



Downstream Processing of Marine Microalgae for the Commercial Scale Production of Biofuels

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Thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

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The University of Adelaide

July 2013

Table of Contents

Table of Contents.....	i
Abstract.....	iii
Declaration.....	vii
Acknowledgement.....	viii
Preface.....	ix
Errata.....	xi
1 Introduction.....	1-1
1.1 Biofuels and biodiesel.....	1-2
1.2 Biodiesel from microalgae.....	1-3
1.3 Primary objectives of thesis.....	1-4
1.4 Structure of thesis.....	1-6
1.5 References.....	1-8
2 Literature Review.....	2-1
2.1 Introduction.....	2-2
2.2 Microalgal harvesting.....	2-2
2.2.1 Harvesting methods.....	2-2
2.3 Cell disruption.....	2-24
2.4 Summary of the objectives of the thesis.....	2-26
2.5 References.....	2-27
3 Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting.....	3-1
4 Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements.....	4-1
5 Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation.....	5-1
6 Harvesting of marine microalgae by electroflocculation: The energetics, plant design, and economics.....	6-1

7	Conclusion	7-1
7.1	Overview	7-2
7.2	Harvesting by microbial and electro flocculation	7-2
7.3	Cell disruption.....	7-2
7.4	Atomic Force Microscopy	7-3
7.5	Recommendations for future work	7-3
Appendix A.....		A1
Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel.....		A1
Appendix B.....		B1
An assessment of large scale microalgal harvesting methods for the production of biodiesel.....		B1
Appendix C.....		C1
Harvesting, Thickening and Dewatering Microalgae Biomass		C1

Abstract

Several species of microalgae have lipid yields that are about 4 to 5 times of that from the highest oil bearing terrestrial plants such as oil palm. Furthermore, marine species can offer the additional advantage of not competing with farm produce for fresh water or arable land. These advantages make marine microalgae appear to be attractive as a feedstock for the production of biofuels; however, despite intensive research, the production cost of microalgal biomass remains high at about A\$ 10 kg⁻¹. In comparison, plantation oil such as canola can be available of about \$1 L⁻¹ and this large cost difference makes algal biofuels uncompetitive. Hence, the sustainability of microalgal biofuel production depends very much on the technical, energetic and economic issues involved with production.

Microalgal lipids and carbohydrates are major feedstock for renewable fuels and the downstream processing can broadly be classified into four steps: harvesting, dewatering, cell disruption and extraction of the desirable products. The specific objectives for the PhD study were harvesting and cell disruption of microalgae; these objectives were structured towards the development of pilot scale production of marine microalgae and included technical, energetic and economic evaluations. Below are details for these objectives:

- I. Harvesting: the objective was to increase the biomass concentration from about 0.2 kg m⁻³ to about 20 kg m⁻³. Such level of concentration would be suitable for the next unit of processing such as cell disruption or secondary dewatering. Flocculation (electro- and microbial-) were applied as the harvesting methods with induced mechanical mixing and electrode separation as the novelties in

energy optimization techniques. The scope of this study included the technical investigation and development of these harvesting methods, evaluation of their energy requirements, plant designs and economic analyses; all these areas were deemed to be necessary for the determination of the viability of such processes.

- II. Cell disruption: disruption processes with potential for the commercial scale biofuel production were investigated with emphasis on the process reliability, energy requirement and disruption efficiencies. The novelty was the determination of disruption energy efficiencies by comparing the process energy input with the theoretically derived values of cell disruption.
- III. Cell mechanics: mechanical cell wall properties that affect the disruption process were investigated. Atomic force microscopy was used to evaluate the disruption energy requirements and determine the efficiencies of various cell disruption processes. The novelty was the measurement of disruption energy requirement for individual cells. From the experimental value obtained, the specific disruption energy on a per kg basis was calculated to determine the energy efficiencies of various disruption methods.

It was found that the mixing of microalgal media during electroflocculation is essential for the reduction in electrical energy requirement, and hydraulic baffles can provide an energy efficient technique for such purpose. The energy required for such mixing is 3.2 kJ kg⁻¹ of the equivalent dry mass based on the design criteria that the value of Camp number is between 10⁴ to 10⁵ and the velocity gradient is between 100 s⁻¹ to 10 s⁻¹. The cost for harvesting by microbial flocculation, including energy, raw material and capital depreciation, was estimated to be A\$ 0.26 kg⁻¹ of the equivalent dry mass. On the other hand, the cost of harvesting by electroflocculation, including electrical energy,

aluminium dissolution and capital depreciation, was estimated to be \$ 0.185 kg⁻¹ of the equivalent dry biomass. The energy consumptions by both types of flocculation have the potential to be further optimised.

For the extraction of lipids for the production of biodiesel, mechanical cell disruption methods are preferred due to the lower risk of contamination of products and the ease of scale up. The drawback is that current mechanical disruption processes have high specific energy consumption that is in excess of that can be available from the combustion of the entire cell mass. The disruption energy may be optimized by selecting processes that are relatively energy efficient and the combination of cell disruption with solvent extraction.

The disruption energy as measured by the use of Atomic Force Microscopy revealed that an average value of 17.4 pJ was required for the disruption of an individual *Tetraselmis sp.* cell, this value is equivalent to specific disruption energy of 673 J kg⁻¹ of dry microalgal biomass. In comparison, disruption by hydrodynamic cavitation, one of the most energy efficient technique, requires specific disruption energy input of 33 MJ kg⁻¹ of the dry biomass. This large difference indicates the low efficiency in the mechanical cell disruption and more innovation is required for the sustainability of such processes in the production of biofuels.

This thesis advances the knowledge in the harvesting and cell disruption of microalgae from the laboratory to pilot scale. In the area of harvesting, advances were made in the scale up on the plant design, energy optimisation and economics of microbial- and electro-flocculation; while in the area of cell disruption, advances were achieved on the understanding of the energy requirements for large scale mechanical cell disruption

processes, and the fundamental cell mechanics with respect to disruption. All these work has been presented as journal publications as detailed in the Preface of this thesis.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Acknowledgement

The completion of this thesis could not have been accomplished without the advice, guidance, support and contribution of many people.

I would firstly like to thank my supervisors, A/Prof Peter Ashman and A/Prof David Lewis for the encouragement and support throughout the years of research. The many hours of discussion, guidance and support are invaluable in the completion of this thesis and the publication of various journal papers.

I would also like to thank Prof Michael Borowitzka from Murdoch University, Western Australia, for his overall co-ordination of the APP Project; Dr Steven Pahl, research fellow from the School of Chemical Engineering, University of Adelaide, for technical assistance with microalgae culturing; Mr. Jason Peake and Mr. Jeffrey Hiorns, workshop technicians, for advice and construction of various experimental rigs; Mrs. Mary Barrow and Mrs. Sue Earle from the School office for everything from stationery to morning tea; and finally all my colleagues for all their help and support.

I am extremely grateful to my dear wife Laifong and two children, Christopher and Alexander; my wife gave me the support necessary to return to university study after an absence of over 30 years and they all have been very patient and provided encouragement throughout the study.

Lastly, I would like to thank Prof James Moore, Head of Colorectal Unit, and Prof Dorothy Keefe, Clinical Director of RAH Cancer Centre, both from Royal Adelaide Hospital for the treatment for my bowel cancer in 2003. Their skill and care in the continuous monitoring of my progress provided me with the most important ingredient of my research, my health.

Preface

This thesis is submitted as a portfolio of publications according to the “Specifications for Thesis 2012” of the University of Adelaide. The journals, in which the papers were published, have high impact factors in the research field of Chemical Engineering. Data on the impact factors of the journals are listed below:

Journal Title	Impact Factors	
	2 year	5 year
Chemical Engineering Research and Design	1.96	2.03
Biomass and Bioenergy	3.64	4.62
Bioresource Technology	4.98	5.35
Applied Energy	5.11	4.45

The main body of work contained in this thesis is within the following four journal papers:

1. **Lee, A.K.**, Lewis, D.M., Ashman, P.J. 2010. Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting. *Chemical Engineering Research and Design*, 88, (8), 988-996.
2. **Lee, A.K.**, Lewis, D.M., Ashman, P.J. 2012. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. *Biomass and Bioenergy*, 46, 89-101.

3. **Lee, A.K.**, Lewis, D.M., Ashman, P.J. 2013. Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation. *Bioresource Technology*, 128, (0), 199-206.
4. **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, (0), 45-53.

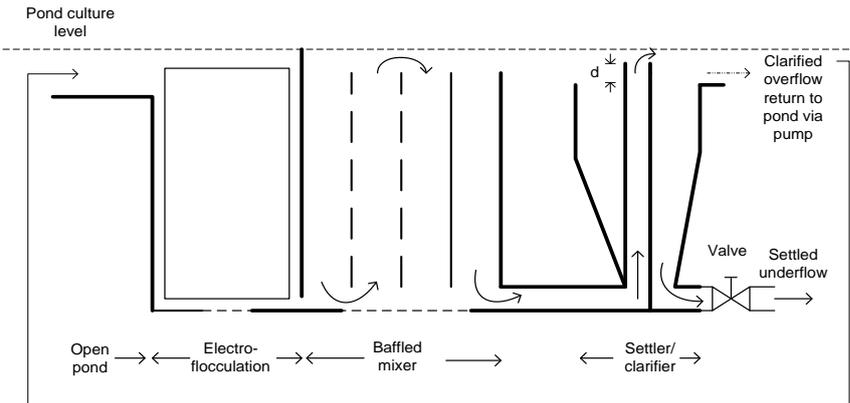
Below are additional publications that are relevant to the present work and included in the appendices of this thesis:

Lee, A.K., Lewis, D.M., Ashman, P.J. 2009. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Journal of Applied Phycology*, 21(5), 559-567.

Andrew K Lee, David M Lewis, Peter J Ashman, 2010. An assessment of large scale microalgal harvesting methods for the production of biodiesel. Proceeding from CHEMECA 2010, Hilton, Adelaide, SA, Australia.

Pahl, S., **Lee, AK.**, Kalaitzidis, T., Ashman, P., Sathe, S., & Lewis, D. (2013). Harvesting, Thickening and Dewatering Microalgae Biomass. In M. A. Borowitzka & N. R. Moheimani (Eds.), *Algae for Biofuels and Energy* (Vol. 5, pp. 165-185): Springer Netherlands.

