Foam Fractionation: An Effective Technology for Harvesting Microalgae Biomass

A Thesis Submitted By

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Abstract

Harvesting and dewatering can account for up to 30% of the overall cost of production of usable microalgae biomass for the biotechnology and bioenergy sectors. Harvesting is particularly challenging due to the small amount of algal biomass produced relative to water volume. This process exacts high energy and cost demands and therefore limits further expansion in the microalgae biomass industry. Foam fractionation has potential to deliver a low cost, low energy harvesting solution. Microalgae cells adsorb to the surface of a stream of fine air bubbles, which then rise up a closed column, discharging the concentrated product at the top. Foam fractionation significantly reduces construction, maintenance, and energy costs compared to other harvesting technologies. In this research, a fractional factorial design of experiments followed by a central composite design were used to determine the optimal levels of major variables influencing the harvest of the freshwater microalga *Chlorella sp*. The effects of bubble size within the liquid pool and foam phase of the harvesting unit were determined, a high concentration factor of 427 as achieved using fluidic oscillation for microbubble generation. The influence of microalgal growth phase on harvest efficiency was investigated to gain insight into the optimal time to harvest during cell cultivation. The effect of surfactant, used to induce foaming, on lipid recovery was examined through methods including total lipid recovery, gas chromatography, energy dispersive x-ray spectrometry and solid phase extraction. The results indicate that the surfactant had the additional benefit of significantly increasing the overall lipid recovery. These encouraging results suggest foam fractionation offers considerable potential as an efficient, low cost, and scalable microalgae biomass harvesting technology.

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Abbreviations and notation

Abbreviations

AOM	Algogenic organic matter
BD	Biodiesel
BSA	Bovine serum albumin
CCAP	Culture collection of algae and protozoa
CCD	Central composite design
CLF	Ceramic plate sparger with a linear air flow input
CN	Cetane number
COF	Ceramic plate sparger with an oscillatory air flow input
CR	Centrifugal recovery
Cst	Centistokes
CTAB	Cetyltrimethylammonium bromide
DAF	Dissolved air flotation
DiAF	Dispersed air flotation
DOE	Department of Energy
DOM	Dissolved organic matter
ECE	Energy conversion efficiency
EPS	Exopolysaccharides
FAME	Fatty acid methyl ester
FF	Foam fractionation
FFA	Free fatty acid
GHG	Greenhouse gas
IMF	International monetary fund
LWLF	Lime wood sparger with linear air flow input
MMBM	Modified methylene blue method
MOM	Methyl orange method
OTAB	Octyltrimethylammonium bromide
PBR	Photo bioreactor
PD	Petroleum diesel
Po	Atmospheric pressure
PP	Pour point
ppm	Parts per million

SOF	Synthetic organic flocculants
SPE	Solid phase extraction
TAG	Triglycerides/ Triglycerol
TFF	Tangential flow filtration
TTS	Total suspended solids

Notation

A	Interfacial area
A_l	Interfacial area within the liquid pool
A_{coloum}	Cross sectional area of column
asc	Anionic surfactant concentration
$A_{sparger}$	Cross sectional area of the sparger used
CF	Concentration factor
csc	Cationic surfactant concentration
d	Bubble diameter
D_1	Dry weight of filter paper after filtration
D_2	Dry weight of filter paper before filtration
d_{32}	Saunter mean diameter
di	Diameter of an individual bubble
E_A	Attachment efficiency
E_c	Collision efficiency
g	Acceleration of gravity
h	Height of fluid in manometer
H_L	Liquid pool height
$MBab$	Absorbance reading of methylene blue -surfactant complex
$MOab$	Absorbance reading of methyl orange -surfactant complex
N	Total number of bubbles sampled
P	Pressure
P_2	Liquid density
Q_{gas}	Volume gas flow
R	Gas constant
R_B	Bubble radius
R_c	Microalgal cell radius

Re_B	Reynolds number
T_1	Temperature
u_c	Liquid velocity
u_D	Superficial gas velocity
V_B	Bubble velocity
W	Specific work
X_1	Column height
X_{12}	Column height x surfactant concentration
X_2	Surfactant concentration

Greek letters

ε	Gas hold up
μ_l	Liquid dynamic viscosity
ρ	Reference liquid density
γ	Ratio specific heat air

Chapter 1

Introduction

1.1 Project background

Microalgae biomass is considered to be one of the most sustainable feedstocks for the production of biodiesel when compared to terrestrial bioenergy crops. This is due to its high oil content and rapid biomass production. A number of downstream processing steps are required to produce and convert microalgal biomass into biodiesel including cultivation, harvesting, dewatering, drying, lipid extraction, and transesterification (Halim et al., 2012). Out of all the steps required for production of microalgal derived biodiesel, harvesting, and dewatering of the biomass remains the one of largest obstacles, specifically with regard to the economics and efficiency of the process (Williams and Laurens 2010, Uduman et al., 2010). Both harvesting and dewatering processes are difficult, due to the dilute nature of microalgal cultures, and are consequently more expensive when compared to terrestrial oilseed crops. Common harvesting methods include gravity: sedimentation, centrifugation, filtration and microscreening, ultra-filtration, flotation, sometimes with an additional flocculation step or with a combination of flocculation–flotation, and electrophoresis techniques (Uduman et al., 2010).The efficiency and energy consumption of the harvesting technique has major implications on the economics of commercial algal biofuel production.

Flotation has been recognised as an efficient and cost-effective method to remove microalgae from waste water treatment sites (Wiley et al., 2009). The purpose of harvesting microalgae biomass feedstock to produce biodiesel is significantly different from that of waste water treatment. The main criteria for waste water treatment being the highest possible percentage removal of the undesired microalgal waste product. However, for biodiesel production biomass concentration is the key performance requirement to reduce the cost of downstream processes and the cost of the raw biomass (Uduman et al., 2010). Dispersed air flotation combined with foam fractionation has been identified as

an effective and economical method to remove over 90% of microalgae from waste water (Wiley et al., 2009; Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998). Foam fractionation originated in the mineral industry as an effective method to separate elements in solution according to the differences in surface activities. It has since been proven to be a useful technology for processes such as the concentration and removal of protein (Du et al., 2002; Du et al., 2001; Du et al., 2000; Brown et al., 1997; Lockwood et al., 1997). The ability to concentrate proteins through the manipulation of controlled variables demonstrates the considerable promise this process could hold with regard to microalgal feedstock harvesting. Foam fractionation has a number of advantages when compared to current algal harvesting technologies including: significantly lower construction, maintenance and energy costs when compared to centrifugal recovery and reductions in the time and space that is required for flocculation and sedimentation. Research into the application of foam fractionation for the harvesting and concentration of microalgal biomass is required to determine its efficiency, economics and potential implementation as a commercial technique.

1.2 Project development

The work presented in this thesis brought together the School of Chemical Engineering and Advanced Materials with the School of Marine Science and Technology, and demonstrates the interdisciplinary knowledge field that is required for this research area. The project initially investigated the effect and influence of controllable variables upon the concentration factor and biomass yield. Subsequently, the appropriateness of foam fractionation for the harvesting of microalgae to be used as a biodiesel feedstock was investigated through the total extractable lipid and fatty acid profile of the harvested microalgae. Attention was paid to the energy consumption of foam fractionation compared to other commonly used harvesting technologies, as an indication of the harvesting economics.

1.3 Aims and objectives

It was hypothesised that through an increased understanding of the effects of key variables, foam fractionation could be utilised as an effective harvesting and concentrating technique for microalgal feedstocks. It is also necessary to ensure that the use of this harvesting technique has no negative implications upon the biodiesel product. The aim of the work described in this thesis is therefore to determine key variable levels to optimise feedstock harvest biomass concentration and yield. It is also imperative to understand the effect of foam fractionation on the lipid content of the harvested biomass. Accordingly, the following objectives were identified:

1. To assess current methods for the production of microalgal feedstocks, specifically focusing on current harvesting technologies.
2. To determine key variable levels and their effects on biomass concentration and yield.
3. To compare the energy consumption of foam fractionation with commonly used harvesting technologies.
4. To assess any potential effect of foam fractionation on biodiesel quality.

1.4 Thesis plan

This thesis is presented as a series of chapters formatted in the style of journal papers for publication. All papers were written by the primary author, Thea Coward, and edited by Dr Jonathan Lee and Dr Gary Caldwell. All experimental work was conducted by Thea Coward unless stated, such as work conducted by masters students under Thea Coward's supervision.

Initially, a literature review was conducted investigating the pros and cons of biofuel derived from microalgae. The literature review starts by broadly establishing the need for microalgae as a feedstock, and then discusses culturing systems and biodiesel production methods. The review then focuses on harvesting, which is commonly described as a major bottle-neck to the

economical establishment, and development of biodiesel derived from microalgae. The harvesting section of the literature review analyses the pros and cons of commonly used harvesting techniques. This allowed identification of all the potential processing stages that may have an effect on or become affected by, a harvesting technique.

Chapter 3 details the design of a flotation column that combines dispersed air flotation and foam fractionation. Fractional factorial experiments were conducted to determine the effect of variables and variable interactions on the harvest process while using the unit. The fractional factorial experiments were initially conducted using negatively charged latex beads to mimic the microalgae and ensure that only known and controllable variables determined the harvesting result. The data were then verified using *Chlorella sp.* microalgae. Utilising the information gained from the fractional factorial design a central composite designed was conducted using *Chlorella sp.*

Chapter 4 builds upon the key variables noted in *Chapter 3* by investigating the effect of bubble size within the system. Bubble size has been described as the most influential factor during the foam fractionation process, and was therefore investigated separately from the other variables. Currently there are two main schools of thought on the effect of bubble size on harvest potential, the first being that small bubbles within the liquid pool increase the interfacial area, increasing the absorption of harvest materials onto the rising air bubbles; the second being that larger air bubbles allow for increased liquid drainage within the foam allowing for a more concentrated product. This chapter investigates to what effect bubble size within the liquid pool and the foam phase has on the concentration factor of a harvest. This chapter also compares the energy consumption of commonly used harvesting technologies with foam fractionation.

Chapter 5 explores the effect of algal growth phase on foam fractionation efficiency. Harvests were performed every 3 days over a period of 3 weeks to establish whether there was an optimal time to harvest. Lipid content was also measured throughout this time period. During the investigations conducted in *Chapter 5* it was noted that microalgae harvested with the foam fractionation method constantly gained higher total lipid levels than those harvested via

centrifugal recovery, despite being cultured under the same conditions. This was dubbed the lipid paradox.

In *Chapter 6,* four theories were developed and investigated to ensure all possible explanations for the occurrence of the lipid paradox were researched. The potential effects that harvesting via foam fractionation may have on biodiesel quality and potential value–added by-products were examined. This enabled the assessment of how suitable foam fractionation is as a feedstock harvesting device.

The overall impact of the research conducted is discussed in *Chapter 7.* The results highlight the huge potential for future work and projects which are also discussed in this chapter, including the harvesting of marine microalgae species and the development of the unit for continuous harvesting from open ponds.

Chapter 2

Literature Review

Abstract

The use of fossil fuels is widely accepted as unsustainable, and this has resulted in the rapid development of the biofuel sector. Biofuels are currently produced from high-grade edible oils; unfortunately there are many ethical and environmental drawbacks related to the use of terrestrial based biofuels. Non-arable feedstocks such as microalgae offer the most promising alternative for the development of biodiesel. Currently biodiesel derived from microalgae is not economically competitive with petroleum based fuels. The greatest constraint to commercialisation is the cost of harvesting and dewatering. Microalgae characteristics greatly affect the harvesting efficiency, and often high biomass concentrations have the associated disadvantage of high energy consumption. Currently there is not a universal, economical, and efficient harvesting technology. This review provides a comprehensive analysis of the biodiesel processing stages from cultivation to transesterification. The harvesting technology chosen can have an effect on one or more of these stages, and therefore can affect the biodiesel quality. In this chapter dispersed air flotation is highlighted as a technique that shows great potential for a low cost harvesting technology, but has thus far only been used in relation to waste water treatment.

2.1 Introduction

Biodiesel is defined as the mono-alkyl esters of fatty acids derived from biological sources such as vegetable oils (e.g. oilseed**,** rapeseed and soya bean), animal fats, waste cooking oils, and algae (Asakuma et al., 2009; Demirbas, 2008; Chisti, 2007). Rudolf Diesel extensively trialled plant oils for use in his eponymous engine, and actively promoted the use of renewable biomass as an energy source (Koonin, 2006). In 1900 an early Diesel engine design was famously demonstrated by the Otto Company at the World Fair,

running purely from peanut oils (Radich, 1998). Diesel was a visionary, saying that "*The use of vegetable oils for engine fuels may seem insignificant today, but such oils may become, in the course of time, as important as the petroleum and coal tar products of the present time*" (Diesel, 1912). Over a hundred years later, due to diminishing petroleum reserves, increasing energy demands, political tension in some oil producing regions and a more environmentally conscious population, the focus has returned to the development of renewable technologies, and Diesel's statement is looking more plausible.

There are currently several sources of renewable energy available to fill the electrical market such as wind, hydroelectric, solar, geothermal, and tidal (Schenk et al., 2008). Nevertheless, the transport industry is limited to only a few readily available renewable fuel substitutes such as biodiesel and bioethanol. Transport activity is a fundamental factor to economic development and human quality of life, which predominately relies on a single fossil fuel resource, with petroleum supplying 95% of the worlds transport markets (Kahn Ribeiro et al., 2007). Opinion is currently divided over the volume and grade of extractable oil remaining worldwide. Estimates of 'proved' world oil reserves range from 930 – 1342 giga barrels (one billion barrels = one giga barrel) (Owen et al., 2010). However, it is clear that conventional oil reserves of easily obtainable oil are quickly depleting, leading to an increase in the use of unconventional oil production methods through the use of oil sands, bitumen and oil shale, which have higher cost and environmental impact when compared to conventional production (Rapier, 2012). Dwindling stocks and increasing dependence on oil from a small number of countries has lead to extreme instability in the price of crude oil, which in turn has had an effect on the global economy. Between July 2008 and July 2009 the price of crude oil has fluctuated from as little as $41.53 (Dec' 08) to as much as $132.55 (July '08) (Oil-Price.net, 2012) (Figure 2.1).

The 2007 Intergovernmental Panel on Climate Change (IPPC) report provided one of the most extensive reviews into the effects of climate change, and emphasised that global greenhouse gas (GHG) concentrations need to be stabilised at around 445 - 490 parts per million (ppm)

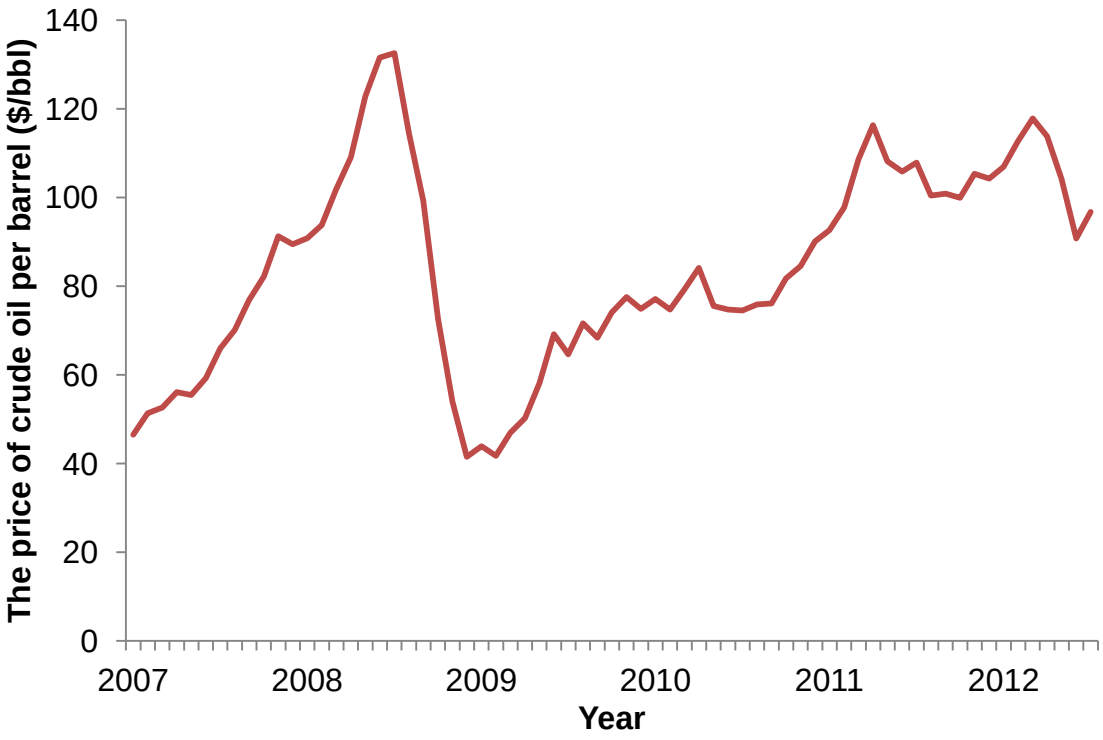

Figure 2.1: The price of crude oil per barrel from January 2007- July 2012 (Oil-Price.net, 2012).

in order to avoid permanent climate disruption (Bernstein et al., 2007). This would require GHG emissions to be slashed by 60-85% by 2050 (Bernstein et al., 2007). However, GHG concentrations are quickly approaching these levels, as the average atmospheric CO_2 concentration now exceeds 390 ppm (Knohl and Veldkamp, 2011). Despite several global campaigns to reduce global carbon emissions, such as the Kyoto Protocol, global carbon emissions continue to rise. The transport sector is providing a significant proportion of the rising GHG emissions. In 2004 road vehicles alone were responsible for 17.25% of the total released GHG emissions. The transport sector provides a difficult and expensive hurdle in the bid to reduce GHG emissions due to its rapid expansion (Kahn Ribeiro et al., 2007). Biofuels have attracted growing interest from world policy makers in recent years because they provide a renewable, biodegradable, alternative fuel that can potentially have a lower life cycle emission profile when compared to petroleum. The biomass feedstocks of biodiesel can theoretically provide a closed carbon cycle, since the burning of them simply returns the carbon to the atmosphere, which plants then extract

during photosynthesis (Puppan, 2002). A considerable appeal of biofuels is that unlike other potential fuels such as hydrogen fuel cells, it can be used immediately, in compression-ignition engines with little or no modifications necessary (Gouveia, 2011; Haas, 2005; Van Gerpen, 2005; Carraretto et al., 2004). In the European Commission's Biomass Action Plan it was highlighted that biofuels provide the "*only direct substitute for oil in transport*" currently available on a significant scale. However, governments plan to reduce dependence on petroleum fuel, with the implementation of bio-fuels targets, have been met with significantly polarised opinions from both the public and scientific communities. This is mainly due to the ethical and environmental drawbacks of the current terrestrial based feedstocks.

The following literature review provides an overview on the background of biodiesel. It pays specific attention to microalgae, a non-arable feedstock, which presents the most promising avenue for the development of biodiesel. Specifically, it aims to define how the development of low cost harvesting technologies could make biodiesel derived from microalgae economically competitive with petro-diesel.

2.2 Biofuel ethics

The bioethics behind biofuels is currently an emotionally charged area; the complexity of the issue is highlighted by both the general public and the scientific community having wide ranging opinions. This section of the review will mainly focus on the ethics behind the production of biofuels from edible terrestrial feedstocks (sugar cane, cereal grains, soybean, rapeseed, and other edible oil seeds) and compare them with non edible feedstocks (lignocellulosic crops, algae, and cyanobacteria). Many bioethical issues related to biofuels, such as deforestation due to expansion of agricultural land or biofuels competing with food production, are directly linked to land availability. There are many conflicting and competing demands for land including food production, conservation, recreation, development, and settlements. In the UK, the pressures are increased due the small area of available land and high population density. For example, 60 million people currently live in the UK, the

total land area is 24.25 Mha, 6 Mha of which is arable farm land and 2.4 Mha is forest (Defra, 2005). The EUs major contributing feedstock to meet the 10% biofuels target by 2020 is rapeseed, *Brassica napus*, (Canakci and Sanli, 2008). Woods et al. (2003) calculated that for the UK to independently produce enough biodiesel to meet a 10% rapeseed methyl ester blend, using the current available technologies, 15 to 36% of UK arable land would be required, which potentially equates to 2.23 Mha. The UK currently has 0.5 Mha of former set-aside land which could be used for rapeseed production. However, this still means that 1.73 Mha (29 %) of UK's arable land needs to be diverted to rapeseed feedstock production. Rapeseed crops also need to be rotated every 2-3 years due to pest and disease implications, thus resulting in a greater percentage of land being affected for biofuel production. Although technological developments will improve crop yield, reducing the amount of land needed, these estimates show that the UK will not become completely self-sufficient for transport biofuels (Pickett, 2008) using only terrestrial based feedstocks. Although this demonstrates the land limitations related to biofuels, the UK is not the best example of land availability issues for terrestrial based feedstocks because of its small size and high population density.

Many studies have been conducted to estimate the potential biomass, for bio-energy, that could be produced on a global scale (Smeets et al., 2007; Obersteiner et al., 2006; Perlack et al., 2005; Wolf et al., 2003). Berndes et al., (2003) reviewed 17 of these studies and concluded that the studies came to "*widely different conclusions about the possible contribution of biomass in the future global energy supply (e.g. from below 100 EJ* yr^{-1} *to above 400 EJ* yr^{-1} *in 2050). The major reason for the differences is that the two most crucial parameters –land availability and yield levels in energy crop production– are very uncertain, and subject to widely different opinions*". Crop yields for example depend on very specific local conditions such as soil types, irrigation, available nutrients, water availability, light etc, these details are almost impossible to apply on a global scale. Some of the assumptions were highly optimistic and implausible. For example, Wolf et al. (2003) came to the conclusion that if high external input systems of agriculture were applied on a global scale by 2050 only 55% of agricultural land would be needed for food production, allowing the remaining 45% to be dedicated to biomass for bio-energy. Compelling evidence

about the lack of available land comes from Gressel (2008), who was able to calculate the arable land needed in the USA to replace 15% of the transport fuel needs. The work was based on the known oil yields from various feedstocks (Table 2.1). If 15% of the USA transport fuel were to be replaced with soybean methyl ester, the most commonly used feedstock in the USA, 67% of the available arable land would have to be dedicated to soybean plantations alone.

Table 2.1: Cropping area needed to replace 15% of transport fuels in the USA from various feedstock sources (Gressel, 2008)

Crop	Oil yield (L ha^{-1})	Land area needed (millions hectares)	Existing US cropping area (%)
Maize	172	462	178
Soybean	446	178	67
Oilseed rape	1,190	67	42
Jatropha	1,892	42	13
Oil palm	5,950	13	7.2
Algae/ cyanobacteria containing 30% oil	59,000	1.3	1.3
Algae/ cyanobacteria containing 70% oil	137,000	0.6	0.6

While the oil yield and productivity are quite optimistic, the work highlights the potential of non arable feedstocks, such as algae and cyanobacteria, as land-saving high lipid feedstocks. Microalgae as a source will be discussed in more detail further into this review.

The evidence shows that in order to meet small targets (such as the EU 2020 10% biofuel blend) with terrestrial biofuel feedstocks, huge areas of existing farm land would have to be dominated by biofuel feedstock crops. This has led to two major implications: the first being the expansion of agricultural land leading to deforestation and habitat destruction, and the second being biofuel crops competing for land and water resources reducing world food-stocks and therefore increasing food prices.

A major aim behind biofuel production is to help mitigate climate change and to reduce the levels of CO_2 being emitted into the atmosphere. However,

the carbon savings created by biofuels are highly dependent on the way they are produced. The expansion of agricultural land for biofuel production or crop production due to existing agricultural land being diverted to biofuel production can have devastating effects on undisturbed ecosystems. Biomass and soils represent huge terrestrial carbon stores, containing ~2.7 times more carbon than the atmosphere (Schlesinger, 1997). In areas such as Brazil, South East Asia and the USA the conversion of rainforests, grasslands, peat-lands, and savannas causes the significant release of CO_2. The CO_2 released over the first 50 years from the converted land is termed the 'carbon debt' (Fargione et al., 2008). To repay this 'carbon debt' the production and combustion of the biofuel must have a net emission that is less than the fossil fuel they displace. Fargione et al. (2008) calculated the time required to repay the 'carbon debt' that is created through the conversion of various habitats. Conversion of large carbon sinks such as peat-land rainforests for the production of palm derived biodiesel in Malaysia would take 423 years to repay, rendering the mitigation factor to zero. The EU plan to meet the 2020 target through domestically grown rapeseed, hoping this will take direct pressure from oil feedstocks produced from environmentally sensitive areas. However, the diversion of massive amounts of edible oil into biofuels leaves a massive gap in the food market. This gap is filled mainly with cheap palm oil imports (Bailey, 2008). The indirect increase in demand for palm oil is linked to a 1.5% annual rate of tropical rainforest deforestation in Indonesia and Malaysia (Hooijer et al., 2006), releasing vast amounts of CO_2 and having massive negative effects on biodiversity (Fitzherbert et al., 2008). In the Gallagher review, as commissioned by the UK government, it was stressed that policies must be enforced to ensure "that agricultural expansion to produce biofuel feedstock is directed towards suitable idle or marginal land..." and that "current policies will lead to net GHG emissions and loss of biodiversity through habitat destruction." (Gallagher et al., 2008).

According to the 2005 Footprint of Nations report each person requires 21.9 hectares of the Earth's surface to supply their needs; whereas the Earth's biological capacity is only 15.7 hectares per person. The demand is ever growing with the expanding population.

Several studies conducted by the International Monetary Fund (IMF), the United Nations (UN), World Bank and Food and Agriculture Organization (FAO)

have all concluded that the increase in demand for biofuels have contributed significantly to the spiralling increase in food prices resulting in the 2007-2008 world food crisis (Bailey, 2008). However, the results vary drastically (Table 2.2). As these reports demonstrate it would be naïve to attribute the full blame of the food price rise to biofuels. It appears that the "perfect storm" of factors culminated to affect the world food prices. These factors include the weak dollar, rising energy prices, unseasonable drought and increasing wealth in Asia creating a demand for a more varied 'Westernised' diet (Islam, 2008; Mitchell, 2008). Terrestrial sourced biofuels add another layer of uncertainty and risk to an already hazardous environment highlighting the need for the expansion of non edible feedstocks.

Table 2.2: A comparison of the studies conducted on the contribution of biofuels to the rising food prices (Bailey, 2008)

Investigating body	Contribution of biofuels to rising food prices (%)
IMF	30
FAO	10
World Bank	65

A final factor that causes concern is the release of nitrous oxide (N_2O) from synthetic nitrogen fertilisers. This has previously been neglected or greatly underestimated in life cycle analysis surveys. However, N_2O is 296 times more effective as a 'greenhouse gas' than CO_2 (Ehhalt et al., 2001). In their revolutionary paper Crutzen et al. (2008) discovered that 3-5 times more N_2O was released from agro-biofuel production than previously assumed in life cycle analysis work.

After analysing the main ethical and environmental drawbacks of terrestrial based biodiesel it is clear that the majority are closely linked to the agricultural phase of the life-cycle. Non-arable feedstocks such as algae may present the most promising avenue for the development of biodiesel production.

2.3 Significance of microalgae as a feedstock

Algae are a hugely diverse group of primitive plant like organisms. Most algae undertake photosynthesis, converting sunlight, CO_2, and water into biomass. Algae have adapted to a wide range of ecological niches, and are found in a wide range of habitats (marine, freshwater, tropical, polar ice, hot springs etc). The exact number species remains unclear, as there is no official inventory of names in current use; however figures as high as 300,000 species have been quoted. They can range in size from a single cell to giant kelp over that can grow to150 feet long. Paleobotanical evidence shows that microalgae are the main constituents of many of the fossil fuel hydrocarbon sources in use today. For example, oil shale in Puertollano, Spain dating back to the Cambrian era has been found to be dominated by the microalga *Botryococcus* (Borrego et al., 1996). In this review microalgae are defined as unicellular microorganisms that can be photosynthetic or heterotrophic. Algae were first considered as a potential fuel in the 1950's for the production of methane gas (Meier, 1955). This concept was fully adopted by the U.S. Department of Energy's (DOE) Office of Fuel Development in 1978. The DOE focused their research on the application of microalgae to develop a renewable transport fuel. However, the work ceased in 1996 due to budget restrictions and the decision for the DOE resources to be focused on the development of bioethanol (Sheehan, 1998). Since then microalgae have again started to be recognised as one of the most promising sources for biodiesel production. For example, Sapphire Energy has secured over $300 million of investment from various sources. They currently have 100 acres of open raceway ponds, with the plan to extend to 300 acres (figure 2.2). Sapphire Energy have stated that their aim to is produce over 100 barrels of oil per day by the end of 2014 (Lamonica, 2012).

Figure 2.2: Sapphire Energy is currently working with 100 acres of traditional raceway ponds, with the plan to expand to 300 acres. The algal farm is located in Columbus, New Mexico. (Picture courtesy of Sapphire Energy).

The advantages of microalgae as a biodiesel feedstock include (Khan et al., 2009; Li et al., 2008a; Schenk et al., 2008; Chisti, 2007; Huntley and Redalje, 2007):

• A high growth rate and a short life cycle; therefore multiple harvests are able to occur throughout the year;
• An oil yield that greatly exceeds the leading terrestrial oil crops;
• An oil content that can be adjusted through changing the growth medium concentration, for example nitrogen limitation;
• High growth rates and oil yields reduce the land requirements to satisfy demand. Microalgae can also be gown on marginal land that is unsuitable for agriculture and therefore does not compete with food production;
• Salt and waste water can be used for cultivation. The waste water can be used for nutrients and can be used as a bioremediation tool, reducing the need for freshwater;
• The release of nitrous oxide is greatly reduced when compared to terrestrial oil crop production;

• Algal ability to tolerate high CO_2 concentrations means that biomass production could be combined with flue gas as a mitigation technique;
• Algae produce high value co-products (e.g. proteins, pigments, fertilisers, animal feed, eicosapentaenoic, and docosaesaenoic acids) that could help offset the cost of biofuel production;
• Cells can utilise nitrogen and phosphorus from wastewaters, removing the need for fertilisers and providing bioremediation for agriculture.

Figure 2.3 gives a conceptual model of microalgae cultivation for the production of biofuels and the potential by-products that can be harnessed. In the following section the advantages of microalgae are discussed in more detail. The factors that have thus far stopped the commercialisation of microalgae feedstocks will also be considered. This field is still in its infancy and there is great potential for costs to be reduced and efficiencies increased.

