need to be validated outside of this environment.

8.3.4 Membrane Systems

The three membranes systems utilised in this research were all different, and none of which were optimised to maximise filtration. The ceramic and PTFE membrane systems were both laboratory scale, and the metal membrane system was a small pilot scale system. The small size of the membrane systems tested would have different hydrodynamics compared to larger scale systems and this could influence the flux and fouling of the membrane. The impact of flux on fouling was not examined in this study and differing flux parameters may enhance filtration. Furthermore, as the experiments were performed in a controlled laboratory environment the operating conditions may be different in a larger industrial environment. Nevertheless, the results still identified a membrane material that did not suffer from fouling when operated at a typical commercial flux

8.3.5 Fungal Flocculation

Due to the infancy of the fungal flocculation knowledge, there were a number of limitations. As the mode of flocculation is poorly understood an assumption that flocculation was caused by both entanglement and adhesion was made. The method utilised to create the fungal pellets requires refinement as the pellets occasionally varied in size and this would have impacted the flocculation efficiency. Furthermore, fungal flocculation has not been trialled in HRAPs and the shape, design and operation of HRAP could impact the flocculation effectiveness of the fungal pellets. Additionally, due to biosafety concerns and restrictions, it was not possible to test the flocculation ability of *A. oryzae* on any cyanobacteria species.

8.4 Recommendations for Future Work

The identification of the productive potential of the HRAP alongside the methods to enhance biomass production and the simple predictive algae growth modelling provide several opportunities for further work. The outcomes of these projects would only enhance the understanding of HRAPs and its capacity to act as a wastewater treatment method while providing a healthy supply of algal

biomass. The outcomes of the membrane filtration and fungal flocculation work also provide a number of opportunities for future work.

8.4.1 Algal Biomass Recommendations

Understanding the productive potential of systems is crucial and examining this over an extended period of time would provide invaluable information. This would provide excellent insight into the operation and biomass productivity of the HRAPs. Furthermore, additional research on optimising the enhancement of algal biomass production would be beneficial. The biggest challenge for growing algae in south-eastern Australia is the high fluctuations in temperature, especially the low temperatures observed during winter. The algal concentrations of the HRAPs were found to be low in winter and it would be beneficial to enhance the biomass concentration, this could potentially be done by adding the correct algal species. This research demonstrated that the growth rate could be improved by adding *Dictyosphaerium* sp. or *S. acuminatus* however, other species could prove better. It is crucial for future research to undergo some bioprospecting in order to identify which algal species would grow the fastest and most efficiently in the cooler winter periods. The operation of the HRAPs in winter may need to be assessed to ascertain if it is economically feasible to run the HRAPs due to the slow algal growth rate and low nutrient removal.

Another major challenge was the pH of the HRAPs, as the elevated pH of the HRAPs diminished the production of algal biomass. To maximise production and control the pH of the HRAPs further testing on the use of organic acid to control the pH is required. In order to be able to utilise organic acid pH control on an industrial scale, it is critical to determine the practicality, feasibility and economics of the process. Furthermore, comparing the addition of organic acid and sparging CO_2 gas to control pH would be beneficial. Additionally, utilising secondary lagoon effluent as the nutrient source for algal growth is problematic as it is commonly low in vital nutrients especially during summer and spring. Filtered primary lagoon effluent, which would be high in nutrients but have low turbidity, could be utilised to overcome this. Testing the effectiveness of utilising filtered primary lagoon effluent as a nutrient source to

replenish the nutrient concentration in the HRAPs during spring and summer may prove advantageous. It would be vital to determine the feasibility and economics of the addition of filtered primary lagoon effluent regarding the filtration and transport of the primary lagoon effluent.

There is potential for the Steele-Secchi model to be enhanced and modified to more accurately predict the algal growth rate. The inclusion of a nutrient limitation equation especially for ammonia would prove beneficial as ammonia was commonly found to be limited towards the end of the runs. Additionally, low nitrogen concentrations have been found to enhance the oil production in algal biomass, so including a nutrient limitation equation in the model may be of interest for these systems. Validating the Steele-Secchi model at other sites in south-eastern Australia and also larger systems is a crucial step in evaluating its effectiveness for use on an industrial scale. Additionally, this would help identify if the model could be successfully implemented into an established system.

8.4.2 Membrane Recommendations

Membrane filtration of algae is not a new concept, and the filtration of algae utilising polymeric membranes is well documented. However, there is minimal work of the use of PTFE and ceramic membranes and virtually none on the use of a metal membrane for algal harvesting. The PTFE membrane was found to be the best membrane tested for the removal of algal biomass. The PTFE membrane was only operated over three cycles at the time and further tests over a longer period of time are required. Furthermore, testing a pilot scale system is crucial in order to examine the difference in the hydrodynamics of the system. Additionally, a techno-economic analysis of the implementation and operation of the PTFE membrane system is required to ensure that it is the most effective and economical filtration system. The research in this study only examined the use of the three membrane material, each in one type of filtration system. Determining the effectiveness of various types of PTFE, ceramic and metal membranes such as flat sheet or hollow fibre, to remove algae from suspension in various different filtration systems would provide valuable

information and could confirm if the PTFE membrane is the most effective membrane for the filtration of algae.

8.4.3 Fungal Flocculation Recommendation

Regarding, fungal flocculation there are a few aspects which would benefit greatly from further research. One of the major recommendations would be to investigate the feasibility of upscaling fungal flocculation. Currently, all the fungal pellet formation has been done in a laboratory, and the largest volume of algae flocculated successfully was 250L. It would also be beneficial to determine if the fungal pellets can be produced on a large scale and economically. Additionally, identifying the flocculation ability of the fungi in a HRAP system is crucial as it has different hydrodynamics to laboratory cultures. Furthermore, as there is already an established understanding of which fungi can flocculate which algae, it would be useful to identify which fungi are also able to flocculate mixed algal communities. Determining if it is possible to flocculate various cyanobacteria such as *Microcystis* sp. or *Anabaena* sp. would also prove valuable, as this could provide an effective and economical method in which to remove these toxic nuisance organisms. It would also be advantageous to work on further understanding the flocculation mechanisms and if it would be possible to induce fungal flocculation of algae utilising any fungal species under the correct conditions. Being able to flocculate algae utilising fungi such as, *Penicillium* sp. would help to alleviate the impact of harmful bacteria and may enhance the scope for which the harvested algal biomass could be utilised. In addition to this, some fungi are known to produce cellulase and other extracellular compounds which could be utilised to help break open algal cell walls. It would be beneficial to ascertain if the fungal flocculation process could act as a pre-treatment step in breaking open the algal cells so that they can be more readily utilised for feedstock for various algal products or for anaerobic digestion, as this would reduce costs and may make certain products more economical.