Errata

Page	Line	Erratum
3.5	Column 1, Paragraph 2, Line 2	Replace “m ² s ⁻¹ ” by “Pa s”
6.8	Section 2.2.2	Replace “air” by “gas”
6.9	Section 3.2	Additional note: “Cell disruption processes such as high pressure homogenizer, bead mill or hydrodynamic cavitation require high shear and impact for the disruption. Algal concentrate with dry mass over 3 % has a consistency of thick cream and will require a significant amount of energy to attain the high velocity necessary to generate such shear”.
6.10	Section 3.3.3	<p>Additional figure and note:</p>  <p>The design of the pilot plant is based on the laboratory batch test results. The flocculation time is expected to be of similar value due to the high turbulence (Eq 11 shows high Reynolds number, hence adequate mixing). The required settling distance, <i>d</i>, as indicated in the figure, should also be similar to the experimental settling value.</p>
6.11	Table 5	Additional note: “Lang factors were not mentioned in the cited literatures in Table 5; hence, such factors have not been used here to compare all these cost estimations on a similar basis.”

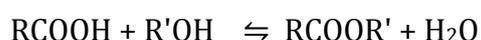
1 Introduction

1.1 Biofuels and biodiesel

The uncertainty associated with the supply of crude oil has increased interest and research in renewable transport fuels, for example: methane from the anaerobic digestion of organic waste (Elliott & Mahmood, 2007), ethanol from the fermentation of cane sugar (Zyl et al., 1988), hydrogen from fermentation of organic waste by bacteria such as *Rhodobacter sphaeroides* (Kapdan & Kargi, 2006) and biodiesel (alkyl esters) from the transesterification of plantation oil or animal fat (Ma & Hanna, 1999). Although some of these fuels appear to be attractive alternatives to fossil fuels, large scale developments are faced with some common problems, for example: the high cost of developing and adopting new technologies, the lack of alternative transport fuel distribution infrastructure and the potential impact on the ecosystem. Some problems are more specific, such as: the low value of combustion energy per unit volume of hydrogen gas results in low mobility range and the competition of biofuel feedstock with food crops for arable land and fresh water (Kleiner, 2007).

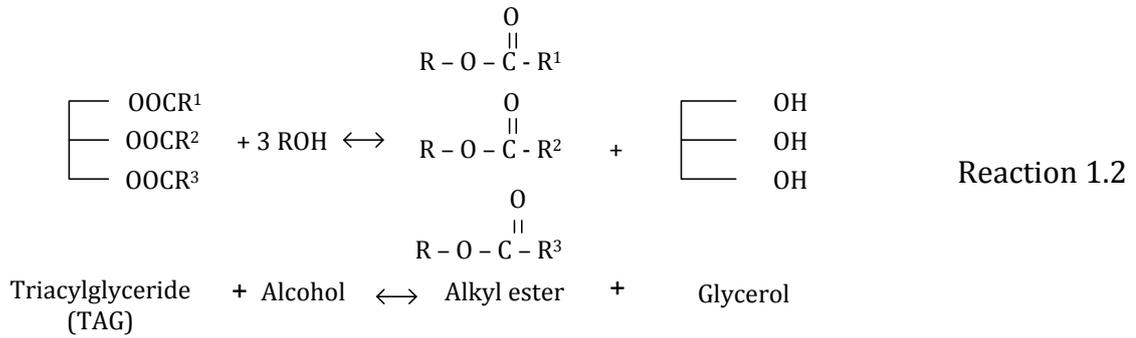
Biodiesel is a renewable fuel that is degradable and has flow and combustion properties similar to petroleum diesel. Comparing with other alternative transport fuels, biodiesel has the advantage of higher energy density (33 - 35 MJ L⁻¹); being able to mix in any proportion with petroleum diesel and does not require modifications of existing fuel distribution systems. In comparison, ethanol can only be blended in petrol with a maximum of 15% without engine modification.

Biodiesel can be derived either from the esterification of free fatty acids (FFA) and alcohols shown by Reaction 1.1:



Reaction 1.1

Or: the trans-esterification of triacylglycerides (TAG) by alcohols shown by Reaction 1.2:



The commercial production of biodiesel from plantation crops such as soy is a proven technology (Gerpen, 2005); however, terrestrial plants have a low solar utilization rate of less than 1%, furthermore, only part of the plant (usually the seeds) contains significant amount of oil. These two factors make land plants less efficient than microalgae as a source of oil supply (Geider et al., 2001); in addition, the diversion of food crops for the production of fuels is also a cause for concern.

1.2 Biodiesel from microalgae

Some microalgae have high lipid yield, for example, the marine microalga, *Pleurochrysis carterae* has a lipid yield of 21.9 t ha⁻¹ y⁻¹ (Moheimani & Borowitzka, 2006) which is about 10 times that of soy or about 4 to 5 times of that from oil palm, a terrestrial plant with one of the highest oil yield. Marine microalgae also have the additional advantage of not competing with farm produce for fresh water or arable land. These advantages make marine microalgae appear to be attractive as feedstock for the production of biodiesel; however, despite intensive research, the production cost of microalgal biomass remains high at about \$ 10 kg⁻¹ (Benemann & Oswald, 1996) and there has been a lack of updated information in the production cost among published literatures. In comparison, plantation oil such as canola can be available at about \$1 L⁻¹ (CCC, 2011), this large cost difference makes algal biofuels economically uncompetitive.

Renewable fuels have a perceived role of reducing the atmospheric carbon dioxide and it is essential for biofuels to have a smallest carbon footprint and positive energy balance, i.e. the energy input for the production throughout its life cycle to be less than the energy available by the combustion of the fuels. Hence, the sustainability of microalgal biodiesel production depends very much on the technical, energetic and economic issues of production.

1.3 Primary objectives of thesis

Major unit process operations for the downstream processing of microalgae from culture media to biofuels are:

1. Harvesting: The separation of microalgal biomass from its surrounding media. Several separation techniques are available, for example: centrifugation, filtration, and flocculation. These techniques can be used singularly or in combination depending on factors such as: the characteristics of microalgae, biomass concentration required by the next processing step or economic considerations.
2. Cell disruption: The breaking open of individual cells for the release of intracellular contents such as lipids or proteins. The process can be mechanical, chemical or physical, single or multi staged depending on the products requirements
3. Extraction: The separation of lipids such as triacylglycerides (TAG) or fatty acids from various cell inclusions such as carbohydrates or proteins. Solvent extraction the current predominant technique for the extraction of lipids with the residue enriched in carbohydrates for ethanol production.

4. Esterification or fermentation: The extracted lipids react with alcohols and to form alkyl esters (biodiesel), various types of catalyst are required for these reactions to proceed at a reasonable rate. Carbohydrates are digested for the fermentation to alcohols.

Much of the processing technology has been developed in related fields such as wastewater treatment. The scope for the PhD study included the harvesting and cell disruption of microalgae; these two topics were structured towards the development of pilot scale production of marine microalgae and included technical, energetic and economic evaluations. Below are details for these processes:

- I. Harvesting: flocculation is the first step in the separation of the biomass from the culture medium, the objective was to increase the biomass concentration from about 0.2 kg m^{-3} to about 20 kg m^{-3} . Such level of concentration would be suitable for the next unit of processing such as cell disruption or further dewatering. Flocculation (electro- and microbial-) were applied as the harvesting methods with induced mechanical mixing and electrode separation as the novelties in energy optimization techniques. The scope of this study included the technical investigation and development of these harvesting methods, evaluation of their energy requirements, plant designs and economic analyses; all these areas were deemed to be necessary for the determination of the viability of such processes.
- II. Cell disruption: disruption processes that are potentially suitable for the commercial scale biofuel production were investigated with emphasis on the process reliability, energy requirement and disruption efficiencies. The novelty was the determination of disruption energy efficiencies by comparing the process energy input with the theoretically derived values of cell disruption.

III. Cell mechanics: mechanical cell wall properties that affect the disruption process were investigated. Atomic Force Microscopy was used to evaluate the disruption energy requirements and the efficiencies of various cell disruption processes were determined. The novelty was the measurement of energy requirement for the disruption of individual cells, and from the experimental value obtained, the specific disruption energy on a per kg basis was calculated to determine the energy efficiencies.

1.4 Structure of thesis

Chapter 2 provides an overview of the current microalgal processing technologies with respect to the special objectives listed in Section 1.3. Additionally, knowledge gaps necessary for the scale up from laboratory to industrial biofuel production are identified.

Chapter 3 is the first of the four journal publications that forms the main body of this thesis; this paper introduces the concept of a baffled hydraulic flocculator as the mixing technique for the large scale microalgal harvesting. The paper also includes the design of the flocculation plant, analysis on energy requirements and estimation of the operating and capital costs of an industrial scale harvesting plant. The results are presented in terms of \$ kg⁻¹ of the dry biomass to assess the process's economic sustainability.

Chapter 4 is the second of the journal publications; it provides a comprehensive review on a range of cell disruption techniques that are potentially suitable for the large scale extraction of lipids for the production of biodiesel. In addition, the energy input required by various mechanical devices are analyzed and compared with the theoretical cell disruption energy requirements. Novelties of this paper are the two different methods

on the theoretical estimation of cell disruption energy requirements and the evaluation of the disruption energy efficiencies of various mechanical cell disruption methods.

Chapter 5 is the third of the journal publications, it reports on the use of Atomic Force Microscope (AFM) for the evaluation on the microalgal cell disruption energy requirements as derived from the force and energy for the indentations and disruption of individual cells. The disruption energy measured as such is one of the fundamental quantities that are required to assess the limit on cell disruption process energy efficiency. Included in this paper is also an evaluation of the spring constant, k , of the AFM cantilever as the accuracy of the force measurement directly depends on the value of k .

Chapter 6 is the fourth of the journal publications for this thesis; it is presented in manuscript form as it is currently under review. This article reports on the effects of electrode separations and induced mechanical mixing on the optimization of electroflocculation energy requirements. Commercial viability of the electroflocculation process is determined by the cost estimation presented as the sum of capital, metal dissolution and energy consumption.

Chapter 7 is a conclusion from the body of work with recommendations for future work in the area of harvesting and cell disruption.

Appendices A to C provide peer reviewed publications that are relevant to this study. Appendix A is a journal paper that precedes Chapter 3 and 7 of this thesis, this paper highlighted the knowledge gap that activated sludge, a microbial flocculation process proposed to be used as microalgal harvesting method, is not suitable for the harvesting

of marine microalgae. This paper demonstrated the modifications necessary for the activated sludge to successfully flocculate microalgae in a marine environment.