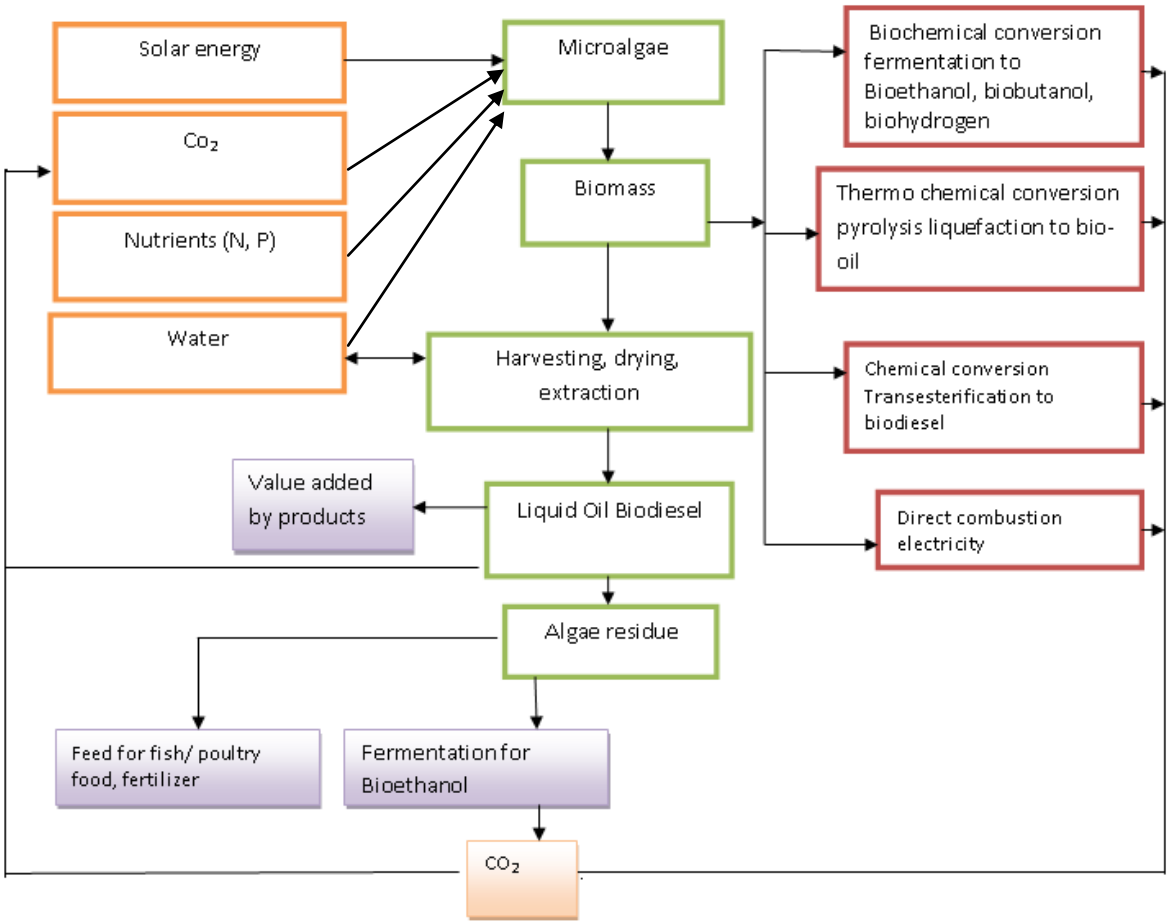

Figure 2.3: Biofuel and by-product production by microalgae biomass (Chakradhar et al., 2008)

2.3.1 Cell efficiency

Autotrophic microalgae are extremely efficient solar energy converters in comparison with terrestrial land crops. The global average for solar energy conversion efficiency (ECE) for terrestrial plants is estimated to be between 1-2% (Vasudevan and Briggs, 2008). In comparison the ECE for microalgae has been observed at between 3-9% in the field (Dismukes et al., 2008; Kebede and Ahlgren, 1996; Zittelli et al., 1996). The reason for these shortcomings of terrestrial crops seems to be linked with factors other than light such as water and nutrient availability. The aquatic environment and the simple structure of the microalgae provides better access to the required elements, increasing the cells photosynthetic efficiency (Vasudevan and Briggs, 2008). Microalgae's high ECE and earth's large water mass has led to microalgae being responsible for the majority of Earth's photosynthetic biomass production. For example diatoms, the dominant phytoplankton in the oceans, are estimated to contribute ~25% to global primary productivity (Scala and Bowler, 2001). Microalgae have a very simplistic reproductive process in comparison to terrestrial plants. In unicellular algae all the reproductive functions are carried out in a single cell, this combined with higher photosynthetic efficiencies and a short life cycle make microalgae the 'fastest growing photosynthetic organisms' (Chakradhar et al., 2008). Microalgae can easily double their biomass in ~24 hrs, but during the exponential growth phase a doubling time of 3.5 hrs is common place (Song et al., 2008; Chisti, 2007). *Arthrospira (Spirulina) maxima* found naturally in volcanic soda lakes between pH 9.5-11 can be grown in open ponds with little worry of contamination from other species. Cultivation of *Arthrospira (Spirulina) maxima* in Mexico has achieved yields of 27 dry metric tonnes ha^{-1} yr^{-1}, which is a 10 fold increase in production when compared to the average yield of rapeseed (Peterson and Hustrulid, 1998). Yield is closely linked to temperature and, microalgae can withstand a temperature 15°C lower than the optimum, but temperatures 2-4°C over the optimum can cause cell death (Gouveia, 2011). Currently, desert conditions in areas such as Israel, are being used to achieve high yields as they provide ample sunlight all year round. Unfortunately, temperature control units are required in the outdoor ponds to offset the cold night temperatures (Gressel, 2008). The additional cost of these units is offset by the extremely high production rates. Annual yields of 60-70 dry metric tons

ha^{-1} yr^{-1} has been achieved and the product can be harvested several times throughout the year. Agriculture in comparison is a slow response sector in the short term and the supply of oil seeds cannot be quickly adjusted to unexpected change in demand because there are only 1-2 harvests per year (Defra, 2008). Algae are able to thrive in a wide variety of environments. With the depth of knowledge on thousands of species of microalgae rapidly growing, the selection of species could be done for specific growth locations. It would be wrong to assume that algal production rates would be the same throughout the year but careful species selection for each site would mean high production and harvesting would be possible throughout the year. Shorter harvesting times will allow for quick and efficient production compared to terrestrial oil crops, therefore, providing a continuous, sustainable and reliable source of biomass feedstock throughout the year.

2.3.2 Oil yield

The high oil content of microalgae is one of the main features that make it particularly attractive as a biofuel feedstock. The DOE research highlighted that 'microalgae are capable of producing 30 times the amount of oil per unit area of land, compared to terrestrial oilseed crops.'(Sheehan, 1998). Quotes as high as 300 times more oil production per unit area have been reported (Schenk et al., 2008). Although highly optimistic these results show there is a high likelihood that production of over 30 times could be achievable. The total content of lipids in microalgae is not always high and varies on a species to species basis; the lipid content of microalgae can range from 1-85% of the dry weight (Rodolfi et al., 2009). The DOE conducted extensive screening of microalgae strains and highlighted 300 species that have promising high oil yields (Sheehan, 1998).

Table 2.3 lists a selection of the most suitable species for biofuel feedstock production and their lipid contents. It can be noted that even within the same species there can be considerable differences within the lipid concentrations. However, the total oil (% dry weight) content can be a misleading figure. The total lipid fraction is often gained though a solvent

extraction which can contain non- transesterifable lipids and non fuel components (e.g. chlorophyll, pigments, proteins, and carbohydrates that make up part of the glycolipids) (Laurens et al., 2012). Therefore, total lipid fractions may not accurately reflect fuel potential. The composition of the total lipid fraction will affect the fatty acid methyl-ester (FAME) yield; Laurens et al. (2012) noted that triglycerides (TAGs) could be totally converted into FAMEs, whereas only 63% of glycolipids could be converted. Laurens et al. also demonstrated that total lipid yields for *Chlorella vulgaris* and *Nannochloropsis sp.,* were significantly higher than the actual fuel yield; with only 30.9% and 51.4% of the extract weight being converted to FAME.

Lipid content has been shown to be affected by factors such as temperature, light and nutrients (Rodolfi et al., 2009; Guschina and Harwood, 2006; Harrison et al., 1990), and fatty acid composition can also vary in response to these culture conditions (Hu et al., 2008). Light is a major physical stimulus, which can modify the fatty acid composition. The formation of polar lipids can be induced by low light, high light intensity can decrease total polar lipids, therefore increasing the amount of neutral lipids, mostly TAGs (Khotimchenko and Yakovleva, 2005; Brown et al., 1996). Fatty acid composition also varies with growth stage, with higher TAGs occurring during the stationary phase (Liang et al., 2006). Cells could therefore be grown or harvested in response to peak TAG production. Extensive research has also been conducted on the many strains of microalgae to assess the effect of nitrogen deficiency on the total lipid content (Illman et al., 2000; McGinnis et al., 1997; Sanchez et al., 1993; Richardson et al., 1969). The consensus is that in nitrogen sufficient conditions growth rates are high but the cells contain low lipid content. In nitrogen deficient conditions growth rates are reduced, yet oil production remains high ‘leading to an accumulation of oil in the cells’ (Sheehan, 1998). The accumulation of lipid can be extreme in some species, for example, in *Chlorella pyenoidosa* the typical lipid content of exponential cultures was ~5%, whereas nitrogen and silicate stress cultures contained up to 85% lipid (Rodolfi et al., 2009). Unfortunately, it appears that the desired combination of high yields and high lipid content is unachievable using current cultivation techniques. Research conducted by Huntley and Redalje (2007) seem to provide an exciting solution. Applying a two-phase system to the production of *Haematococcus pluvialis* gained on average an annual production of >420 GJ

ha^{-1} yr^{-1}. This equates to 6 times more energy per hectare than rapeseed, annual production is likely to be improved as a maximum yield of 1014 GJ ha^{-1} yr^{-1} has been achieved. The work was conducted on a major scale (i.e. 2 ha) over several years. The first stage of production was nitrogen sufficient and, the algae were grown to high densities in a photo-bioreactor. In the second stage, the algae were transferred to nutrient starved open ponds for a few days. This was enough time to increase lipid content but not enough for contamination to occur. The limitations to this technique are closely linked to the capital costs of photo-bioreactors and the fact that there are currently no low cost microalgae harvesting technique- these limitations will be discussed further.

Table 2.3: The oil content of some of the most suitable species for biofuel feedstock production (Meng et al., 2009; Chisti, 2007; Liu and Zhao, 2007; Illman et al., 2000)

Microalga	**Oil content (% dry weight)**
Botryococcus braunii	25-75
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp	31-68
Neochliris oleoabundans	35-54
Nitzchia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23
Chlorella vulgaris	18-56.6
Chlorella emersonii	29-63
Chlorella sorokiniana	20-22
Chlorella minutissima	31-57

From table 2.1 it can be seen that even at 15% oil content (toward the lower end of lipid concentrations listed in table 2.3), with a 40% conversion of total lipid to fatty acid methyl esters (FAMEs), using microalgae would still dramatically reduce the land requirements for biofuel production compared to the leading terrestrial crops. Microalgae can be grown on land unsuitable for agriculture. If a marine species is being cultivated the production site can be by the sea in lagoons or salt flats utilising free ocean water (Ginzburg, 1993). Freshwater cultivation can be undertaken on marginal land and be linked to waste water bioremediation. It is important to define the term marginal land as it is often used as a sweeping generalised term in many reports. For this review marginal land is land that is unsuitable for agriculture and has poor biodiversity. It is imperative that when marginal land is used in developing countries it is done in a responsible manner. In India marginal land contributes ¼ to poor household's incomes (Bailey, 2008). Large projects should therefore be developed with the community backing if they are to be successful, for example, community projects should be created.

2.3.3 Water usage

The water usage of microalgae has been shown to be less or comparable to that required to grow terrestrial crops (Chakradhar et al., 2008; Dismukes et al., 2008; Sheehan, 1998). The amount of water required for microalgae biomass production depends on the cultivation technique i.e. open raceway ponds will lose more water than closed photobioreactors, environmental conditions i.e. wind speed, temperature, humidity, harvesting method and, water recycling capabilities. If marine species are being cultivated the water requirements are of little consequence, as long as the production area is sited on a coastal region or near a saline ground water supply. Approximately 72% of the Earth's surface is covered by ocean water, providing a massive water reserve. However, ocean water must be pre-treated to reduce the chance of contamination. Freshwater species can be cultivated without placing pressure upon precious freshwater resources, as microalgae are able to utilise nutrient laden wastewaters. Microalgae have often been used for enhanced removal of organic

contaminates, heavy metals, and most significantly nutrients. Coupling wastewater treatment with biomass production provides economical incentives, by reducing the amount of chemical nutrients required and environmental benefits, such as reducing the fertiliser requirements, so reducing the N_2O released (see *2.2 Biofuel ethics*). Utilising sea/wastewater decreases water requirement by 90% and eliminates the need of all the nutrients except phosphate (Yang et al., 2011). The DOE highlighted that utilising wastewater improves the economics of microalgae feedstock production. They also noted that a 'dual' purpose system would provide a plausible near term application for getting microalgae biofuels into the market. However, Schenk (2008) raised some concerns that wastewater containing metal contaminants would reduce production. Heavy metals have been shown to inhibit photosynthesis and can cause morphological changes to the microalgae cell (Muñoz and Guieysse, 2006), although, *Chlorella, Ankistrodesmus* and *Scenedesmus* have all been used to biodegrade hazardous pollutants contained in oil and paper mill wastewater (Pinto et al., 2003; Tarlan et al., 2002). Selecting species that have a higher tolerance to hazardous pollutants would increase the chances of obtaining a monoculture in open pond cultures. Unfortunately by-products from feedstock biomass produced in wastewater, such as high value chemicals and animal feed is unlikely to reach the high standards required, however, the by-products can still be used for biogas and bioethanol production (Muñoz and Guieysse, 2006).

2.3.4 Carbon capture

Microalgae have mastered the art of carbon capture and conversion. Higher plant and animal growth is significantly reduced when CO_2 levels are above 5%, whereas many microalgae can thrive with CO_2 concentrations of up 40% (Gressel, 2008; Wang et al., 2008). Although wastewater provides adequate nitrogen (N) and phosphorus (P) for microalgae growth carbon, (C) is often the limiting factor. For C, N, and P the optimum ratio for growth is C: N: P = 50:8:1, wastewater general provides C: N: P = 20:8:1 (Lundquist, 2008). The atmosphere currently contains 0.03-0.06% CO_2 (Wang et al., 2008), therefore combining wastewater that has a high nutrient content with industrial flue gas

would provide the right ratio for optimum growth. Flue gas contains ~14% CO_2 (Wang et al., 2008). Microalgae are so efficient at CO_2 conversion that 1g of algae biomass fixes ~1.83 g of CO_2 (Chisti, 2008), therefore, if the theoretical average for microalgae biomass production in an open pond system is 50 g m^{-2} day^{-1}, 1 hectare could fix up to 1 ton of CO_2 day^{-1} (Schenk et al., 2008).

Flue gases from power stations produce more than 7% of the total world CO_2 emissions (Laurens et al., 2012). Microalgae can therefore provide a promising mitigation tool to help stabilise atmospheric CO_2. Biomass that is left over from oil production can be turned into agri-char via pyrolysis (Downie, 2007). Agri-char is extremely useful in returning carbon to the soil, increasing crop productivity.

2.4 Mass cultivation of microalgae

Comprehensive work has been conducted into the mass culture of microalgae since the 1940's. However, large scale commercial culture has only really occurred within the past 40 years (Borowitzka, 1999). During the short history of microalgae mass culture two major approaches have evolved: (i) open-ponds and (ii) closed bioreactors, but neither are without their disadvantages. The perfect production system would be efficient, easy to operate, have economical set up and running costs (Becker, 1994). Unfortunately, with the current production systems it is a choice between capital cost or rate of production and quality of product. This has sparked much debate as to which cultivation technique will advance microalgae biofuels into the commercial market.

2.4.1 Open systems

The majority of microalgae biomass is produced in open systems. This is purely due to economics, as closed systems have a high initial set up cost and are difficult to scale up (Borowitzka, 1999). There are four types of open ponds currently being used: shallow big ponds, tanks, circular ponds and raceway ponds, the most commonly employed being the raceway type. Raceway ponds

have changed very little since their development in 1950 by Kohlenbiologische Forschungsstation (Huntley and Redalje, 2007). These systems are an oblong shape and are divided by a central wall (single type), or a winding set of horizontal channels linked by a recirculation channel (Figure 2.4). The optimal width for each channel is 2-3 m and the optimal depth is 10-30 cm. The pond depth is a compromise between allowing enough sunlight to each cell and preventing osmotic stress due to evaporation and allowing an adequate depth for mixing. A paddle wheel is employed to continuously mix the culture; baffles guide the flow and to increase turbulence therefore further improving mixing. Dimensions commonly range from 1000-5000 m^2 (Muñoz and Guieysse, 2006), although the largest raceway pond occupies an area of 440,000 m^2 (Spolaore et al., 2006). Nutrients and water are continuously supplied; while mature algae are removed (the pros and cons of current harvesting methods are discussed in section *2.7 Harvesting*). A variety of materials can be used for the construction such as Polyvinyl chloride (PVC), clay, or asphalt (Muñoz and Guieysse, 2006); these materials are all fairly easy to clean should there be a build-up of biofilm (Schenk et al., 2008). The simple design of raceway ponds means that construction is easy and requires little technical skill therefore keeping capital investment low in comparison to closed systems.

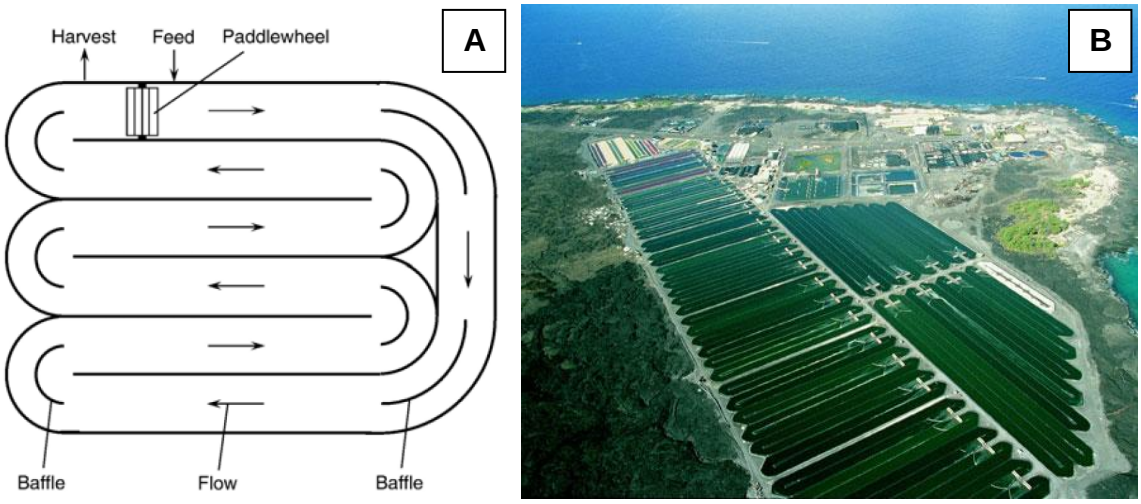

Figure 2.4: A) A schematic aerial view of a group of raceways joined by a recirculation channel (Chisti, 2007); **B)** Single raceway ponds of the Cyanotech Corporation Kona, Hawaii (picture courtesy of Canotech Corporation)

One of the main disadvantages to open culture is the risk of contamination. New ponds are inoculated with the desired species but over time new faster growing strains will take hold reducing the yield. This risk can be reduced by growing algae in batch or semi batch systems (Borowitzka, 1999) or by culturing 'extremophiles' (Schenk et al., 2008). As the name suggests extremophile strains are able to grow in extreme environmental conditions such as high or low pH, high salinity or high nutrients (Lee, 2001), thus limiting the environment for possible contaminator species. These extreme strains include *Dunaliella* (high salinity), *Spirulina* (high alkalinity) and *Chlorella* (high nutrition) (Harun et al., 2010b; Lee, 2001).

Compelling evidence for the use of open systems for the cultivation of algae to be used for fuel production comes from Sapphire Energy, which has opted to produce microalgae in open raceway ponds. Sapphire Energy produces microalgae that are resistant to fungal infection, and is grown in high pH levels that many predators cannot survive in. Tim Zenk, the company's vice president of corporate affairs states 'In the low-margin fuel industry, cost is everything and the advantages of bioreactors don't justify their added expense' (Lamonica, 2012).

2.4.2 Closed systems

The need to achieve greater control over the culture medium led to the development of enclosed photo-bioreactor systems. Photo-bioreactors (PBR) are currently used in industry for high value products, such as enzymes, isotopically labelled chemicals and pharmaceuticals (Pulz and Gross, 2004; Becker, 1994). In order to produce these products either a sterile or highly controlled environment is required (Becker, 1994). A variety of PBR have been constructed to regulate specific or multiple environmental conditions (Sierra et al., 2008). The designs cover a huge range (Figure 2.5) including horizontal tubular, helical tubular, vertical flat panels and bubble columns. The basic concept consists of a transparent solar collector through which the medium is pumped. Degassing equipment is required to remove the oxygen produced

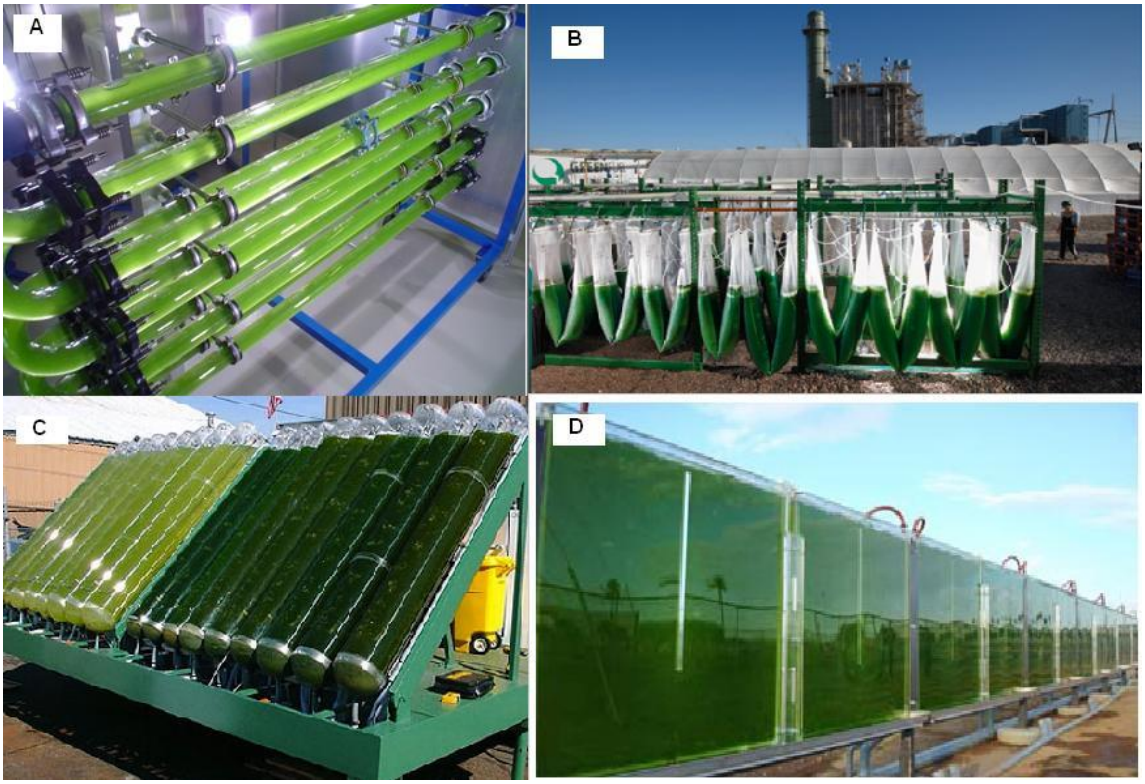

Figure 2.5: A selection of photo-bioreactor systems showing the diversity of design; **A)** Horizontal tubular, (Photo courtesy of Dan Brookshear) **B)** 'Big bag' culture Redhawk power plant, Phoenix (Photo courtesy of Robert Clark) **C)** Vertical tubular (Photo courtesy of Steve Jurvetson) **D)** Vertical flat plate at Arizona State University.

during photosynthesis. The most "scalable" systems are designed around a tubular network (Molina Grima et al., 1999). However, the term scalable is used loosely. Molina Grima et al. (1999) calculated that at present PBR systems should have a pipe diameter no larger than 0.1 m, and a continuous tube length of no more than 80 m. The maximum scalable limits are due to the accumulation of oxygen and the depletion of CO_2 creating pH inconsistencies, also any further increase in tube diameter would increase the number of dark zones and therefore reduce yield (Molina Grima et al., 1999). PBR have the advantage of saving water, chemicals, reducing contamination risk and achieving up to a fivefold biomass increase in comparison to open systems (Schenk et al., 2008). Despite these advantages Lee (2001) concluded that closed photo-bioreactors are no better at "illuminated area, volumetric

productivity and cost of production" when compared to open systems. Lee's conclusion was drawn from the analysis of 25 years worth of data from flat plate and tubular photo-bioreactors. In order to achieve a 27% increase in oil yield using a PBR a 10 fold capital cost increase would have to occur (Table 2.4). This would not make economical sense for a low value product such as microalgae feedstock. As discussed previously (*2.3.2 Oil yields*), a two phase system may provide the solution to providing a high yield and high lipid concentration.

Table 2.4: Comparison of photo-bioreactor and raceway production systems (Chisti, 2007)

Variable	Photo-bioreactor	Raceway ponds
Annual biomass production (kg)	100,000	100,000
Volumetric productivity (kg m^{-3} d^{-1})	1.535	0.117
Biomass concentration in media (kg m^{-3})	4.00	0.14
Dilution rate (d^{-1})	0.384	0.250
Area needed (m^2)	5681	7828
Oil yield (L ha^{-1})	136,900[1] 58,700[2]	99,400[1] 42,600[2]
Annual CO_2 consumption (kg)	182,333	182,333
System geometry	132 parallel tubes/ unit; 80 m tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30m deep
Number of units	6	8
Capital costs per m^{-2} (US$)	100[3]	9.40[4]

[1] Based on 70% by wt oil in biomass
[2] Based on 30% by wt oil in biomass
[3] Based on estimations from Hallenbeck and Benemann (2002)
[4] Based on estimations form Huntley and Redalje (2007)

2.5 Microalgae biofuel production

Biofuels are quickly making the transition from research topic to the petrol station forecourt. Currently the biofuels market is dominated by two main liquid fuels (Laurens et al., 2012; Chisti, 2008; Defra, 2008; Gressel, 2008):-

- Bioethanol – an alcohol derived from sugar crops and digested starchy grains (e.g. wheat, corn, sugar beets, barley, switchgrass)
- Biodiesel- consists of fatty acid methyl esters made by the transesterification of naturally produced oils (feedstocks include: palm oil, soybean oil, rapeseed, algae, jatropha) with an alcohol to remove the glycerine.

The EU is actively championing the use of biodiesel (BD), with 80% of the 768 million gallons of biofuel produced in the EU during 2004 being biodiesel (Schnepf et al., 2006). This is in part due to the fact that biodiesel has energy efficiency extremely close to that of petroleum based diesel (PD), when comparing the conversion of primary energy to fuel product energy (80.55% BD vs. 83.28% PD) (Sheehan et al., 2000). The high energy efficiencies means that biodiesel can be successfully used in a wider range of transport vehicles including cars, buses (Carraretto et al., 2004), trains (Chisti, 2008) and even aircraft (Laurens et al., 2012; Cunningham, 2007). In comparison, bioethanol only has ~ 64% of the energy content of biodiesel (Chisti, 2008), this requires more frequent refuelling, reducing the number of applications.

Pure vegetable oils can be directly used in diesel engines, for example, during World War II *Jatropha* oil was used in blends and as a direct substitute for petroleum diesel (Demirbas, 2009; Canakci and Sanli, 2008; Shah et al., 2004). Although initial results were positive, using pure oils quickly caused several operational problems, including poor fuel atomisation (Knothe and Steidley, 2007; Barnwal and Sharma, 2005), incomplete burning, which results in carbon deposits being formed (Demirbas, 2003), injector chocking (Raneses et al., 1999), and piston ring sticking (Agarwal, 2007). The majority of complications associated with the use of pure algal oils are due to their high viscosity and low volatility. These problems are particularly bad in modern direct

injection engines, which are more vulnerable to poor fuel quality. Viscosity has been described by Canakci and Sanli (2008) as one of the most crucial fuel features. The viscosity of microalgae oils is 33.06 CentiStokes (cSt) mm^2/s compared to 3.03 cSt mm^2/s for petroleum diesel at 40°C (Chen et al., 2011b), therefore microalgae oils are over 10 times more viscous. The high viscosity of vegetable oils is a result of their molecular structure and weight, the molecular weight is ~ 20 times greater than that of diesel (Barnwal and Sharma, 2005). To become legally road worthy biofuel needs to meet stringent kinematic viscosity specifications (at 40°C). The American standard ASTM D6751 requires 1.9 - 6.0 cSt mm^2/s; the European standard EN 14214 requires 3.5 - 5.0 cSt mm^2/s. To meet these standards and to reduce the number of operational problems the algal oil need to be chemically modified into biodiesel, there are three main processes, thermal cracking (pyrolysis), microemulsion, and transesterification, (Ma and Hanna, 1999).

2.5.1 Pyrolysis

Pyrolysis is the process of heating organic materials, such as vegetable oils, in the absence of oxygen or nitrogen (Fukuda et al., 2001). Pyrolysis can also be applied to a wide variety of organic materials including waste food, forestry waste, and microalgae. The heating produces compounds such as alkenes, alkanes, aromatics, alkadienes, and carboxylic acids (Demirbas, 2009; Fukuda et al., 2001; Ma and Hanna, 1999). The end product of the biomass can be split into three states, a liquid phase (pyrolysis oil or bio-oil), a carbon phase (charcoal), and a gas phase (Aresta et al., 2005). The liquid and gas phases from microalgae are of particular interest (Jena and Das, 2011; Brennan and Owende, 2010; Amin, 2009). The product can be controlled and affected by the temperature, residence time, and heating rate (Onay and Kockar, 2003). Demirbąs (2006) found that during slow pyrolysis temperature had a significant effect on the bio-oil faction gained from *Chlorella protothecoides*, with yields ranging from as little as 5.7% at 251°C to as much as 53.3% at 501°C. Jena and Das (2011) noted a similar pattern using *Spirulina platensis* stating that higher temperatures resulted in higher bio-oil and gaseous yields. However, currently bio-oil produced from pyrolysis is not of sufficient standard to be

integrated into the transport market, this is due to unacceptable levels of carbon, ash, and a poor pour point (PP) (Sharma et al., 2008; Fukuda et al., 2001). Despite these poor attributes pyrolysis offers some major advantages, such as making it possible to produce biofuels using existing refineries; this has the potential of producing a lower cost fuel, integrating existing infrastructure and, could potentially attract major oil companies.