8.4.4 Industrial Applications and Recommendations

The industrial use of HRAPs to produce algal biomass while treating wastewater needs further research. The main areas which require further

research are the methods in which to enhance or optimise biomass production. In areas such as south-eastern Australia, there are large fluctuations in temperature throughout the year. Research into the feasibility and economics of various methods to heat and control the temperature of HRAPs would be of great significance. The size of HRAPs plays a large role in its ability to maintain and disperse heat. The use of hot flue gas from a nearby power plant could be sparged into a HRAP. This could both heat the water while also providing CO_2 gas to help control the pH and enhance growth. Additionally, clear glass solar panels (ClearVue), which utilise ultraviolet and infrared light instead of visible light, could be placed over the HRAPs, and the energy provided from these solar panels could be utilised to heat the HRAPs, power the paddlewheel and power the pH control mechanisms (Vasiliev and Alameh, 2018). However this technology is quite new, and this may not currently be economical. The algal biomass produced in HRAPs utilising wastewater is contaminated by bacteria and other organic compounds and cannot be used for pharmaceuticals and several other products. To enhance the feasibility of HRAPs, further research into the uses of the algal biomass grown on wastewater is required. Currently, the production of algal biomass for biodiesel production is not economical and investigation into other products such as biogas production or animal feeds are currently a more feasible pathway. The energy from the biogas produced through anaerobic digestion of the algal biomass could be recycled into the treatment plant to reduce its carbon footprint. Furthermore, it may be possible for the algal biomass to be utilised as a feedstock for poultry, livestock or aquatic animals, however, the biosafety implications and potential benefits are unknown. The production of algal products generally creates a number of by-products and research on the reuse of these by-products could be useful. For example, unused disrupted algal cells could be digested, and the biogas produced could be utilised as a power source or recycled back into the HRAPs to supplement nutrients.

8.5 Closing Statement

The outcomes of this research offer further insight into the operation and application of HRAPs in the wastewater industry. The main objective of a

wastewater treatment plant is the economical and reliable treatment of water. This research demonstrated that HRAPs were capable of reliably treating the wastewater in a controlled and effective manner. The developed model and biomass enhancement methods examined have the potential to help predict and improve the biomass production potential of HRAPs. The simple and easy to access data utilised in the model allows for it to be easily applied to an established HRAP system. Furthermore, the accuracy of the model in the south-eastern Australian climate and in an elevated pH environment has not been previously achieved and can be of great importance for the development of new wastewater treatment plants. The success of both the PTFE algal harvesting membrane system and the fungal flocculation offer many opportunities for industrial application.

The production potential results, biomass enhancement techniques, modelling, membrane filtration research and the work on fungal flocculation all provide invaluable information for the successful operation of HRAPs to treat wastewater and produce algal biomass simultaneously.

References

ABDEL-RAOUF, N., AL-HOMAIDAN, A. A. & IBRAHEEM, I. B. M. 2012. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences,* 19**,** 257-275.

ACIÉN, F. G., GÓMEZ-SERRANO, C., MORALES-AMARAL, M. M., FERNÁNDEZ-SEVILLA, J. M. & MOLINA-GRIMA, E. 2016. Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? *Applied Microbiology and Biotechnology,* 100**,** 9013-9022.

AHMAD, A. L., MAT YASIN, N. H., DEREK, C. J. C. & LIM, J. K. 2012. Crossflow microfiltration of microalgae biomass for biofuel production. *Desalination,* 302**,** 65-70.

AL-HOTHALY, K. A., ADETUTU, E. M., TAHA, M., FABBRI, D., LORENZETTI, C., CONTI, R., MAY, B. H., SHAR, S. S., BAYOUMI, R. A. & BALL, A. S. 2015. Bio-harvesting and pyrolysis of the microalgae *Botryococcus braunii*. *Bioresource technology,* 191**,** 117-123.

ALAM, M. A., VANDAMME, D., CHUN, W., ZHAO, X., FOUBERT, I., WANG, Z., MUYLAERT, K. & YUAN, Z. 2016. Bioflocculation as an innovative harvesting strategy for microalgae. *Reviews in Environmental Science and Bio/Technology,* 15**,** 573-583.

AMINI, H., WANG, L. & SHAHBAZI, A. 2016. Effects of harvesting cell density, medium depth and environmental factors on biomass and lipid productivities of Chlorella vulgaris grown in swine wastewater. *Chemical Engineering Science,* 152**,** 403-412.

APHA/AWWA/WEF 1998. Standard methods for the examination of water and wastewater. *American Public Health Association. American Water Works Association and World Environment Federation. 20th Edition, Washington DC*.

AZOV, Y. & GOLDMAN, J. C. 1982. Free ammonia inhibition of algal photosynthesis in intensive cultures. *Applied and environmental microbiology,* 43**,** 735-739.

BARKAN, C. M., DELUCA, K. A. & PRESTON, M. R. 2018. Design of A Flexible, High-volume Direct Flue Gas-to-Algae Conversion Process for Value-added Bioproducts.

BARROS, A. I., GONÇALVES, A. L., SIMÕES, M. & PIRES, J. C. M. 2015. Harvesting techniques applied to microalgae: A review. *Renewable and Sustainable Energy Reviews,* 41**,** 1489-1500.

BASHAR, R., GUNGOR, K., KARTHIKEYAN, K. & BARAK, P. 2018. Cost effectiveness of phosphorus removal processes in municipal wastewater treatment. *Chemosphere,* 197**,** 280-290.

BEAL, C. M., GERBER, L. N., SILLS, D. L., HUNTLEY, M. E., MACHESKY, S. C., WALSH, M. J., TESTER, J. W., ARCHIBALD, I., GRANADOS, J. & GREENE, C. H. 2015. Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment. *Algal Research,* 10**,** 266-279.

BÉCHET, Q., CHAMBONNIÈRE, P., SHILTON, A., GUIZARD, G. & GUIEYSSE, B. 2015. Algal productivity modeling: A step toward accurate assessments of full-scale algal cultivation. *Biotechnology and Bioengineering,* 112**,** 987-996.