Appendix B is a conference paper on the economics of microalgal dewatering; the biomass concentrated by flocculation still contains large amount of water and this paper compares various industrial dewatering equipments and evaluates their performance based on their overall operation efficiency and cost.

Appendix C is a book section on microalgal harvesting methods. It provides a critical review on various separation processes and their potential as industrial scale microalgal harvesting methods.

1.5 References

- Benemann, J. R., & Oswald, W. J. (1996). *Systems and economic analysis of microalgae ponds for conversion of CO₂ to biomass. Final report to Department of Energy*. Pittsburgh: Pittsburgh Energy Technology.
- CCC. (2011). Canola Council of Canada, Seed, Oil and Meal Prices, accessed 16 May 2012. from <http://www.canolacouncil.org/canolaprices.aspx>
- Elliott, A., & Mahmood, T. (2007). Pretreatment technologies for advancing anaerobic digestion of pulp and paper biotreatment residues. *Water Research*, 41, 4273-4286.
- Geider, R. J., Delucia, E. H., Falkowski, P. G., Finzi, A. C., Grime, J. P., Grace, J., et al. (2001). Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global Change Biology*, 7(8), 849-882.
- Gerpen, J. V. (2005). Biodiesel processing and production. *Fuel Processing Technology*, 86(10), 1097-1107.
- Kapdan, I. K., & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*, 38(5), 569-582.
- Kleiner, K. (2007). *The backlash against biofuels*. New York: Nature Reports / Climate Change / vol 2/ Jan 2008 doi:10.1038/climate.2007.71.

- Ma, F., & Hanna, M. A. (1999). Biodiesel production: a review. *Bioresource Technology*, 70(1), 1-15.
- Moheimani, N., & Borowitzka, M. (2006). The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds. *Journal of Applied Phycology*, 18(6), 703-712.
- Zyl, C., Prior, B., & Preez, J. (1988). Production of ethanol from sugar cane bagasse hemicellulose hydrolyzate by *Pichia stipitis*. *Applied Biochemistry and Biotechnology*, 17(1-3), 357-369.

2 Literature Review

2.1 Introduction

The purpose of Chapter 2 is to provide a brief review of the current microalgal harvesting and disruption processes; the emphasis is on the scale up from that of laboratory to commercial. To be viable as a renewable fuel with the intention of atmospheric CO₂ reduction, it is essential for these processes to have a positive energy balance, a reduction in carbon footprint and a cost structure that is competitive with plantation oil such as soy or canola. Therefore, estimation of energy requirement and economic analysis are essential to all these techniques. In addition to the low cost, the harvesting of microalgal lipids for biodiesel production must also be reliable and yield a product that is relatively free from contaminants. Finally, as discussed in Section 1.2, marine species of microalgae have the advantage of not competing with food crops for fresh water and arable land; hence, in this chapter, microalgal processing techniques are discussed with respect to marine microalgae.

2.2 Microalgal harvesting

2.2.1 *Harvesting methods*

Overview

Commercially available solid/liquid separation techniques include filtration, centrifugation and flocculation (including sedimentation and flotation), these techniques can be used either individually or in combination with each other. However, there are certain difficulties for these techniques to be used for the harvesting of microalgae, these problems include:

- Most microalgae are of small sizes between 3 µm - 20 µm in diameter (except for the filamentous species such as *Spirulina sp*) (Winter & Siesser, 1994); in

addition, these cells are often compressible, these two factors make filtration energy consuming and ineffective.

- Microalgae grow in very dilute media with a typical dry mass concentration between 200-600 mg L⁻¹ under autotrophic conditions. These cells also have high water content, and thus a large amount of water needs to be removed for a small amount of algal mass.
- Cell surfaces are negatively charged to keep the cell bodies in suspension, making settling difficult. This, together with the high dilution, makes flocculation difficult without the addition of large amount of chemicals.
- The density of a typical microalgal cell is usually only slightly higher than that of the surrounding medium; the small size and density difference make centrifugation inefficient (Becker 1995; Benemann 1996).
- The microalgal biomass residue after lipid extraction still contains high concentrations of proteins and carbohydrates. These by products may have economic values. The presence of inorganic flocculants such as Al³⁺ may preclude such use.

Despite these difficulties, the commercial harvesting techniques mentioned above are effective in separating the biomass from the media. However, the economic recovery of microalgae biomass is challenging and costs must be reduced to an acceptable level before microalgae based biofuels can be commercially viable. For the harvesting methods suitable for the production of microalgal biofuels, they need to be low cost, high capacity and reliable enough to be operated continuously throughout the year. The literature in relation to large scale harvesting of microalgae has recently been reviewed by (Pahl et al., 2013), this reference is reproduced here as Appendix C. An edited summary of this information is provided below:

Centrifugation

Centrifugation improves the settling process by generating centrifugal forces to increase the settling rates. Some of the common types of centrifuges include disc stack centrifuges, perforated basket centrifuges, imperforated basket centrifuges and decanters (or scroll centrifuges). The discharge solid concentrations depend on feed properties such as particle size and particle-fluid density difference. Operating parameters include the feed flow rate, the rotational speeds of the bowl and biomass concentrations. The decanter or scroll centrifuge is more suitable for the industrial recovery of microalgae as they can be operated continuously, have high capacity and lower maintenance requirements while the basket type centrifuges are only suitable for batch operations.

Decanters

Decanters essentially consist of two concentric rotating elements surrounded by a stationary casing or cover. The tapered outer rotating element, or 'bowl', and the inner element, or 'screw', rotate at slightly different speeds. Solids entering the decanter settle on the bowl wall and are conveyed along the bowl wall to the discharge, while the clarified liquid is discharged at the opposite end. The solids concentration on discharge is dependent on the feed properties (particle size, particle-fluid density difference) and can be modified by altering the feed flow rate, the rotational speeds of the bowl and screw and the bowl length. The maximum discharge concentration typically achieved in continuous centrifugation processes are 10 % -20 % solids with an energy consumption rate of about 1 kWh m⁻³ (Mohn, 1988).

Hydrocyclones

One type of centrifuge that involves no moving parts is the hydrocyclone. A typical hydrocyclone consists of an upper cylindrical section joined to a conical base. Feed is injected tangentially through an inlet opening near the top of cylindrical section and the particles experience the radial centrifugal force. Hydrocyclones have no moving parts, are relatively cheap but somewhat inflexible once installed. The separation performance is sensitive to the fluid and particle dynamics. While hydrocyclones have shown some promise for the primary concentration of microalgae, their reliability is poor and inflexible for species of microalgae or floc particles with different settling characteristics (Mohn, 1988).

Although centrifugation is a proven technology for the effective harvesting of microalgae, its high capital, energy and operational costs must be considered in conjunction with the scale and value of the product; hence it is suitable only for high value products such as dietary supplements (Becker, 1995). A cost comparison with other harvesting methods is presented in Table 2.1 at the end of this section on microalgal harvesting.

Filtration

Most industrial filters are either pressure filters or vacuum filters with screening of particles through a mesh or a bed of coarse particles such as sand. However, slimy or very fine particles can form a dense, impermeable cake that will quickly plug any filter media. In such cases, filter aids such as pre-coating are often necessary. The most common industrial filtration equipment include belt filters, drum filters, tangential (cross) flow membrane filters, mechanical presses (rotary or screw) and filter presses. These types of filtration, with the exception of filter press, are suitable for continuous

operation and many can be operated on a large scale for waste water treatment and potentially suitable for the processing of microalgal biofuels.

Belt filters

Belt filters are generally continuously operated and consist of a fabric mesh (filter media) that moves over rollers. Microalgae feed streams usually need to be flocculated to increase the efficiency and blades are required to remove the slurry to increase the amount of drainage. Belt filters generally have relatively low power consumption and capital cost with a maximum output solid concentration of about 5 % - 7 %. The extent of dewatering depends on the drainage time, belt tension, floc characteristics and the way that the water is bound to the microalgae (Tchobanoglous, 1997).

Tangential (cross) flow filtration

During cross flow filtration, the shear force created by the flow parallel to the membrane surface minimises the cake formation and keeps the membrane surface clean; however, there is a concern that the high shear force may also result in cell damages. Membranes for either ultra-filtration (UF; pore size 1nm -100 nm) or micro-filtration (MF; pore size 100nm-10 μ m) can be used with the flux determined by the membrane technology, pore size, feed rates, feed concentration, trans-membrane pressure and any membrane fouling. Permeate fluxes of between 15 - 60 L m⁻² h⁻¹ are typical with UF membranes operating at low pressure and low velocity (Rossignol et al., 1999). Both freshwater and marine microalgae have been separated in the laboratory scale using this technology (Danquah et al., 2009; Petrussevski, 1994) with a reported energy consumption of 0.38-0.51 kWh m⁻³, permeate fluxes of approximately 20 L m⁻² h⁻¹ and concentration factors of 20-46 (Danquah et al. 2009). A major problem is the membrane fouling due to the

formation of biofilms during long-term uses, the fouling reduces permeate fluxes, membrane life and possibly product quality.

Mechanical presses

There are two main types of mechanical presses, namely, the screw and the rotary. Both are suitable for continuous operation, requiring less back-washing water than belt filters. This type of press makes use of slowly rotating screens or screws to generate high pressure to dewater slurries. Such presses generally have higher throughputs and lower footprints. In a rotary press the solid suspension is fed through a channel between two parallel revolving screens. The retained solids are pushed forward inside the channel by rotating blades and eventually form a cake. Screw press consists of a rotating screw with a reducing pitch inside a perforated screen. As the screw rotates, the suspension is subjected to gradually increasing pressure and the liquid is forced out through the perforated screen, whilst the retained solids moves toward the exit end of the press. The moisture content in the dewatered cake can be controlled by the discharge pressure and rotational speeds. Porous water absorbing membranes are usually used to aid in the dewatering process.

Vacuum filters (rotary drum)

Vacuum filters almost exclusively employ a rotating drum filters. Important design variables include the size and type of filter, cake discharge mechanism, vacuum level, cycle time and feed conditioning. Feed streams are flocculated with water removal typically range between 40 % - 60 % with a biomass recovery rate over 90 %.