2.5.2 Micro-emulsification

Microemulsion forms micrometer-sized droplets in the range of 1-50 µm (Wellert et al., 2008; Agarwal, 2007) improving the viscosity and increasing the cetane number (CN) (Ma and Hanna, 1999). The CN is the measure of the time taken between the start of the fuel injection and the start of ignition. Microemulsions have the advantage of avoiding the production of unpurified glycerol, unlike the transesterification reaction which produces glycerol as a co-product. Glycerol is expensive to purify and the current high production yields has dramatically reduced the price of glycerol as a value-added product, it may also have disposal problems, which may lead to environmental concerns (Attaphong et al., 2011). Microemulsions are formed by the mixing of two immiscible liquids such as vegetable oils (triglycerides esters) and short to medium chain alcohols such as methanol, ethanol, and propanol with one or more ionic amphiphiles (Fukuda et al., 2001; Ma and Hanna, 1999). The ionic surface active substance increases the kinetic stability of the microemulsion by controlling the curvature of the interfacial surfactant film when the short chain alcohol is added (Wellert et al., 2008). Despite improving the viscosity and the CN, prolonged usage has been found to cause the injector needle to stick, incomplete combustion and the formation of carbon deposits (Fukuda et al., 2001; Ma and Hanna, 1999). The PP is the lowest temperature at which the fuel will flow; it helps to give an indication as to how easily a fuel can be pumped. Recent studies have been applying microemulsion to methyl esters (biodiesel) to improve the pouring point at lower temperatures (Fernando and Hanna, 2004).

2.5.3 Transesterification

Transesterification is the most commonly applied technique to reduce viscosity and to create biodiesel on an industrial scale (Ma and Hanna, 1999). It converts lipids into fatty acid alkyl esters via three consecutive steps: triglyceride (the lipid) to diglyceride, monoglycerides, and finally glycerol (the by-product) where an alkyl ester (biodiesel) is formed at each stage. The lipids are reacted with an alcohol and usually the reaction is catalyzed by alkalis, acids, or enzymes. Methanol is the most commonly used alcohol due to its low cost and availability (Ma and Hanna, 1999). Methanol is also a simple polar liquid which gives it physical and chemical advantages when compared to ethanol (Fukuda et al., 2001; Ma and Hanna, 1999). The use of methanol will yield fatty acid methyl esters (FAMEs).

The type of catalyst used during the reaction is dependent on the free fatty acid (FFA) content of the oil feedstock. For an oil sample with more than 2 wt % FFA content an acidic catalyzed reaction is required, which will reduce the formation of soap and water. Several studies have been conducted using an acid catalyst to convert algal oil (Li et al., 2007; Miao and Wu, 2006; Xu et al., 2006), due to high FFA contents of the oil. However, the use of acid catalysts have several drawbacks including being corrosive to industrial equipment (Barnwal and Sharma, 2005), having a slow reaction time and may require a high reaction temperature of over 100°C (Kulkarni and Dalai, 2006).

Reactions using alkaline catalysts (such as KOH, NaOH and $NaOCH_3$) are ~ 4000 times faster than acid catalyzed reactions (Fukuda et al., 2001), taking around 30 minutes to complete. Consequently alkali catalysts are most often used for industrial scale transesterification (Fjerbaek et al., 2009; Chisti, 2008). Recently research attention has turned to the conversion of algal oils with alkaline catalysts. Velasquez-Orta et al. (2011) demonstrated FAME recoveries of up to 77.6% in a 75 min time period using an *in situ* transesterification reaction even though the *Chlorella vulgaris* had a FFA content of 3.2 ± 0.2 wt % of total lipids. Chen et al. (2011b) converted *Chorella protothecoides* oil into FAMEs using KOH as an alkaline catalyst. The microalgae oil methyl esters satisfied most biodiesel specifications including

ASTM D6751 in the United States and EN 14214 in Europe (Table 2.5). The low freezing point of microalgae oil methyl esters are of particular interest to the aviation industry. On January 8th 2009 Continental Airways successfully flew a Boeing 737 twin-engine jet powered by a 50/50 blend of kerosene and biofuel derived from algae and jatropha. The fuel passed all tests without the need for engine modification and it was noted that the fuel had advantageous low-freezing points and flash points when compared to other biofuels (Gouveia, 2011).

Table 2.5: Properties of microalgae oil methyl esters compared to the biodiesel specifications of United States (ASTM D6751) and Europe (EN 14214)

Property (unit)	**microalgae oil methyl esters**	**ASTM D6751**	**EN 14214**
Acid value (mg KOH g)	0.29	0.5 max	0.5 max
CFPP (°C)	-13	Not specified	Not specified
Density at 15°C (kg/m^3)	882	Not specified	860-900
Ester content (% m/m)	97.7	Not specified	96.5 min
Monoglyceride content (% m/m)	0.128	Not specified	0.8 max
Diglyceride content (% m/m)	0.023	Not specified	0.2 max
Triglyceride content (% m/m)	0.045	Not specified	0.2 max
Free glycerol (% m/m)	0.0	0.02 max	0.02 max
Total glycerol (% m/m)	0.196	0.24 max	0.25 max
Iodine value (gI_2/100g)	112.2	Not specified	120 max
Kinematic viscosity at 40°C (mm^2/s)	4.43	1.9-6.0	3.50-5.0
Oxidation stability, 110°C (h)	4.52-4.53	3.0 min	6.0 min

During conventional extraction and transesterification method it is crucial that the water content of the feedstock oil is kept to an absolute minimum as its presence can reduce the product yield and increase the formation of froth, gels (leading to an increase in viscosity), and the formation of soaps making further processing such as glycerol removal more difficult (Demirbas, 2009; Fjerbaek et al., 2009; Vasudevan and Briggs, 2008; Ma and Hanna, 1999). The energy required for the dewatering (harvesting and drying) process can account for 84.9% of the total energy consumption (Xu et al., 2011), therefore to reduce the cost of production recent research attention has turned to direct transesterification of the wet algal biomass (Teixeira, 2012; Patil et al., 2011; Kita et al., 2010; Johnson and Wen, 2009).

Patil et al. (2011) demonstrate a one-step supercritical methanol process, which uses the water in wet algae as a tunable co-solvent, to accelerate the conversion of oils to fatty acid methyl esters (FAMEs) and increases solubility and acidity (Patil et al., 2011). Although it is still not economically viable it demonstrates huge potential to provide the economical production of biodiesel from microalgae biomass.

Post transesterification the vessel chamber contains the by-product glycerine, the unused catalyst and some soap. Generally, for every 4.5 kg of biodiesel produced ~0.45 kg crude glycerol is produced as a co-product (Chen and Walker, 2011). The water and the alcohol are removed to produce 80-88% pure glycerine. This can be further processed to produce >99% pure glycerine (Power, 2008), which can be sold for use in the food market, pharmaceuticals, and within explosives. However, biodiesel production has oversaturated the world glycerine markets, resulting in a price crash, between 2004 and 2005 the price decreased from $0.25/lb to $0.025/lb (Yazdani and Gonzalez, 2007). Chen and Walker (2011) have demonstrated that crude biodiesel glycerol can be used as an alternative carbon source for the heterotrophic growth of microalgae, providing an economical feedstock for biodiesel removing many of the problems associated with crude glycerol disposal.

2.5.4 Biofuels from residual biomass

Bioethanol is an established fuel that could be made from carbohydrates that remain after biodiesel production. Bioethanol is usually achieved via alcoholic fermentation; the biomass is pre-treated with acids or enzymes which cause cell wall disruption improving yields (Harun et al., 2010a). Starch is hydrolyzed by enzymes to form glucose which can be readily fermented by yeast into ethanol. Distillation and dehydration purifies the ethanol. *Tetraselmis suecica* and *Spirulina sp.* have been shown to have high starch quantities when the cells are grown in both nutrient replete and deplete conditions (Dismukes et al., 2008) demonstrating the potential to combine biodiesel production with bioethanol production post transesterification. The remaining microalgal biomass can then be subject to anaerobic digestion to produce methane (Ueda et al., 1996). Mixtures of bacteria are used to hydrolyze organic biopolymers into monomers, which produce a gas containing 50-75% methane and 25-50% carbon dioxide. The methane can be removed and used for electrical generation and the carbon dioxide can be recycled back into the cell growth. Both these biofuels can be produced via established methods using low cost technologies (Gouveia, 2011), providing valuable co-products and an economical way to dispose of the biomass waste.

2.6 Economics of microalgae oil

The public's perception of a product is extremely important. Often public support is closely linked to the price and ethics of a product (see *2.2 biofuel ethics* to understand the benefits of microalgae). A number of studies have been conducted to estimate the cost of algae oil per barrel. The range is massive from as little as $49.28 US/bbl (Benemann and Oswald, 1996) (increased from $32 US/bbl due to inflation from 1996 to 2011) to as much as $209 US/bbl (Schenk et al., 2008). Benemann and Oswald (1996) calculated their estimates on theoretical potentials of a 400 ha open pond, utilising CO_2 from a coal power station. Productivity was assumed to be 30-60 g m^{-2} day^{-1} with a 50% lipid yield; this is an extremely optimistic combination and, although possible, has yet to be

achieved. These idealistic assumptions resulted in a low estimate of $49.28 US/bbl. Huntley and Redalje (2007) used the same infrastructure costs assumptions as Benemann and Oswald (1996) when estimating the cost of oil per barrel produced from their two stage system. Due to the initial photo-bioreactor stage, productivities of 70.4 g m^{-2} day^{-1} were achieved; this combined with the nutrient stress stage in the open pond gave a high lipid yield of 35%. Due to the high operational costs the photo-bioreactor microalgae oil price is $84 US/bbl. From figure 2.1 it can be seen that crude oil prices peaked at $133 US/bbl '08 –'09 and are currently still above $100 US/bbl (July 2012). If the crude oil price remained at this level the Huntley and Redalje (2007) algae oil prices would be commercially viable. However, in 2009 oil prices levelled out to between $41-$70 US/bbl (Oil-Price.net, 2012), making algae oil seem very expensive in comparison. For microalgae produced biofuel to be fully accepted it must consistently undercut crude oil prices.

Williams and Laurens (2010) analysed the economics of the whole production system, and it was concluded that for biodiesel derived from microalgae to be sold at the low price of $75 US/bbl valuable residual biomass needs to be sold in conjunction to offset the price. Residuals such as protein and carbohydrate mixes need to be sold for a minimum value of $500/tonne and protein alone needs to sell for a minimum value of $800/tonne. Importantly Williams and Laurens (2010) highlighted the uncertainties related to estimating the economics of biomass and biofuel production due to the immaturity of the subject, however, the work does demonstrate the need to have integrated co-production to ensure a profitable system.

Currently, the most economical route for feedstock production is through raceway ponds, therefore research and development should be focused on increasing the efficiency and improving the economics of open systems. Many researchers have discussed genetic engineering as a positive way to improve biomass yield and improve the cost of production (Radakovits et al., 2010; Meng et al., 2009; Li et al., 2008a; Schenk et al., 2008; Chisti, 2007; Spolaore et al., 2006). Genetic and metabolic engineering can be used for a number of modifications such as reducing photosaturation and photoinhibition, therefore increasing photosynthetic efficiency and increasing biomass output in open systems. The recent advances in genetic engineering have equipped scientists with new tools that enable the analysis and manipulation of metabolic pathways

with extreme procession (Radakovits et al., 2010). Genetic engineering is an important element to algae-to-biofuel research, and its advances will help to quickly reduce the cost of production. However, as mentioned earlier it is vital for microalgal derived biofuels to have public support. At this time society has a negative view of genetic modification, especially within Europe (FOEEurope.org, 2008); however public views may quickly change once algae base biofuels become economically competitive. Concerns are also sparked at the potential devastating ecological effects that could occur if genetically modified microalgae were inadvertently released into the natural environment. It is therefore critical to ensure the proper management of genetically modified microalgae to prevent any potential environmental damage, and loss of consumer support.

A possibility to significantly reduce capital costs would be to increase the efficiency of biomass harvesting. It has often been highlighted that efficiency, energy consumption or cost of harvesting is of significant concern (Uduman et al., 2010; Chisti, 2007; Li et al., 2008b; Molina Grima et al., 2003) and has been quoted to add as much as 30% onto the cost of production (Molina Grima et al., 2003). Finding a harvesting method that is fast and inexpensive would have a massive positive impact on the economics of algae biofuels.

2.7 Harvesting

The technology employed for the recovery of microalgae is considered to have the most influential effect on the economy of microalgae production (Brennan and Owende, 2010; Danquah et al., 2009a; Becker, 1994; Shelef et al., 1984). Many techniques have been devised over the past four decades, yet none have proved to be economical for the large scale removal of microalgae biomass. The recovery of microalgae biomass is particularly difficult due to:

- Microalgae occur in very dilute concentrations within the medium, commonly between 0.3- 5 g L^{-1} (Gouveia, 2011; Li et al., 2008b; Wang et al., 2008; Ginzburg, 1993). In industrial conversion operations concentrations of 300-400 g L^{-1} dry weight is required, which means up to a thousand times concentration is often needed.

- The small concentration means that large volumes of culture must be handled to obtain the biomass.
- Most microalgae are extremely small ranging from 5-50 µm diameter (Shelef et al., 1984), with densities that are only slightly greater than the culture medium (Becker, 1994).
- The zeta potential on the surface of the microalgae surface and algogenic organic matter (AOM) creates a stable cell suspension (Danquah et al., 2009a).

The selection of harvesting technology is dependent on many factors including cell density, size, and value of the end product (Brennan and Owende, 2010; Olaizola, 2003). The harvesting process can generally be broken down into a one or two stage process; During the primary or bulk harvesting the biomass is concentrated to 2-7% total suspended solids (TSS), this can be achieved using flocculation, flotation or gravitational separation (Brennan and Owende, 2010; Uduman et al., 2010). This is followed by a secondary dewatering or thickening step, which produces an algal cake with 15-25% TSS, this is achieved with filtration or centrifugation (Brennan and Owende, 2010; Uduman et al., 2010). Unfortunately there is currently no universal technology for the recovery of microalgae (Gouveia, 2011).

2.7.1 Gravitational sedimentation

The stable suspension of many microalgae is both a help and a hindrance for the production of biomass. During the growth phase suspension is vital in reducing dark zones and allowing high photosynthetic efficiencies. However, it provides a difficult challenge during harvesting. The stability of the suspension is affected by the negative charge on the cell surface and the buoyant nature of the cells. Inter-particular repulsive force between the algae cells and between the cells and the water body influence the stability (Greenwell et al., 2009). Zeta potential is a measure of the electric potential. The majority of algal cells have a negative cell surface (Danquah et al., 2009b; Henderson et al., 2008a; Phoochinda and White, 2003; Liu et al., 1999). The zeta potential in microalgae has huge range typically from -5 to -40 mV (Greenwell et al., 2009),

electronegative values as low as -43.2 ± 0.7 mV have been measured for *Tetraselmis suecica* (Danquah et al., 2009b). Table 2.6 shows the effects of zeta potential on the stability of the suspension.

Table 2.6: The effects of electronegative values on the stability behaviour of the colloid (Huang et al., 2010)

Zeta potential (mV)	Stability behavior of the colloid
From 0 to ±5	Rapid coagulation or flocculation
From ± 10 to ± 30	Incipient instability
From ± 30 to ± 40	Moderate stability
From ± 40 to ± 60	Good stability
More than ±61	Excellent stability

Gravitational sedimentation is common in wastewater treatment as an inexpensive separation technique. As the microalgae cells often have a marginally greater density than the medium they will sink due to gravity, but due to the liquid surroundings, drag will reduce the speed at which they sink. Gravitational sedimentation is both time and space consuming, making it an inappropriate choice for biodiesel feedstocks (Schenk et al., 2008). The rate of sedimentation can be increased by flocculation.

2.7.2 Flocculation

Flocculation promotes the agglomeration of cells in a colloidal suspension, increasing relative particle size and weight, therefore increasing the settling efficiency. Flocculation is routinely used in industrial applications as a primary harvesting technique, improving the effectiveness of secondary applications such as sedimentation, filtration, centrifugal recovery and flotation (Barany and Szepesszentgyörgyi, 2004). The methods used for flocculation can be classified into several groups:

- Chemical flocculants
  - Inorganic flocculants

- Organic flocculants/ polyelectrolyte flocculants

• Electrolytic flocculation

• Natural flocculants

• Autoflocculation

The ideal flocculation process should be inexpensive, effective in small concentrations, non-toxic, biodegradable and able to be used over a wide range of environmental conditions. This section of the review will explore to what extent each group meets these criteria.

2.7.2.1 Inorganic chemical flocculants

The majority of inorganic chemical flocculants (ICF) are based on multivalent cations such as aluminium sulphate, ferric chloride and ferric sulphate (Phoochinda et al., 2004; Molina Grima et al., 2003; Bare et al., 1975). These positively charged molecules interact with the algal cell surface reducing or neutralising the electronegative surface charge, which prevents aggregation (Christenson and Sims, 2011). Under the appropriate conditions, many ICF react with the water in the culture to form metallic hydroxides, which cluster together to form a mesh like structure trapping the algae cells (Becker, 1994). In salinities greater than 5 kg m^{-3} (seawater being ~ 37 kg m^{-3}) ICF flocculating ability is significantly inhibited (Molina Grima et al., 2003). The salinity of seawater can often be expressed by ionic strength. The concentration of flocculent required in the marine environment can linearly be linked with the increases in the ionic strength (Sukenik et al., 1988). Sukenik et al. (1988) and Danquah et al. (2009a) have both concluded that flocculation in high salinities could be increased by following an ICF with a synthetic organic flocculent (SOF) such as Zeteg 7650. ICF such as alum and ferric chloride are effective in freshwater but are expensive to apply on a large scale application (Schenk et al., 2008; Oh et al., 2001), requiring large concentrations and producing huge amounts of sludge (Chen et al., 2011a). ICF are also highly sensitive to pH levels and do not work for all microalgal species. The residual aluminium from flocculants will contaminate the feedstock making it unsuitable to utilise for

many high value by-products such as animal feed, fertilisers, and nutraceuticals (Chen et al., 2011a; Mulbry et al., 2005).

2.7.2.2 Organic flocculants/ polyelectrolyte flocculants

SOF are derived from polyacrylamide or polyethylene imine (Li et al., 2008b). They have proved to be more cost effective and achieve greater efficiencies when compared to ICF. In addition to stabilising the algal cells electronegative charge SOF adsorb macromolecules onto the surface of cell walls linking and binding cells together, this process is known as bridging (Uduman et al., 2010; Barany and Szepesszentgyörgyi, 2004; Molina Grima et al., 2003). It occurs due to the attachment of the polymer molecule onto the microalgal surface by electrostatic/chemical forces. During the bridging process the polymer is able to adsorb onto the cell due to one or a combination of interactions including: Coulombic interactions, dipole-dipole interactions, hydrogen bonding, and van der Waals interactions (Uduman et al., 2010). Polymer dosage has been found to significantly affect the flocculation efficiency, less than the optimum amount will result in weak bridging, resulting in flocs that will easily be broken up. If dosage is too high bridging potential can be reduced due to electrostatic/static hindering (Tenney et al., 1969). The optimum dosage of the polymer can be decreased by increasing molecular weight, as lower molecular weight polyelectrolytes are more difficult to adsorb (Uduman et al., 2010). Polyelectrolytes can be cationic, anionic, or nonionic, however the latter two fail to induce flocculation in microalgae due to electro repulsion, whereas a high charge interaction unfolds the polymer molecule, improving its bridging (Molina Grima et al., 2003).

However SOF have been shown to have poor decomposition rates (Wu and Ye, 2007; Salehizadeh and Shojaosadati, 2001), and some of their degraded monomers have been shown to have a strong toxic or carcinogenic potential (Oh et al., 2001; Shih et al., 2001). These disadvantages have lead to research and development into alternative, safe, biodegradable flocculants.

2.7.2.3 Electrolytic flocculation

Electrolytic flocculation has the advantages of not requiring the addition of flocculants, having few moving parts and only requiring electricity as an input (Ofir et al., 2007). Flocculation is achieved via electrodes which are placed vertically in the culture, the negatively charged microalgae move to the anode, causing the cell to lose its charge, enabling it to form aggregates (Uduman et al., 2010; Poelman et al., 1997). There is very limited research regarding the application of this technology, however, Poelman et al. (1997) demonstrate one of the few applications of electrolytic flocculation, achieving up to 96% separation of cells while only consuming 0.3 kWh m^3 in a 75 minute time period. It was found that although decreasing the voltage lead to slower algae removal rates, it also drastically reduced the energy consumption of the process. Increasing the distance between the anode and cathode also improved the energy requirements (Poelman et al., 1997). Poleman et al. (1997) concluded that the biomass could be used for animal feed or food due to the lack of flocculent contamination; however Pearsall et al. (2011) noted that electro-flocculation leaves residual metals in the algal concentrate, therefore further investigation is needed to establish the by-products that could be gained from using this harvesting method. Disadvantages to this process include; cathode fouling and maintenance, the research gap relating to electrode design and arrangement.

2.7.2.4 Natural flocculants

Chitosan has many of the characteristics of chemical flocculants without many of the disadvantages. It is a polymer of acetylglucosamine that can be produced from chitinous waste such as prawn peelings (Molina Grima et al., 2003; Divakaran and Pillai, 2002). It is a non-toxic, biodegradable cationic flocculant. Chitosan can easily be manufactured and is effective at flocculating many freshwater species. The flocculating efficiency is highly sensitive to pH, with the optimum cell recovery occurring at pH 7.0 for freshwater species (Divakaran

and Pillai, 2002). The flocculation efficiency is also dramatically reduced when used to recover marine species with flocculation efficiency dropping quickly at relatively low ionic strengths (Molina Grima et al., 2003). The decrease in flocculating efficiency can be slightly compensated by increasing the dose but this will have repercussions on the cost of harvesting, for example to recover the freshwater phytoplankton *Scenedesmus obliquies* requires an optimum dose of 50 mg L^{-1} (Becker, 1994), where as to recover the marine species *Chaetoceros muelleri* 150 mg L^{-1} is needed (Heasman et al., 2000).

Bioflocculation has been described as the "the tendency of normally repulsive microalgae to aggregate in large flocs…The mechanisms of bioflocculation involve extracellular polymers excreted by the algae" (Sheehan, 1998). Aggregation of cells has been linked to an increase in exopolysaccharides (EPS) which play an important role in the 'sticking' of microalgae in nature (Thornton, 2002; Shipin et al., 1999; Mopper et al., 1995). The 'stickiness' of EPS has been stated to be in response to environmental and nutritional stimuli, with many diatom blooms creating mass aggregations at the end of the cycle (Mari, 2008; Mopper et al., 1995). Laboratory investigations have demonstrated that nutrient limitation increases the production of EPS in diatoms (Thornton, 2002; Dethier et al., 1993), however it is still a poorly understood natural phenomena (Thornton, 2002; Sheehan, 1998; Kiørboe et al., 1990). The flocculation process is well known in bacteria and can be utilised for the production of bioflocculants to assist in algae aggregation (Kiørboe et al., 1990). Bacteria have been found to produce a wide range of biopolymer flocculants (Deng et al., 2003; Oh et al., 2001; Salehizadeh and Shojaosadati, 2001; Shih et al., 2001). In the past major disadvantages of bioflocculants was due to low flocculating efficiencies and large dosage requirements (Deng et al., 2003). Recently, a wide range of efficient microbial bioflocculants have been produced. As stated previously the efficiency of a flocculent is closely related to the molecular weight, bioflocculants with high molecular weights have been found, up to 200,000 Da for flocculants produced by *Aspergillus sojae,* creating stronger bridging and flocculating activity *(Salehizadeh and Shojaosadati, 2001).* The bacterium *Paenibacillus* sp. AM49 was able to produce a bioflocculant that efficiently recovered 83% of the cells between pH 5-11 (Oh et al., 2001). Bioflocculants produced from bacteria can be grown on a large scale converting nutrients in the culture medium to high weight polymers. The bioflocculant either

occurs in the surface of the cell or is excreted into the medium (Deng et al., 2003).

Cationic starch has been demonstrated as an efficient bioflocculant for freshwater species in jar text experiments (Vandamme et al., 2009). It is advantageous when compared to traditional inorganic flocculants as it requires lower doses, it also does not contaminate biomass and has been approved for food contact (Vandamme et al., 2009; Krentz et al., 2006). Although it requires higher dosages when compared to chitosan, cationic starch is cheaper to produce and the efficiency is not dependent on pH of the culture.

2.7.2.5 Autoflocculation

Autoflocculation is induced by a high pH interacting with divalent cations caused by the consumption of dissolved carbon dioxide (Christenson and Sims, 2011; Muñoz and Guieysse, 2006; Sheehan, 1998). An increase of pH causes supersaturation of calcium and phosphate ions, resulting in a positively charged calcium phosphate precipitate which, will result in a neutralisation of the negatively charged algae cells (Christenson and Sims, 2011). An increase in pH can be reduced by stopping the paddle wheels or the CO_2 supply (Becker, 1994), which could provide a cost-effective harvesting method. In freshwater systems the addition of lime provides Ca^{2+} and increases the pH quickly creating large flocs (Elmaleh et al., 1996). It has been shown that a pH greater than 10 creates rapid aggregates. Knuckey et al. (2006) noted that above pH 10 the flocs formed had a more “robust” structure and settled faster than those of a lower pH, efficiencies of 97 ± 2% were also achieved at pH 10 for *Scenedesmus*. Although often demonstrated on a lab scale, autoflocculation still needs to be tested on a large scale (Rodolfi et al., 2009) and a greater understanding of its mechanisms and how to control them is required. Also, if autoflocculation is to be applied on a large scale for improved sedimentation it would require the pond system to stop production for harvesting to take place or for the culture to be fed into a second settlement pond, although improved, settlement would still require several hours before effective harvesting can occur.

2.7.3 Filtration

During filtration a pressure drop must be applied across the surface of the medium. Gravity, vacuum, pressure or centrifugal force can be employed to drive filtration, where medium passes through and the solid is captured onto permeable medium (Uduman et al., 2010; Shelef et al., 1984). Filtration is commonly applied, but success is dependent on the size of the microalgae being harvested. In a bid to improve algal recovery a wide range of filtration methods have been tested, designs range from simple gravitational filtration to complex vacuum and pressure systems (Bruton et al., 2009). As the system complexity increases so does the cost of harvesting. With all forms of filtration its application is only effective when used for larger species such as *Coelastrum proboscideum* and *Spirulina platensis,* but fails to recover commercially important strains such as *Chlorella, Scenedesmus* and *Dunaliella* (Molina Grima et al., 2003). However, filtration of larger microalgae strains is still not without difficulty. The filtration pores must be small enough to retain the algae however, they can quickly become clogged. Blockages and clogging can dramatically affect the potential run and therefore efficiency of filtration (Henderson et al., 2008a), for example, even extremely low concentrations of (250-1000 cells ml^{-1}) of the diatom *Synedra acus* were able to block filters greatly reducing run time from 35 hrs to 23.5 hrs (Jun et al., 2001).

Microstrainers are low speed (4-7 rpm) rotating drum filters. They use gravitational methods for microalgae recovery (Polprasert, 1996). The filtering fabric can be made from stainless steel, for the recovery of larger strains, or polyester for smaller strains. Conventional microstrainers have a mesh size within the range of 23 – 60 µm (Polprasert, 1996; Becker, 1994). However, microstrainers down to 1µm have been developed, unfortunately low recovery rates of ~50% were typically achieved and there was an extremely short service life of 1.5 yrs (Becker, 1994). Microstraining has often been found to be too coarse for many microalgae, the effect of rubbing can cause the cells to leak their valuable contents (Kim et al., 2005; Polprasert, 1996), and reduce the cell quality affecting any potential by-product production.

Tangential flow filtration (TFF) involves the media travelling tangentially across the surface of the filter, rather than into the filter, keeping the cells in suspension. Small particles pass through the membrane pores, particles larger than the membrane pores are retained (Petrusevski et al., 1995). Danquah et al. (2009a) combined polymer flocculation with TFF; this method concentrated the feedstock 357 times. The process is relatively energy intensive compared to other filtration methods, consuming 2.06 kWh m^{-3} where as pressure filters only consume 0.88 kWh m^{-3} (Danquah et al., 2009a; Molina Grima et al., 2003). TFF has the advantage of maintaining the structure, mobility and properties of the harvested microalgae (Petrusevski et al., 1995). The high cost contributed to the continuous replacement of filters, due to fouling, and pumping combined with a low recovery rate and long processing times make filtration an unattractive harvesting technique for large scale processes such as biodiesel feedstocks.

2.7.4 Centrifugal recovery

The recovery of biomass by centrifugation involves the use of the centrifugal force to accelerate the rate of sedimentation. Centrifugation offers many advantages over the previously mentioned methods such as:

- Recovered biomass is free from flocculants or chemicals
- Centrifugation can be applied to all strains of microalgae.
- High recovery rate and concentrates are easily, predicatively and quickly achieved.

There are many different designs for centrifuges, however, the machines based on continuous harvesting are most appropriate for large scale applications, as batch type centrifuges must be regularly stopped and manually cleaned (Shelef et al., 1984). The most attractive centrifuge for biodiesel feedstock recovery is the decanter type. Decanters are able to achieve high solid fractions while continuously discharging the product (Wiley et al., 2011; Becker, 1994; Shelef et al., 1984). The decanter has been proven able to obtain

22% total suspended solids (TSS) from a 2% TSS culture (Shelef et al., 1984), however, Becker (1994) reports that it is not suitable for all types of microalgae, such as the commonly grown *Chlorella sp*.

Heasman et al. (2000) gained an 88-100% recovery rate when using centrifugal recovery on a wide range of microalgae at 13,000 x g, yet a rapid decline in harvest efficiency occurred at lower acceleration factors. The high acceleration rates required are closely linked to the excessive energy consumption, and it has been estimated to be as high as 3,000 kWh/ton (Schenk et al., 2008). Although 'low cost' centrifuges are often presented at many algal biofuel conferences, centrifugation is the extremely costly of recovery process, which makes it unsuitable for harvesting low value feedstocks.