BÉCHET, Q., SHILTON, A. & GUIEYSSE, B. 2013. Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. *Biotechnology Advances,* 31**,** 1648-1663.

BÉCHET, Q., SHILTON, A. & GUIEYSSE, B. 2016. Maximizing Productivity and Reducing Environmental Impacts of Full-Scale Algal Production through Optimization of Open Pond Depth and Hydraulic Retention Time. *Environmental Science & Technology,* 50**,** 4102-4110.

BÉCHET, Q., SHILTON, A., PARK, J. B., CRAGGS, R. J. & GUIEYSSE, B. 2011. Universal temperature model for shallow algal ponds provides improved accuracy. *Environmental science & technology,* 45**,** 3702-3709.

BENEMANN, J. R. 2003. Bio-fixation of CO_2 and greenhouse gas abatement with microalgae-technology roadmap. *Final Report to the US Department of Energy. National Energy Technology Laboratory*.

BERNARD, O. & RÉMOND, B. 2012. Validation of a simple model accounting for light and temperature effect on microalgal growth. *Bioresource Technology,* 123**,** 520-527.

BERNER, T., DUBINSKY, Z., SCHANZ, F., GROBBELAAR, J., RAI, H., UEHLINGER, U. & FALKOWSKI, P. 1986. The measurement of primary productivity in a high-rate oxidation pond (HROP). *Journal of plankton research,* 8**,** 659-672.

BEUCKELS, A., SMOLDERS, E. & MUYLAERT, K. 2015. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Research,* 77**,** 98-106.

BHATTACHARYA, A., MALIK, A. & MALIK, H. K. 2017a. A mathematical model to describe the fungal assisted algal flocculation process. *Bioresource Technology,* 244**,** 975-981.

BHATTACHARYA, A., MATHUR, M., KUMAR, P., PRAJAPATI, S. K. & MALIK, A. 2017b. A rapid method for fungal assisted algal flocculation: Critical parameters & mechanism insights. *Algal Research,* 21**,** 42-51.

BHAVE, R., KURITZ, T., POWELL, L. & ADCOCK, D. 2012. Membrane-based energy efficient dewatering of microalgae in biofuels production and recovery of value added co-products. *Environmental science & technology,* 46**,** 5599-5606.

BILAD, M. R., ARAFAT, H. A. & VANKELECOM, I. F. J. 2014a. Membrane technology in microalgae cultivation and harvesting: A review. *Biotechnology Advances,* 32**,** 1283-1300.

BILAD, M. R., DISCART, V., VANDAMME, D., FOUBERT, I., MUYLAERT, K. & VANKELECOM, I. F. J. 2014b. Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR). *Bioresource Technology,* 155**,** 410-417.

BILAD, M. R., VANDAMME, D., FOUBERT, I., MUYLAERT, K. & VANKELECOM, I. F. J. 2012. Harvesting microalgal biomass using submerged microfiltration membranes. *Bioresource Technology,* 111**,** 343-352.

BOROWITZKA, M. A. & VONSHAK, A. 2017. Scaling up microalgal cultures to commercial scale. *European Journal of Phycology,* 52**,** 407-418.

BOUGARAN, G., BERNARD, O. & SCIANDRA, A. 2010. Modeling continuous cultures of microalgae colimited by nitrogen and phosphorus. *Journal of Theoretical Biology,* 265**,** 443-454.

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**,** 557-577.

CAI, T., PARK, S. Y. & LI, Y. 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews,* 19**,** 360-369.

CALDWELL, D. 1946. Sewage oxidation ponds: performance, operation and design. *Sewage Works Journal,* 18**,** 433-458.

CARNEY, L. T., WILKENFELD, J. S., LANE, P. D., SOLBERG, O. D., FUQUA, Z. B., CORNELIUS, N. G., GILLESPIE, S., WILLIAMS, K. P., SAMOCHA, T. M. & LANE, T. W. 2016. Pond Crash Forensics: Presumptive identification of pond crash agents by next generation sequencing in replicate raceway mass cultures of *Nannochloropsis salina*. *Algal Research,* 17**,** 341-347.

CASSARDO, C. & JONES, J. A. A. 2011. Managing water in a changing world. Molecular Diversity Preservation International.

CHAN, Y. J., CHONG, M. F., LAW, C. L. & HASSELL, D. G. 2009. A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal,* 155**,** 1-18.

CHEN, F., LIU, Z., LI, D., LIU, C., ZHENG, P. & CHEN, S. 2012. Using ammonia for algae harvesting and as nutrient in subsequent cultures. *Bioresource Technology,* 121**,** 298-303.

CHEN, G., ZHAO, L. & QI, Y. 2015. Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. *Applied Energy,* 137**,** 282-291.

CHEN, J., LENG, L., YE, C., LU, Q., ADDY, M., WANG, J., LIU, J., CHEN, P., RUAN, R. & ZHOU, W. 2018. A comparative study between fungal pellet- and spore-assisted microalgae harvesting methods for algae bioflocculation. *Bioresource technology*.

CHEN, S., CHEN, X., PENG, Y. & PENG, K. 2009. A mathematical model of the effect of nitrogen and phosphorus on the growth of blue-green algae population. *Applied Mathematical Modelling,* 33**,** 1097-1106.

CHEN, Z., ZHANG, X., JIANG, Z., CHEN, X., HE, H. & ZHANG, X. 2016. Light/dark cycle of microalgae cells in raceway ponds: Effects of paddlewheel rotational speeds and baffles installation. *Bioresource Technology,* 219**,** 387-391.

CHENG, J., YANG, Z., YE, Q., ZHOU, J. & CEN, K. 2016. Improving CO_2 fixation with microalgae by bubble breakage in raceway ponds with up–down chute baffles. *Bioresource Technology,* 201**,** 174-181.

CHISTI, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances,* 25**,** 294-306.

CHISTI, Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology,* 26**,** 126-131.

CHISTI, Y. 2016. Large-Scale Production of Algal Biomass: Raceway Ponds. *In:* BUX, F. & CHISTI, Y. (eds.) *Algae Biotechnology: Products and Processes.* Cham: Springer International Publishing.

CHOI, Y.-N., CHO, H. U., UTOMO, J. C., SHIN, D. Y., KIM, H. K. & PARK, J. M. 2016. Efficient harvesting of *Synechocystis* sp. PCC 6803 with filamentous fungal pellets. *Journal of Applied Phycology***,** 1-7.