Filtration is applicable only for filamentous or colony forming microalgae such as *Arthrospira platensis* or *Micractinium sp.* The majority of the microalgal species are unicellular microorganisms with pliable cell walls, these structures tend to block the

filter medium, making filtration a labour and energy intensive process. Flocculation by the use of multivalent metal salts such as alum tends to contaminate the algal biomass with metal ions such as Al^{3+} (Mohn, 1988) while flocculation by cationic polymers can be inhibited by the high ionic strength of sea water (Bilanovic & Shelef, 1988). Other types of flocculation, such as bio-flocculation, which is induced by environmental stresses such as extreme pH, temperature or nutrient depletion, may cause cell composition changes and is generally considered as too unreliable to be economical on a commercial scale (Benemann and Oswald, 1996).

Filter presses

Filter press consists of sets of plates interspaced with filter media where the suspension is pumped into the press compartment with the flows perpendicular to the filter media. Particles retained by the filter media build up to form a *cake*. When the cake consumes the void between the plates, the filter press is full and filtration ceases. The filtration process must be temporarily stopped to allow the plates to be separated and the cake discharged. While filter presses can be automated to minimise operator requirements they are infrequently used to recover microalgae. Energy costs of 0.38-0.51 kWh m⁻³ with permeate fluxes of ~20 L m⁻² h⁻¹ and concentration factors of 20-46 have been reported (Danquah et al. 2009). However, membrane fouling especially with long-term use, which reduces permeate fluxes, membrane life and possibly product quality, is a significant problem in many other industries and remain a major concern.

Flocculation

Coagulation and flocculation refer to two distinct processes: coagulation involves the initial destabilizing of the suspended particles and flocculation involves the aggregation of destabilised particles to form flocs. Coagulation is followed by flocculation almost

instantaneously and the two terms are often used interchangeably. Flocculation can be induced by the addition of flocculants including inorganic salts of Al or Fe, polymers such as Polyacrylamide or through processes such as autoflocculation, bioflocculation, ultrasound and electrocoagulation procedures. Flocculants destabilise the microalgal cells in suspension by reducing or neutralising the cell surface charge, the mechanisms can take four forms including (1) electrical double-layer compression, (2) adsorption and charge neutralisation, (3) adsorption and inter-particle bridging, and (4) entrapment in a precipitate, or “sweep floc”. As flocculation relies on the interaction between the negatively charged microalgae and the flocculant used; the extent of recovery and cost are dependent on the microalgal species, initial biomass concentration, surface charge of the cells, the types and dose of flocculants used, degree of mixing and media parameters such as alkalinity, ionic strength, pH and temperature.

Inorganic (chemical) flocculation

Many inorganic chemicals can be used to flocculate microalgae with soluble aluminium and iron salts as common choices due to their relatively low cost and high efficiency in removing colloidal particles. The dissolution of these inorganic salts in water initiates the formation and precipitation of their hydroxides. In addition to neutralising the negative charge on the microalgae, these precipitates may also act as nucleation sites for microalgae attachment and microalgae removal may also be accomplished via entrapment (Shammas, 2005). The anions present in the coagulants have also been shown to influence the extent of biomass recovery, with the chloride salts of aluminium, iron and zinc being more effective than the sulphate salts in flocculating cultures of the freshwater green alga *Chlorella minutissima* (Papazi et al., 2010).

The optimal dose depends on the individual system and is usually determined by the use of standard jar test based on the recovery efficiency or the concentration factor. The ionic strength of the cultivation media has a significant effect on the optimal coagulant dose with higher ionic strength systems requiring higher doses and subsequently higher operational cost. In many wastewater treatment processes the Alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$) dose varies between 50 to 600 mg L⁻¹ (Crites, 2006).

Commercially, the use of flocculants is often combined with dissolved air flotation for a more compact plant footprint area and higher loading rate (Crossley & Valade, 2006). The major concerns with the use of inorganic coagulants for the recovery of microalgae for biofuels are high operating costs, residual metal salts dissolved in the growth media (which must be reused) and the metal salts incorporated in the recovered biomass. For example, the high aluminium concentrations typical in Alum flocculated biomass renders any residual biomass unsuitable for use as animal feed. In 1998, the Aquatic Species Program (ASP) concluded that inorganic flocculation was too expensive for the production of biofuels from microalgae (Sheehan et al., 1998).

Organic (chemical) flocculation

Organic flocculants can be derived either naturally occurring substances (e.g. chitosan) or synthetic (Polyacrylamide), these flocculants can broadly be divided into three types, namely, cationic, anionic and non-ionic. Organic flocculants can be used to recover microalgae by themselves but more frequently they are used in conjunction with inorganic coagulants to aid in inter-particle bridging.

A number of organic coagulants are commercially available; they vary in molecular weight and charge density. Organic coagulants are generally considered to be non-toxic. Cationic polyelectrolytes have generally received the most attention in the recovery of

microalgae as they can facilitate bridging and assist in neutralising the negative surface charge on microalgae. Whilst anionic polyelectrolytes can destabilise negative colloids (Levy et al., 1992; Shamma, 2005), their efficiency when tested with microalgae was poor (Tilton et al., 1972). However, similar to inorganic coagulants, the effectiveness of many polyelectrolytes decrease as the ionic strength of the water is increased. This decrease in flocculation effectiveness is a result of the polyelectrolyte structure collapsing or folding (Bilanovic & Shelef, 1988) and this change in shape reduces the extent of bridging.

While organic coagulants are more expensive than inorganic coagulants, this cost can be generally be offset by lower dose (Shamma, 2005). The optimal dose of inorganic coagulations, similar to inorganic coagulants, needs to be determined on a case-by-case basis. The maximum performance of organic coagulants can also be hampered by their narrow operability window, with concentrations lower than required resulting poor flocculation and concentrations higher than required resulting in charge-reversal and re-stabilisation (Shamma, 2005). In saline systems, microalgae recoveries of between 70% and 95% have been achieved when Chitosan was dosed at 40 and 150 mg L⁻¹ (Heasman et al., 2000), while recoveries around 70% were reported with 1 mg L⁻¹ Praestol® (Pushparaj et al., 1993).

Autoflocculation

Under stressful environments, microalgae may flocculate without the addition of chemicals. Such conditions are usually induced under non-ideal growth conditions, e.g. high pH, low light intensity and the presence of phosphate and divalent cations (Sheehan et al., 1998). Autoflocculation occurs through a co-precipitation of magnesium or calcium salts that provide a positive surface charge (Becker, 1995). The performance of

auto-flocculation is difficult to predict and is species dependent (Becker, 1995). Furthermore, as the precipitation of magnesium and calcium carbonate salts generally only occur when the pH>10 (Sukenik & Shelef, 1984), this method is unsuitable for continuous cultures which need to be maintained near optimal pH for maximum productivity.

Microbial flocculation

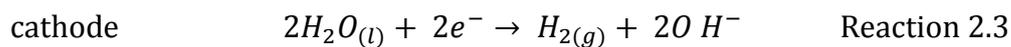
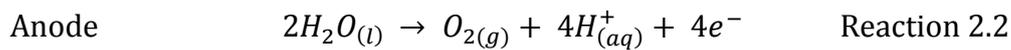
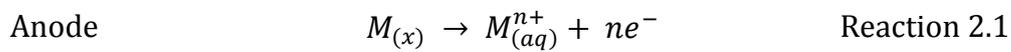
Microbial flocculation has been practised in industries such as wastewater treatment (Al-Shahwani et al., 1986; De la Noüe et al., 1992; Noüe et al., 1992) as activated sludge or in fermentation for clarifying the culture media (Suh et al., 1997). This process was suggested as a harvesting technique for microalgae by Benemann and Oswald (1996). Further evidence supporting its use in the present application may be found in the literature: for example, it has been reported that all known microalgae produce extracellular polymeric substances (EPS) such as uronic or pyruvic acid, which are indistinguishable from those produced by bacteria (Shipin et al., 1999), and that these polymers are responsible for the adhesion of cells (Frølund et al., 1996). Furthermore, microalgae embedded in the polymer matrix did not show signs of stress or lyses over an extended period (Shipin et al., 1999). Although the exact composition of the EPS may vary according to the species (Choi et al., 1998) or extraction methods (Comte et al., 2006), the fact that all bacterial and microalgal surfaces are negatively charged with similar polysaccharides (Liao, 2001) suggests that bacterial flocculants may be used to aggregate microalgae.

Marine microalgae have been flocculated successfully in the laboratory by stressing the co-existing bacteria, *Pseudomonas stutzeri* and *Bacillus cereus* (A K Lee et al., 2009) or microalgae, *Scenedesmus obliquus* (Salim et al., 2011) to produce *in situ* the EPS

necessary for flocculation. At The only published literature published regarding the use of microbial flocculation as a microalgal harvesting technique prior to 2009 was Lee et al. 2009

Electroflocculation

Electroflocculation of microalgae can be achieved by passing an electrical current between a sacrificial anode (commonly aluminum or iron) and a cathode. Half-reactions occur at each electrode are shown in Reactions 2.1, 2.2 and 2.3.



Hydrogen gas is generated at the cathode and the sacrificial anode releases cations which can destabilize microalgae by reducing or neutralizing the negative surface charge. The destabilized microalgae can then be flocculated. Depending on the design of the harvesting system, the flocs may settle to the bottom of the vessel, or attach to the gas bubbles and float to the surface.

In comparison with auto-, bio- or microbial flocculation, electroflocculation is a physical/chemical process that has the advantages of being non-species specific, simpler to operate and more predictable results. Unlike chemical flocculation such as the use of alum or ferric chloride, electroflocculation does not introduce unnecessary anions such as SO_4^{2-} or Cl^{-} which can result in the lowering of pH (Vandamme et al., 2011). The construction of the electroflocculation cell is also relatively simple; it consists of a

container with electrode plates and a direct current power supply, and hence involves modest capital investment.

Some of the gases formed at the electrodes during electroflocculation, such as hydrogen or oxygen, may be useful byproducts; these gases may be collected and converted to electrical energy. However, the commercial use of these gases will involve the additional processes of collection, compression, purification and storage; these steps involve substantial capital and operational costs and have to be justified on economic basis. In addition, operational conditions such as applied current, voltage and characteristics of the electrical current should be optimized to produce the maximum amount of Al^{3+} ions while minimizing the gas production. Future work should be carried out in the optimization of the dissolution of electrodes rather than collection of gases as by-products.