2.7.5 Electrolytic coagulation

Electrolytic coagulation is similar to electrolytic flocculation, however there is key difference due to the presence of sacrificial electrodes, such as iron or aluminium, that produce coagulating ions 'in situ' (Uduman et al., 2010; Mollah et al., 2004). Coagulants are produced when Fe/Al dissolves from the anode producing metal ions, which hydrolyze to produce polymeric iron (for Fe) or aluminium hydroxide (for Al). These polymeric hydroxides combine with the negatively charged algae, which are then carried towards the anode due to electrophoretic motion (Mollah et al., 2004). The processes can be broken down into three clear stages (Gouveia, 2011; Bukhari, 2008; Mollah et al., 2004):

• Electrolytic oxidation of sacrificial electrode generating coagulants

• Destabilisation of particulates and colloid suspension

• Aggregation of the destabilised particles forming flocs

Gao et al. (2010) combined electrolytic coagulation with flotation, achieving 100% algae removal under optimum conditions, while only using 0.4 kWh/m^3. The results indicated that aluminium was a preferential material to use over iron, and that cell removal is improved at low to neutral pH (Gao et al., 2010). Wang et al. (2012) found that electrolytic coagulation can be superior to

chemical coagulation when comparing the removal of chlorophyll-a, UV254 matter and the quantity of sludge production. Uduman et al. (2011) demonstrated that electrolytic coagulation can be effective at harvesting marine species of algae as well as freshwater. However, the effectiveness of cell removal was greatly dependent on water temperature and salinity. At the starting temperature of 5°C only 5% of *Chlorococcum sp*. cells and 68% of the *Tetraselmis sp*. cells were removed. This increased to 96 and 94% for *Chlorococcum sp*. and *Tetraselmis sp.* respectively at a temperature of 60°C. Increasing the temperature increases the kinetic energy of the microalgae, increasing the likelihood of cell collision and therefore floc formation, increasing the temperature also increases electrical conductivity of the liquid (Uduman et al., 2011). Uduman at al. (2011) concluded that higher temperatures should reduce the power requirements of cell removal. However, this does seem to account for the huge energy requirements needed to heat the culture up to 60°C and the effect on the cell this will have, such as cell death. Therefore currently, although achievable, electrolytic coagulation is not the appropriate harvesting technique for marine microalgae.

The main disadvantages of electrolytic coagulation is the contamination of the biomass by increased doses of the electrode metal and the need to regularly replace the sacrificial anode (Uduman et al., 2011; Mollah et al., 2004).

2.7.6 Flotation

Harvesting via flotation uses the low density of microalgae to its advantage, as algae particles can float upwards much more rapidly than sediment downwards. This is due to the microalgae developing methods to reduce sedimentation rates such as gas vesicles, setae to increase drag, and high lipid contents which are positively buoyant. Some microalgae naturally float due to the presence of gas vesicles; they include cyanobacteria such as *Microcystis*, *Anabaena* and *Spirulina* (Oliver and Ganf, 2000). An 80% removal rate of *Spirulina platensis* was achieved through gentle stirring during the logarithmic growth phase (Kim et al., 2005). However for the majority of algal species air or gas particles are used. Bubbles are introduced at the bottom of the liquid, where

the algae are collected from the liquid suspension and carried to the surface where it can be removed. It has been used as a method of particle separation for over 100 years in the mineral industry. Since its introduction flotation has been widely adapted and successfully used in many commercial applications such as the removal of oil from water, the removal of ink from paper fibres, and waste water treatment (Phoochinda et al., 2004; Phoochinda and White, 2003; Jameson, 1999).

For flotation to be successful the cell must be hydrophobic, this can be achieved through the addition of surfactants (sometimes referred to as collectors) or coagulants. The attachment of air bubbles to the particle depends on many factors including particle size, the probability of collision and the probability of adhesion. Decreasing particles size increases the likelihood for the particle to be lifted to the surface. However, it has been shown that a decrease in particle size also decreases the probability of the cells colliding with the bubble. Increasing the contact angle between the air and the solid particle and increasing the likelihood for the air and solid to adhere, this can be achieved by the addition of surfactants (Uduman et al., 2010; Shelef et al., 1984). The main methods used to induce flotation including dissolved air flotation (DAF), electrolytic flotation and dispersed air flotation (DiAF).

2.7.6.1 Dissolved air flotation

DAF is widely used around the world as a clarification treatment method for drinking water (Edzwald, 2010) (Figure 2.6). It occurs in several stages. The first stages occur in the saturator. A compressor is used to supersaturate the water with air. This water is then released into a flotation tank at atmospheric pressure. The dissolved air precipitates out of the water forming small bubbles (10-100 µm), which adhere to the suspended matter carrying them to the surface (Wiley et al., 2011; Uduman et al., 2010; Wiley et al., 2009; Chung et al., 2000). Traditionally the waste water is pre-treated with coagulants, typically cationic metal coagulants, to increase the size of the algal aggregates, therefore increasing the likelihood of collision between the bubbles and cells (Edzwald, 2010; Henderson et al., 2008b), however, combing flocculation and flotation can

be problematic, if the flocs produced become too large they are more likely to detach requiring multiple bubble attachments to reduce the increase in density caused by flocculation (Edzwald, 2010). Henderson et al. (2008b) gained high removal rates of 87%, the cyanobacteria *Microcystis aeruginosa,* by addition of cationic surfactants into the saturator. The addition of cationic surfactants producing small positively charged bubbles which result in electrostatic interactions between the bubbles and the cell surface, improving flotation. Henderson et al. (2008b) concluded that the addition of surfactants were advantageous, as it reduces the cost incurred from large coagulant doses. It was also suggested that the residual surfactant could be removed via ozone treatment, followed by granular activated carbon which can commonly be found on sites that treat algae (Henderson et al., 2008b).

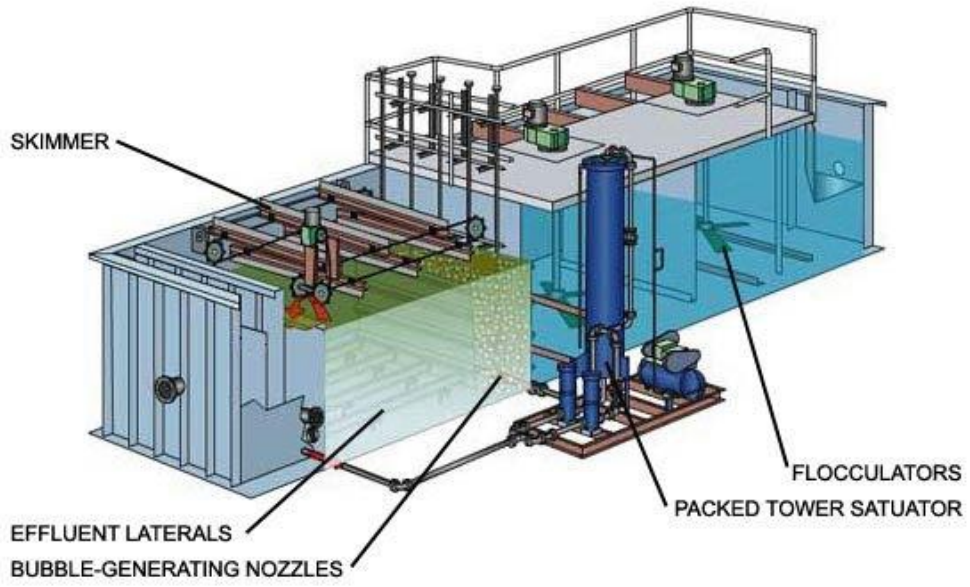

Figure 2.6: A standard dissolved air flotation (DAF) tank that is used in waste water treatment. (Diagram courtesy of Asia Water Business).

Although commonly used in wastewater treatment, DAF units are expensive to operate, due to the energy intensive compressor, to create typical saturator pressure of 500 kPa (72.5 psi) (Edzwald, 2010), resulting in high energy requirements of 7.6 kWh/m^3 (Wiley et al., 2009), however, combining wastewater treatment with the production of algae based biofuels and by-

products would be beneficial for both industries (Christenson and Sims, 2011). Surfactant based DAF should be used to reduce the likelihood of sludge production as chemical coagulants transforms the biomass into waste sludge which requires disposal (Christenson and Sims, 2011; Henderson et al., 2008b).

2.7.6.2 Electrolytic flotation

During electrolytic flotation hydrogen microbubbles are produced at an inert metal cathode (electrochemically non-depositing) entrapping the algae cells and carrying them up to the surface (Alfafara et al., 2002). Electrolytic flotation is often combined with electrolytic flocculation by using an active polyvalent anode, to produce flocculating metal hydroxides, with an inactive metal cathode to induce flotation. Alfafara et al. (2002) investigated the use of electrolytic flotation for the removal of microalgae in both continuous and batch systems. A polyvalent aluminium anode and an inactive titanium alloy cathode were used. The results demonstrated that removal of cells can be increased by increasing the electrical input; however this had the disadvantage of also causing a large increase in the temperature and pH. It was also found that electrolytic flotation could not be used as the sole removal technique as it only achieved a maximum removal of 40-50% of the cells.

2.7.6.3 Dispersed air flotation

DiAF uses a technique similar to DAF to harvest biomass; however, DiAF eliminates the need for an expensive energy intensive compressor by generating bubbles and foam with the addition of a surfactant and a low pressure sparger or agitator (Wiley et al., 2011; Wiley et al., 2009). Surfactants or ‘collectors’ are used to improve the interfacial tension between the cells and the liquid, increasing the foaming potential and the contact angle. The efficiency of the surfactant is, however, related to the surface properties of the microalgae, as this affects the hydrophobicity and charge of the cell (DeSousa et al., 2006).

As discussed previously most microalgae are negatively charged and therefore cationic surfactants such as cetyltrimethylammonium bromide (CTAB) have proved to be extremely effective for cell removal (up to 90%) (Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998). It has often been shown that anionic surfactants can be used just as effectively as cationic surfactants if the culture is adjusted to a more acidic pH (Chen et al., 1998; Bare et al., 1975; Levin et al., 1962), a reduction in pH reduces the algae's cell charge improving the absorption of anionic surfactants (Phoochinda and White, 2003). DiAF may provide a promising tool for harvesting high volume-low value biomass such as microalgal feedstock due to the extremely low energy requirement. Wiley et al. (2009) found that DiAF only required 0.003 kWh/m^3 compared to 7.6 kWh/m^3 for DAF; this is one the lowest energy requirements that has been quoted for a harvesting technology thus far. Also, compared to DAF, filtration, and centrifugal technologies, DiAF has relatively few mechanical parts which require maintenance or replacement (Csordas and Wang, 2004; Phoochinda and White, 2003), compared to DAF and flocculation DiAF has very low land requirement (Liu et al., 1999).

Due to the addition of surfactants DiAF can be combined with foam fraction (often referred to as a foam column in literature). This can be employed to concentrate and physically separate microalgae by using a column of rising foam which is then deposited into a collection vessel (Figure 2.7 A and B). This technique has two main benefits when compared to traditional harvesting methods; firstly, unlike flocculation and sedimentation the physical separation of biomass eliminates the need for additional energy intensive pumping to remove the residual water which is covering the biomass. Secondly, unlike flocculation and DAF foam fractionation it will remove the chemical additive, concentrating and separating the surfactant from the original culture, potentially enabling both the water and the surfactant to be recycled (Boonyasuwat et al., 2003).

Boonyasuwat et al. (2003) found that a number of variables such as decreasing air and flow rate, increasing foam height, and increasing the number of stages in a multistage foam fractionation could result in surfactant removal as high as 100%.

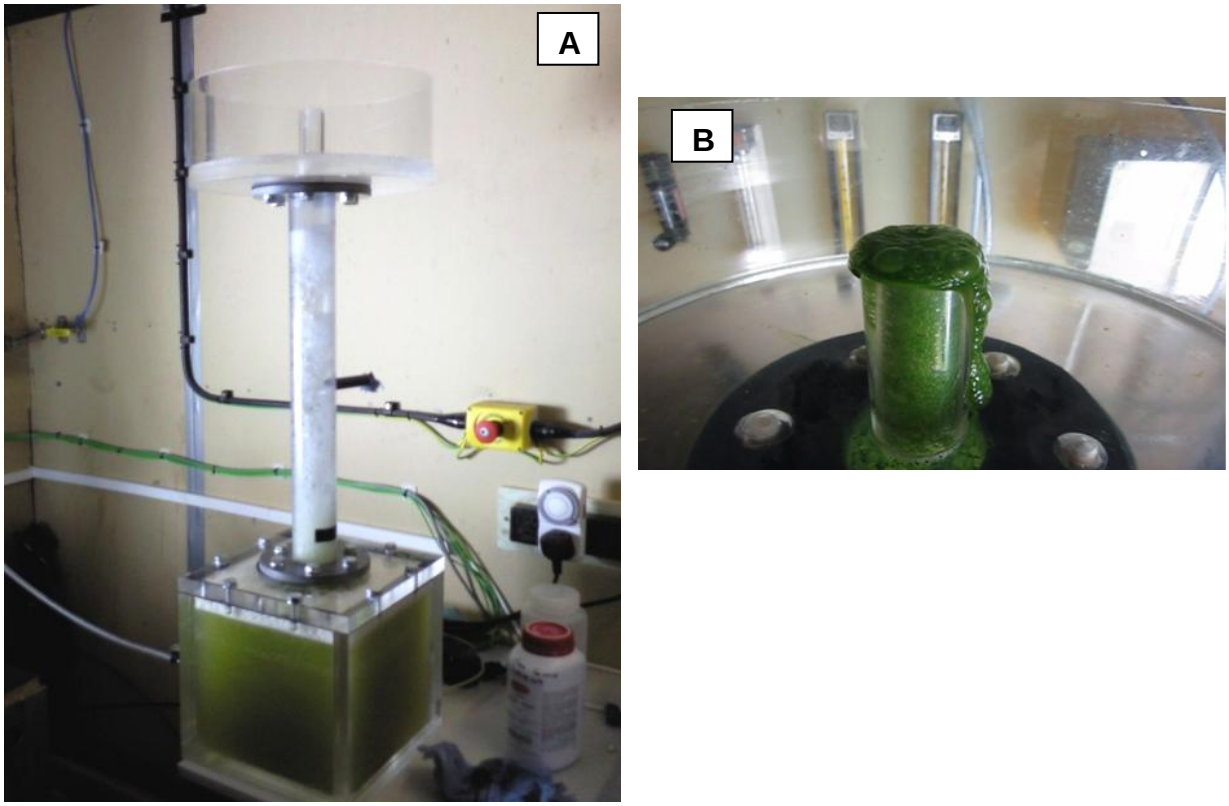

Figure 2.7 A) A flotation system combining DiAF and foam fractionation

B) Concentrated and separated algal product.

Many researchers have successfully achieved high algal removal rates of up to 90% combining DiAF and foam fractionation (Table 2.7). It must be noted (Table 2.7) that from the limited research conducted specifically on the recovery of microalgae the majority of it has been conducted on an extremely small scale (between 30-40 cm). It is evident that there is a clear research gap with regard to scaling up to larger projects, also to investigating the effect of column size and height.

Table 2.7: The cell removal success of DiAF technologies using synthetic surfactant foaming agents

Algae species concentrated	Removal (%)	Scale of foam fractionation column (cm x cm)	Surfactant used	Reference
Scenedesmus quadricauda	90	4 x 30	SDS	Phoochinda et al. (2004)
Microcystis aeruginosa	87	-	OTAB	Henderson et al. (2008b)
Scenedesmus quadricauda	90	3 x 40	CTAB	Chen et al. (1998)
Chlorella vulgaris	90	3 x 40	CTAB	Liu et al. (1999)
Scenedesmus quadricauda	90	4 x 30	CTAB	Phoochinda and White (2003)
Chaetoceros sp.	90	45.7 x 157	Naturally secreted from algae	Csordas and Wang (2004)
Chlorella sp.	NA	8.9 x 21	MicroFroth™	Wiley et al. (2009)

In 1981 Zutic et al. demonstrated that marine microalgae are able to produce and secrete bio-surfactants. Csordas and Wang (2004) used this information to their advantage and collected 90% of the *Chaetoceros sp*. cells in 12% of the total culture without the addition of synthetically produced flocculants or surfactants. It should be noted that a 1219 x 457 mm column was used. This is the largest column size that has been used to research into the separation of microalgae using a DiAF and foam fractionation combination. It must also be noted that this is one of the few papers which quotes the end volume of the concentrated medium. This offers great potential as ionic strengths >0.2 M

dramatically reduces the efficiency of synthetically produced surfactants (Liu et al., 1999; Chen et al., 1998). High bio-surfactant producing marine stains such as *Cryptomonas sp.* could be added to high lipid content marine cultures to improve the harvesting potential via foam fractionation. After harvesting, the remaining culture may have a few cells left behind, which could quickly out-compete the desired species - it is therefore recommended that UV light treatment is used before the water and nutrients are returned to the culture. Csordas and Wang (2004), however, noted large variances in harvesting efficiencies, which was due to the changes in bio-surfactant levels that occur throughout the microalgae's lifecycle. Further research needs to be conducted on the control and prediction of bio-surfactants produced from microalgae. For freshwater culture systems bio-surfactant secreting bacteria such as *Pseudomonas* sp. DSM 2874 (Chen et al., 2006) could be grown in conjunction with the algae. Research must be conducted into the effects of this on algal growth, but this could prove to be an effective and economical way of harvesting.

DiAF potentially offers the most economical way to harvest a wide range of microalgae. DiAF can be combined with foam fractionation to provide many advantages over the currently used harvesting technologies. However, work on this area has only been conducted on a small number of variables and on a small scale. If synthetic surfactants are to be used for a low cost biodiesel feedstock harvesting method further research into the effects of surfactants on the composition of cell lipid, and the effects on potential by-products must be investigated.

2.8 Conclusion

The need for cheap, biodegradable, renewable fuel sources has never been greater. Biodiesel provides an attractive alternative to petroleum, offering a potentially inexhaustible source of energy. Unfortunately, there is no possibility that first generation terrestrial oilseed crops, such as rapeseed and soya bean, could come close to meeting the demand for diesel. There are also many ethical and ecological disadvantages linked to the use of these crops for

biodiesel. Algal feedstocks are able to produce huge amounts of oil and appear to be the only current feedstock source that could meet the massive global oil demand in a sustainable manner. Their production removes the agricultural phase and gives the opportunity to achieve the much desired renewable fuel without any of these negative consequences. However the cost of production of algal oil needs to be slashed from \$2.80 L^{-1} to \$0.48 L^{-1} to make biodiesel derived from microalgae economically competitive with petroleum. At the moment a significant factor in the high cost of production, up to 30% of the total, is the expense of harvesting. There is currently no economical, universal harvesting technique. It is evident that if we are to achieve an economically viable fuel from this source, a cheap and efficient harvesting technology must be developed. Comparing the current harvesting technologies has shown that DiAF combined with foam fractionation and auto/bio flocculation could provide an economical way to harvest a wide range of microalgae. Auto/bio flocculation can be achieved at low cost and can be applied to large quantities of culture. These flocculants are non-toxic and have no secondary pollutants related to them. DiAF combined with foam fractionation has significantly lower construction, maintenance, and energy costs when compared to centrifugal recovery and reduces the time and space required for flocculation and sedimentation. It is also able to physically separate off the biomass product reducing the need for energy intensive pumps and, it could potentially improve water and chemical recycling. Further research must be conducted on both of these harvesting mechanisms to gain knowledge in the following key areas:

- How to gain a constant and predictably high harvest yield
- Improve economics of microalgal harvesting
- The effect of harvesting on lipids and co-products.
- Scaling up of low cost technologies.

Chapter 3

Development of a foam flotation system for harvesting microalgae biomass

Abstract

The lack of efficient and cost-effective technologies for harvesting bulk quantities of microalgae biomass is a major obstacle to commercialisation of algae-derived biofuels. This chapter demonstrates the efficacy of a foam harvester that combines dispersed air flotation with foam fractionation to allow harvesting, concentration, and physical separation of particles in suspension. Fractional factorial experiments using polystyrene latex beads were combined with trials using microalgae to determine the relative importance of key design and operational variables (air flow rate, batch run time, foam column height, surfactant concentration, and surfactant type) on the particle concentration factor. The model revealed that highest concentration factors were gained using the following variables and viable interactions: cationic cetyl trimethylammonium bromide (CTAB), lower surfactant concentrations, and CTAB combined with high column heights. Variables that increased foam residence time produced the greatest concentration factors. Foam flotation may prove to be an economical means to harvest microalgae.

3.1 Introduction

The microalgae biotechnology sector is at an early stage of commercial development and deployment. A number of microalgae-derived products have entered the marketplace with some degree of commercial success (Apt and Behrens, 1999), however, those that have proved commercially viable tend to be high added-value products that can tolerate the high costs of production,

harvesting and downstream processing. In stark contrast industries supporting high volume-low value products such as lipids for biodiesel conversion are in a nascent state and face an uncertain future (Sheehan, 1998). It is intuitive that ventures entering bulk commodity markets, particularly those targeting the biofuels sector, must produce substantial biomass at a competitive price. From a bioenergy perspective microalgae are, in theory, a highly attractive route for sustainable biomass production (Demirbas and Fatih Demirbas, 2011). Discounting the not inconsiderable costs and difficulties associated with biomass production, the process of harvesting and dewatering has often been cited as one of the major factors preventing a scalable industry.

The recovery of microalgae biomass from solution is a particular challenge, largely due to the small biomass concentration relative to the volume of liquid - low biomass to liquid ratio - (typically between 0.3-5 g L^{-1}) (Li et al., 2008a; Wang et al., 2008). In industrial conversion operations concentrations of 300-400 g L^{-1} dry weight is required, necessitating up to a thousand fold concentration. The low biomass to liquid ratio is further compounded by the small cell sizes, commonly with cell sizes < 30 µm diameter, with specific gravities very similar to that of the culture medium (Becker, 1994). Further, the zeta potential of the surface of the microalgae cell creates a stable cell suspension (Becker, 1994; Danquah et al., 2009a) making settlement and flocculation problematic.

The technology employed for the recovery of microalgae biomass is considered to have a major influence on the economy of microalgae production (Brennan and Owende, 2010; Danquah et al., 2009a; Schenk et al., 2008). As yet none of the commonly deployed technologies have proved economical at scale. The principal harvesting techniques include: centrifugation; filtration; flocculation; sedimentation; and electrophoresis (Molina Grima et al., 2003; Uduman et al., 2010). Centrifugal recovery is the fastest and most reliable method of biomass recovery for a wide range of species. However, the rotational speeds required to generate the acceleration required for separation are high and are closely linked to excessive energy consumption. The energy input required for centrifugal recovery is as high as 3000 kWh t^{-1} (Schenk et al., 2008).

Filtration is common, but success is dependent on the size of the microalgae being harvested. As a technique it has been demonstrated to be excessively abrasive for many microalgae inducing cell rupture and loss of, or reduction in the quality of the cellular content (Kim et al., 2005). Further, operational costs are high, due to pumping and the frequent replacement of filters. When coupled with a low recovery rate and long processing times, filtration proves unattractive for large scale processing. Likewise, flocculation is currently uneconomical. The most effective commercial flocculants to be found thus far are aluminium sulphate and some specific organic cationic polyelectrolyte flocculants (Molina Grima et al., 2003; Uduman et al., 2010). Unfortunately the quantities and therefore costs of flocculent required for large scale operation are prohibitive, though there have recently been a number of promising developments related to autoflocculation (Brennan and Owende, 2010) that may alter this outlook.

Gravity sedimentation is generally used for biomass recovery from sewage cultured algae; however it is time consuming and requires large land areas for settling ponds and tanks. The biomass gained from sedimentation has a high moisture content which increases the cost of further downstream processes (Molina Grima et al., 2003).

Electrocoagulation and electroflotation employ the electrochemical oxidation of a consumable metal electrode to destabilise algae suspensions causing the cells to float. There is currently limited understanding regarding using this technique to harvest microalgae (Uduman et al., 2010).

Foam flotation, also known as foam fractionation or protein skimming, is a process for removing surface active chemicals from water and dewatering dilute solid-liquid mixtures. Foam flotation as a means of removing algae from a growth system was first studied during the 1960s (Levin et al., 1962). The foam is normally generated by adding a surface-active chemical to the solid-liquid mixture and then injecting air bubbles to create stable foam. The bubbles can range in size from 10 to 3000 µm in diameter depending on the method used (Csordas and Wang, 2004; Uduman et al., 2010). Hydrophobic solids adsorb to the air bubbles and are separated from the suspension. The foam is essentially a complex network of interconnected channels surrounding the bubbles. As

liquid moves through these networks gravity promotes drainage, which is retarded by capillary action and friction at the channel walls. Adsorbed particles are recovered from the top of the foam body and as a consequence of drainage within the foam will be dewatered to some extent. Other processes occurring within the foam, such as bubble coalescence, may enhance dewatering by reducing the channel area (Neethling et al., 2003) or counteract it by releasing solids previously held at the gas-liquid interface into the liquid draining from the foam (Neethling, 2008).

The aim of this chapter is to evaluate the harvesting potential of a simple, yet robust foam flotation device. Initial optimisation trials of foam column performance were conducted using latex beads as a proxy for microalgae cells. Subsequent trials were conducted on cultures of *Chlorella* sp. Polystyrene latex micro particles have previously been used by Mari and Robert (2008) to simulate diatoms, as they have buoyancy and colloidal properties similar to those of many microalgae. For this study the substitute beads were determined to have the following characteristics in common with a 'generic' microalgae cell: similar in size and shape thereby giving comparable settling velocities; a negative surface charge; and similar density and specific gravity. Polystyrene latex micro-beads meet these criteria. They are spherical and available in a broad size range and can be used to represent different algal cells by varying the particle size to account for shape differences. The density of the beads is 1050 kg m^{-3} compared to typical algal cell densities of 1020 kg m^{-3}. Zeta potential is –40 mV (Brown and Zhao, 1993) compared to values for algae cells in the range –25 mV to –45 mV (Danquah et al., 2009b).

3.2 Materials and methods

3.2.1 Design and construction of harvesting unit

The majority of work that has previously used foam flotation to harvest, or remove microalgae has been conducted in batch systems at a bench scale, harvesting between 500 mL and 1.2 L, and using small column sizes (Table 2.7)

(Wiley et al., 2009; Phoochinda and White, 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998), with the notable expectation of Csordas and Wang's (2004) design, which was able to handle up to 220 L. The harvesting unit used throughout this body of work was designed to use easily sourced, economical and durable materials. Due to limitations linked to the amount of algae that could feasibly be grown in the laboratory the reservoir was designed to only hold 10.2 L at any one time and work on a batch system. However, Csordas and Wang's (2004) scale was kept in mind so that the unit could be adapted to work on the large scale in a continuous or semi continuous manner.

3.2.2 Column dimensions

The foam harvester (Figure 3.1) was constructed from polymethyl methacrylate; Lucite® or Perspex® sheet and tube. The reservoir had a working volume of 10.2 L. A lime wood sparger was used to generate bubbles with a mean diameter of 1.13 ± 0.14 mm. Bubble size was determined using a combination of a high-speed digital video camera (Olympus i-speed 3) and image processing software Image J (National Institutes of Health, Bethesda, Maryland, USA). The columnar section had a modular construction with each module consisting of tubular sections of 500 mm in length and 46 mm internal diameter. A series of tubular modules could be attached in sequence to increase the column height from 500 to 2500 mm in 500 mm or 250 mm increments. At the apex of the column the foam passed through a restriction of 15 mm in diameter and was received by the foam collection cup. The purpose of the restriction was to increase the bubble diameter within the foam thereby minimising the time needed to collapse the foam within the collection cup. The ratio of the restriction diameter to the column diameter was such that the water content of the foam was unaffected (Xie et al., 2004). The foam harvester was designed to combine dispersed air flotation with foam fractionation to allow harvesting, concentration and physical separation of the biomass.

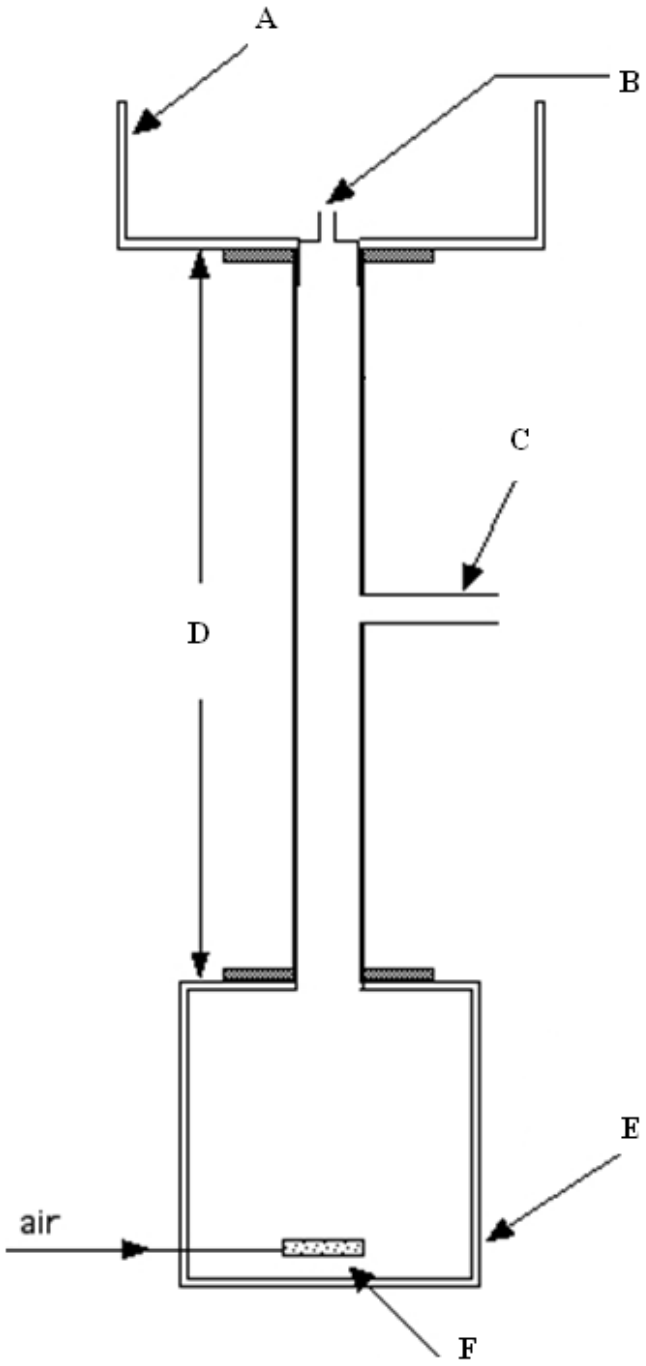

Figure 3.1 Dimensions of the foam column used in the experiments: A- Foam collection cup, 300 mm diameter, 100 mm tall; B- Foam restriction, 15 mm; C- Sample tube located 250 mm from A; D- One tubular module, 500 mm tall, 50 mm outer diameter, 46 mm inner diameter; E- Culture container inner dimensions: 220 mm X 220 mm X 211 mm = 10.2 L working culture volume; F- Lime wood sparger.