CHRISTENSON, L. & SIMS, R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances,* 29**,** 686-702.

COLE, G. A. & WEIHE, P. E. 2015. *Textbook of Limnology*, Waveland Press.

COSTA, J. A. V. & DE MORAIS, M. G. 2011. The role of biochemical engineering in the production of biofuels from microalgae. *Bioresource technology,* 102**,** 2-9.

CRAGGS, R., PARK, J., HEUBECK, S. & SUTHERLAND, D. 2014. High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of Botany,* 52**,** 60-73.

CRAGGS, R., SUTHERLAND, D. & CAMPBELL, H. 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater

treatment and biofuel production. *Journal of Applied Phycology,* 24**,** 329-337.

CROMAR, N. J. & FALLOWFIELD, H. J. 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. *Journal of Applied Phycology,* 9**,** 301-309.

CSIRO MARINE RESEARCH. *Media Recipes used in CMAR* [Online]. Available: http://www.marine.csiro.au/microalgae/methods/Media%20CMARC%20recipes.htm 2016].

DE GODOS, I., MENDOZA, J. L., ACIÉN, F. G., MOLINA, E., BANKS, C. J., HEAVEN, S. & ROGALLA, F. 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresource Technology,* 153**,** 307-314.

DECOSTERE, B., DE CRAENE, J., VAN HOEY, S., VERVAEREN, H., NOPENS, I. & VAN HULLE, S. W. H. 2016. Validation of a microalgal growth model accounting with inorganic carbon and nutrient kinetics for wastewater treatment. *Chemical Engineering Journal,* 285**,** 189-197.

DOE., U. S. D. O. E. 2016. National Algal Biofuels Technology Review. *In:* U.S. DEPARTMENT OF ENERGY, O. O. E. E. A. R. E., BIOENERGY TECHNOLOGIES OFFICE (ed.).

DREXLER, I. L. C. & YEH, D. H. 2014. Membrane applications for microalgae cultivation and harvesting: a review. *Reviews in Environmental Science and Bio/Technology,* 13**,** 487-504.

EDMUNDSON, S. J. & HUESEMANN, M. H. 2015. The dark side of algae cultivation: Characterizing night biomass loss in three photosynthetic algae, Chlorella sorokiniana, Nannochloropsis salina and Picochlorum sp. *Algal Research,* 12**,** 470-476.

EDWARDS, K. F., THOMAS, M. K., KLAUSMEIER, C. A. & LITCHMAN, E. 2016. Phytoplankton growth and the interaction of light and temperature: A synthesis at the species and community level. *Limnology and Oceanography,* 61**,** 1232-1244.

ELCIK, H. & CAKMAKCI, M. 2017. Harvesting microalgal biomass using crossflow membrane filtration: critical flux, filtration performance, and fouling characterization. *Environmental Technology,* 38**,** 1585-1596.

ESPINOSA-ORTIZ, E. J., RENE, E. R., PAKSHIRAJAN, K., VAN HULLEBUSCH, E. D. & LENS, P. N. L. 2016. Fungal pelleted reactors in wastewater treatment: Applications and perspectives. *Chemical Engineering Journal,* 283**,** 553-571.

EUSTANCE, E., WRAY, J. T., BADVIPOUR, S. & SOMMERFELD, M. R. 2016. The effects of cultivation depth, areal density, and nutrient level on lipid accumulation of *Scenedesmus acutus* in outdoor raceway ponds. *Journal of Applied Phycology,* 28**,** 1459-1469.

FALLOWFIELD, H., TAYLOR, M., BAXTER, K., LEWIS, J. & BUCHANAN, N. 2016. Comparison of the performance of high rate algal ponds fed wastewater and wastewater enriched with CO_2 recovered from biogas at Melbourne Water, Western Treatment Plant.

GARCÍA, J., MUJERIEGO, R. & HERNÁNDEZ-MARINÉ, M. 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. *Journal of Applied Phycology,* 12**,** 331-339.

GOLUEKE, C. G., OSWALD, W. J. & GOTAAS, H. B. 1957. Anaerobic digestion of algae. *Applied microbiology,* 5**,** 47.

GONZÁLEZ-FERNÁNDEZ, C. & BALLESTEROS, M. 2012. Microalgae autoflocculation: an alternative to high-energy consuming harvesting methods. *Journal of Applied Phycology,* 25**,** 991-999.

GULTOM, S. O., ZAMALLOA, C. & HU, B. 2014. Microalgae harvest through fungal pelletization—co-culture of *Chlorella vulgaris* and *Aspergillus niger*. *Energies,* 7**,** 4417-4429.

HACH 2008. *Procedure Manual,* Hach, Loveland, CO.

HALLMANN, A. 2007. Algal transgenics and biotechnology. *Transgenic Plant J,* 1**,** 81-98.

HARDING, W. R. 1997. Phytoplankton primary production in a shallow, well-mixed, hypertrophic South African lake. *Hydrobiologia,* 344**,** 87-102.

HAZLEBECK, D. Commercial Zobi Harvester Operations. 8th International Conference on Algal Biomass, Biofuels and Bioproducts, 2018 Seattle USA. Elsevier.

HE, J. & CHEN, J. P. 2014. A comprehensive review on biosorption of heavy metals by algal biomass: Materials, performances, chemistry, and modeling simulation tools. *Bioresource Technology,* 160**,** 67-78.

HEAVEN, S., SALTER, A. M., CLARKE, D. & PAK, L. N. 2012. Algal wastewater treatment systems for seasonal climates: Application of a simple modelling approach to generate local and regional design guidelines. *Water Research,* 46**,** 2307-2323.

HENA, S., FATIHAH, N., TABASSUM, S., LALUNG, J. & JING, S. 2015. Magnetophoretic harvesting of freshwater microalgae using polypyrrole/$_{Fe}$3O4nanocomposite and its reusability. *Journal of Applied Phycology***,** 1-13.

HU, B., MIN, M., ZHOU, W., LI, Y., MOHR, M., CHENG, Y., LEI, H., LIU, Y., LIN, X., CHEN, P. & RUAN, R. 2012. Influence of Exogenous CO2 on Biomass and Lipid Accumulation of Microalgae *Auxenochlorella protothecoides* Cultivated in Concentrated Municipal Wastewater. *Applied Biochemistry and Biotechnology,* 166**,** 1661-1673.