The major factors which affect the electroflocculation of microalgae include voltage, current, residence time, electrode materials and system design. High biomass recoveries are possible, with biomass recovery up to 99% and 98% being observed under laboratory conditions for *Tetraselmis* sp. and *Chlorococcum* sp., respectively (Uduman et al., 2010). The greatest issues surrounding the use of electroflocculation is the contamination of the recovered biomass and growth media with salts formed from the metal ions from the sacrificial anode; the high cost of anode replacement and the formation of an insulating deposit on the cathode. While the energy consumed during electroflocculation can be calculated based on the applied current, voltage, residence time and volume of growth media, the reported energy consumptions vary significantly and depend on the system design and operational parameters. The initial biomass concentration, mode of biomass recovery (i.e. flotation or sedimentation), percent

biomass recovery and the salinity of the media have a significant bearing on the operational cost. Freshwater systems generally have a higher energy demand over saline systems as the electrical resistance in freshwater systems is higher and a higher voltage is required in order to achieve the same current.

Mixing

Mixing increases the probability for the charged particles to collide with each other to form larger particles for easier settling. For microalgal harvesting, both the demand for energy and the necessary economy of scale require the microalgal culture to be processed in the order of thousands of cubic meters per hour (A. K. Lee et al., 2010). On such a scale, energy efficient mixing is essential and can be provided by the use of baffled hydraulic flocculators, such system offers advantages such as simplicity in construction, no moving parts, do not induce short circuiting and low maintenance.

“The extent of mixing necessary for a proper flocculation can be quantified by the velocity gradient, G , in the range 100 to 10 s^{-1} and Camp number Ca , in the range 10^4 - 10^5 (Camp & Stein, 1943; Kolarik & Booker, 1995).

The velocity gradient, G , is given by Eq 2.1

$$G = \sqrt{\frac{P}{\nabla\mu}} \quad 2.1$$

Where P is the power consumed by the fluid media ($kg\ m^2\ s^{-3}$), μ is the dynamic viscosity (Pa s), and ∇ is the total volume of fluid in the flocculator (m^3).

Although the conventional interpretation of G is the velocity gradient, Eq 2.1 indicates that it may also be considered as the root mean of the power dissipated per unit volume and is therefore correlated to the energy consumed due to flocculation.

Camp number, Ca , is the product of G (s^{-1}) and the fluid retention time, θ (s), in the flocculator Eq 2.2

$$Ca = G \theta \quad 2.2$$

Ca gives an indication of the total energy dissipated by each individual volume as it flows through the flocculator.

An additional advantage is that baffles and electrodes can be integrated into one single unit to simplify the plant construction; however, it remains to be demonstrated that the cost of constructing these mixing units can be justified by the increase in harvesting efficiency.

Electrode separation

The total overpotential, η_n , that is necessary for the flow of electric current between the electrodes in the flocculation tank, comprises of three components as indicated by Eq 2.3

$$\eta_n = \eta_k + \eta_m + \eta_{IR} \quad 2.3$$

Where: η_k is the kinetic overpotential due to contributing factors such as flow dynamics or gas evolution, η_m the mass transfer overpotential due to the diffusion of ions, and η_{IR} is the internal potential drop due to the resistance of solutions and electrode deposits. Values of η_{IR} are inversely proportional to the distance of electrode separation d_s , but in an industrial scale electroflocculation plant, a larger number of electrodes correspond to

higher installation and maintenance costs. For this reason, it is necessary to optimize d_s and hence the number of electrodes required. A search on available literature reviews that d_s had been chosen arbitrarily by various authors and there is no apparent correlation between the energy consumption and d_s (Alfajara et al., 2002; Gao et al., 2010; Il'in et al., 2002; Poelman et al., 1997; Vandamme et al., 2011). Finally, these experiments were performed in an essentially fresh water environment and the energy requirement in a marine environment is expected to be lower due to the higher electrical conductivity.

Cost of electroflocculation

In addition to the electrolytic dissolution as determined by Faraday's Law, many metal can be chemically dissolved during electrolysis, these electrodes are corroded by chemical reactions where the electrons released by the sacrificial metal are not part of the electrolytic current. The electrical dissolution efficiency for Aluminium, D_{al} has a value between 1.4 to 2, i.e., it will be dissolved at a rate 1.4 to twice of that determined by Faraday's Law (Gu et al., 2009).

Eq 2.4 was developed by Donini et al. (1994) to estimate the operating cost of electroflocculation, C_{op} , in \$ m⁻³.

$$C_{op} = 0.03917 (0.02127 V + D_{al}) \times \frac{I}{Q_L} \quad 2.4$$

Where: V is the applied voltage, V; D_{al} is the aluminum dissolution efficiency, no unit; I is the current, A and Q_L is the volumetric flow rate, L min⁻¹.

This model was based on an electrical energy cost of \$ 0.05 kWh⁻¹ and aluminium cost of \$ 7 kg⁻¹ (Donini et al., 1994). The first term on the right hand side of Eq 2.4 represents

the electrical energy cost while the second term represents the aluminium dissolution cost. This equation suggests that for a typical applied voltage between 10 V to 20 V and a typical D_{al} value of 1.5, the aluminium dissolution cost is 3.5 to 7 times more than the energy cost and such cost need to be taken into consideration during the production.

Bioflocculation

Bioflocculation can be achieved through the use of biologically excreted organic compounds, often termed extracellular polymeric substances or extracellular polysaccharides (EPS). Some microalgae and bacteria can be induced to excrete EPS, which are usually polymers of uronic or pyruvic acids under extreme temperature, pH or nutrient stress conditions (A K Lee et al., 2009; Mishra & Jha, 2009). While microalgae are known to excrete EPS, the EPS generally only occur under non-ideal growth conditions and this method is unsuitable for continuous cultures which are grown under optimal conditions for maximum productivity. As previously mentioned EPS can come from a range of other sources and while the addition of a crude or purified EPS is unlikely to be economically viable, due to the complexity and high costs associated with EPS separation and purification, several trials have been successful to co-culture bacteria for EPS production with microalgae (Fukami et al., 1997; A K Lee et al., 2009), unlike autotrophic microalgae, require a suitable organic carbon source as a substrate for growth, and this requirement can be exploited without affecting the microalgae biomass productivity. While the use of EPS prevents the risk of contamination by inorganic coagulants and can achieve similar flocculating efficiencies (Lee et al. 2009), the performances are often unpredictable (A. K. Lee et al., 2010; Li & Yang, 2007) estimated that the cost of bio-flocculation could be AUS\$ 0.13 m⁻³ of culture.

Ultrasound

Standing acoustic waves can cause material to flocculate by forcing the material into the pressure nodes. Ultrasound induced flocculation can be advantageous in some situations as it can be operated continuously, has a small footprint, lack of freely moving parts and the flocculation mechanism does not rely on the addition of chemicals Bosma et al. (2003); however, the energy requirement is too high and the concentration factor is too low for the economical recovery of microalgae. The scale up from laboratory bench top to pilot size operation also presents a technical challenge due to the maximum size of the sonicator is only 1000 L day⁻¹ (Bosma et al., 2003).

Sedimentation

Sedimentation is one of the simplest forms of solid-liquid separation. The major advantages of sedimentation processes are low power consumption, low design cost and low requirement for skilled operators. The major disadvantages of sedimentation include the low solids concentration achieved and slow sedimentation rates; hence and the large footprint required. The sedimentation rate is controlled by the net gravitational force acting on the microalgae. For a small inert spherical isolated particle in a Newtonian fluid the sedimentation rate is described by Stokes Law with the terminal velocity of the particle, V , given by Eq 2.5:

$$V = \frac{g (\rho_s - \rho_f) d^2}{18 \mu} \quad 2.5$$

Where: g is the gravitational acceleration (m s⁻²), ρ_s is the density of the particle (kg m⁻³), ρ_f is the density of the fluid (kg m⁻³), d is the equivalent diameter of the particle (m) and μ is the fluid viscosity (Pa s).

The actual sedimentation rate of microalgae can also depend on cell motility, cell composition and water turbulence. As most microalgae are not spherical a suitable

equivalent spherical diameter must be used. The sedimentation rates of most microalgae are too low for practical operations.

Lamellar settler

The settling rate in a sedimentation tank can be increased through the use of inclined channels, plates or tubes. The term Lamella® is a registered trademark (held by Parkson Corp, Fort Lauderdale, Florida) for a settling tank with inclined plates to promote enhanced-gravity sedimentation. Unlike traditional sedimentation where the distance particles need to travel is large, these gravity-enhanced settlers use a series of angled plates or tubes to minimise the distance a particle needs to travel before hitting a surface. Once particles hit the surface, they may slide down. The ease at which particles slide depends on the nature of the particles, material of the plate surface and the angle of the plates or tubes. A recent report suggested that Lamella® settlers could be used to recover some microalgae (Nakamura et al., 2004).

Whilst enhanced settling is commonly used in waste water treatment processes, it does rely on there being significant density differences between the liquid and suspended solids. The density difference between individual cells and the growth media is not sufficient in many biological systems, but may be enhanced through the use of flocculants.

Flotation

Flotation is a separation process based on air or gas bubbles adhering to the particles or flocs, which are then rise to the liquid surface to be skimmed off. Flotation-flocculation is frequently used in wastewater treatment and mineral processing industry. Gas bubbles are usually introduced or generated within the flotation cell and often used in conjunction with flocculants to increase efficiencies. Common types of flotation

separation processes include dissolved air flotation (DAF), bioflotation and electrolytic flotation. Smaller gas bubbles are more efficient than larger gas bubbles, as they have a larger surface area per unit volume and lower buoyancy (Crossley & Valade, 2006); thus increasing the likelihood of collisions between an air bubble and a particle. The major operational parameters for all flotation systems include the air to solids ratio, hydraulic loading, solids loading, weir overflow rate and handling of floated solids and any sediment.

Dispersed Air Flotation

Air can be continuously pumped into a flotation cell through a process of froth or foam flotation. The bubble size in dispersed air flotation systems is typically large (often >1 mm diameter) and results in low flotation efficiencies. While concentration factors of between 50 and 200 have been reported for mineral processing (Levin & Shaheen, 1967), dispersed air flotation is not widely used for the recovery of microalgae.