3.2.3 Surfactant types

Two surfactants were used to enhance foam formation and stability: the cationic cetyl trimethylammonium bromide (CTAB) and the anionic Ecover©, which is a mixture of biodegradable surfactants (15% anionic surfactant, 5% non ionic surfactant) produced from yeasts, glucose, and rapeseed oil (J.Sunter, Ecover© personal communication, 16/07/2009). CTAB was selected as it is a commonly used, readily available cationic surfactant, and has been used effectively in waste water treatment. Ecover© was selected as it is one of the few surfactants that is derived from plant material, therefore potentially reducing any environmental impact.

3.2.4 Fractional factorial experiments

For initial optimisation trials 10 µm polystyrene latex beads (Sigma UK, 55463-10ML-F) were used in preference to microalgae to circumvent any potential issues associated with algae inter-batch variability. A fractional factorial method (Box et al., 2005) was applied to determine the key process variables and the most advantageous combination thereof. The variables of interest were: air flow rate, batch run time, column height, surfactant concentration, and surfactant type. A resolution V fractional factorial design (2^{5-1}) was used as it reduces confounding to only occur at higher interaction levels and importantly no confounding occurs at two level interactions. The fractional factorial design generated 20 trails with 16 different combinations of the variables and 4 centre points (Tables 3.2 and 3.4).

During each trial 4 mL of beads were added to 10 L of freshwater in the reservoir. The beads were mixed thoroughly and a sample taken to determine the initial bead concentration (See Appendix 1). The combinations of variables, as defined by the fractional factorial model were applied. After the trail the collected foam was allowed to collapse, the volume of liquid was measured, and samples were taken from the harvested material. The bead density was determined using an improved Neubauer hemocytometer. The concentration factor was defined using equation (1).

$$CF = \frac{\left(\text{cells cm}^{-3}\right)_{collector}}{\left(\text{cells cm}^{-3}\right)_{culture\,chamber}} \quad (1)$$

Pareto charts, normal probability plots and analysis of variance (ANOVA) were used to determine the statistical significance of each individual variable, their combinations and to exclude insignificant variables at an alpha level of 0.1 using Minitab software (release 15, Minitab Inc., State College, PA).

Table 3.1 Independent variables for the fractional factorial design. The '+' and '-' apply to the high and low factor levels in the corresponding rows. Surfactant concentration is coded as follows for CTAB (mg L^{-1}) and Ecover© (mL L^{-1}): **A)** 20, 0.15 **B)** 30, 0.20

Variable			Variable range
(A)	Air flow rate (L h^{-1})	+	100
		-	50
(B)	Batch run time (min)	+	45
		-	30
(C)	Column height (m)	+	1.5
		-	1
(D)	Surfactant concentration	+	B
		-	A
(E)	Surfactant type	+	CTAB
		-	Ecover©

3.2.5 Verification of fractional factorial experiments

The data generated from trials using the latex beads were directly compared against trials harvesting microalgae. A stock culture of *Chlorella* sp. was obtained from Blades Biological Ltd, Kent, UK and grown using a proteose

peptone medium as recommend by the Culture Collection of Algae and Protozoa (CCAP). The medium contained the following (per litre): 0.2 g $MgSO_4.7H_2O$, 0.2 g K_2HPO_4, 2 g KNO_3 and 1 g proteose peptone (oxoid L85) (Sigma Aldrich). The principle organic compounds present in domestic wastewater are proteins, carbohydrates, lipids and products of their decomposition (Raunkjær et al. 1994). A proteose peptone media, can therefore emulate many features of wastewater unlike many commonly used sterile media types. The cultures were grown in 20 L polycarbonate carboys (Nalgene), at 19°C in a non-sterile environment. The light regime (16 L: 8 D) comprised of a combination of warm and cold fluorescent lights with a mean luminance of between 2200–2800 Lux. Mixing and gas transfer was facilitated by an aquarium pump. The operating conditions using latex beads that yielded the highest, mid and lowest concentration factors were replicated for foam harvesting experiments using the microalgae at similar cell densities to the latex beads. During each harvest 2 litres of concentrated microalgae culture were added to 7.5 litres of tap water to achieve a cell concentration of $4.1x10^7 \pm 9.6x10^6$ and a dry weight of 0.11 ± 0.08 g L^{-1}, which is similar to the biomass productivity produced currently in open pond systems (Adesanya et al., 2012; Lardon et al., 2009).

3.2.6 Central composite design

Once verification using microalgae had occurred a central composite design (CCD) with five centre points was used to optimise the significant factors identified in the screening test (Table 3.2). Table 3.2 displays the five coded levels (i.e. -0.71, -1.00, 0, +0.71, +1.00) and their actual associated values of the significant factors gained from the screening tests using the latex beads.

Table 3.2 Coded levels and their actual associated values tested in the central composite design (CCD)

X (coded levels)	***X_1 (column height, m)***	***X_2 (surfactant concentration, mg L^{-1})***
-1.00	0.29	7.93
-0.71	0.50	10.00
0.00	1.00	15.00
+0.71	1.50	20.00
+1.00	1.71	22.07

The experimental results were fitted to a second order polynomial equation in order to predict the optimal points; equation (2) is to be used for two parameters:

$$\textit{Predicted response} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (2)$$

In which the experimental variables are represented by X_1 and X_2; β_1, β_2, β_{11}, β_{22}, and β_{12} are the coefficients of the model equation and ε represents the error term (Tukai et al., 2002); β_{11} and β_{22} values represent the square effect of each of the parameters. A backward stepwise elimination technique was used to determine the statistical significance of each coefficient (Bosma., 2003). The probability value (P-value) generated by each coefficient was used to distinguish noise signals from legitimate signals. All the coefficients produced were put into the polynomial model equation and then the coefficient with the highest p-value was selectively removed. Only coefficients with a p-value equal to or less than 0.10 should remain (working with an alpha value of 0.10), unless they are needed within a significant hierarchy term. The statistic test factor (F-test), coefficient of multiple determination (R^2) was used to evaluate the quality of fit for the polynomial equation. During each trial air flow rate was held at 100 L h^{-1}, the batch run time was 15 minutes and CTAB was used as the surfactant type. The ideal harvest should have a high concentration factor, a low total

harvest volume yield and a high biomass yield, for the CCD these were measured. The concentration factor and harvest volume was measured as described previously in this chapter. The harvest yield is measured by drying pre prepared low ash filter papers (Whatman No. 42) at 102°C for a minimum of 2 hours, or until a constant weight was gained. The papers were then left to cool in a desiccator and weighed to the nearest 0.1 mg using a Mettler® Toledo AL54 precision balance (Mettler, Columbus, OH). A minimum volume of 1 mL of harvested material was filtered and the volume used noted. The filter papers were then placed back in the oven for a further 2 hours at 102°C, left to cool in a desiccator and weighed to the nearest 0.1 mg. The biomass yield is calculated using equation (3):

$$\text{mg /ml} = \frac{(D2-D1)}{Volume\ of\ harvest\ material\ filtered} \quad (3)$$

Where D1 is the dry weight of the filter paper before filtration and D2 is the dry weight of the filter paper post filtration.

3.3 Results and discussion

3.3.1 Screening of the significant factors

Screening tests are often used to identify the most significant effecting variables from those less important (Myers and Montgomery, 2002). During this work a fractional factorial design (2^{5-1}) was used in batch trials to determine the influence of five variables on the product concentration factor (air flow rate, batch run time, foam column height, surfactant concentration, and surfactant type). Table 3.3 shows the design matrix in coded units and experimental results (dependent variables) for the concentration factors achieved. ANOVA revealed that the obtained model was of significance, this was evaluated by p-

value in the lack-of-fit test. A p-value lower than 0.05 shows lack-of-fit, in the data of the analytical curve a p-value of 0.539 was generated (Table 3.4).

Pareto charts have previously been described as a useful tool for highlighting the most significant variables (Haaland, 1989; Nasri Nasrabadi and Razavi, 2010), therefore they were applied along with a normal probability plot to determine the most significant factors (Figures 3.3 (A) and (B)). An R^2 (= 90.83%) shows that only 9.17% of the total variability cannot be explained by the model. The adjusted determination coefficient was also high (R^2_{adj} = 85.48%), which suggests the experimental data and any predicated value of response are in agreement. Due to the R^2 and R_{adj} values an alpha level of 0.10 was chosen in order to highlight all possible significant variables and variable interactions.

In a Pareto chart the length of each bar is defined as being proportional to the absolute value of its associated regression coefficient or estimate effect (Nasri Nasrabadi and Razavi, 2010). The bars are ordered accordingly to the size of the effect. The vertical line represents the 90% limit, therefore any bar crossing the line is of statistical significance. Figures 3.3 (A) and (B) reveal that of the five variables tested only surfactant type, column height and surfactant concentration had a significant effect (Table 3.4).

Figure 3.3(B) demonstrates that surfactant concentration has a negative effect on the concentration factor; therefore at higher surfactant concentrations lower harvest concentration factors were gained. Higher surfactant concentrations produce wetter foams made up of small spherical shaped bubbles (Figure 3.2). The wetter foams occur as increasing the surfactant concentration results in a reduction of surface tension; this reduces bubble sizes resulting in the production of more foam, increasing the amount of liquid trapped between the foam lamellae. Neethling (2002) demonstrated with modelling a clear link between liquid velocity within the foam and the proportion of liquid within the foam.

Table 3.3 Fractional factorial design matrix and experimental results. Variables as described in Table 3.1

	Variable					
Experimental trial number	**A**	**B**	**C**	**D**	**E**	**Concentration factor**
1	-1	+1	-1	-1	+1	20.19
2	+1	+1	+1	-1	-1	18.03
3	+1	+1	+1	+1	+1	74.22
4	+1	+1	-1	-1	+1	22.19
5	+1	+1	-1	+1	-1	1.03
6	0	0	0	0	-1	6.88
7	-1	+1	+1	-1	+1	125.25
8	-1	+1	+1	+1	-1	5.82
9	+1	-1	+1	+1	-1	3.32
10	0	0	0	0	+1	72.61
11	-1	-1	+1	-1	-1	22.36
12	-1	-1	+1	+1	+1	70.20
13	+1	-1	+1	-1	+1	85.53
14	0	0	0	0	+1	98.62
15	+1	-1	-1	-1	-1	15.08
16	-1	-1	-1	-1	+1	35.05
17	-1	+1	-1	1	1	42.62
18	-1	-1	-1	+1	-1	1.66
19	0	0	0	0	-1	4.11
20	+1	+1	-1	-1	+1	22.19

Table 3.4 Analysis of variance (ANOVA) of the estimated effects and coefficients obtained for concentration factor according to the experimental design in Table 3.3

Source of variation	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Main effects	5	20375.9	4075.2	20.49	<0.001
Two-way interactions	1	2943.9	2943.9	14.80	0.002
Residual error	12	2386.6	198.9		
Lack-of-fit	10	2044.5	204.5	1.20	0.539
Pure error	2	342.1	171.0		
Total	19	26024.8			

Determination coefficient (R^2) = 90.83%

Adjusted determination coefficient (R^2_{adj}) = 85.48%

Variable	Coefficient	p-value
Constant	35.581	<0.001
Air flow rate	-4.813	0.197
Batch run time	3.088	0.398
Column height	15.011	0.001
Surfactant concentration	-7.379	0.058
Surfactant type	27.728	<0.001
Column height*Surfactant type	13.564	0.002

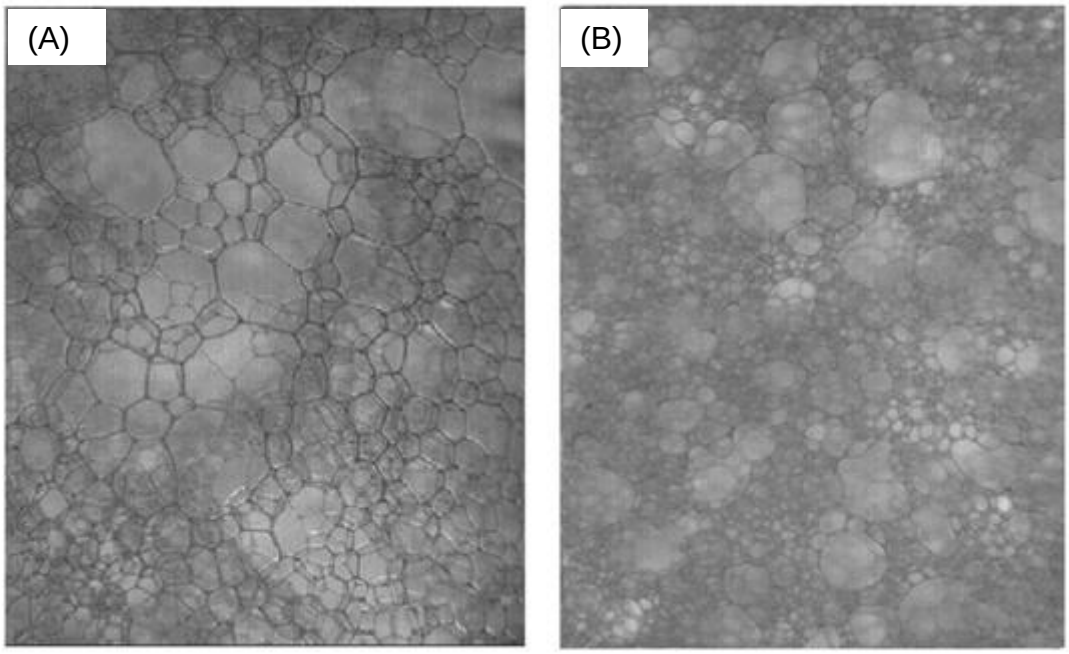

Figure 3.2 Foam characteristics are significantly different with changing surfactant concentration. **A)** 0.1mL L^{-1} of Ecover© produces bubbles with a polyhedral shape **B)** 0.2 mL L^{-1} of Ecover© produces bubbles with a much more spherical shape.

It is therefore evident that using lower levels of surfactant allow for dry foams, made up of polyhedral shaped bubbles carrying lower volumes of liquid between the lamellae. Foams produced with lower surfactant concentrations have a lower liquid content increasing the residence time of the foam within the column, which allows more opportunities for drainage of the liquid in the films. A number of previous studies have been conducted on the affect that surfactant concentration has on the separation of algal cells using foam flotation (Chen et al., 1998; Liu et al., 1999; Phoochinda and White, 2003). In all of these studies the harvest efficiency was measured in percentage of algal cells removed. It was concluded that the removal percentage increased with increasing surfactant concentration. Csordas and Wang (2004) summarised the difference between harvest efficiency and concentration factor by stating; harvesting efficiency is an increasing function of foam volume and concentration factor is a decreasing function of foam volume.

The variable with the largest effect was surfactant type (p=<0.001) the normal probability plot (Figure 3.3(B)) shows that the surfactant type affects the concentration factor in a positive way. Therefore using cationic CTAB instead of the anionic Ecover© significantly increased the concentration factor. Cationic CTAB can be adsorbed onto the negative surface of particles (Paria and Khilar, 2004); this therefore increases the hydrophobicity of the once hydrophilic solid–liquid interface. The cationic surfactant may also create electro-static interactions between the gas bubbles and the harvest particles improving flotation (Liu et al., 1999). This result confirms the findings of previous studies, which investigated the effect of surfactant type on the separation of algae via foam flotation (Chen et al., 1998; DeSousa et al., 2006; Henderson et al., 2008b; Liu et al., 1999; Phoochinda and White, 2003; Phoochinda et al., 2004).

Column height had the second largest effect on concentration factor (p=0.001). The normal probability plot (Figure 3.3 (B)) shows that the column height affects the concentration factor in a positive way. Therefore a taller column significantly increased the concentration factor. In previous work conducted on protein removal it was concluded that the concentration of protein increases with foam height (Brown et al., 1999a; Uraizee and Narsimhan, 1996). This was because the greater foam heights allow greater residence times, therefore allowing improved opportunity for liquid drainage (Morgan and Wiesmann, 2001). Standardised effects are used to convey the size of an effect relative to the variability in the trails.

The interaction between column height and surfactant type was also significant (p=0.002). Contour plots (Figure 3.4) containing interpolated data are an effective way of demonstrating the interaction between key variables. In Figure 3.4 column height and surfactant concentration change within the experimental ranges, while batch run time and air flow rate are fixed. The contour plots are broken down into two to show the changes in concentration factor using the two different surfactant types.

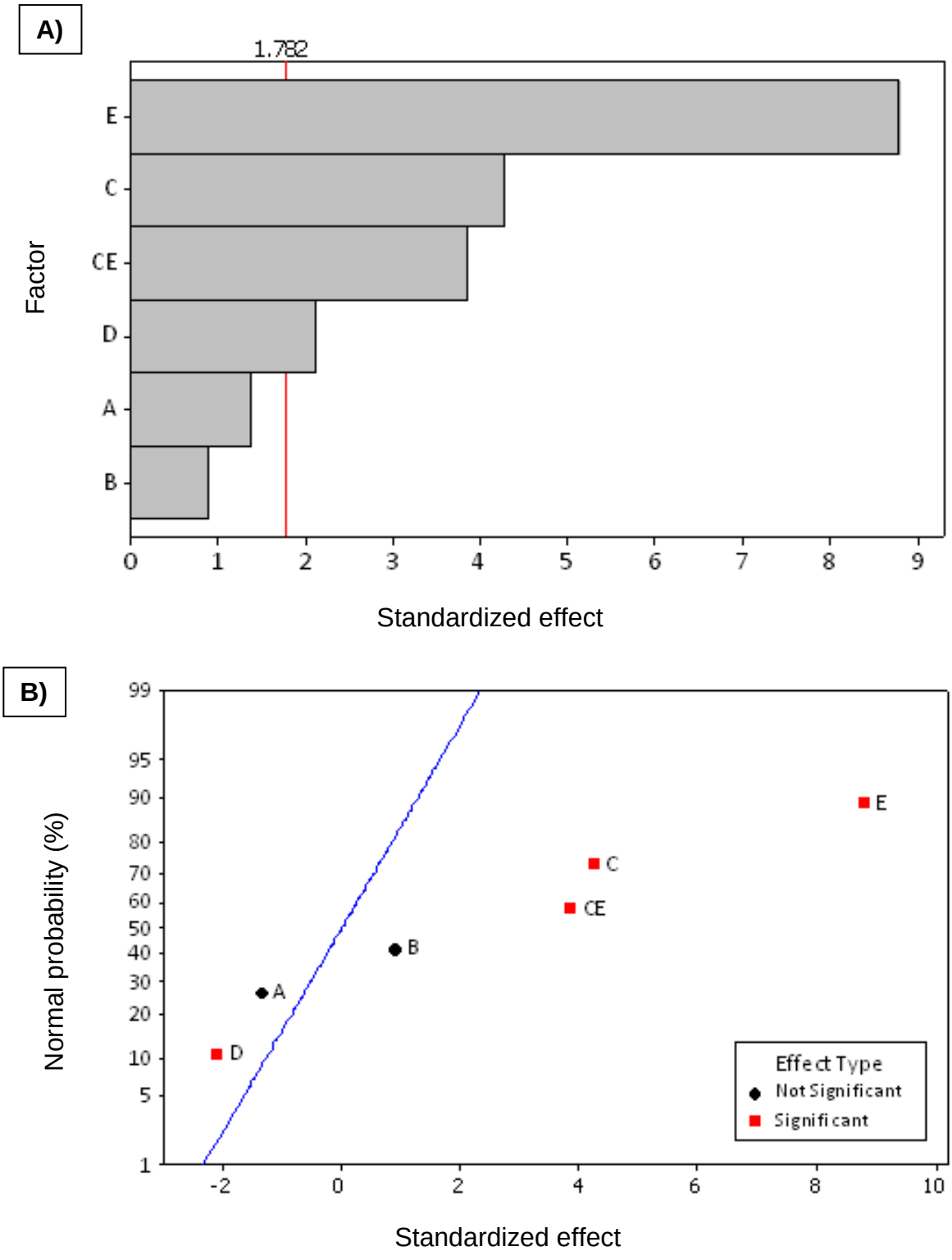

Figure 3.3 A) Pareto chart of the standardised effects gained (Alpha = 0.1). **B)** Normal Plot of the main effects. Symbols: Circles are non-significant factors ($p>0.10$) and squares are significant ($p < 0.10$). Factors are coded as follows: A: air flow; B: batch run time; C: column height; D: surfactant concentration; E: surfactant type; and CE: interaction between column height and surfactant type.

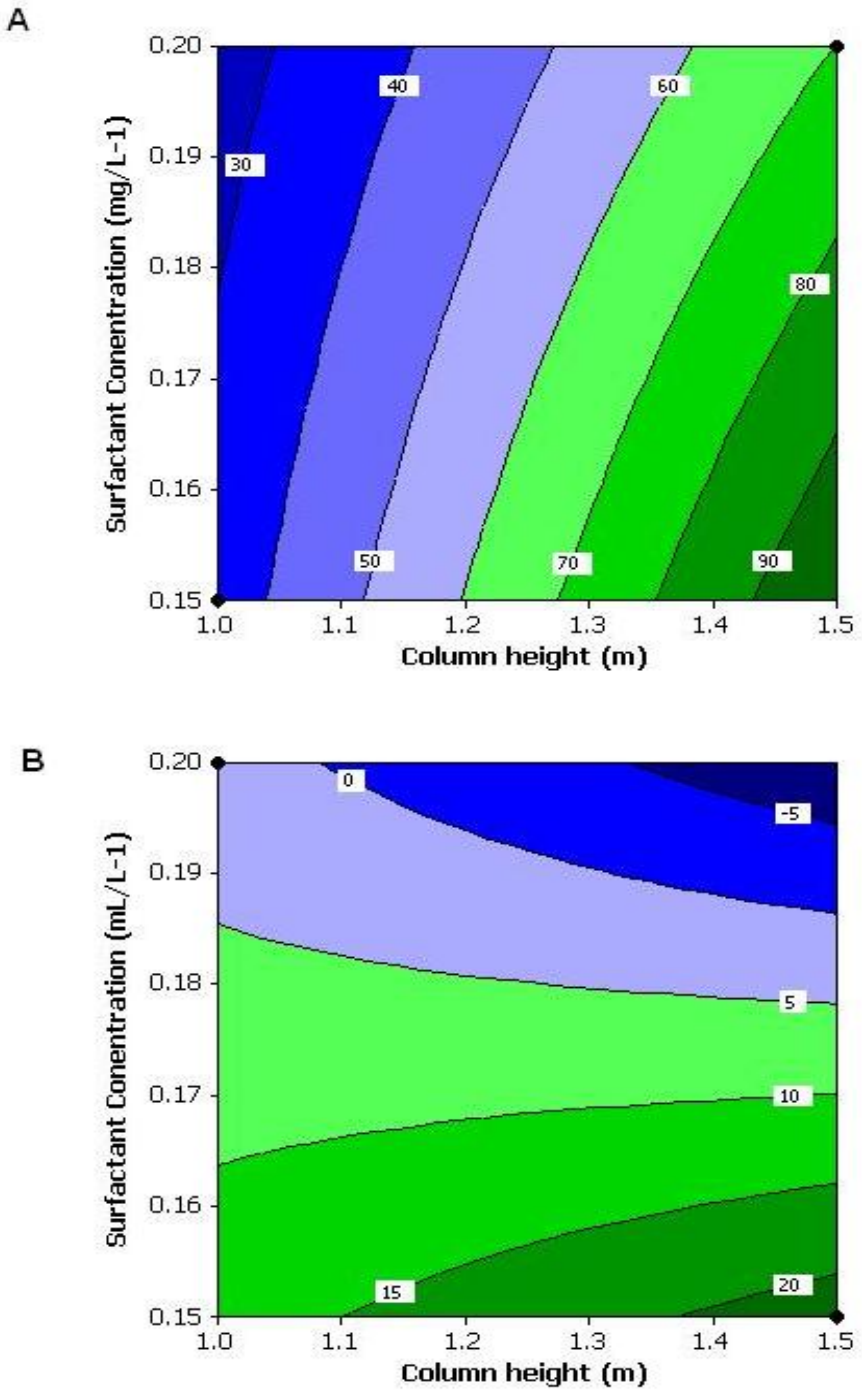

Figure 3.4 Contour plots for the model equation fitted to the data. **A)** Hold values: air flow = 50, run time = 30 min, surfactant type = CTAB; **B)** hold values: air flow = 50, run time = 30 min, surfactant type = Ecover©. The dark green to blue colour gradient represents changes in the concentration factors achieved. Dark green represents the highest concentration factors while dark blue represents the lowest.

The contour plots reveal that the combination of CTAB and high column heights yields concentration factors of over 90. The plots also reveal that to a certain extent an increase of column height can counteract the effects high surfactant concentrations have on the harvest concentration factor. Again this is due to an increase in the foam residence time.

3.3.2 Validation of the fractional factorial design using microalgae

To assess the validity of using the polystyrene latex beads and to confirm the work of the fractional factorial designs, the trials that yielded the highest, mid and lowest concentration factors were replicated using microalgae. The trials were conducted in replicates of four, the variables that were used for each trial can been seen in Table 3.5. The results from the microalgae trials were compared to those gained using the polystyrene latex particles under the same variable conditions (Figure 3.5). The concentration factors with the microalgae were not significantly different to the latex bead trials. This means there is a correlation with the significant variables shown in the fractional factorial model with the microalgae. Large variance of concentration factor occurred while harvesting microalgae under the same conditions; this is due to the addition of unknown variables within the culture. This was also noted by Csordas and Wang (2004) using foam flotation to harvest *Chaetoceros spp.* Concentration factors as high as 225 were recorded while harvesting the microalgae which suggest that the concentration factor could be increased even further by adjusting the other variables within the algal cell culture such as cell age, lipid content and growth media.

Table 3.5 The variable levels that yielded the highest, mid and lowest concentration factors during the fractional factorial design as used during microalgae trails

Variable	Yield		
	High	**Mid**	**Low**
Air flow rate (L h^{-1})	50	50	100
Run time (mins)	45	45	45
Column height (m)	1.5	1	1
Surfactant concentration (mg L^{-1})	20	30	0.3
Surfactant type	CTAB	CTAB	Ecover
Concentration factor gained with beads	125.25	42.62	1.03

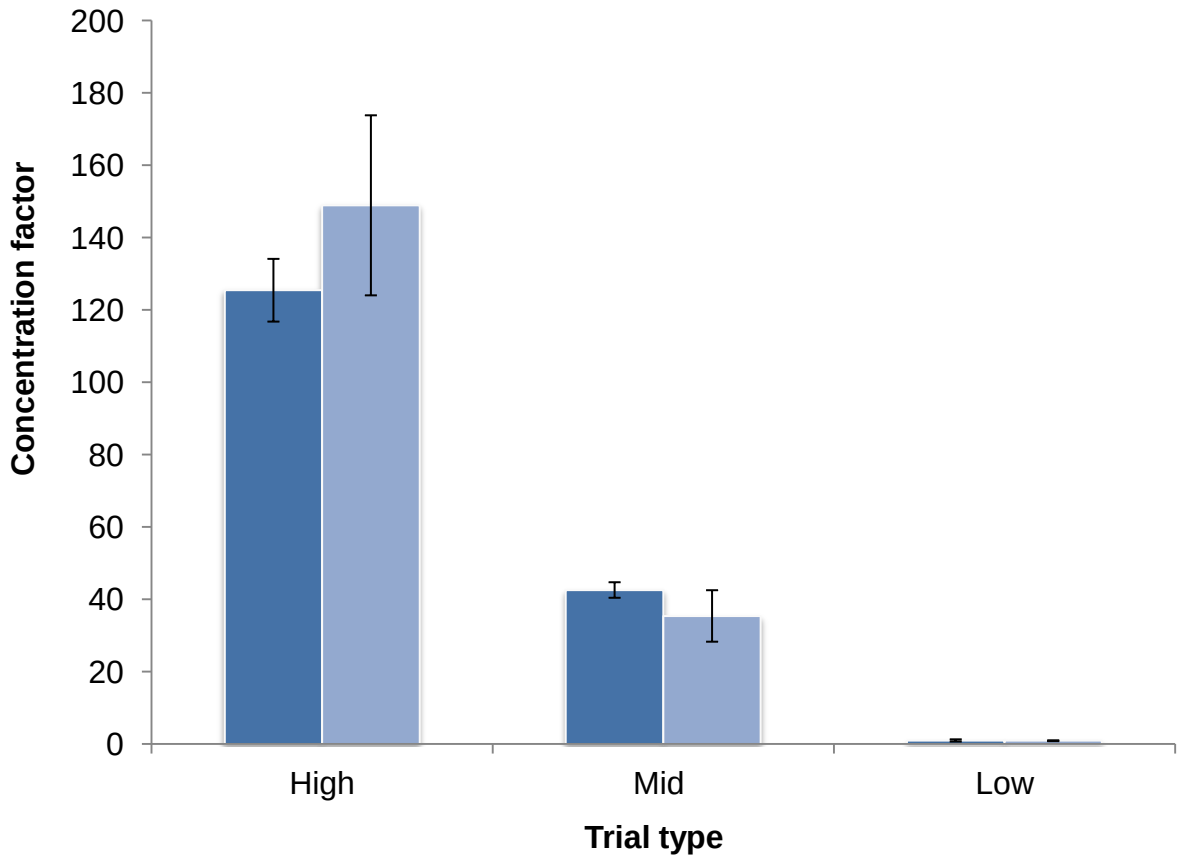

Figure 3.5 A comparison of the concentration factors gained from the microalgae trials (light blue) with those gained using the polystyrene latex (dark blue) particles under the same variable conditions.