HUESEMANN, M., CROWE, B., WALLER, P., CHAVIS, A., HOBBS, S., EDMUNDSON, S. & WIGMOSTA, M. 2016. A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures. *Algal Research,* 13**,** 195-206.

HUESEMANN, M. H., VAN WAGENEN, J., MILLER, T., CHAVIS, A., HOBBS, S. & CROWE, B. 2013. A screening model to predict microalgae biomass growth in photobioreactors and raceway ponds. *Biotechnology and bioengineering,* 110**,** 1583-1594.

HUSSAINY, S. U. 1979. Ecological studies of lagoons at Werribee: removal of biochemical oxygen demand, nitrogen and heavy metal. *Progress in water technology,* 11**,** 315-37.

HUSSAINY, S. U. 2018. Type to WREDE, D.

HUTCHINSON, G. E. 1957. A Treatise on Limnology Volume 1- Geography, Physics, and Chemistry. J. Wiley & Sons.

JAYARAMAN, S. K. & RHINEHART, R. R. 2015. Modeling and Optimization of Algae Growth. *Industrial & Engineering Chemistry Research,* 54**,** 8063-8071.

JOHIR, M. A. H., VIGNESWARAN, S., KANDASAMY, J. & SLEIGH, R. 2013. Coupling of physico-chemical treatment and steel membrane filtration to enhanced organic removal in wastewater treatment. *Desalination and Water Treatment,* 51**,** 2695-2701.

JUNEJA, A., CEBALLOS, R. & MURTHY, G. 2013. Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: A Review. *Energies,* 6**,** 4607.

KIM, J.-O., JUNG, J.-T. & CHUNG, J. 2007. Treatment performance of metal membrane microfiltration and electrodialysis integrated system for wastewater reclamation. *Desalination,* 202**,** 343-350.

KUMAR, K., MISHRA, S. K., SHRIVASTAV, A., PARK, M. S. & YANG, J.-W. 2015. Recent trends in the mass cultivation of algae in raceway ponds. *Renewable and Sustainable Energy Reviews,* 51**,** 875-885.

KUMAR, R. & ISMAIL, A. 2015. Fouling control on microfiltration/ultrafiltration membranes: Effects of morphology, hydrophilicity, and charge. *Journal of Applied Polymer Science,* 132.

LAAMANEN, C. A. & SCOTT, J. A. 2017. Development of heat-aided flocculation for flotation harvesting of microalgae. *Biomass and Bioenergy,* 107**,** 150-154.

LEE, A. K., LEWIS, D. M. & ASHMAN, P. J. 2013. Harvesting of marine microalgae by electroflocculation: The energetics, plant design, and economics. *Applied Energy,* 108**,** 45-53.

LEITE, G. B., ABDELAZIZ, A. E. & HALLENBECK, P. C. 2013. Algal biofuels: challenges and opportunities. *Bioresource technology,* 145**,** 134-141.

LI, Y., XU, Y., LIU, L., LI, P., YAN, Y., CHEN, T., ZHENG, T. & WANG, H. 2017. Flocculation mechanism of *Aspergillus niger* on harvesting of *Chlorella vulgaris* biomass. *Algal Research,* 25**,** 402-412.

LIAO, Y., BOKHARY, A., MALEKI, E. & LIAO, B. 2018. A review of membrane fouling and its control in algal-related membrane processes. *Bioresource Technology*.

LIU, B., QU, F., LIANG, H., VAN DER BRUGGEN, B., CHENG, X., YU, H., XU, G. & LI, G. 2017. Microcystis aeruginosa-laden surface water treatment using ultrafiltration: Membrane fouling, cell integrity and extracellular organic matter rejection. *Water Research,* 112**,** 83-92.

LIZARRALDE, I., BROUCKAERT, C. J., VANROLLEGHEM, P. A., IKUMI, D. S., EKAMA, G. A., AYESA, E. & GRAU, P. Incorporating aquatic chemistry into wastewater treatment process models: A critical review of different approaches. Proceedings 4th IWA/WEF Wastewater Treatment

Modelling Seminar. WWTmod2014, Spa, Belgium, March 30–April 2 2014, 2014. 227-232.

LOFRANO, G. & BROWN, J. 2010. Wastewater management through the ages: A history of mankind. *Science of The Total Environment,* 408**,** 5254-5264.

LOW-DÉCARIE, E., FUSSMANN, G. F. & BELL, G. 2011. The effect of elevated CO2 on growth and competition in experimental phytoplankton communities. *Global Change Biology,* 17**,** 2525-2535.

LUNDQUIST, T., SPIERLING, R., POOLE, K., BLACKWELL, S., CROWE, B., HUTTON, M. & LEHR, C. 2018. Development Of Nutrient And Water Recycling Capabilities In Algae Biofuels Production Systems. Final Summary Report. California Polytechnic State Univ.(CalPoly), San Luis Obispo, CA (United States).

LYNCH, F., SANTANA-SÁNCHEZ, A., JÄMSÄ, M., SIVONEN, K., ARO, E.-M. & ALLAHVERDIYEVA, Y. 2015. Screening native isolates of cyanobacteria and a green alga for integrated wastewater treatment, biomass accumulation and neutral lipid production. *Algal Research,* 11**,** 411-420.

MACKAY, S., GOMES, E., HOLLIGER, C., BAUER, R. & SCHWITZGUÉBEL, J.-P. 2015. Harvesting of Chlorella sorokiniana by co-culture with the filamentous fungus *Isaria fumosorosea*: A potential sustainable feedstock for hydrothermal gasification. *Bioresource Technology,* 185**,** 353-361.

MAHAPATRA, D. M., CHANAKYA, H. N. & RAMACHANDRA, T. V. 2014. Bioremediation and lipid synthesis through mixotrophic algal consortia in municipal wastewater. *Bioresource Technology,* 168**,** 142-150.

MANI, D. & KUMAR, C. 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *Int J Environ Sci Te,* 11.

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**,** 217-232.

MATHUR, M., BHATTACHARYA, A. & MALIK, A. 2017. Advancements in Algal Harvesting Techniques for Biofuel Production. *In:* GUPTA, S. K., MALIK,

A. & BUX, F. (eds.) *Algal Biofuels: Recent Advances and Future Prospects.* Cham: Springer International Publishing.

MAYNARD, H. E., OUKI, S. K. & WILLIAMS, S. C. 1999. Tertiary lagoons: a review of removal mechanisms and performance. *Water Research,* 33**,** 1-13.