Dissolved Air Flotation (DAF)

Higher flotation efficiencies can be obtained if air-supersaturated water is injected under pressure into a flotation cell, upon which small air bubbles (with diameters of 60 μm -200 μm) are formed due to the reduction in pressure. This process, known as dissolved-air flotation (DAF), is often chosen in preference to sedimentation as higher volumetric capacities per unit area are achievable. A typical DAF unit has approximately 5-20% of the effluent recycled, supersaturated with air and used as the pressurised flow. Particles attach to the small air bubbles and float to the surface. In addition to the increase in volumetric capacity, the solid concentration typically achieved with DAF systems is higher of about 7%, compared to 2% - 3% for sedimentation. Operational

costs of DAF systems are generally higher than sedimentation due to the high energy cost of supersaturating the water with air under pressure (Féris & Rubio, 1999).

Suspended Air Flotation (SAF)

Suspended air flotation (SAF) is similar to dissolved air flotation in that small bubbles are used to float particles to the surface of the water. However, SAF units utilise chemicals (often cationic surfactants) to create the small bubbles, eliminating the need for a compressor and saturator thus reducing energy costs (Wiley et al., 2009).

In addition, the flotation bubbles can be electrically-charged to increase the stability of the float. The increased stability of the float can significantly increase the acceptable hydraulic loading rate and solid loading rate. Suspended air flotation is a relatively new technology and at the present time it is not known whether the chemicals used to create the flotation bubbles, although often non-toxic, will effect subsequent medium recycling.

Autoflotation

Mechanical methods used to provide the flotation gas are often energy intensive and this disadvantage may be overcome by using photosynthetic oxygen (autoflotation) (Arbelaez et al., 1983; Koopman & Lincoln, 1983). Dissolved oxygen (DO) concentrations exceeding 14 to 16 mg L⁻¹ are essential for successful autoflotation (Arbelaez et al. 1983) and can be obtained by careful withdrawal of the oxygen saturated surface layer in unmixed and stagnant ponds. As the autoflotation process requires highly dissolved oxygen concentrations, it cannot be used as a method to continuously harvest algae; therefore, DO flotation is not a feasible option in recovering microalgae biomass for biofuels.

Cost comparison of various harvesting methods

In order to compare the cost of different harvesting methods directly, their values (including centrifugation) have been converted to \$ kg⁻¹ equivalent biomass, updated to their current values and presented in Table 2.1:

Table 2.1 Cost comparison for different harvesting methods

Separation processes	References	Cost (\$ kg ⁻¹ biomass)		Items included in the processing cost
		Original ^c	2012	
Centrifugation – self cleaning plate	Becker, 1995	1.71	2.58	Plant depreciation, maintenance and energy
	Mohn, 1988	0.86 ^a	1.68	Equipment depreciation, maintenance and energy
Flocculation - sedimentation	Mohn, 1988	0.37 ^a	0.72	Plant depreciation, maintenance, flocculant and energy
	Benemann, 1996	1.25	1.83	Plant depreciation, maintenance, flocculant and energy
Flocculation - flotation	Becker 1995	1.39	2.10	Plant depreciation, maintenance, flocculant and energy
	Mohn, 1988	0.91 ^a	1.78	Plant depreciation, maintenance, flocculant and energy
Electro-flocculation	Poelman et al, 1997	0.22	0.31	Energy only
Microbial flocculation	Lee et al, 2010	0.29 ^{b,d}	0.31	Plant depreciation, maintenance, raw materials and energy
Electro-flocculation			0.19	Plant depreciation, electrode dissolution and energy

^a US \$ 1 = DM 1.85 in 1988

^b US\$ 1 = A \$ 1.1 in 2010

^c US Consumer Price Index in 1988, 1995, 1996, 1997, 2009 and 2012 are 115.7, 150.3, 154.4, 159.1, 211.1 and 226.6 respectively.

^d Typical biomass concentration is assumed to be 0.5 kg m⁻³

Plant design, energy requirements and economic analysis

The success of microbial flocculation depends on the production of EPS from the bacteria and the attachment of the microalgae onto the polymers to form flocs. Mixing increases the contact necessary for the attachment of microalgal cells onto EPS and also provides a uniform distribution of nutrients and oxygen gas necessary for the growth of the bacteria. Therefore mixing is one of the essential factors in microbial flocculation. In the laboratory, mixing can be provided by shake tables, but on a commercial scale, where the daily mixing of mega or giga litres of cultures are required, the technique for the mixing on such a scale, the amount of energy required, the operational and capital costs need to be investigated.

2.3 Cell disruption

All known species of microalgae, with the exception of *Botryococcus braunii* (Banerjee et al., 2002; Largeau et al., 1980), have their lipids located inside the cells. These inclusions, and other cell components, are bound by membranes and/or cell walls (Barsanti et al., 2007; Simon & Helliwell, 1998), such that the lipids are not readily available for extraction from intact cells. Thus, the recovery of lipids from microalgal cells can be improved by up to 3 to 4 times by disrupting the cells (Cravotto et al., 2008; J. Y. Lee et al., 2010). However, these cell boundaries are usually quite strong and so most disruption processes are highly energy intensive. For the production of algal lipids or pigments for pharmaceutical or nutraceutical uses, the high disruption energy required can be justified by the high product values, and process energy is an important but not critical factor. However, for microalgae as a potential source of alternative renewable fuel, the energy input for disruption is a critical consideration since a net negative energy output is clearly not sustainable.

Despite the importance of microalgal cell disruption in relation to the extraction of lipid, cell disruption has received relatively little attention. In some microalgal biofuels review papers, cell disruption was either not mentioned (Chisti, 2007; Fajardo et al., 2007; Lardon et al., 2009), or only briefly described in a paragraph or two (Brennan & Owende, 2010; Mata et al., 2010). Some authors have wrongly assumed that microalgal lipids can be extruded in mechanical press similar to that used for plantation crops such as soy (Amin, 2009); however, due to the small size of typical microalgae cells (3 μm to 15 μm) and the tough but pliable cell walls, microalgae cells will simply be squeezed through a typical mechanical press without being disrupted. Some authors have experimented with apparently undisrupted microalgal cells for biodiesel production; however in these cases a cell disruption step has been incorporated into the solvent extraction step and so the effects of cell disruption and solvent extraction are not distinguishable (Carrero et al., 2011).

While much research has been done on the disruption of microorganisms such as yeast or bacteria, very little has been done on microalgae and even less in relation to the production of biodiesel from microalgae. The energy requirements for the various cell disruption processes are unknown; hence the sustainability of such processes in the production of renewable fuels cannot be assessed. The energy efficiency of these disruption processes, i.e. the fraction of energy input that is actually received by the cells for disruption, are also unknown.

Disruption energy requirements

A review of the scientific literature showed that microalgal cell disruption processes have energy requirements ranging from 33 MJ kg^{-1} by hydrodynamic cavitation to 529 MJ kg^{-1} by high pressure homogenizers (Andrew K. Lee et al., 2012). In comparison, the

energy available by the combustion of the entire microalgal biomass is estimated to be approximately 29 MJ kg⁻¹ (Andrew K. Lee et al., 2012, 2013). This net negative energy balance indicates current mechanical cell disruption processes are not sustainable for microalgal biofuels production. Some of the obvious questions are: is there any potential for further reductions in the disruption process energy or have the current disruption processes already reached the theoretical limit. To answer these questions, the actual amount of energy required by the cells for disruption needs to be compared with the total energy input during the cell disruption processes. While the total energy input during cell disruption can be measured by the electrical energy consumed during each individual process, the energy required by the cells for disruption are unknown and need to be determined.

2.4 Summary of the objectives of the thesis

The primary objective of this thesis is to provide the technical and economic information necessary for the development of a pilot scale microalgal biofuel production plant. The fact that biofuels are low cost commodities requires innovations throughout this production process to reduce energy consumptions and capital requirements.

Details of the objectives are:

1. To reduce harvesting cost by selecting low cost harvesting methods; namely, microbial and electroflocculation, and improve on their harvesting efficiencies and energy requirements by an appropriate level of mixing. In the case of electroflocculation, the electrode separations are to be determined to optimise the energy consumption and the cost of electrode dissolution is to be incorporated in the operational cost estimation.

2. To determine the feasibility of such processes by detailed plant designs, estimation on the energy consumptions, capital and operational cost.
3. To evaluate and select cell disruption methods which are suitable for the large scale extraction of lipids. The selection is based on the energy consumption, non contamination of products and process reliability.
4. To provide a fundamental understanding of the cell walls mechanical characteristics so as to determine the energy efficiencies of various cell disruption processes. In particular, the force and energy requirements as related to cell disruption.

2.5 References

- Al-Shahwani, M., Jazrawi, S., & Al-Rawi, E. (1986). Effects of bacterial communities on floc sizes and numbers in industrial and domestic effluents. *Agric Wastes*, 16(4), 303-311.
- Alfajara, C. G., Nakano, K., Nomura, N., Igarashi, T., & Matsumura, M. (2002). Operating and scale-up factors for the electrolytic removal of algae from eutrophied lakewater. *Journal of Chemical Technology & Biotechnology*, 77, 871-876.
- Amin, S. (2009). Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management*, 50(7), 1834-1840.
- Arbelaez, J., Koopman, B., & Lincoln, E. P. (1983). Effects of Dissolved Oxygen and Mixing on Algal Autoflotation. *Journal (Water Pollution Control Federation)*, 55(8), 1075-1079.
- Banerjee, A., Sharma, R., Chisti, Y., & Banerjee, U. C. (2002). *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. *Critical Reviews in Biotechnology*, 22(3), 245-279.
- Barsanti, L., Evangelista, V., Frassanito, A. M., Vesentini, N., Passarelli, V., & Gualtieri, P. (2007). Absorption microspectroscopy, theory and applications in the case of the photosynthetic compartment. *Micron*, 38(3), 197-213.
- Becker, E. W. (1995). *Microalgae biotechnology and microbiology*. Cambridge: Cambridge University Press.
- Bilanovic, D., & Shelef, G. (1988). Flocculation of microalgae with cationic polymers-effects of medium salinity. *Biomass*, 17, 65-76.