Microalgae are sources of natural surfactants, although the extent and form of production varies between species and in response to culture age and condition (Zutic et al., 1981). Production is believed to be maximal during senescence when cell architecture and membrane permeability can be significantly compromised. Nevertheless, Zutic et al., (1981), using a number of marine species concluded that surfactants were produced *in situ* during exponential growth. Using a *Chlorella* sp. Levin and co-workers (1962) found that by adjusting pH to around 3, algae-derived surfactants produced sufficient foam for effective harvesting. In addition they found that low air flow rates and low feed concentrations produced the largest cell concentration changes between the foam column inlet and outlet. Csordas and Wang (2004) through pH regulation successfully harvested *Chaetoceros* sp. from a large scale photo-

bioreactor via foam flotation using *in situ* surfactant production only. Whereas harvesting based on *in situ* surfactant production is technically feasible, to conduct this at scale would require a closed system to achieve adequate pH control. From the perspective of bulk biomass production, currently the only economically viable production technologies involve open systems such as raceways and lagoons (Borowitzka, 1999; Schenk et al., 2008) that present significant logistical problems for large-scale pH manipulation. Even within a closed system the culture will be subject to a number of variables that may complicate reliable surfactant production including pH, cell age and zeta potential. Csordas and Wang (2004) concluded that the large variances in their harvest data were attributable to unmeasured variables caused by changes in cell age. Unknown interactions may confound *in situ* surfactant production and consequently impact harvesting efficiency.

3.3.3 Optimisation of significant variables: central composite design

Optimisation of significant variables was determined with a central composite design using *Chlorella* sp. The design matrix and results gained for the central composite design are presented in table 3.4. Column height and surfactant concentration (obtained in the screening tests) were used to optimise the harvesting results, each at five levels (-1.00, -0.71, 0, +0.71, +1.00) and with five replicates at the centre point (Table 3.6). The replication at the centre points is a necessary feature to account for pure internal error (Myers and Montgomery, 2002).

For concentration factor a model F-value of 4.07 was found, which implies that the model was significant. There is a 4.42% chance that a model F-value this large could be gained due to noise. Only one linear term, column height, was significant ($p= 0.0288$), however, the coefficient of multiple determination (r^2) is 0.57, indicating that the variables do not account for a significant proportion of the variation within the data. The polynomial model describing the correlation between concentration factor and the two variables was represented as follows:

$$\textit{Concentration factor} = 43.05 + 41.2X_1 - 24X_2 - 39.7X_{12} \quad (4)$$

Where X_1 is column height, X_2 is surfactant concentration and X_{12} is the interaction between column height and surfactant concentration.

Table 3.6 Central composite design experimental matrix and results (X_1: column height, X_2: surfactant concentration)

Trial number	X_1	X_2	Concentration factor	Biomass yield (mg mL^{-1})	Total harvest Volume (mL)
1	-0.71	-0.71	25.4	2.01	17
2	0.71	-0.71	230.4	24.7	1
3	-0.71	0.71	8.2	0.85	169
4	0.71	0.71	54.3	2.01	53
5	-1.00	0.00	8.8	0.76	210
6	1.00	0.00	64.2	1.53	48
7	0.00	-1.00	0	0	0
8	0.00	1.00	1	0.61	400
9	0.00	0.00	31.6	1.35	73
10	0.00	0.00	29.8	1.45	74
11	0.00	0.00	30.9	1.51	71
12	0.00	0.00	33.2	1.16	70
13	0.00	0.00	33.1	1.17	71

For the total harvest volume a model F-value of 5.63 signifies the model is significant and that there is only a 1.88% chance that a model F-value this large would occur due to noise. One linear term, Surfactant concentration, was significant (p= 0.0051). Again, a low r^2 value is gained, 0.65, indicating the variables do not account for a significant proportion of the variation within the data. The polynomial model describing the correlation between total harvest volume and the two variables was represented as follows:

$$\textit{Total Harvest Volume} = 96.69 - 45.1X_1 + 96.2X_2 - 25X_{12} \quad (5)$$

Where again, X_1 represents the column height, X_2 represents surfactant concentration and X_{12} represents the interaction between column height and surfactant concentration.

For biomass yield a model F-value of 3.07 suggests that the model is significant and that there is only an 8.36% chance that the model F-value could have occurred due to noise. The two-way interaction between column height and surfactant concentration is the only significant term. A low r^2 value of 0.51 also indicates that the variables do not account for a significant proportion of the variation within the data. The polynomial model describing the correlation between biomass yield and the two variables was represented as follows:

$$\textit{Biomass yield} = 3.01 + 3.12X_1 - 2.87X_2 - 5.38X_{12} \quad (6)$$

Again, X_1 represents the column height, X_2 represents surfactant concentration and X_{12} represents the interaction between column height and surfactant concentration.

To show the influence of the independent factors on concentration factor, total harvest volume and biomass yield the quadric models were used to create response surface graphs (Figure 3.6). It is evident that the optimum for all the responses, i.e. high concentration factor (Figure 3.6 (A)), up to 230 times, high biomass yield (Figure 3.6 (B)), up to 25 mg mL^{-1} and low total volume (Figure 3.6 (C)), as little as 1 mL was produced using the highest column height of 1.5 meters and the lowest surfactant concentration of 10 mg L^{-1}.

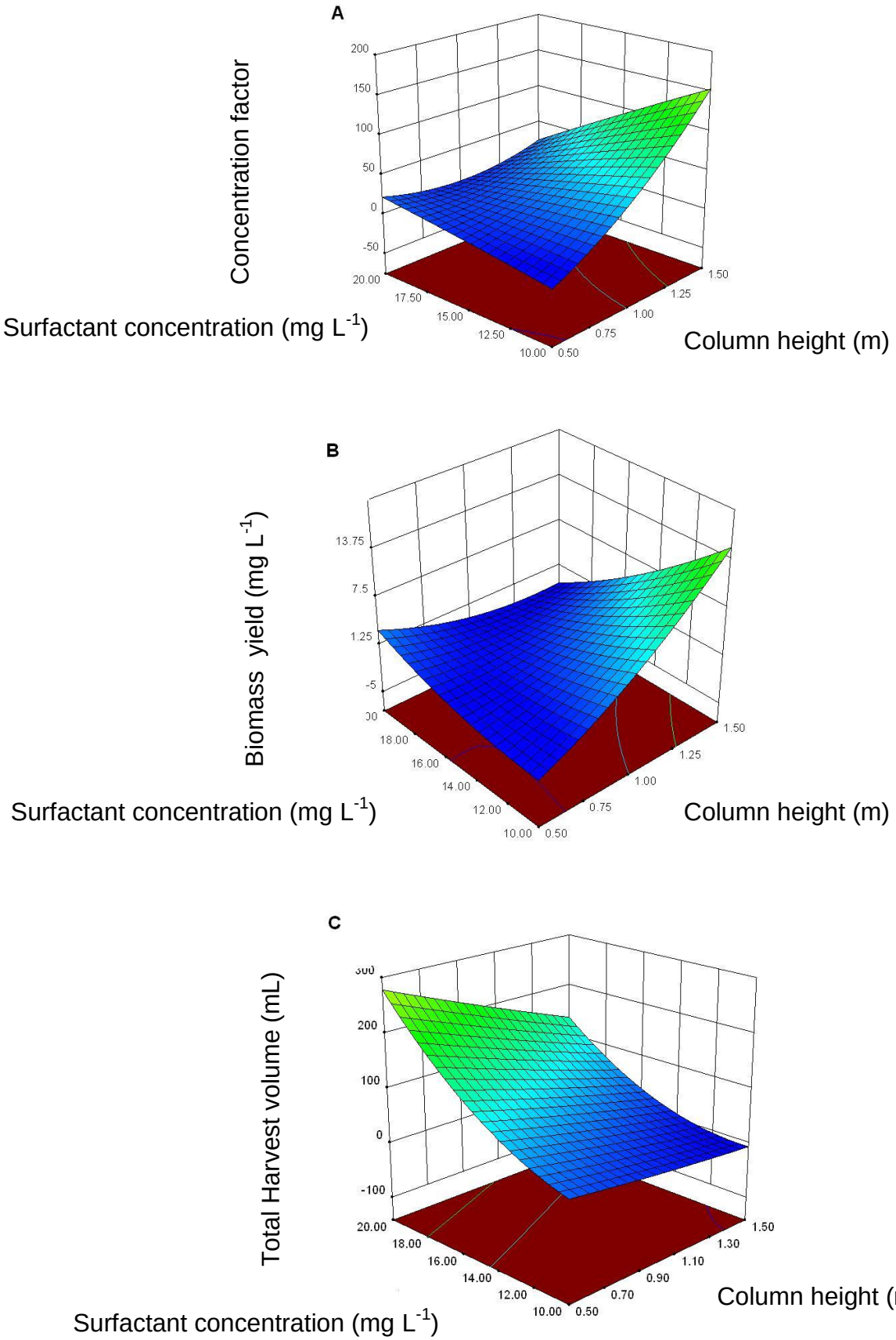

Figure 3.6 Surface plots of the model equations fitted to the data.

Examination of lack of fit, which measures the fitness of the model, gave a significant F-value for concentration factor, total harvest volume, and biomass yield, therefore the model cannot be used for predicting experimental data. Although concentration factor, total harvest volume, and biomass yield all had significant models, the presence of non significant terms, which cannot be removed from the model equation, is the most likely cause of a significant lack of fit (Tukai et al., 2002).

3.4 Conclusion

Fractional factorial experiments using latex beads combined with additional microalgae trials demonstrate that foam flotation can greatly improve the concentration factor of harvesting algal biomass, and may therefore be a potentially economical way to harvest microalgae. The fractional factorial model using the beads revealed that the highest concentration factors can be gained using the following variables and viable interactions: cationic CTAB, taller column, lower surfactant concentrations, CTAB combined with high column heights. The CCD revealed that concentration factors of up to 230 times can be produced using column heights of 1.5 meters and surfactant concentrations of 10 mg L^{-1}. This research highlights that for effective harvesting of microalgae for further processing foam residence time is an important factor, which thus far has rarely been considered.

Chapter 4

Combining micro-flotation, dispersed air flotation and foam fractionation for algal separation

Abstract

Concentration factor and biomass yield of *Chlorella sp.* were examined as a product of a foam fractionation harvesting unit. The effects of three sparger types and air supply input operating factors, affecting the bubble size, were investigated. The sparger set-ups were, lime wood sparger with linear air flow input, ceramic flat plate sparger with linear air flow input and ceramic plate sparger with an oscillatory air flow input. Bubble size, bubble rise velocity, gas hold-up, and interfacial area within the liquid pool were calculated for each operating condition. The smallest average bubble size of 621.8 ± 58.5 μm was produced using fluidic oscillation designed for microbubble generation. The ceramic spargers produce small bubbles in dense clouds, which had fast bubble rise velocities when compared to the larger bubbles produced by the lime wood sparger. The fast rise velocities of the bubbles produced by the ceramic spargers were correlated to smaller bubble residence times, gas hold-ups, and interfacial areas with the liquid pool. Concentration factor and biomass yield increased with decreasing bubble size, with the maximum concentration factor of 426.5 ± 58.5 being gained with a ceramic sparger operating under oscillatory supply flow. Higher product yields were associated with collection efficiency that increased with decreasing bubble size. Analyses of the harvest economics revealed that a foam fractionation unit using a ceramic sparger operating under a linear air flow supply could provide an extremely cost competitive bulk harvesting technology.

4.1 Introduction

Bubble size and distribution has been highlighted as one of the most important factors within the study of foam and foam fractionation (Csordas and Wang, 2004; Du et al., 2003; Wong et al., 2001). For this reason a study into the effects of bubble size as a variable within the liquid pool and the foam phase was carried out separately from the investigation of the other variables. It was thought that this variable would have an overwhelming influence on the foam fractionation process.

The bubble size within the liquid pool determines the interfacial area affecting mass transfer between the gas and liquid phases. The interfacial area, *a,* (m^2 area/m^3 of gas) can be determined via a single bubble's diameter, assuming it is spherical in shape, by the following equation:

$$a = \frac{4\pi R_B^2}{(\frac{4}{3})\pi R_B^3} = \frac{3}{R_B} = \frac{6}{d} \tag{7}$$

where R_B is the bubble's radius and d the diameter (Du et al., 2001). However during the foam phase liquid drainage occurs, which commonly causes bubbles to form a more dodecahedron-like shape (Uraizee and Narsimhan, 1996), therefore equation (7) should be modified to (Du et al., 2001):

$$a = \frac{6.59}{d} \tag{8}$$

From equations (7) and (8) it is evident that smaller bubble diameters provide a larger interfacial area, and therefore have advantageous mass transfer rates when compared to larger bubbles. Zimmerman et al. of Sheffield University have been extremely influential in the area of micro-bubble generation over recent years (Al-Mashhadani et al., 2012; Hanotu et al., 2012;

Zimmerman et al., 2011; Zimmerman et al., 2009; Zimmerman et al., 2008). Zimmerman's work is based on the Coanda effect, which uses a fluidic oscillator to alter a continuous air flow into an oscillatory flow (Hanotu et al., 2012; Tesar and Bandalusena, 2011; Zimmerman et al., 2011; Zimmerman et al., 2009; Zimmerman et al., 2008). During a linear air flow an anchoring force attaches the bubble to the exit pore of the sparger. In order to overcome this force the bubble will grow until the point where the buoyant force is greater than the anchoring force. This results in the generation of bubbles that are significantly larger than the exit pore of the sparger (Zimmerman et al., 2008). During an oscillatory flow a pulse is created, which provides a lifting force enabling the bubble to break away from the anchoring force at an earlier stage than the linear flow bubbles, producing a significantly smaller bubble (Hanotu et al., 2012).

Within the rising foam phase, smaller bubbles are closely linked to a decrease in foam drainage. This leads to dilute harvest (Li et al., 2011); resulting in an increase in energy requirements and further processing of the biomass. The foam phase therefore plays a critical role in determining the grade and recovery of the microalgae harvested (Neethking et al. 2002). Brown et al. (1999b) found that during the continuous harvest of bovine serum albumin (BSA) using foam fractionation, larger bubbles within the foam phase reduced liquid hold up, concentrating the BSA. This conclusion was also reached by Wong et al. (2001) whilst harvesting proteins. It was noted that coalescence (which occurs when the thin film between two small bubbles break, forming one larger bubble) enriched the foam, promoting higher concentrations. Coalescence is more likely to occur at higher points along the foam column as bubble sizes get larger within the foam phase (Du et al., 2001; Brown et al., 1999b; Uraizee and Narsimhan, 1996). However, when a film ruptures during coalescence there is a loss of surface area. The algae previously attached to this film either attach onto a neighbouring interface or move into Plateau boarders and can therefore act as freely moving practices, therefore coalescence may result in a fraction of the microalgae falling back into the culture chamber. Neethling et al. (2002) have been able to calculate simple estimates of material recovery using foam fractionation and noted that there is a critical height within the column, particles that fall off bubbles in this region are recovered and concentrated, however particles below this critical height will be lost.

Hanotu et al. (2012) were able to generate microbubbles as small as 86 µm using an oscillatory flow and a stainless steel baffle distributor sparger. The bubbles were extremely effective at separating *Dunaliella salina*, resulting in up to a 99.5% removal yield. However, the experiments required high metallic coagulant dosages of 150 mg L^{-1} of ferric chloride. The algal removal was conducted in a flotation cell and therefore no foam phase was formed, and the biomass concentration was not noted. In *Chapter 2* it was demonstrated that *Chlorella sp*. can be successfully harvested using a combination of foam fractionation and dispersed air flotation using as little as 10 mg L^{-1} of CTAB. Combining foam fractionation and dispersed air flotation with microbubble generation would be an attractive low cost method to harvest, concentrate, and physically separate the biomass requiring significantly less chemical additives when compared to the Hanotu et al. (2012) flotation cell technique. Using the current prices of each chemical additive (£8.60 per 100g for ferric chloride and £68.00 per 100g for CTAB), using CTAB at 10 mg L^{-1} instead of ferric chloride at 150 mg L^{-1} would reduce the cost of chemicals from £0.013 per litre to be treated to £0.007 per litre to be treated. Due to the wide range of bubble sizes that affect the characterisation of the foam fractionation process it is clear that a single average bubble size would not reflect what is occurring within the column. Therefore it is important to report the bubble size distributions in the two key areas; the liquid pool and the foam phase. It has not been reported to what extent the bubble size and bubble production method affect the bubble size in the foam phase. The ideal separation would occur when small bubbles are produced in the liquid pool and large bubbles form in the foam phase.

This chapter explores the effects of bubble size and distribution on the concentration factor, yield, and potential further processing of microalgae biomass.

4.2 Materials and methods

Bubble size can be determined by several methods; however the most commonly used are optical and acoustical techniques (Hanotu et al., 2012). Although bubble characterisation via optical techniques has been described as tedious and time consuming (Hanotu et al., 2012; Du et al., 2001) it has the

advantage of being able to measure bubble size and distribution, and to track individual bubbles over a sequence of images to determine the bubbles velocity.

4.2.1 Sparger set-ups

Three different sparger and air flow supply set-ups where used to generate differing bubble sizes; lime wood sparger with linear flow (LWLF), ceramic plate sparger with linear flow (CLF), and a ceramic plate sparger and fluidic oscillator to create an oscillatory flow (COF). In order to induce an oscillatory flow, a steady supply flow rate of 5000 L h^{-1} must be supplied to the fluidic oscillator (Hanotu et al., 2012). The air flow entering the foam fractionation unit for each sparger set-up was regulated to 100, 150 or 200 L h^{-1} with the use of a flow meter.

4.2.2 Sparger location

The liquid pool was held in the culture tank with dimensions of 22 cm x 22 cm x 21 cm (Figure 4.1). During all experimental trials the bulk liquid pool height started at a height of 20.7 cm. A clear Perspex® column with an I.D of 4.6 cm, an O.D of 5 cm, and a length of 50 cm was used in this study. Two sparger types where used; the first being lime wood with the dimensions of 2 cm x 2 cm x 5 cm in height, width, and length, respectively, with a mean pore diameter of 35.0 µm (Yang et al., 2004); The second being a ceramic flat plate sparger, with the dimensions of 2 cm x 11 cm x 11 cm in height, width, and length, respectively. The ceramic sparger had a mean pore diameter of 20.0 µm (Al-Mashhadani et al., 2012). Both sparger types were located centrally in the culture chamber with the air entering the spargers 2 cm from the bottom of the liquid pool. However, the ceramic flat plate sparger had an inbuilt elbow to distribute the air evenly across the ceramic plate; this resulted in the sparger being located 6 cm from the bottom of the unit (Figure 4.1). Therefore the effective column height between the top of the lime wood and top of the restrictor was of 79 cm compared to 69 cm for the ceramic flat plate sparger.

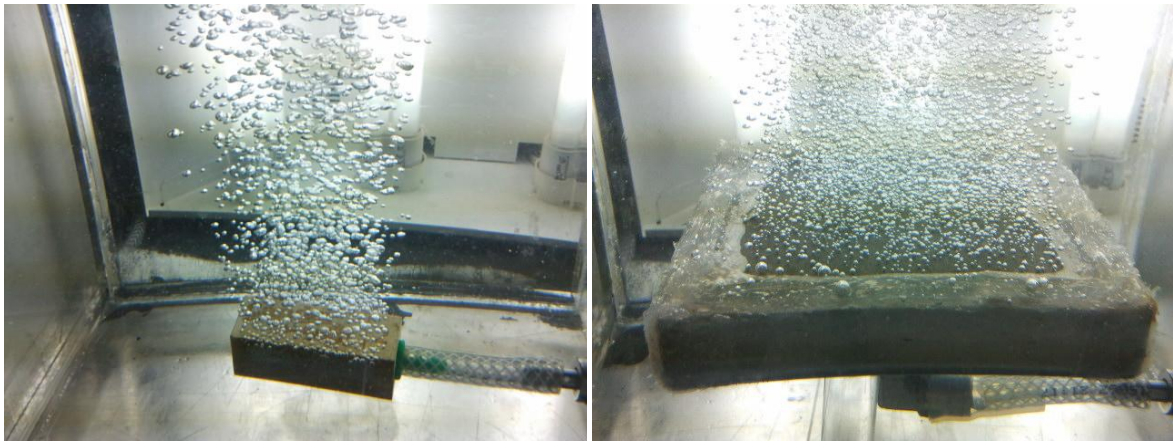

Figure 4.1 Fine bubbles (1.20 ± 0.155 mm) are produced from the lime wood sparger with linear flow (left). Microbubbles (0.621 ± 0.059 mm) are produced from a ceramic flat plate sparger with an oscillatory flow (right).

4.2.3 Bubble photographs

An Olympus *i*-Speed 2 camera coupled to a Computar 18-80 lens (Nº. 5001568) were used to photograph the bubbles from a distance of 22.8 ± 0.23 cm. A ruler was placed on the outside wall of the liquid chamber or column to set the scale of all photographs. The camera typically took an image of 800 x 600 pixels. To avoid the magnification effect only bubbles nearest the walls were photographed (Wong et al., 2001). For all trials 10 mg L^{-1} of CTAB was dissolved into the water. A schematic representation of the flotation unit set-ups to be used with an oscillatory flow is shown in Figure 4.2. Initially photographs of the liquid pool were taken; the camera was set-up 4 cm above the bottom of the chamber and a minimum of 200 pictures where recorded at 1000 frames per second (fps). For each sparger and air flow set-up, pictures were taken of the bubbles formed at flow rates of 100 L h^{-1}, 150 L h^{-1}, and 200 L h^{-1}. Each air flow rate and sparger set-up were then repeated at the camera height of 46 cm above the bottom of the chamber, the midpoint of the column. Again a minimum of 200 pictures where recorded at 1000 fps. Pictures were taken at 0-30 minutes at 5 minute intervals to gain information on how bubble size within the foam phase changes as the surfactant becomes exhausted, and if the bubbles

in the foam phase are affected by the bubble size produced within the liquid pool.

When photographing the bubbles, the flotation unit was back-lit using ‘Nebula 4’ hydroponic plant lights, which are each fitted with four Philips PL-L 4P 55W florescent lamps, and the lights are mounted vertically. This helped when focussing the camera and gaining a well-defined image.

4.2.4 Analysis of bubble size and velocity

Pictures of the bubbles were converted into high-quality JPEG images using Olympus i-SPEED Software Suite. The JPEG images were then opened with the visual image analysis software Image J (National Institutes of Health, Bethesda, Maryland, USA). As soon as the images were opened they were calibrated by measuring the known length of the ruler and equating this to the measured length in pixels. Usually 200-300 bubbles were measured with care taken not to measure the same bubble more than once. Image J can only display the measurement as the bubble’s circumference; this was then converted into the bubble’s diameter. The bubble velocity for bubbles within the liquid pool and the foam phase was determined from the images by recording distance moved by the bubble in a known time frame. Each bubble velocity value reported is an average of 40 values.

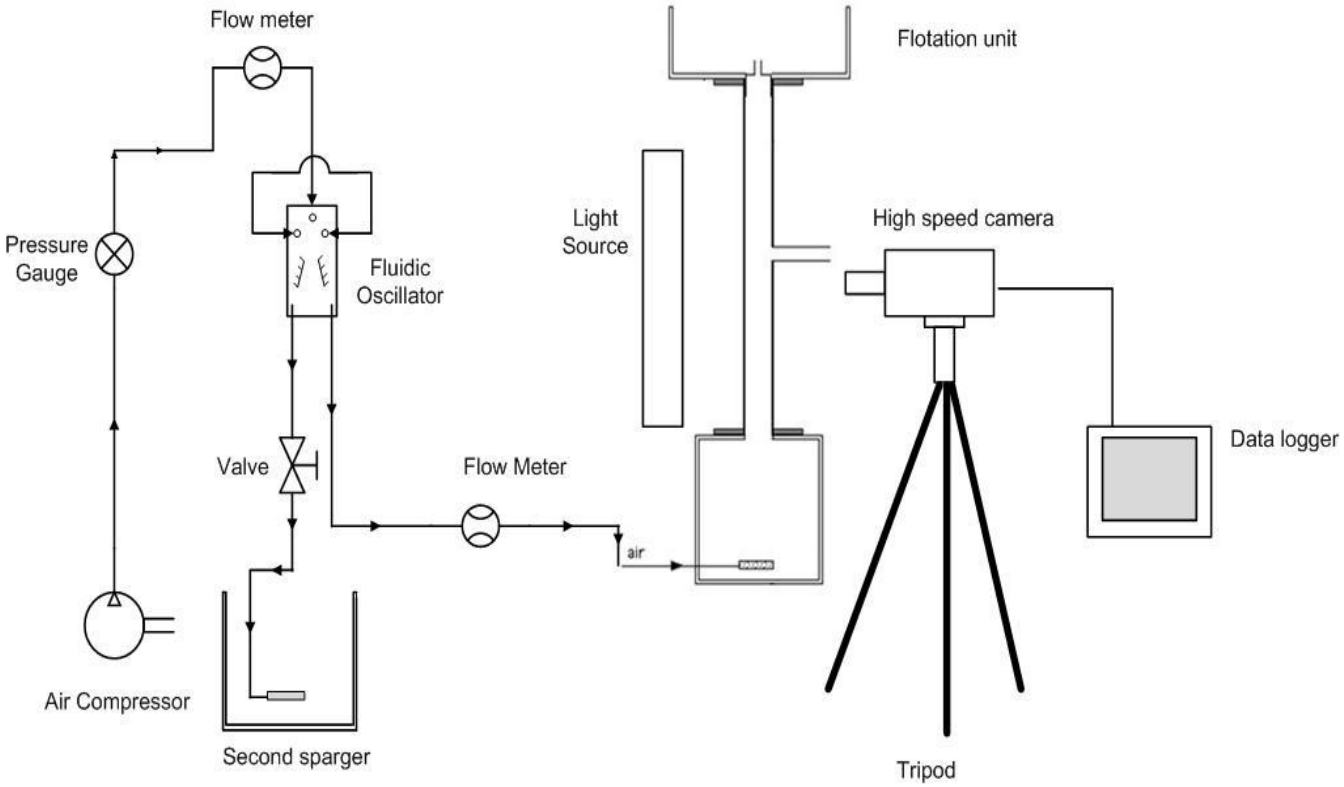

Figure 4.2 Experimental set-up used to measure the bubble size distribution and velocity. This set-up is for use with an oscillatory flow. For a linear flow the air would go from the compressor to the pressure gauge, through one flow meter and into the bottom of the flotation unit.

4.2.5 Gas hold up and interfacial area

As described previously, the interfacial area is a key parameter for the absorption of solutes (Du et al., 2001). Within the liquid pool the interfacial area (A, m^2) is related to the gas holdup to calculate the area of bubbles within the liquid dispersion. This can be achieved using equation (9) modified from Wong et al. (2001):

$$A_l = \frac{6A_{sparger}H_L\varepsilon}{d_{32}} \quad (9)$$

where H_L is the liquid pool height, which is measured from the top of the sparger to the top of the liquid pool, in this case 0.167 m for the lime wood sparger and 0.127 m for the ceramic flat plate sparger. The bubble distribution is not dispersed evenly throughout the liquid pool, but instead the bubbles rise as a column from the sparger (Figure 4.1). Therefore if $A_{sparger}$ is the cross sectional area of the sparger used, this would be 0.001 m^2 for the lime wood sparger and 0.0121 m^2 for the ceramic sparger. The volume fraction of the gas holdup is represented by ε. The average Saunter mean diameter of the bubble size distribution, or d_{32}, can be calculated using equation (10), where N is the total number of bubbles sampled and d_i is the diameter of individual bubbles (Du et al., 2001).

$$d_{32} = \frac{\sum_{i=1}^{N} d_i^3}{\sum_{i=1}^{N} d_i^3} \tag{10}$$

The volume fraction of the gas holdup represented by ε in equation (9), and can be estimated using equations (11), (12), and (13) (Nedeltchev and Schumpe, 2008; Guy et al., 1986):

$$u_D = \frac{Q_{gas}}{A_{sparger}} \tag{11}$$

$$\frac{u_D}{\varepsilon} - \frac{u_c}{(1-\varepsilon)} = V_B \tag{12}$$

$$\varepsilon = \frac{u_D}{V_B} \tag{13}$$

in which u_D is the superficial gas velocity and Q_{gas} is the volume flow of gas, m^3/s^{-1}. In equation (12) u_c is the liquid velocity and V_B represents the bubble velocity. u_c was assumed to be 0, due to the lack of facilities to measure the actual velocity, therefore ε is only ever considered as an estimate. Equation (12) can be simplified into equation (13), where the bubble velocity, V_B was calculated via the method previously discussed in *4.2.4.* Equations (9) and (10) assumed that the bubble is spherical. If a number over 1 is calculated for the gas holdup, ε then 1 will be used in equation (9). To compensate for the dodecahedron shape of bubbles within the foam phase equation (9) can be modified to:

$$A_l = \frac{\mathbf{6.59} A_{column} C_L}{d_{32}} \quad (14)$$

Here ε is assumed to be 1 because the faction of water within the column will be insignificant compared to the fraction of air. A_{column} is the cross-sectional area of the column, which in this case is 0.00166 m^2 and C_L is the column length, which is 0.5 m.

4.2.6 Microalgae separation

The growth conditions for the microalgae used have been previously described (see 3.2 materials and methods). During foam flotation 2 litres of concentrated microalgae culture were added to 7.5 litres of tap water to give a cell concentration of $4.1 \times 10^7 \pm 9.6 \times 10^6$. The process set variables were: air flow rate = 100 L h^{-1}, batch run time = 30 minutes, surfactant concentration = 10 mg/L, surfactant type = cationic CTAB. Three different sparger types and flow type set-ups (as described in 4.2.2) were used to determine the effect of bubble size on microalgae separation. Each harvest was conducted with 4 replicates at room temperature.

4.2.7 Performance criteria

Foam fractionation performance of microalgae harvest/removal is often quoted in terms of recovery; the fraction of microalgae recovered from the culture (Hanotu et al., 2012; Phoochinda and White, 2005; Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998). The correct performance criteria should be used for the separation process (Wong et al., 2001). Recovery is commonly used when removing microalgae from wastewater during water treatment and purification, where high recovery and low algal residues are desired. However, for feedstock recovery, high yield and biomass concentration are desired. Yield (mg mL^{-1}) and concentration factor, also commonly referred to as enrichment, are therefore, the most appropriate performance criteria to use.

After each the trial the collected foam was allowed to collapse, the volume of liquid was measured, and samples were taken from the harvested material. The cell density was determined using an improved Neubauer hemocytometer. The concentration factor was calculated using equation (1) (page 63). The harvest yield is gained by drying pre-prepared low ash filter papers (Whatman No. 42) at 102°C for a minimum of 2 hours, or until a constant weight was reached. The papers were then left to cool in a desiccator and weighed to the nearest 0.1 mg using a Mettler® Toledo AL54 precision balance (Mettler, Columbus, OH). A minimum volume of 1 mL of harvested material was filtered and the volume used noted. The filter papers were then placed back in the oven for a further 2 hours at 102°C, and then left to cool in a desiccator, then weighed to the nearest 0.1 mg. The biomass yield is calculated using equation (15):

$$\text{mg /ml} = \frac{(D2-D1)}{Volume\ of\ harvest\ material\ filtered} \quad (15)$$

where D_1 is the dry weight of the filter paper before filtration and D_2 is the dry weight of the filter paper post filtration.