MEHRABADI, A., CRAGGS, R. & FARID, M. M. 2015. Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. *Bioresource technology,* 184**,** 202-214.

MEHRABADI, A., FARID, M. M. & CRAGGS, R. 2016. Variation of biomass energy yield in wastewater treatment high rate algal ponds. *Algal Research,* 15**,** 143-151.

MENNAA, F. Z., ARBIB, Z. & PERALES, J. A. 2015. Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability. *Water Research,* 83**,** 42-51.

MILLEDGE, J. J. & HEAVEN, S. 2013. A review of the harvesting of micro-algae for biofuel production. *Reviews in Environmental Science and Bio/Technology,* 12**,** 165-178.

MIRANDA, A., TAHA, M., WREDE, D., MORRISON, P., BALL, A., STEVENSON, T. & MOURADOV, A. 2015. Lipid production in association of filamentous fungi with genetically modified cyanobacterial cells. *Biotechnology for Biofuels,* 8**,** 179.

MO, W., SOH, L., WERBER, J. R., ELIMELECH, M. & ZIMMERMAN, J. B. 2015. Application of membrane dewatering for algal biofuel. *Algal Research,* 11**,** 1-12.

MONTEMEZZANI, V., DUGGAN, I. C., HOGG, I. D. & CRAGGS, R. J. 2015. A review of potential methods for zooplankton control in wastewater treatment High Rate Algal Ponds and algal production raceways. *Algal Research,* 11**,** 211-226.

MURADOV, N., TAHA, M., MIRANDA, A. F., WREDE, D., KADALI, K., GUJAR, A., STEVENSON, T., BALL, A. S. & MOURADOV, A. 2015. Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. *Biotechnology for biofuels,* 8**,** 1-23.

MURRELL, M. C., CAFFREY, J. M., MARCOVICH, D. T., BECK, M. W., JARVIS, B. M. & HAGY, J. D. 2018. Seasonal Oxygen Dynamics in a Warm Temperate Estuary: Effects of Hydrologic Variability on Measurements of Primary Production, Respiration, and Net Metabolism. *Estuaries and Coasts,* 41**,** 690-707.

NAGAPPAN, S. & VERMA, S. K. 2016. Growth model for raceway pond cultivation of Desmodesmus sp. MCC34 isolated from a local water body. *Engineering in Life Sciences,* 16**,** 45-52.

OLGUIN, E., RODRIGUEZ, D., SANCHEZ, G., HERNANDEZ, E. & RAMIREZ, M. 2003. Productivity, protein content and nutrient removal from anaerobic effluents of coffee wastewater in *Salvinia minima* ponds, under subtropical conditions. *Acta biotechnologica,* 23**,** 259-270.

OLSSON, N. 2015. The mechanism of microalgal adhesion to fungal pellets and its applications.

OSWALD, W., GOTAAS, H., GOLUEKE, C., KELLEN, W., GLOYNA, E. & HERMANN, E. 1957. Algae in waste treatment [with discussion]. *Sewage and Industrial Wastes,* 29**,** 437-457.

OXYMEM. 2018. *OxyMem* [Online]. Available: https://oxymem.com/ [Accessed 10/09/2018.

PANDEY, A., SELVAKUMAR, P., SOCCOL, C. R. & NIGAM, P. 1999. Solid state fermentation for the production of industrial enzymes. *Current science***,** 149-162.

PARK, C. J., AHN, H. M., CHO, S. C., KIM, T. H., OH, J. M., AHN, H. K., CHUN, S. H. & GYE, M. C. 2014. Developmental toxicity of treated municipal wastewater effluent on *Bombina orientalis* (Amphibia: Anura) embryos. *Environmental toxicology and chemistry,* 33**,** 954-961.

PARK, J., CRAGGS, R. & SHILTON, A. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource technology,* 102**,** 35-42.

PARKER, C., JONES, H. & TAYLOR, W. 1950. Purification of sewage in lagoons. *Sewage and Industrial Wastes***,** 760-775.

PAWAR, S. 2016. Effectiveness mapping of open raceway pond and tubular photobioreactors for sustainable production of microalgae biofuel. *Renewable and Sustainable Energy Reviews,* 62**,** 640-653.

POWELL, N., SHILTON, A., CHISTI, Y. & PRATT, S. 2009. Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. *Water Research,* 43**,** 4207-4213.

PRAGYA, N., PANDEY, K. K. & SAHOO, P. K. 2013. A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renewable and Sustainable Energy Reviews,* 24**,** 159-171.

PRAJAPATI, S. K., BHATTACHARYA, A., KUMAR, P., MALIK, A. & VIJAY, V. K. 2016. A method for simultaneous bioflocculation and pretreatment of algal biomass targeting improved methane production. *Green Chemistry,* 18**,** 5230-5238.

PRAJAPATI, S. K., KUMAR, P., MALIK, A. & CHOUDHARY, P. 2014. Exploring pellet forming filamentous fungi as tool for harvesting non-flocculating unicellular microalgae. *BioEnergy Research,* 7**,** 1430-1440.

PREISENDORFER, R. W. 1986. Secchi disk science: Visual optics of natural waters. *Limnology and oceanography,* 31**,** 909-926.

PRUSSI, M., BUFFI, M., CASINI, D., CHIARAMONTI, D., MARTELLI, F., CARNEVALE, M., TREDICI, M. R. & RODOLFI, L. 2014. Experimental and numerical investigations of mixing in raceway ponds for algae cultivation. *Biomass and Bioenergy,* 67**,** 390-400.

PULZ, O. 2001. Photobioreactors: production systems for phototrophic microorganisms. *Applied microbiology and biotechnology,* 57**,** 287-293.

PULZ, O. & GROSS, W. 2004. Valuable products from biotechnology of microalgae. *Applied microbiology and biotechnology,* 65**,** 635-648.

PUTT, R., SINGH, M., CHINNASAMY, S. & DAS, K. C. 2011. An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. *Bioresource Technology,* 102**,** 3240-3245.

QUINN, J. C. & DAVIS, R. 2015. The potentials and challenges of algae based biofuels: A review of the techno-economic, life cycle, and resource assessment modeling. *Bioresource technology,* 184**,** 444-452.

QUIROZ ARITA, C. E., PEEBLES, C. & BRADLEY, T. H. 2015. Scalability of combining microalgae-based biofuels with wastewater facilities: A review. *Algal Research,* 9**,** 160-169.