- Bosma, R., van Spronsen, W. A., Tramper, J., & Wijffels, R. H. (2003). Ultrasound, a new separation technique to harvest microalgae. *Journal of Applied Phycology*, 15(2), 143-153.
- Brennan, L., & Owende, P. (2010). Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2), 557-577.
- Camp, T. R., & Stein, P. C. (1943). Velocity gradients and internal work in fluid motion. *Journal of Boston Society of Civil Engineers*, 85, 219-237.
- Carrero, A., Vicente, G., Rodríguez, R., Linares, M., & del Peso, G. L. (2011). Hierarchical zeolites as catalysts for biodiesel production from *Nannochloropsis* microalga oil. *Catalysis Today*, 167(1), 148-153.
- Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25(3), 294-306.
- Choi, C., Yoo, S., Oh, I., & Park, S. (1998). Characterization of an extracellular flocculating substance produced by a planktonic cyanobacterium, *Anabaena sp.* *J Biotech L*, 20(7), 643-646.
- Comte, S., Guibaud, G., & Baud, M. (2006). Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties Part I. Comparison of the efficiency of eight EPS extraction methods. *Enzy Microbial Tech*, 38(1-2), 237-245.
- Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., & Cintas, P. (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics Sonochemistry*, 15(5), 898-902.
- Crites, R. (2006). Integrated planning for wastewater treatment and recycling for a small community development. *Proceedings of the Water Environment Federation*, 2006(5), 6460-6467.
- Crossley, I., & Valade, M. (2006). Review of the technological development of DAF. *J Water Sup Res Tech—AQUA*, 55(7-8), 479-491.
- Danquah, M. K., Ang, L., Uduman, N., Moheimani, N., & Forde, G. M. (2009). Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *Journal of Chemical Technology & Biotechnology*, 84(7), 1078-1083.
- De la Noüe, J., Laliberté, G., & Proulx, D. (1992). Algae and waste water. [10.1007/BF02161210]. *Journal of Applied Phycology*, 4(3), 247-254.
- Donini, J., Kan, J., Szykarczuk, J., Hassan, T. A., & Kar, K. (1994). The operating cost of electrocoagulation. *The Canadian Journal of Chemical Engineering*, 72(6), 1007-1012.
- Fajardo, A. R., Cerdán, L. E., Medina, A. R., Fernández, F. G. A., Moreno, P. A. G., & Grima, E. M. (2007). Lipid extraction from the microalga *Phaeodactylum tricornutum*. *European Journal of Lipid Science and Technology*, 109(2), 120-126.

- Féris, L. A., & Rubio, J. (1999). Dissolved air flotation (DAF) performance at low saturation pressures. *Filtration & Separation*, 36(9), 61-65.
- Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, 30(8), 1749-1758.
- Fukami, K., Nishijima, T., & Ishida, Y. (1997). Stimulative and inhibitory effects of bacteria on the growth of microalgae. *Hydrobiologia*, 358(1-3), 185-191.
- Gao, S., Yang, J., Tian, J., Ma, F., Tu, G., & Du, M. (2010). Electro-coagulation-flotation process for algae removal. *Journal of Hazardous Materials*, 177(1-3), 336-343.
- Gu, Z., Liao, Z., Schulz, M., Davis, J. R., Baygents, J. C., & Farrell, J. (2009). Estimating dosing rates and energy consumption for electrocoagulation using Iron and Aluminum electrodes. *Industrial & Engineering Chemistry Research*, 48(6), 3112-3117.
- Heasman, M., Diemar, J., O'Connor, W., Sushames, T., & Foulkes, L. (2000). Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. *Aquaculture Research*, 31(8-9), 637-659.
- Il'in, V. I., Kolesnikov, V. A., & Parshina, Y. I. (2002). Purification of highly concentrated industrial sewage from the porcelain and faience industry by the electric flotation method. *Glass and Ceramics*, 59(7), 242-244.
- Kolarik, L., & Booker, N. (1995). *Modern techniques in water and wastewater treatment - Fundamentals of coagulation-flocculation in water treatment*. Melbourne, Vic., Australia: CSIRO Publishing
- Koopman, B., & Lincoln, E. P. (1983). Autoflotation harvesting of algae from high-rate pond effluents. *Agricultural Wastes*, 5(4), 231-246.
- Lardon, L., Helias, A., Sialve, B., Steyer, J., & Bernard, O. (2009). Life-cycle assessment of biodiesel production from microalgae. *Environmental Science and Technology*, 43(17), 6475-6481.
- Largeau, C., Casadevall, E., Berkaloff, C., & Dhamelinourt, P. (1980). Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *Phytochemistry*, 19(6), 1043-1051.
- Lee, A. K., Lewis, D. M., & Ashman, P. J. (2009). Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Journal of Applied Phycology*, 21(5), 559-567.
- Lee, A. K., Lewis, D. M., & Ashman, P. J. (2010). Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting. *Chemical Engineering Research and Design*, 88(8), 988-996.
- Lee, A. K., Lewis, D. M., & Ashman, P. J. (2012). Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. *Biomass and Bioenergy*, 46 89-101.

- Lee, A. K., Lewis, D. M., & Ashman, P. J. (2013). Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation. *Bioresource Technology*, 128(0), 199-206.
- Lee, J. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., & Oh, H. M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresource Technology*, 101(1, Supplement 1), S75-S77.
- Levin, G. V., & Shaheen, D. G. (1967). Metabolic removal of phosphate from sewage effluent. *Biotechnology and Bioengineering*, 9(4), 457-470.
- Levy, N., Magdassi, S., & Bar-Or, Y. (1992). Physico-chemical aspects in flocculation of bentonite suspensions by a cyanobacterial bioflocculant. *Water Research*, 26(2), 249-254.
- Li, X., & Yang, S. (2007). Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research*, 41(5), 1022-1030.
- Liao, B., Allen DG, Droppo IG, Leppard GG, Liss SN. (2001). Surface properties of sludge and their role in bioflocculation and settleability. *Water Res*, 35(2), 339-350.
- Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14(1), 217-232.
- Mishra, A., & Jha, B. (2009). Isolation and characterization of extracellular polymeric substances from micro-algae *Dunaliella salina* under salt stress. *Bioresource Technology*, 100(13), 3382-3386.
- Mohn, F. (1988). Harvesting of microalgal Biomass. In M. Borowitzka & L. Borowitzka (Eds.), *Micro-algal Biotechnology*, Ch 15 (pp. 488). New York: Cambridge University Press.
- Nakamura, T., Olaizola, M., & Masutani, S. (2004). *Recovery and sequestration of CO₂ from stationary combustion systems by photosynthesis of microalgae*. Pittsburgh, PA, USA: National Energy Technology Laboratory (US).
- Noüe, J., Laliberté, G., & Proulx, D. (1992). Algae and waste water. *Journal of Applied Phycology*, 4(3), 247-254.
- Pahl, S., Lee, A., Kalaitzidis, T., Ashman, P., Sathe, S., & Lewis, D. (2013). Harvesting, Thickening and Dewatering Microalgae Biomass. In M. A. Borowitzka & N. R. Moheimani (Eds.), *Algae for Biofuels and Energy* (Vol. 5, pp. 165-185): Springer Netherlands.
- Papazi, A., Makridis, P., & Divanach, P. (2010). Harvesting *Chlorella minutissima* using cell coagulants. [10.1007/s10811-009-9465-2]. *Journal of Applied Phycology*, 22(3), 349-355.

- Petrussevski, B., Bolier G, Van Breemen AN, Alaert GJB. (1994). Tangential flow filtration: A method to concentrate freshwater algae. *Water Res*, 29(5), 1419-1424.
- Poelman, E., De Pauw, N., & Jeurissen, B. (1997). Potential of electrolytic flocculation for recovery of micro-algae. *Resources, Conservation and Recycling*, 19(1), 1-10.
- Pushparaj, B., Pelosi, E., Torizillo, G., & Materassi, R. (1993). Microbial biomass recovery using a synthetic cationic polymer. *Biores Tech*, 43(1), 59-62.
- Rossignol, N., Vandanjon, L., Jaouen, P., & Quéméneur, F. (1999). Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacultural Engineering*, 20(3), 191-208.
- Salim, S., Bosma, R., Vermuë, M., & Wijffels, R. (2011). Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology*, 23(5), 849-855.
- Shammas, N. (2005). Coagulation and Flocculation. In L. Wang, Y.-T. Hung & N. Shammas (Eds.), *Physicochemical Treatment Processes* (Vol. 3, pp. 103-139): Humana Press.
- Sheehan, J., Dunahay, T., Benemann, J., & Roessler, J. (1998). *A look back at the U.S. Department of Energy's Aquatic Species Program-biodiesel from algae*. Golden, Colorado 80401-3393: National Renewable Energy Laboratory, U.S. Department of Energy, Office of Fuels Development.
- Shipin, O. V., Meiring, P. G. J., Phaswana, R., & Kluever, H. (1999). Integrating ponds and activated sludge process in the PETRO concept. *Water Research*, 33(8), 1767-1774.
- Simon, D., & Helliwell, S. (1998). Extraction and quantification of chlorophyll a from freshwater green algae. *Water Research*, 32(7), 2220-2223.
- Suh, H., Kwon, G., Lee, C., Kim, H., Oh, H., & Yoon, B. (1997). Characterization of bioflocculant produced by *Bacillus* sp. DP-152. *Journal of Fermentation and Bioengineering*, 84(2), 108-112.
- Sukenik, A., & Shelef, G. (1984). Algal autoflocculation—verification and proposed mechanism. *Biotechnology and bioengineering*, 26(2), 142-147.
- Tchobanoglous, G., Burton F. (1997). *Wastewater engineering - treatment, disposal and reuse* (third ed.). New York: Metcalf & Eddy, Inc, Mcgraw- Hill Inc.
- Tilton, R. C., Murphy, J., & Dixon, J. K. (1972). The flocculation of algae with synthetic polymeric flocculants. *Water Research*, 6(2), 155-164.
- Uduman, N., Qi, Y., Danquah, M. K., & Hoadley, A. F. A. (2010). Marine microalgae flocculation and focused beam reflectance measurement. *Chemical Engineering Journal*, 162(3), 935-940.

- Vandamme, D., Pontes, S. C. V., Goiris, K., Foubert, I., Pinoy, L. J. J., & Muylaert, K. (2011). Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater microalgae. *Biotechnology and Bioengineering*, *108*(10), 2320-2329.
- Wiley, P. E., Brenneman, K. J., & Jacobson, A. E. (2009). Improved Algal Harvesting Using Suspended Air Flotation. *Water Environment Research*, *81*(7), 702-708.
- Winter, A., & Siesser, W. (1994). *Coccolithophores*. Cambridge, UK: Cambridge University Press.