4.2.8 Statistical analysis

Data was tested for normality using a Kolmogorov-Smirnov test. Normally distributed data was compared using an analysis of variance (ANOVA) test. Data that was not normally distributed was compared using the Mood's median nonparametric test.

4.3 Results and discussion

4.3.1 Bubble size distribution within the liquid pool and foam phase

Due to the optical density of the microalgae, bubble characterisation was performed prior to the harvesting of the microalgae. Bubbles from within the foam phase were characterised 5 minutes into the flotation run time. Previous studies have demonstrated that the majority of microalgae, up to 85%, are removed within the first 5 minutes (Liu et al., 1999; Chen et al., 1998), therefore characterisation at this time period is of particular interest. Foam phase characterisation at 0 and 10-30 minutes, at 5 minute intervals, can be found in appendix 2 A2.1. Figure 4.3 presents the distribution of bubble sizes within the liquid pool and foam phase generated under linear and oscillatory air flow conditions. Under linear flow the lime wood sparger (represented in blue) generated bubbles with a wide size distribution over all flow rates. The smallest bubble size produced was 673 μm at 150 L h^{-1}, while the largest was 2427 μm at 200 L h^{-1}. Each peak shows a negative skew to the distribution and highlights a dominance of bubbles with the size range between 1200 -1400 μm, with the highest peak being generated at 150 L h^{-1} representing only 31.2% of the whole distribution. The average bubble diameter was 1206 μm, 1232 μm, and 1316 μm for 100L h^{-1}, 150 L h^{-1}, and 200 L h^{-1} respectively, with the bubble sizes generated at 200 L h^{-1} being significantly different from those generated at 100 L h^{-1} and 150 L h^{-1} ($p=<0.001$). Keeping a linear flow but reducing the exit pore size from 35μm to 20μm by using a ceramic flat plate sparger (represented by red Figure 4.3) significantly changes the bubble diameter and distributions

produced. The bubble distribution is more confined leading to higher percentage dominance, with 800-1000 μm representing 46.9% and 50.3% for air flow rates of 100L h^{-1} and 150L h^{-1} respectively, and 38.7% for bubble sizes between 1000-1200 μm for air flow rates of 200 L h^{-1}. The smallest bubble diameter was 437 μm generated at 100 L h^{-1} and the largest was 1687 μm generated at 200 L h^{-1}. The average bubble diameter was 860 μm, 864 μm, and 1040 μm for air flow rates of 100 L h^{-1}, 150 L h^{-1}, and 200L h^{-1} respectively. Again the bubble sizes generated at 200 L h^{-1} was significantly larger than those generated at 100 L h^{-1} or 150 L h^{-1} ($p = <0.001$).

Changing the flow type to an oscillated air supply again dramatically alters the bubble sizes and distribution. Although the bubble sizes presented in this study are significantly larger than those produced by Hanotu et al. (2012) using a stainless steel mesh sparger, the data in this study is closely related to that produced by Al-Mashhadani et al.(2012), using a similar sparger type to the flat plate ceramic sparger used in this study. Figures 4.3 (A) and (B) show a positive skew to the bubble size distribution generated using a COF (represented by green), while Figure 4.3 (C) demonstrates that at flow rates of 200 L h^{-1} the bubble size distribution becomes more negatively skewed. However each peak shows a dominance of 600 - 800 μm representing 54.7%, 65.7% and 53.3% for air flow rates of 100 L h^{-1}, 150 L h^{-1}, and 200 L h^{-1} respectively. The smallest bubble produced was 285 μm in diameter, generated during an air flow of 150 L h^{-1}, the largest bubble size generated was 1250 μm generated under an air flow of 200 L h^{-1}. Bubble sizes generated at 200 L h^{-1} were significantly larger than those generated at 100 L h^{-1} and 150 L h^{-1}. The average bubble size produced over the three production methods, LWLF, CLF, and COF were significantly different ($p = <0.001$). Over all, the bubbles produced using the oscillatory air flow had a more uniform size when compared to bubbles produced under a linear flow. This is due to regular detachment of the bubble from the exit pore, reducing coalescence at the pore as bubbles form (Hanotu et al., 2012).

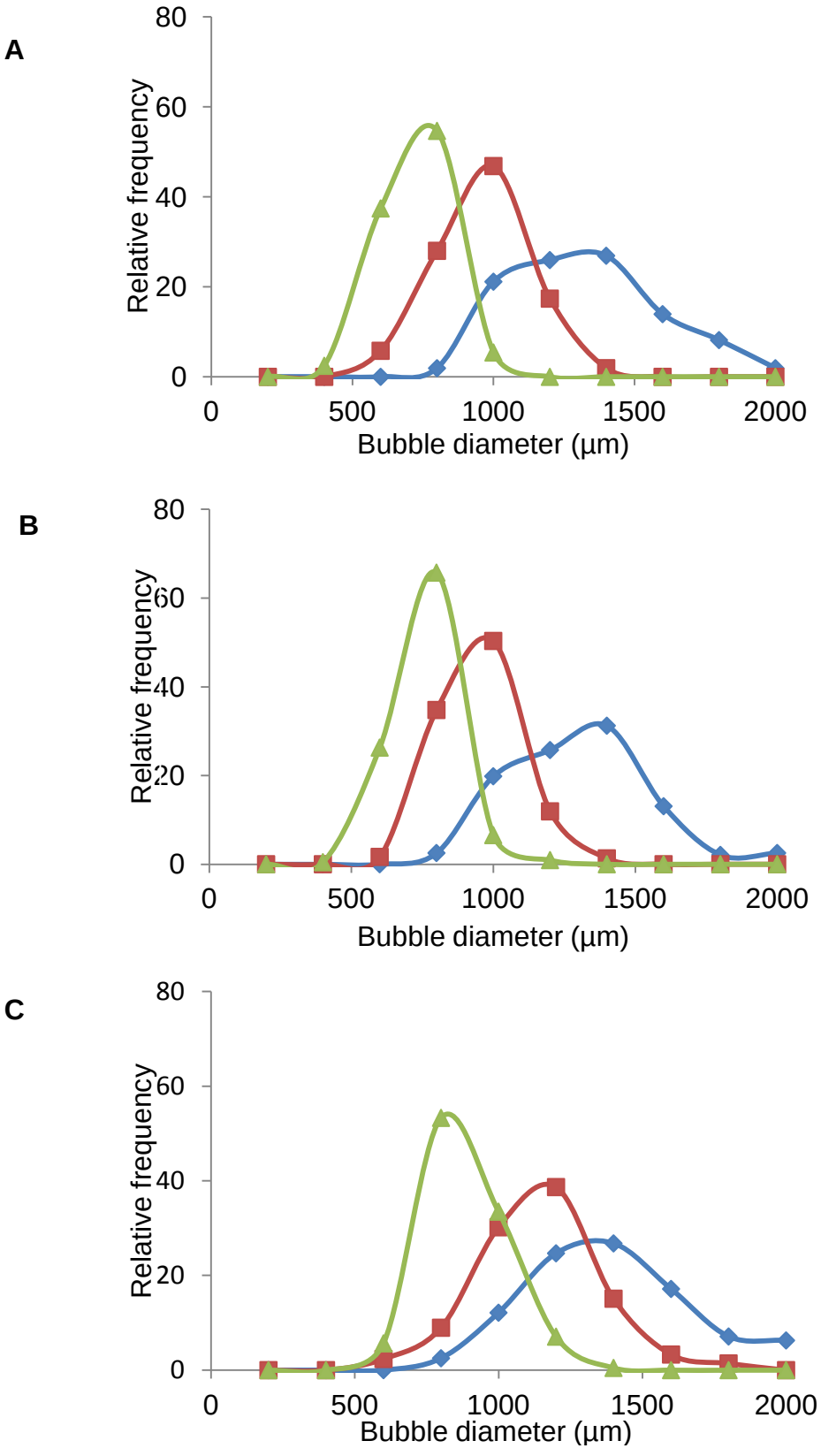

Figure 4.3 Bubble size distribution within the liquid pool. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear

flow by red and the ceramic flate plate sparger with oscillatory flow by green. **A)** was performed at 100 L h^{-1}, **B)** at 150 L h^{-1}, and **C)** 200 L h^{-1}.

During the foam phase (Figure 4.4) the bubble distribution for all sparger types and air flow set-ups peaked between bubble sizes 1000- 1500 μm at 100 L h^{-1} flow rate, with similar bubble frequencies of 34.3%, 33.6%, and 34.1% for the LWLF, CLF, and COF set-ups respectively. However, the LWLF had a significantly different distribution to the CLF and COF set-ups (p=<0.001). The COF had a main peak being followed by several smaller peaks (Figure 4.4 (A)), this is reflected in the average bubble diameters of 1481 μm, 1708 μm, and 2305 μm for the LWLF, CLF, and COF set-ups respectively. During air flow rates of 150 L h^{-1} (Figure 4.4 (B)) the highest peak for all sparger type and air flow set-ups occurs between 500-1000 μm representing 40.8%, 72.1%, and 36.8% for the LWLF, CLF, and COF sparger set-ups respectively. Again the oscillatory air flow rate produces a peak followed by two smaller peaks. Gaining average bubble diameters of 1317 μm, 990 μm, and 1337 μm for the LWLF,CLF, COF set-ups respectively, with the foam being produced by the LWLF and COF being significantly larger than the foam being produced by the CLF (p= <0.001). At 200 L h^{-1} (Figure 4.4 (C)) the bubbles size frequency again peaks at the same point, this time between 1000- 1500 μm representing similar frequencies of 28.3%, 33.8%, and 34.9% for the LWLF, CLF, COF set-ups respectively. The main peak produced by the ceramic oscillatory sparger is only followed by one smaller peak. The average bubble sizes gained in the foam phase is 1800 μm, 1705 μm, and 1620 μm for the LWLF, CLF, and COF set-ups respectively, with the LWLF being significantly larger than the CLF and COF (p=0.016).

Within the foam phase bubbles were significantly larger and had a wider size range when compared to bubbles within the liquid phase, which may have resulted due to bubble coalescence during liquid drainage (Du et al., 2002). The average bubble sizes produced over the three production methods revealed that the foam phase produced by the two linear flow rates - lime wood and ceramic flat plate - were extremely similar (p=0.866) however the foam produced by the ceramic flat plate sparger with an oscillatory air flow produced bubbles that were significantly larger (p= <0.001).

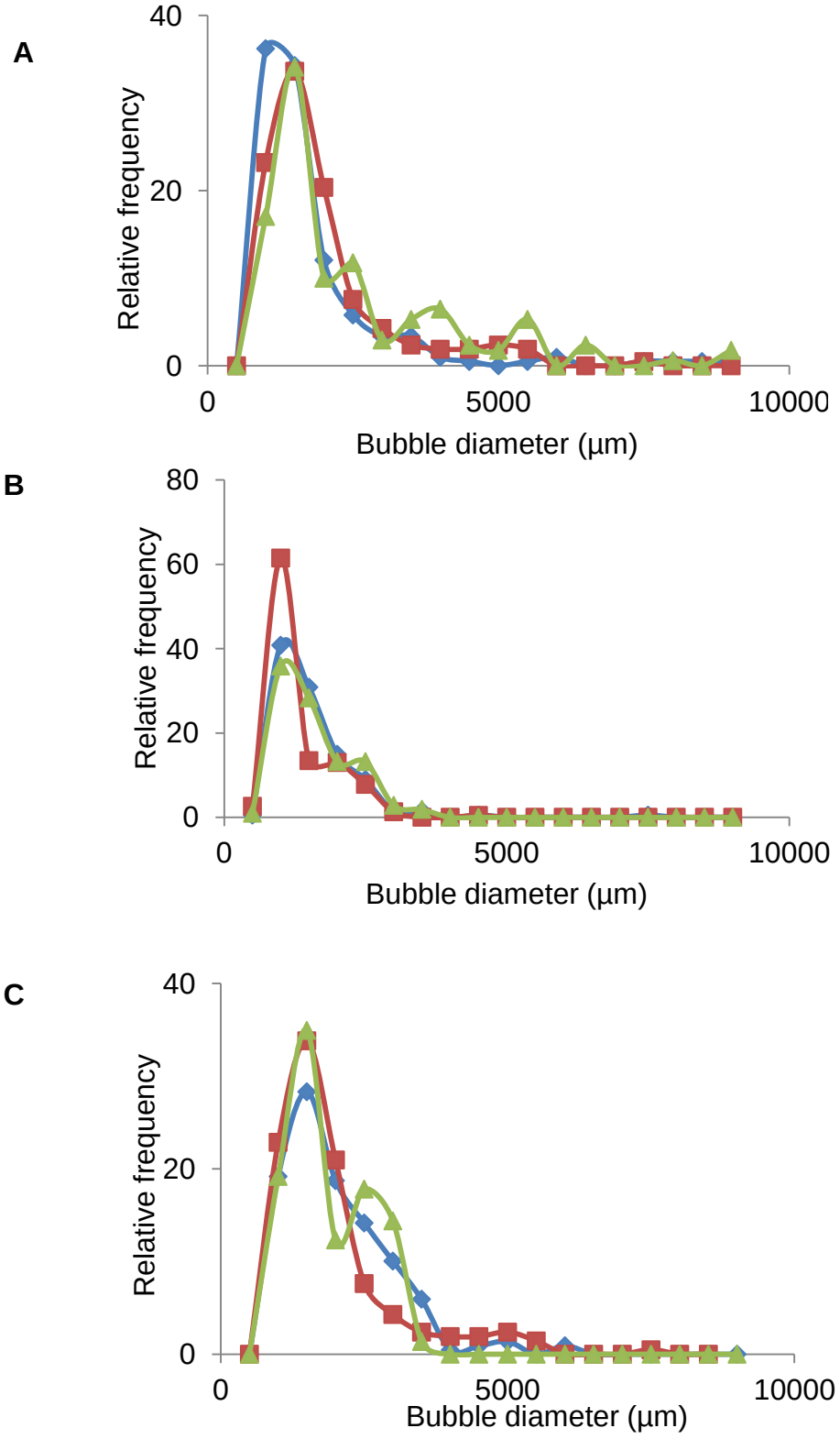

Figure 4.4 Bubble size distribution within the foam phase. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger

with linear flow by red and the ceramic flate plate sparger with oscillatory flow by green. **A)** was performed at 100 L h^{-1}, **B)** at 150 L h^{-1}, and **C)** 200 L h^{-1}.

At 100 L h^{-1} the COF produced the best combination of bubble sizes, with the smallest average bubble size within the liquid pool creating the largest average bubble within the foam phase. The COF therefore appears to be creating the ideal combination of bubble sizes for increased biomass yield and concentration factor.

4.3.2 Bubble rise velocity

The rise velocity of air bubbles within liquid dispersions is a key parameter, as it describes the gas residence time and therefore affects the contact time for interfacial transport (Kulkarni and Joshi, 2005). It is, however, commonly an unreported feature. Within the liquid pool (solid lines - Figure 4.5) the LWLF produced bubbles with a significantly slower bubble rise velocity when compared to the CLF and the COF sparger set-ups (p value = <0.001). Within a stationary continuous phase the rise velocity of a bubble is normally dependent upon the bubble size, as larger bubbles have a greater buoyant-force : drag force ratio than smaller bubbles, therefore reducing the bubble size reduces the bubble rise velocity, increasing the residence time within the liquid phase and increasing the gas hold up (Azgomi et al., 2007). When considering the bubble size data (see section 4.3.1) produced by all three sparger set-ups it is appears to oppose this theory. This can be explained through the momentum transfer that occurs in clouds of rising bubbles. The ceramic spargers operating under both linear and oscillatory flow cover a larger surface area than the lime wood sparger; they also produce dense clouds of rising bubbles. These clouds of smaller bubbles increase the surface area to volume ratio of the bubble phase within the liquid. Momentum is transferred due to shear stress that occurs across the surface area of the bubble, and flux of the momentum therefore increases with a decrease in bubble size. As the surface area increases, more liquid is dragged into the bubble cloud resulting in the mixing of the continuous phase (Zimmerman et al., 2009; Zimmerman et al., 2008).

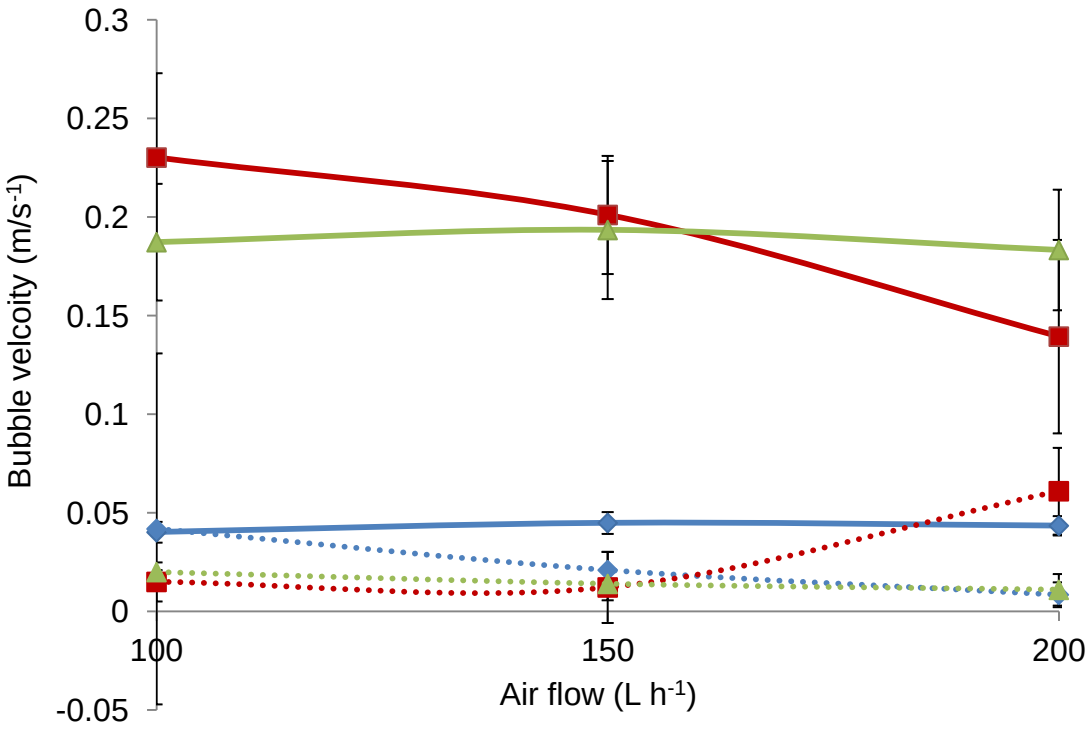

Figure 4.5 Bubble velocity within the liquid pool (soild lines) and foam phase (dashed lines). The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatory flow by green.

The average bubble rise velocity of the CLF sparger describes a downward trend with the average bubble rise velocity decreasing as air flow increases, whereas the bubbles produced under oscillatory flow has a uniform bubble rise velocity. This can be related to the changes in bubble size (refer to section 4.3.1) during linear flow the ceramic sparger produced an average bubble that dramatically increased in size at an airflow rate of 200 L h^{-1}, therefore reducing the surface area and the momentum transfer, which thus reduced the bubble rise velocity produced during linear flow at 200 L h^{-1}. The oscillatory sparger produced bubbles with a more uniform size range resulting in a more uniform average bubble rise velocity when compared to the CLF sparger.

Figure 4.5 displays the rise velocity of bubbles within the foam phase at 5 minutes into the run time, the bubble rise velocity at 0-30 minutes can be found in appendix 2 A2.2. The bubbles within the foam phase have restricted movement and therefore do not respond to the changing airflow regime in the

same manner as the freely moving bubbles within the liquid pool, however, each sparger set-up did behave differently to the changing air flow. Increasing the air flow rate had a negative effect on the bubble rise velocity within the foam produced by the LWLF sparger; however it had the opposite effect on foam produced from the CLF, with bubble rise velocities increasing with air flow rate. The bubble rise velocities produced by the oscillatory sparger were uniformly slow with the increasing air flow rates. Using the oscillatory sparger would therefore produce foam with the best liquid drainage over all air flow rates.

4.3.3 Gas holdup and interfacial area

The gas holdup (ε) was estimated as a function of volume input gas (L h^{-1}) - the results are displayed in figure 4.6. Under a given gas flux, the gas hold up is determined by the bubble rise velocity, highlighting that the gas holdup (ε) determines the gas residence time within the liquid pool (Nedeltchev and Schumpe, 2008). A common assumption is that an increase in gas hold up is related to a decrease in bubble velocity associated with a decrease in bubble size (Ramezani et al., 2012; Azgomi et al., 2007). However, as explained in section 4.3.2, momentum transfer increases with decreasing bubble size, increasing the velocity of the bubbles produced by the ceramic plate sparger and results in the mixing of the continuous liquid phase. The mixing of the continuous phase that was noted in section 4.3.2 highlights some inaccuracies in the calculation of the gas hold up (ε) using equations (12) and (13) as the liquid velocity (u_C) was assumed to be 0, due to the assumption that the continuous phase was stationary. Further work is required to accurately measure the liquid velocity for each sparger set-up. A clear correlation between an increase in bubble rise velocity (Figure 4.5) and a reduction in gas holdup (Figure 4.6) can be made, resulting in the LWLF sparger gaining the highest gas holdup. Gas hold up (ε) generally increases with an increase in superficial gas velocity u_D. However, a sparger area above 1x10^3 m has a

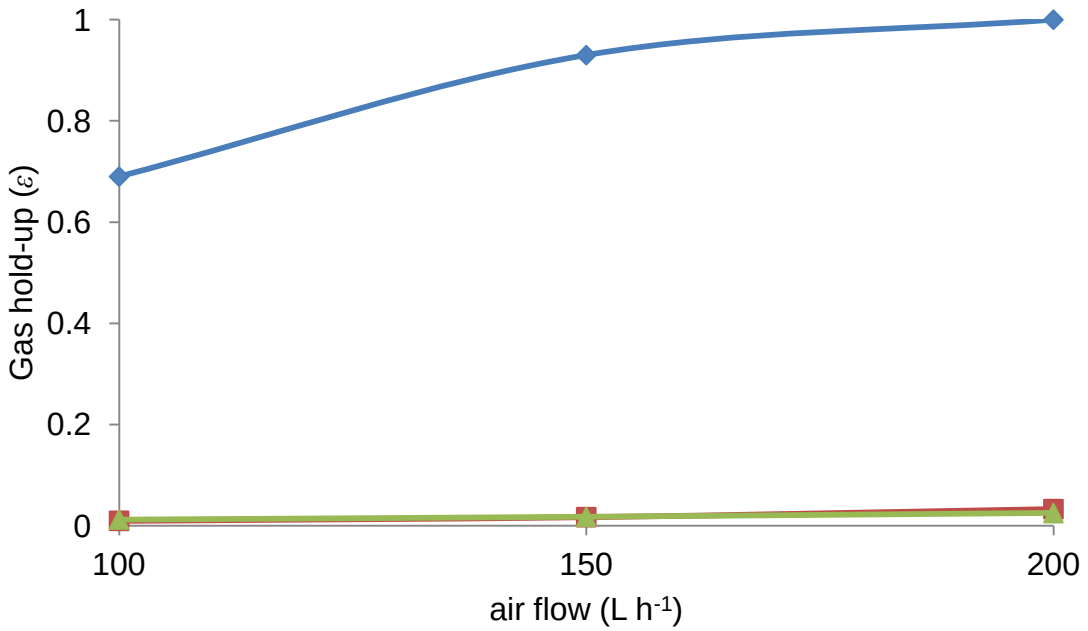

Figure 4.6 The volume fraction of the gas holdup within the liquid pool. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatory flow by green.

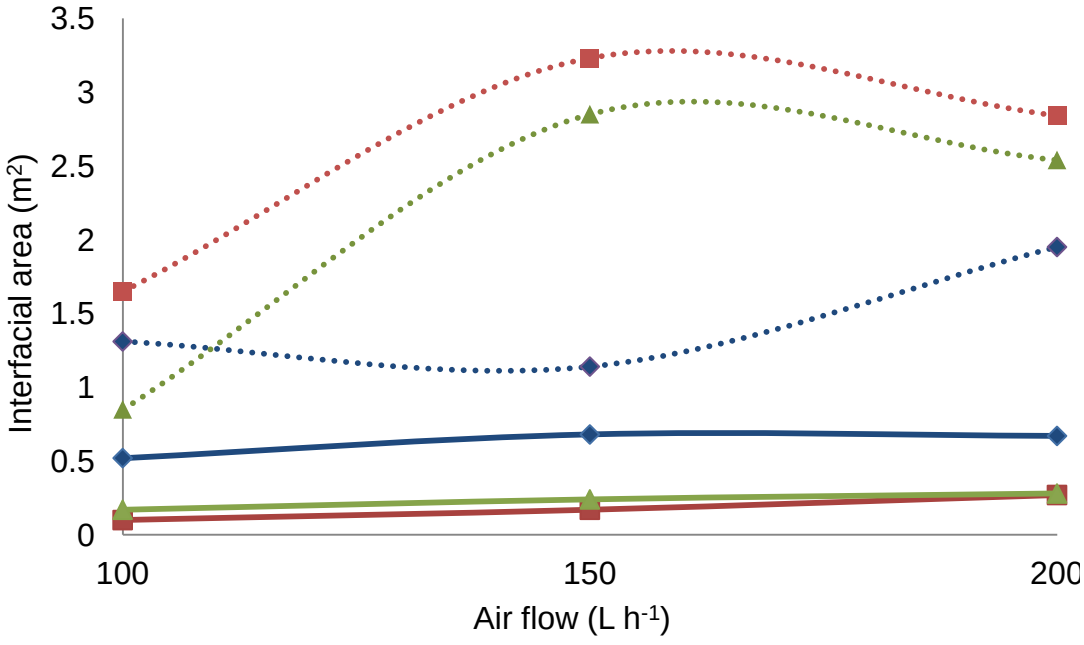

Figure 4.7 The interfacial area liquid pool (soild lines) and foam phase (dash lines). The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatory flow by green.

pronounced influence on the superficial gas velocity u_D (Wilkinson et al., 1992), with superficial gas velocity u_D significantly increasing with a decrease in sparger diameter. More details on the calculations made to determine gas holdup, superficial gas velocity and interfacial area can be found in appendix 2 A2.3.

Within the liquid pool the interfacial area (Figure 4.7 solid lines) is related to the gas holdup (ε), Saunter mean diameter and the bubble distribution area (equation 9). Despite having a significantly smaller Saunter mean diameter and a larger bubble distribution area, the interfacial area produced by the ceramic spargers under linear and oscillatory flow is significantly smaller than the interfacial area produced by the lime wood sparger under a linear air flow, therefore the reduced gas holdup is the main influential factor when comparing the two sparger types. Within the liquid phase the interfacial area generally increases with increasing air flow rates, due to an increase in the gas holdup. Within the foam phase the interfacial area is less affected by changes in air flow rate, since the gas holdup was assumed to be 1, due to the closely packed volume of bubbles compared to the small volume of liquid, therefore, within foam phase the Saunter mean diameter is the most significant effect on interfacial area.

It is difficult to make good comparisons between the lime wood sparger and the ceramic plate sparger because of the difference in distribution area, which affects many significant variables. The effect of air flow into the sparger on the interfacial area can be better understood when comparing the same sparger type, as they will have the same superficial velocities over the range of air flow rates, and cover the same distribution area (Figure 4.8). At 100 and 150 L h^{-1} the COF produces bubbles of a smaller Saunter mean diameter and with a slower rise velocity, resulting in a higher interfacial area. At 200 L h^{-1}, both flow types produce extremely similar interfacial areas - the COF produces a significantly smaller Saunter diameter (823 µm compared to 1127 µm), whereas the CLF produces a bubble with a significantly slower bubble rise velocity (500.4 m/h^{-1} compared to 658.8 m/h^{-1}), thus increasing the gas holdup.

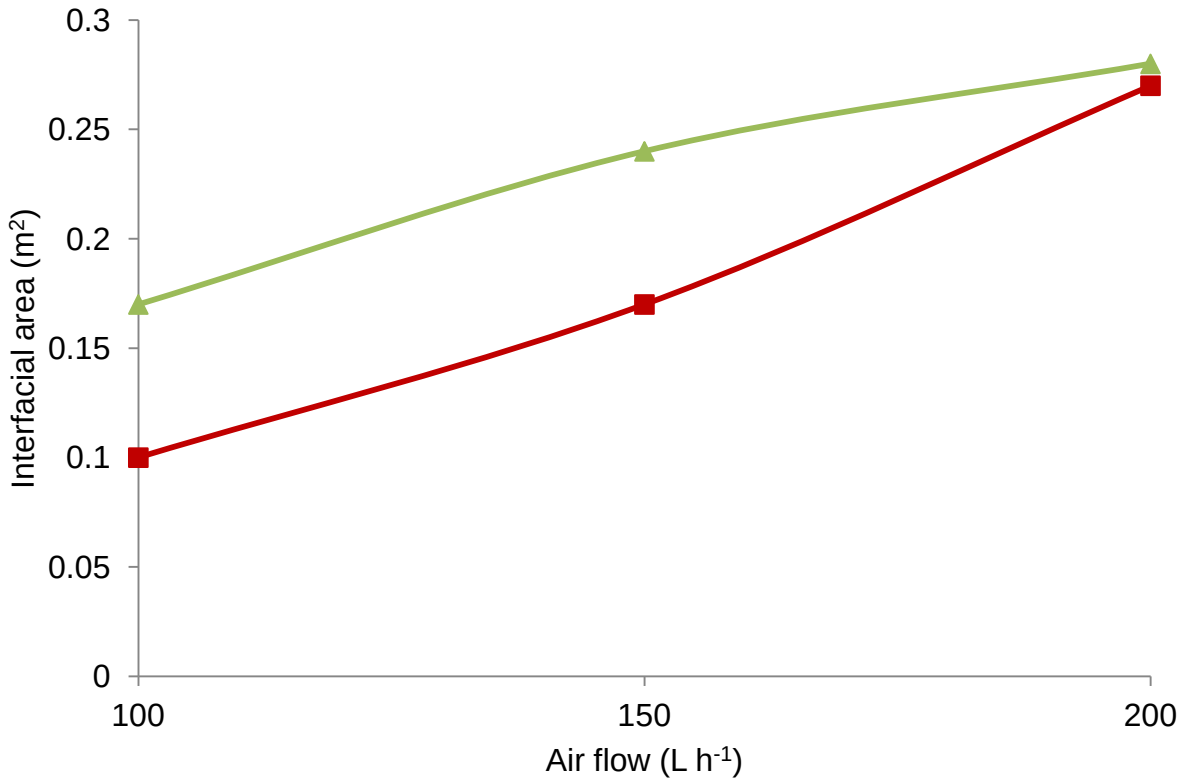

Figure 4.8 The interfacial area liquid pool for the ceramaic sparger only. Linear flow is represented in red and the oscillatory flow by green.