RAWAT, I., RANJITH KUMAR, R., MUTANDA, T. & BUX, F. 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy,* 103**,** 444-467.

RAZZAK, S. A., ALI, S. A. M., HOSSAIN, M. M. & DELASA, H. 2017. Biological CO_2 fixation with production of microalgae in wastewater – A review. *Renewable and Sustainable Energy Reviews,* 76**,** 379-390.

REDFIELD, A. C. 1958. The Biological Control of Chemical Factors in the Environment *American Scientist,* 46**,** 230A-221.

REYNOLDS, C. S. 1984. *The ecology of freshwater phytoplankton*, Cambridge University Press.

ROGERS, J. N., ROSENBERG, J. N., GUZMAN, B. J., OH, V. H., MIMBELA, L. E., GHASSEMI, A., BETENBAUGH, M. J., OYLER, G. A. & DONOHUE, M. D. 2014. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research,* 4**,** 76-88.

ROSEMOND, A. D., MULHOLLAND, P. J. & BRAWLEY, S. H. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences,* 57**,** 66-75.

RUIZ-MARIN, A., MENDOZA-ESPINOSA, L. G. & STEPHENSON, T. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology,* 101**,** 58-64.

SALIM, S., BOSMA, R., VERMUË, M. H. & WIJFFELS, R. H. 2011. Harvesting of microalgae by bio-flocculation. *Journal of applied phycology,* 23**,** 849-855.

SAMSON, R. A., HOEKSTRA, E. S. & VAN OORSCHOT, C. A. 1981. *Introduction to food-borne fungi*, Centraalbureau voor Schimmelcultures.

SATHE, S. & DURAND, P. M. 2015. A low cost, non-toxic biological method for harvesting algal biomass. *Algal Research,* 11**,** 169-172.

SHEKHAR, M., SHRIWASTAV, A., BOSE, P. & HAMEED, S. 2017. Microfiltration of algae: Impact of algal species, backwashing mode and duration of filtration cycle. *Algal Research,* 23**,** 104-112.

SINGH, G. & PATIDAR, S. K. 2018. Microalgae harvesting techniques: A review. *Journal of Environmental Management,* 217**,** 499-508.

SINGH, S. P. & SINGH, P. 2015. Effect of temperature and light on the growth of algae species: A review. *Renewable and Sustainable Energy Reviews,* 50**,** 431-444.

SOMMER, U. 1991. A comparison of the Droop and the Monod models of nutrient limited growth applied to natural populations of phytoplankton. *Functional Ecology***,** 535-544.

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER 20TH EDN 1998. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

STEELE, J. H. 1962. Environmental control of photosynthesis in the sea. *Limnology and oceanography,* 7**,** 137-150.

SUN, X., WANG, C., TONG, Y., WANG, W. & WEI, J. 2013. A comparative study of microfiltration and ultrafiltration for algae harvesting. *Algal Research,* 2**,** 437-444.

SUTHERLAND, D. L., HEUBECK, S., PARK, J., TURNBULL, M. H. & CRAGGS, R. J. 2018. Seasonal performance of a full-scale wastewater treatment enhanced pond system. *Water Research,* 136**,** 150-159.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2014a. Seasonal variation in light utilisation, biomass production and nutrient removal by wastewater microalgae in a full-scale high-rate algal pond. *Journal of Applied Phycology,* 26**,** 1317-1329.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2015a. The effects of CO_2 addition along a pH gradient on wastewater microalgal photo-physiology, biomass production and nutrient removal. *Water Research,* 70**,** 9-26.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2015b. Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology,* 184**,** 222-229.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2015c. Frequency of CO_2 supply affects wastewater microalgal photosynthesis, productivity and nutrient removal efficiency in mesocosms: implications for full-scale high rate algal ponds. *Journal of Applied Phycology,* 27**,** 1901-1911.

SUTHERLAND, D. L., TURNBULL, M. H. & CRAGGS, R. J. 2014b. Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. *Water Research,* 53**,** 271-281.

SUTHERLAND, D. L., TURNBULL, M. H. & CRAGGS, R. J. 2017. Environmental drivers that influence microalgal species in fullscale wastewater treatment high rate algal ponds. *Water Research,* 124**,** 504-512.

TAKAHASHI, M. & ICHIMURA, S. E. 1968. Vertical distribution and organic matter production of photosynthetic sulfur bacteria in Japanese lakes. *Limnology and Oceanography,* 13**,** 644-655.

TALBOT, P., THÉBAULT, J. M., DAUTA, A. & DE LA NOÜE, J. 1991. A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. *Water Research,* 25**,** 465-472.

TALUKDER, M. M. R., DAS, P. & WU, J. C. 2014. Immobilization of microalgae on exogenous fungal mycelium: A promising separation method to harvest both marine and freshwater microalgae. *Biochemical Engineering Journal,* 91**,** 53-57.

TRAN, D.-T., LE, B.-H., LEE, D.-J., CHEN, C.-L., WANG, H.-Y. & CHANG, J.-S. 2013. Microalgae harvesting and subsequent biodiesel conversion. *Bioresource technology,* 140**,** 179-186.

UMMALYMA, S. B., GNANSOUNOU, E., SUKUMARAN, R. K., SINDHU, R., PANDEY, A. & SAHOO, D. 2017. Bioflocculation: An alternative strategy for harvesting of microalgae – An overview. *Bioresource Technology,* 242**,** 227-235.

UNDURRAGA, D., POIRRIER, P. & CHAMY, R. 2016. Microalgae growth kinetic model based on the PSII quantum yield and its utilization in the operational curves construction. *Algal Research,* 17**,** 330-340.

VANDAMME, D., FOUBERT, I. & MUYLAERT, K. 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in Biotechnology,* 31**,** 233-239.

VANNI, M. J. & TEMTE, J. 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology and Oceanography,* 35**,** 697-709.

VASILIEV, M. & ALAMEH, K. 2018. Spectrally-Selective Energy-Harvesting Solar Windows for Public Infrastructure Applications. *Applied Sciences,* 8**,** 849.

VERSPAGEN, J. M., VAN DE WAAL, D. B., FINKE, J. F., VISSER, P. M. & HUISMAN, J. 2014. Contrasting effects of rising CO_2 on primary production and ecological stoichiometry at different nutrient levels. *Ecology letters,* 17**,** 951-960.

WAN, C., ALAM, M. A., ZHAO, X.-Q., ZHANG, X.-Y., GUO, S.-L., HO, S.-H., CHANG, J.-S. & BAI, F.-W. 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. *Bioresource Technology,* 184**,** 251-257.