3 Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting

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Chemical Engineering Research and Design 88 (2010) 988–996

Statement of authorship

Lee AK, Lewis DM, Ashman PJ (2010)

Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting. Chemical Engineering Research and Design 88: 988-996

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I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

Signed Date

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Manuscript evaluation

I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

Signed Date

Note:

This publication is included on Pages 3-4 to 3-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available on line to authorised users at

<http://www.sciencedirect.com/science/article/pii/S026387621000050X>

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2010) Energy requirements and economic analysis of a full-scale microbial flocculation system for microalgal harvesting.
Chemical Engineering Research and Design, v. 88(8), pp. 988-996

NOTE:

This publication is included on pages 3-4 to 3-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1016/j.cherd.2010.01.036>

4 Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements

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Biomass and Bioenergy 46 (2012) 89-101

Statement of authorship

Lee AK, Lewis DM, Ashman PJ (2012)

Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. *Biomass and Bioenergy* 46, 89-101

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Note:

This publication is included on Pages 4-4 to 4-16 in the print copy of the thesis held in the University of Adelaide Library.

It is also available on line to authorised users at

<http://dx.doi.org/10.1016/j.biombioe.2012.06.034>

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2012) Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. *Biomass and Bioenergy*, v. 46(November), pp. 89-101

NOTE:

This publication is included on pages 4-4 to 4-16 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1016/j.biombioe.2012.06.034>

5 Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation

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Bioresource Technology, (2013) 128, 199-206.

Statement of authorship

Lee AK, Lewis DM, Ashman PJ (2013)

Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation. *Bioresource Technology*, 128, 199-206.

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Signed Date

Note:

This publication is included on Pages 5-4 to 5-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available on line to authorised users at

<http://dx.doi.org/10.1016/j.biortech.2012.10.032>

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2013) Force and energy requirements for microalgal cell disruption: An atomic force microscope evaluation.
Bioresource Technology, v. 128(January), pp. 199-206

NOTE:

This publication is included on pages 5-4 to 5-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1016/j.biortech.2012.10.032>

6 Harvesting of marine microalgae by electroflocculation: The energetics, plant design, and economics

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Statement of authorship

Lee AK, Lewis DM, Ashman PJ (2012)

Harvesting of marine microalgae by electroflocculation: The energetics, plant design, and economics. Applied Energy, 108, 0, 45-53.

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Signed Date

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Signed Date

Note:

This publication is included on Pages 6-4 to 6-13 in the print copy of the thesis held in the University of Adelaide Library.

It is also available on line to authorised users at

<http://dx.doi.org/10.1016/j.apenergy.2013.03.003>

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2013) Harvesting of marine microalgae by electroflocculation: The energetics, plant design, and economics. *Applied Energy*, v. 108(August), pp. 45-53

NOTE:

This publication is included on pages 6-4 to 6-13 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1016/j.apenergy.2013.03.003>

7 Conclusion

7.1 Overview

This thesis advances the knowledge in the harvesting and cell disruption of microalgae from the laboratory to pilot scale. In the area of harvesting, advances were made in the scale up on the plant design, energy optimisation and economics of microbial- and electro-flocculation; while in the area of cell disruption, advances were achieved on the understanding of the energy requirements for large scale mechanical cell disruption processes, and the fundamental cell mechanics with respect to disruption. All these work has been presented as journal publications or manuscript under review as detailed previously in the Preface of this thesis.

7.2 Harvesting by microbial and electro flocculation

Hydraulic baffles can provide an energy efficient technique for the mixing requirements during the flocculation of microalgae. This technique is suitable on a scale for the production of biofuels due to the simplicity in construction, low maintenance and no short circuiting during mixing. The pressure head required for the mixing using such a flocculator of surface area 1 km² is equivalent to 0.1m of hydraulic head or 0.9 kWh tonne⁻¹ of the equivalent dry mass when Camp number and velocity gradient are taken into consideration.

The cost for harvesting by microbial flocculation, including energy, raw material and capital depreciation, is estimated to be \$ 0.13 m⁻³ of the culture medium or \$ 0.26 kg⁻¹ of the equivalent dry mass. On the other hand, the cost of harvesting by electroflocculation, including electrical energy, aluminium dissolution and capital depreciation, is estimated to be \$ 0.185 kg⁻¹ of the microalgal biomass. Both cost estimations have the potential to be further optimised.

7.3 Cell disruption

For the production of biodiesel, mechanical cell disruption methods are preferred due to the lower risk of contamination of products and the ease of scale up for the production of biofuel. The drawback is that current disruption processes have high specific energy consumptions with the most energy efficient technique, hydrodynamic cavitation, has an energy consumption of 33 MJ kg⁻¹. This rate of energy consumption is 5 orders of magnitude higher than that required by the theoretical estimation. This disruption process energy is also in excess of that can be available from the combustion of the entire cell mass, showing that more innovation and research is required for the disruption processes to be sustainable.

7.4 Atomic Force Microscopy

The energy required for the disruption of one single microalgal cell in a liquid environment can be measured by the use of atomic force microscopy (AFM). The value obtained by this technique varied according to the exact location on the cell surface due to the underlying cell structures. The average energy for disruption of an individual cell has been measured to be 17.4 pJ, which is equivalent to specific disruption energy of 673 J kg⁻¹ of dry microalgal biomass. In comparison, disruption by hydrodynamic cavitation, one of the most energy efficient techniques, requires specific disruption energy input of 33 MJ kg⁻¹ of the dry biomass. This large difference indicates the low efficiency in mechanical cell disruption processes and more innovation is required for the sustainability of such processes in the production of biofuels.

7.5 Recommendations for future work

As mentioned previously, the results of this thesis advance the knowledge in the areas of large scale microalgal harvesting, cell disruption and microalgal cell wall characteristics. However, further studies in the areas of flocculation and post disruption treatment are

necessary for these two processing units to be sustainable for microalgae to be suitable as a feedstock for renewable biofuels. The major areas suggested for future works are listed below:

1. During electroflocculation, the electrode surfaces are gradually deactivated by the formation of a passive layer of gas bubbles and mineral deposits. Constant mechanical removal of this layer is required to prevent the increase in the electrical resistance and the subsequent loss of electrical energy. On a large scale, such maintenance is labour intensive and electrical current in the form of AC pulse may be investigated as an alternative to reduce the effects of electrode fouling.
2. Mechanical mixing of the media is essential for the proper floc formation and the turbulence created during mixing may be incorporated in the removal of passive layers mentioned in the previous paragraph.
3. The biomass harvested by flocculation still contains a large amount of moisture, and the biomass will begin to decompose in a matter of hours under warm tropical climate. Studies are required to delay the decomposition of biomass for the potential logistical problem.
4. The choice of harvesting and dewatering units depends on the requirement for the next step of processing, i.e. the type of lipid extraction; therefore, it would be better if the study on harvesting was to be carried out in conjunction with lipid extraction to determine a suitable dewatering level.
5. The cost of electrical energy for the electroflocculation is calculated to be \$ 0.09 kg⁻¹ for settling and \$ 0.15 kg⁻¹ for flotation. This cost is still too high considering

there are other processing steps involved in the production of algal lipids, and further energy/cost optimization or product integration is necessary.

6. The microalga, *Pleurochrysis carterae*, was used during the study of harvesting by microbial flocculation; however, the interactions between microalgae and bacteria are often species specific and the characteristics of the flocculation, such as reaction rate or recovery efficiency, may vary according to species and the mixing time and reactor volume need to be optimized. Therefore, experiments need to be performed on a range of microalgal species.
7. Mechanical cell disruptions are energy intensive processes and more innovative research is required to minimize the disruption energy requirement or develop a completely new approach to replace the disruption/extraction step. The integration of hydrodynamic cavitation and solvent extraction into one single unit process has been proposed to improve the overall disruption/extraction energy efficiency.

Appendix A

Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel

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Journal of Applied Phycology (2009) 21: 559-567

Statement of authorship

Lee AK, Lewis DM, Ashman PJ (2009)

Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel

Journal of Applied Phycology (2009) 21: 559-567

Andrew K Lee (Candidate)

Design and performed experiments; interpreted and processed data, wrote manuscript.

Signed Date

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Manuscript evaluation

I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

Signed Date

Peter J Ashman

Manuscript evaluation

I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

SignedDate

Note:

This publication is included on Pages A-4 to A-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available on line to authorised users at

<http://dx.doi.org/10.1007/s10811>

-008-9391-8

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2009) Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Journal of Applied Phycology*, v. 21(5), pp. 559-567

NOTE:

This publication is included on pages A-4 to A-12 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1007/s10811-008-9391-8>

Appendix B

An assessment of large scale microalgal harvesting methods for the production of biodiesel

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An assessment of large scale microalgal harvesting methods for the production of biodiesel

Proceedings from CHEMECA 2010, Hilton Adelaide, Adelaide, SA, Australia, 26-29 September, 2010

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Design and performed experiments; interpreted and processed data, wrote manuscript.

Signed Date

David M Lewis

Manuscript evaluation

I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

Signed Date

Peter J Ashman

Manuscript evaluation

I give consent for Andrew K Lee to present this paper for examination towards the Doctor of Philosophy.

SignedDate

Note:

This publication is included on Pages B-4 to B-18 in the print copy of the thesis held in the University of Adelaide Library.

Lee, A.K., Lewis, D.M. & Ashman, P.J. (2010) An assessment of large scale microalgal harvesting methods for the production of biodiesel.

Proceedings from CHEMECA 2010, Hilton Adelaide, pp. 1-15

NOTE:

This publication is included on pages B4 to B-18 in the print copy of the thesis held in the University of Adelaide Library.

Appendix C

Harvesting, Thickening and Dewatering Microalgae Biomass

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Harvesting, thickening and dewatering microalgae biomass

Citation

Pahl, S., **Lee, AK.**, Kalaitzidis, T., Ashman, P., Sathe, S., & Lewis, D. (2013). Harvesting, Thickening and Dewatering Microalgae Biomass. In M. A. Borowitzka & N. R. Moheimani (Eds.), *Algae for Biofuels and Energy* (Vol. 5, pp. 165-185): Springer Netherlands.

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http://dx.doi.org/10.1007/978-94-007-5479-9_10

Pahl, S.L., Lee, A.K., Kalaitzidis, T, Ashman, P.J., Sathe, S. & Lewis, D.M. (2013) Harvesting, thickening and dewatering microalgae biomass.
In, Algae for Biofuels and energy, Springer Science and Business Media, pp. 165-185

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