WANG, J.-H., ZHANG, T.-Y., DAO, G.-H., XU, X.-Q., WANG, X.-X. & HU, H.-Y. 2017. Microalgae-based advanced municipal wastewater treatment for reuse in water bodies. *Applied Microbiology and Biotechnology***,** 1-17.

WANG, L., MIN, M., LI, Y., CHEN, P., CHEN, Y., LIU, Y., WANG, Y. & RUAN, R. 2010. Cultivation of Green Algae *Chlorella* sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. *Applied Biochemistry and Biotechnology,* 162**,** 1174-1186.

WANG, S.-K., STILES, A. R., GUO, C. & LIU, C.-Z. 2015. Harvesting microalgae by magnetic separation: A review. *Algal Research,* 9**,** 178-185.

WIELGAT-RYCHERT, M., RYCHERT, K., WITEK, Z. & ZALEWSKI, M. 2017. Calculation of the Photosynthetic Quotient (PQ) in the Gulf of Gdańsk (Southern Baltic). *Baltic Coastal Zone,* 21**,** 51-60.

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*.

WREDE, D., HUSSAINY, S. U., RAJENDRAM, W. & GRAY, S. 2018. Investigation and modelling of high rate algal ponds utilising secondary effluent at Western Water, Bacchus Marsh Recycled Water Plant. *Water Science and Technology,* 78**,** 20-30.

WREDE, D., TAHA, M., MIRANDA, A. F., KADALI, K., STEVENSON, T., BALL, A. S. & MOURADOV, A. 2014. Co-Cultivation of Fungal and Microalgal Cells as an Efficient System for Harvesting Microalgal Cells, Lipid Production and Wastewater Treatment. *PLoS ONE,* 9**,** e113497.

WU, Y.-H., LI, X., YU, Y., HU, H.-Y., ZHANG, T.-Y. & LI, F.-M. 2013. An integrated microalgal growth model and its application to optimize the biomass production of *Scenedesmus* sp. LX1 in open pond under the nutrient level of domestic secondary effluent. *Bioresource Technology,* 144**,** 445-451.

XIA, G., JIN, C., ZHOU, J., YANG, S., ZHANG, S. & JIN, C. 2001. A novel chitinase having a unique mode of action from Aspergillus fumigatus YJ-407. *The FEBS Journal,* 268**,** 4079-4085.

XIE, M., SHON, H. K., GRAY, S. R. & ELIMELECH, M. 2016. Membrane-based processes for wastewater nutrient recovery: Technology, challenges, and future direction. *Water Research,* 89**,** 210-221.

XIE, S., SUN, S., DAI, S. Y. & S.YUAN, J. 2013. Efficient coagulation of microalgae in cultures with filamentous fungi. *Algal Research,* 2**,** 28-33.

XIE, Y., LI, X., CHAI, T., REN, J., DING, L., ZHU, Y., HAN, J. & ZHU, T. 2015. Filtration performance comparison of a metal membrane and an organic membrane in bioreactor. *Desalination and Water Treatment,* 54**,** 3566-3574.

YEHOSHUA, Y. & GOPHEN, M. 2018. Seasonal Partitioning of Primary Production and Biomass between Phytoplankton and Metaphyton in a Shallow Lake Agmon, Hula Valley (Israel). *Open Journal of Modern Hydrology,* 8**,** 66.

YOUNG, P., TAYLOR, M. & FALLOWFIELD, H. J. 2017. Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment. *World Journal of Microbiology and Biotechnology,* 33**,** 117.

ZAMALLOA, C., GULTOM, S. O., RAJENDRAN, A. & HU, B. 2017. Ionic effects on microalgae harvest via microalgae-fungi co-pelletization. *Biocatalysis and Agricultural Biotechnology,* 9**,** 145-155.

ZHANG, G., WANG, B., ZHANG, P., WANG, L. & WANG, H. 2006. Removal of algae by sonication-coagulation. *Journal of Environmental Science and Health Part A,* 41**,** 1379-1390.

ZHANG, J. & HU, B. 2012. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. *Bioresource Technology,* 114**,** 529-535.

ZHANG, J. & ZHANG, J. 2016. The filamentous fungal pellet and forces driving its formation. *Critical reviews in biotechnology,* 36**,** 1066-1077.

ZHANG, S., QU, Y., LIU, Y., YANG, F., ZHANG, X., FURUKAWA, K. & YAMADA, Y. 2005. Experimental study of domestic sewage treatment with a metal membrane bioreactor. *Desalination,* 177**,** 83-93.

ZHANG, X., FAN, L. & RODDICK, F. A. 2013. Understanding the fouling of a ceramic microfiltration membrane caused by algal organic matter released from *Microcystis aeruginosa*. *Journal of Membrane Science,* 447**,** 362-368.

ZHAO, F., CHU, H., YU, Z., JIANG, S., ZHAO, X., ZHOU, X. & ZHANG, Y. 2017. The filtration and fouling performance of membranes with different pore sizes in algae harvesting. *Science of The Total Environment,* 587–588**,** 87-93.

ZHENG, H., GAO, Z., YIN, J., TANG, X., JI, X. & HUANG, H. 2012. Harvesting of microalgae by flocculation with poly (γ-glutamic acid). *Bioresource technology,* 112**,** 212-220.

ZHOU, W., CHENG, Y., LI, Y., WAN, Y., LIU, Y., LIN, X. & RUAN, R. 2012. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. *Applied Biochemistry and Biotechnology,* 167**,** 214-228.

ZHOU, W., MIN, M., HU, B., MA, X., LIU, Y., WANG, Q., SHI, J., CHEN, P. & RUAN, R. 2013. Filamentous fungi assisted bio-flocculation: A novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Separation and Purification Technology,* 107**,** 158-165.

ZHU, L., HILTUNEN, E., SHU, Q., ZHOU, W., LI, Z. & WANG, Z. 2014. Biodiesel production from algae cultivated in winter with artificial wastewater through pH regulation by acetic acid. *Applied Energy,* 128**,** 103-110.

ZOLLER, S. & LUTZONI, F. 2003. Slow algae, fast fungi: exceptionally high nucleotide substitution rate differences between lichenized fungi Omphalina and their symbiotic green algae Coccomyxa. *Molecular Phylogenetics and Evolution,* 29**,** 629-640.