4.3.4 Algal harvest

The influence of sparger type and air flow set-up on the harvest concentration factor and biomass yield was investigated. Figure 4.9 demonstrates that the ceramic oscillatory air flow was able to produce extremely high concentration factors of 427 ± 35. Figure 4.10 determines the volume of harvest culture that needs to be processed relative to the biomass gained; this is an important consideration that has a significant effect on the economics of further processing. The ceramic sparger combined with the fluidic oscillatory air flow provides the most favourable biomass to volume culture yield within the batch system. If scaled to a continuous system, assuming the same efficiencies, this would equate to 27.8 g L^{-1} (based on cellular dry weight).

The interfacial area is usually a good indicator of transfer between the liquid and gas phases e.g. momentum, mass, and energy (Lehr and Mewes,

2001). However, from the data gained in section 4.3.3 it is evident that the microalgae are being collected with a greater efficiency when using the COF. The efficiency of a microalgal cell being collected in the liquid pool of the harvest unit can be given by:

$$E_K = E_c.E_A \tag{16}$$

where E_K is the collection efficiency, E_C is the collision efficiency and E_A is the attachment efficiency. Equation (16) assumes the bubbles within the liquid pool rise as a ridged sphere, which is a reasonable assumption within a surfactant solution, such as that used within the foam fractionator (Lee and Lee, 2002). The microalgal cells are also assumed to be spherical; this again is a reasonable assumption for *Chlorella sp*. Collision efficiency (E_C) is determined by the hydrodynamics of the system and is strongly affected by the particle size, bubble size and the turbulence within the liquid pool. Attachment efficiency (E_A) is also affected by hydrodynamics but is more difficult to predict as it largely linked to the surface chemistry of microalgal cell and the bubble (Yoon, 2000).

For Stokes flow conditions, where the Reynolds number is significantly less than 1 (see appendix 2 A2.4), the collision efficiency can be estimated by:

$$E_c = \frac{3}{2}\left(\frac{R_c}{R_B}\right)^2 \tag{17}$$

where R_C is the microalgal cell radius and R_B is the bubble radius. The microalgal cell radius is assumed to be 6μm for all calculations, (Berberoglu et al., 2009). The collision efficiency of the bubbles produced by the three sparger/air flow set-ups at 100 L h^{-1} is shown in figure 4.11. The results closely mirror those gained for the concentration factors (Figure 4.9). Decreasing the bubble size results in an increase in the number of particles collected per bubble as a function of the cross-sectional area. The cross-sectional area of a small bubble is greater than that of a larger bubble (Kouachi et al., 2010; Reay and Ratcliff, 1973).

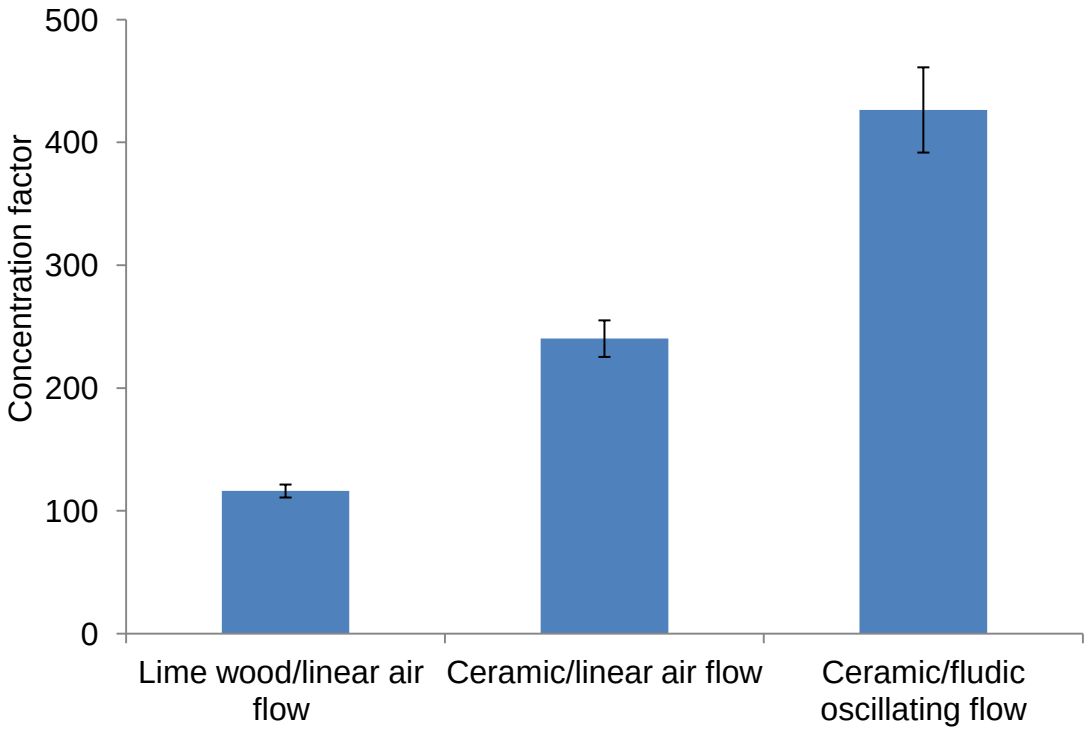

Figure 4.9 The harvest concentration factor gained under the three different sparger/air flow set-ups.

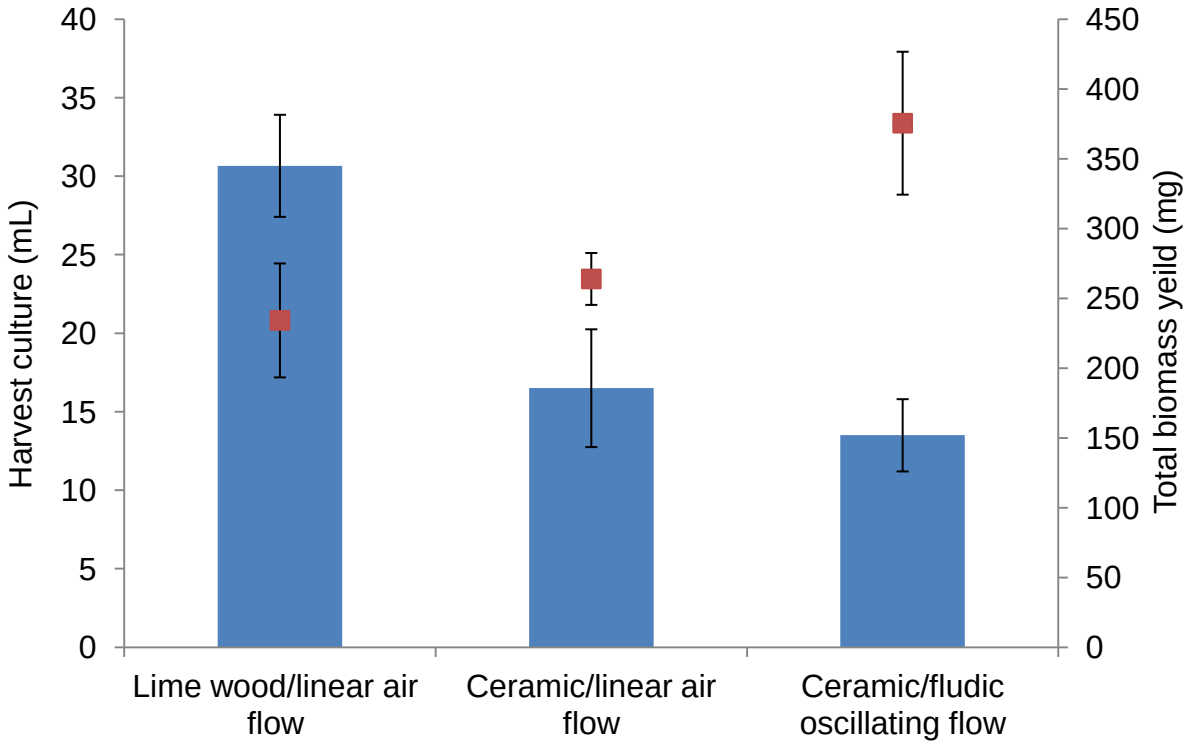

Figure 4.10 The volume of harvest culture relative to the biomass gained for the three different sparger/air flow set-ups. The blue bars signify the volume of harvest culture and the red squares relate to the total biomass yield gained.

Collision between a bubble and a microalgae cell does not necessarily result in attachment. A number of processes must occur; firstly the thin layer of liquid between the cell and the bubble needs to drain away. Once it reaches a critical thickness it ruptures. This rupture allows the three - phase contact line (the boundary that occurs between the solid cell, the receding liquid, and the advancing gas phase), which must then adjust until a stable wetting perimeter is established. This sequence of drainage, rupture and adjustment of the contact line allows for a stable cell-bubble union (Ralston et al., 1999).

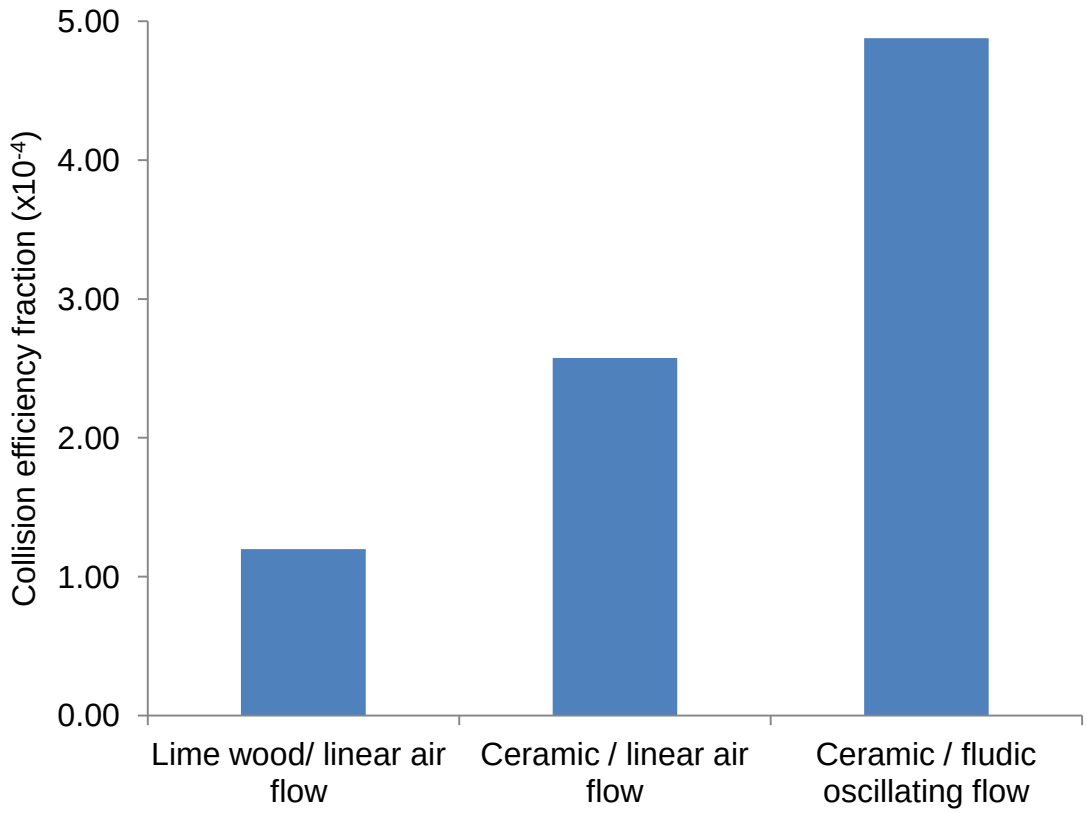

Figure 4.11 The collision efficiency fraction gained for each sparger types/air flow set-up at 100 L h^{-1}.

A number of studies have been conducted on the effect of bubble size on the attachment efficiency of particles (Ralston et al., 1999; Hewitt et al., 1995; Yoon and Luttrell, 1989; Ahmed and Jameson, 1985), and in all studies it was concluded that under similar conditions smaller bubbles always gave higher attachment efficiency than larger bubbles. Yoon and Luttrell (1989) noted that

for bubbles larger than 0.35 mm, attachment efficiency decreased with increasing bubble diameter.

It can therefore be concluded that although the ceramic spargers create a smaller interfacial area, the microalgal cells are more efficiently collected. This is due to an increase in collision efficiency and attachment efficiency as a function of bubble size.

4.4 Harvest economics

Although several harvesting methods exist, currently there is no universal harvesting technique. Often the disadvantage of a harvesting method is linked to incompatibilities between efficiency and economics. Table 4.1 demonstrates the ranges of efficiency and energy consumption for common harvesting methods. Small changes within a harvest system, such as air pressure required or air flow rate can dramatically affect the work required for an air compressor, therefore affecting the energy consumption and economics of the harvest system. The specific work of an air compressor can be calculated using the following equation:

$$W = \frac{RT_1}{\gamma - 1}\left[\left(\frac{P_2}{P_1}\right)^{\frac{\gamma-1}{\gamma}} - 1\right] \quad (18)$$

Where W is the work in J/mol^{-1}, R is the gas constant, assumed to be 8.314 J mol^{-1}K^{-1}, T_1 is the temperature in K, assumed to be 293 K, P is pressure and γ is the ratio specific heat air and is assumed to be 1.4. The pressure required for each air flow and sparger set-up was recorded using either a pressure gauge (RS, 0-6 bar) or a manometer. The manometer was filled with mercury (density = 13.534 g/cm^3) and the height differential was measured when connected to a working harvesting unit. Gauge pressure can be calculated using the following equation:

$$\Delta P = P - P_0 = \rho g h \quad (19)$$

Where P_0 is atmospheric pressure, ρ is the reference liquids fluid density, g is the acceleration of gravity (9.81m/s^2) and h is the height of the fluid in the column.

Table 4.1 A comparison of the efficiency and in energy consumption of commonly used harvesting technologies

Harvest technology	Energy consumption (kWh/m^3)	Suspended solids (%) in concentrate
Filtration		
Tangential flow filtration (Danquah et al., 2009a, Danquah et al., 2009b)	0.38-2.06	2.5-8.9
Chamber filter (Molina Grima et al., 2003)	0.88	22-27
Vacuum filters (Molina Grima et al., 2003)	5.9	18
Flocculation		
Polymer flocculation (Poelman et al., 1997)	14.81	15
Electrolytic flocculation	0.331	Not determined
Centrifugation		
Nozzle discharge centrifuge (Molina Grima et al., 2003)	0.9	2-15
Decanter bowl centrifuge (Molina Grima et al., 2003)	8.00	22
Hydrocyclone (Molina Grima et al., 2003)	0.3	0.4
Flotation		
Dissolved air flotation (Wiley et al., 2009)	7.6	5
Dispersed air flotation (Wiley et al., 2009)	0.003	4.8

Table 4.2 shows the energy consumption required for an air compressor to provide air for 3 different sparger type set-ups. The efficiency of an air compressor is never 100% due to leaks and energy being converted to heat, therefore 50% efficiency is assumed for the foam fractionation harvesting unit. Table 4.2 demonstrates that the suspended solids (%) in concentrate gained due to harvesting via foam fractionation is comparable to, or better than, those achieved using tangential flow filtration Danquah et al., 2009b), nozzle discharge centrifuge, hydrocyclone centrifuge (Molina Grima et al., 2003), dissolved air flotation and dispersed air flotation (Wiley et., 2009). However, foam fractionation using LWLF or CLF demonstrates advantageous energy consumption over all the harvesting technologies previously listed, apart from dispersed air flotation. COF provides a poor cost-benefit relationship, due to the higher pressures and air flow rates required for the fluidic oscillator, used in the COF set-up, to induce the oscillatory air flow. Therefore, a CLF sparger set-up within a foam fractionation may reduce financial barriers associated with many commonly used bulk harvesting techniques.

Table 4.2 The energy consumption required for an air compressor to provide air for 3 different sparger type set-ups used in foam fractionation

Sparger set-up in foam fractionation unit	Energy consumption of air compressor (100% efficiency) (kWh/m^3)	Energy consumption of air compressor (50% efficiency) (kWh/m^3)	Suspended solids (%) in concentrate
LWLF	0.008	0.015	0.63-0.9
CLF	0.009	0.019	1.48-1.71
COF	2.742	5.485	2.4-3.16

4.5 Conclusion

The performance of a foam fractionation microalgal harvesting unit was analysed in terms of bubble size generated by three different sparger and air input set-ups; lime wood sparger with linear air flow input, ceramic flat plate sparger with linear air flow input and ceramic plate sparger with an oscillatory air flow input. Bubble size was greatly dependent on air flow rate, and sparger set up. The smallest average bubble size was 621.8 ± 58.5 μm and was produced by the COF at 100 L h^{-1}. Interfacial area within the liquid pool for bubbles generated via CLF and the COF was lower than expected, this was due to bubble residence times within the liquid pool. The small bubbles generated by the ceramic spargers did not conform to the theory that smaller bubbles travel at slower velocities, due to momentum transfer that occurs when clouds of bubbles are generated. The higher bubble velocities reduce the bubble's residence times, reducing the gas hold-up within the system and therefore reducing the interfacial area within the liquid pool. High concentration factors and biomass yields gained with the COF are associated to an increase in collection efficiency due to a decrease in bubble size, however, when considering the energy requirements for each sparger set-up it is evident that CLF provides an extremely advantageous benefit-cost relationship, out-competing many commonly used bulk harvesting technologies.

Chapter 5

The effect of growth stage on the efficiency of foam fraction of *Chlorella sp.*

Abstract

The aim of this study was to determine the influence of growth phase on the efficiency of harvesting of *Chlorella sp.* via foam fractionation. In order to determine the optimal time to harvest the effect of growth phase and foam fractionation on the total lipid content was also investigated. The highest concentration factors and biomass yields were found during the exponential growth phase. The total lipid content of microalgae harvested by foam fractionation was significantly higher than cells harvested via centrifugal recovery. The results indicate that foam fractionation has a significant influence on the lipid recovery of the microalgae.

5.1 Introduction

Microalgae provide several advantages for biofuel production when compared with terrestrial based crops, producing a high yielding feedstock for biodiesel, ethanol, and aviation fuel without competing with food supplies, arable land, or rainforests (Christenson and Sims, 2011; Demirbas and Fatih Demirbas, 2011; Chisti, 2007). Microalgae, therefore, represent one of the few sustainable feedstock options for biodiesel production. As a consequence of the recognition of this there has been an increased interest and a rise in the number algae fuel projects and reviews. Unfortunately the commercialisation of biofuels using algal biomass has been significantly held back due to two major economic challenges: the ability to produce high yielding biomass on a large scale and harvesting large quantities of feedstock (Christenson and Sims, 2011; Uduman et al., 2010). Improvements in production, harvesting and integrating co-production of higher-value products/processes are critical in the bid to reduce

algal oil production cost (Demirbas and Fatih Demirbas, 2011; Schenk et al., 2008).

In recent years considerable research efforts have been focussed on the application of harvesting technologies to improve biomass yield, however the majority have demonstrated disadvantageous effects with regard to the cost-benefit relationship (Table 4.1). Chapters 3 and 4 have thus far demonstrated that foam fractionation can successfully be employed as an extremely efficient low energy harvesting technology and that the cost-benefit relationship can be greatly influenced by the choice of sparger type, bubble size within the liquid phase, bubble size within the foam phase, and air flow rate. Danquah et al. (2009) and Zhang et al. (2012) have shown a significant knowledge gap with regard to the effect of natural growth properties on the effectiveness and efficiency of biomass harvesting. Selecting the most advantageous growth phase to harvest may significantly increase the effectiveness of foam fractionation, and unlike the other influential features previously listed (sparger type etc), would create little significant effect on energy requirement. The growth phase of the microalgal culture is particularly influential due to the changes in the cell surface electrochemical properties. The electrochemical properties are mainly affected by the algal surface functional groups, which decrease with growth phase, and the dissolved organic matter (DOM), which increases with growth phase; therefore affecting the microalgal cell stability (Zhang et al., 2012).

The effect of DOM is greatly dependent upon the microalgal species. Henderson et al. (2010) reported that DOM contributed to 84%, 3%, and 30% of the total charge density for *Chlorella vulgaris*, *Microcystis aeruginosa* and *Asterionella formosa* respectively. However in the absence of DOM, the functional group concentration determines the charge density and therefore the harvest efficiency (Zhang et al., 2012). Functional groups include carboxyl, phosphate, and amine groups; deprotonation of functional groups confer electrostatic charge to the cell wall periphery and thus give the cell wall anionic characteristics (Hadjoudja et al., 2010).

To date, the influence of growth phase on the settling efficiency, tangential flow filtration efficiency (Danquah et al., 2009), flocculation efficiency (Lee et al., 1998), and the coagulant dosage required for dissolved air flotation (DAF) has been investigated. Despite this, to my knowledge, no information is

available about the possible effects of algal growth phase on the efficiency of foam fractionation.

The aim of this study was to establish the influence of *Chlorella sp.* growth phase on the efficiency of foam fractionation, whilst maintaining optimal lipid levels.

5.2 Materials and methods

5.2.1 Cultivation of algae

A mixed *Chlorella sp.* was obtained from Blades Biological Ltd, Kent, UK and grown using a proteose peptone medium as described in section 3.3.5.

5.2.2 Determination of growth

The growth of the *Chlorella sp.* was determined by taking samples every 3 days over a 21 day culture period from the culture carboys. The cell number was calculated using a haemocytometer, counting at least 12 replicate haemocytometer slides per culture.

5.2.3 Harvesting experiments

Algae from the same culture were harvested at 3 day intervals between 0-21 days, via foam flotation and centrifugal recovery. During foam flotation 2 litres of microalgae culture were added to 7.5 litres of tap water to give an initial dry weight of 126.14 ± 10.06 mg L^{-1}, which is similar to the dry weights currently being produced in paddle-wheel raceway open culture systems (Borowitzka, 1999). All foam flotation harvests were conducted under the following conditions: air flow rate = 100 L h^{-1}, batch run time = 30 minutes, surfactant concentration =

10 mg L^{-1}, surfactant type = cationic cetyl trimethylammonium bromide (CTAB). Each harvest was conducted with 4 replicates. The concentration factor of each harvest was calculated using equation (1) (available in chapter 3). The biomass yield was calculated as described in chapter 4 (section 4.2.7)

The harvest from foam flotation was then further concentrated via centrifugation using a *Sigma 3K18C* centrifuge for 20 minutes at 10733 g. The cell pellet was washed twice with distilled water and then freeze dried using a Christ Alpha 1-4 LD Plus (SciQuip, UK). To compare harvest techniques 2 litres of culture was also harvested using only centrifugation for 20 minutes at 10733 g and then freeze dried.

5.2.4 Total lipid extraction

The freeze dried microalgae from the two harvest methods were ground to a fine power using a pestle and mortar. A known quantity of microalgae, 0.095-0.33 g, was first homogenized with methanol followed by chloroform, the total homogenate volume was 30 times that of the tissue weight (Christie, 1982) at a 1:2 v/v. A modified version of the Folch method (Folch et al., 1957) was used. The mixtures were centrifuged for 20 minutes at 10733 g to remove the cell debris. The lipid fraction was carefully transferred by pipette into a clean test tube, a weak salt solution of potassium chloride (0.88% KCl) was then added at 25% of the starting volume to wash the lipid fraction and remove any non-lipid contaminants. The final biphasic system was centrifuged, and the resulting mixture left to separate into two phases. The lower phase was transferred into a pre-weighed glass tube and evaporated to dryness under a stream of oxygen free nitrogen at 37°C. The weight of the crude lipid obtained from each sample was determined gravimetrically.

In the presence of water, small molecules can often become dissolved into the organic solvents; these contaminants include pigments, lipophilic amino acids, polypeptides and hydrophobic proteins. The washing procedure in the Folch method can remove the majority of contaminants, however pigments remain. Therefore when total extracted lipid is referred to throughout this thesis

it is an estimate as there will most likely be contamination form chlorophyll compounds (Bahmaei et al., 2005).

5.2.5 Statistical analysis

The changes in growth phase and the total lipid contents from the microalgae harvested via foam fractionation and centrifugal recovery were compared using an analysis of variance (ANOVA) test. An alpha level of 0.05 was used to determine the significance of the data.

5.3 Results and discussion

5.3.1 Growth of Chlorella sp.

The growth curve of *Chlorella sp.* was determined from parallel cultures all starting with the same inoculums. The changes in cell number are shown in figure 5.1. A lag phase occurs between days 3-9, this was indicated by no significant increase in cell number ($p=0.009$). An increase in cell number was observed between days 9-15, peaking at a concentration of $4.44 \times 10^8 \pm 6.01 \times 10^7$ by the end of the exponential phase. The stationary phase occurred between days 15-18, which is indicated by no significant change within the cell concentration ($p=0.604$) during this period. A significant decline in the cell number occurred at day 21, when compared to the height of the exponential phase at day 15 ($p=0.001$), representing a 32.9% decline in the cell number. The four growth phases indentified during *Chlorella sp.* cultivation have been indicated in figure 5.1.

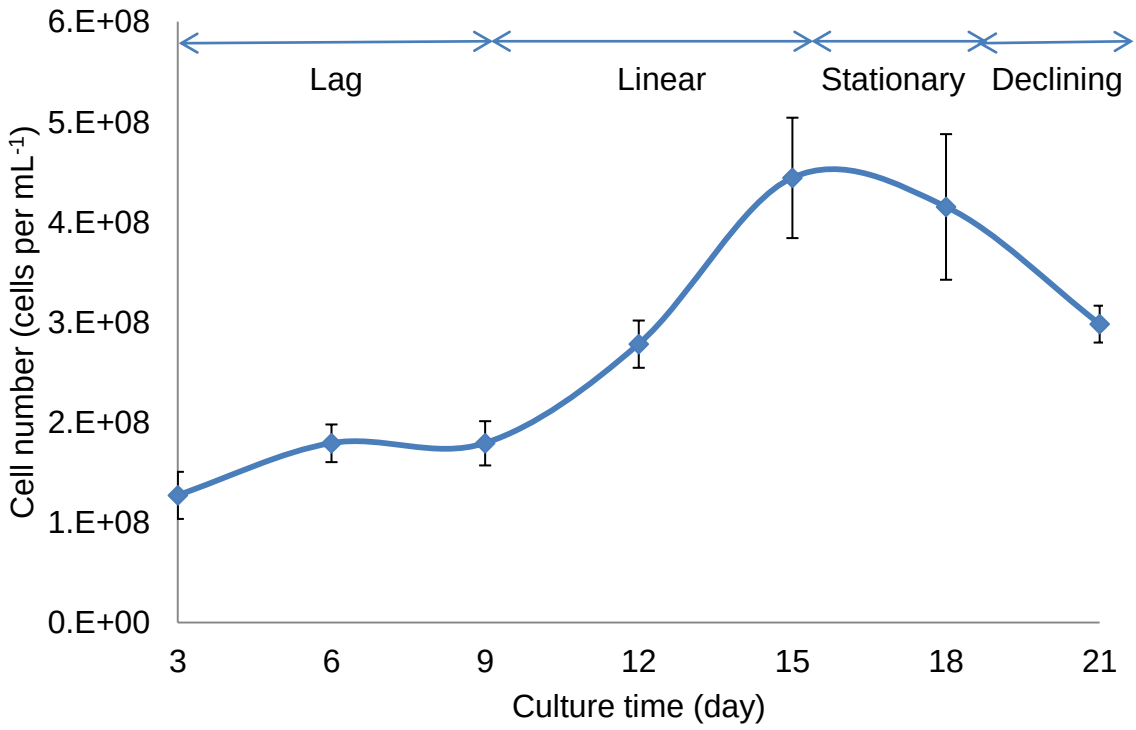

Figure 5.1 Changes in cell number during a 21 day cultivation of *Chlorella sp.*

5.3.2 Concentration factor

Figure 5.2 demonstrates the effect of culture age on the concentration factor of cells harvested via foam fractionation. The results generally demonstrate that variation in concentration factor can be related to microalgal growth phases. The highest concentration factor, of up to 335.08 ± 49.35, occurs during the linear growth phase (day 12), this is significantly higher ($p = <0.001$) than the concentration factors gained during the lag (day 0-6), stationary (day 15-18) and declining (day 21) phase of the growth cycle. Danquah et al. (2009b) reported that cultures harvested during a low growth rate period of the cell cycle have increased settling efficiencies when compared to cultures harvested during a high growth period, due to a reduction in cell stability caused by a decline in zeta potential during the low growth rate phase. The decrease in zeta potential has been found to be linked to DOM, which increases with the age of the algae, and can play an important role in promoting or inhibiting floc formation (Zhang et al., 2012; Henderson et al., 2008a). Zhang et al. (2012) reported that DOM competed for the flocculent Al^{3+}, while harvesting using dissolved air flotation,

resulting in the stationary and declining growth phases requiring more Al^{3+} than the exponential growth phase. Unfortunately, the zeta potential was not measured during the experimental period, due to a lack of access to a zeta-meter. A previous study has demonstrated that the zeta potential of *Chlorella zofingiensis* varies significantly with a change in growth phase, with the zeta potential declining from -20.6 ± 0.9, to 13.2 ± 3.0, and 12.2 ± 0.5 mV during the exponential, stationary, and declining growth phase respectively (Zhang et al., 2012). Compiling the available information with the data presented in figure 5.2, it can be concluded that the most advantageous growth phase for improving harvest efficiency is dependent upon the harvesting technology used. Low levels of DOM and a higher zeta potential, which occur during the exponential/ linear growth period, improve the efficiency of cell flotation. However, a reduction in zeta potential, which occurs due to an increase of DOM and a reduction in major functional groups in the stationary and declining growth phases, improves settling efficiency. Higher cell zeta potential may also increase the electro-static interactions which occur between the cationic CTAB and the negative cell interface. CTAB can be adsorbed onto the negative surface of particles (Paria and Khilar, 2004); this therefore increases the hydrophobicity of the once hydrophilic solid–liquid interface. The cationic surfactant may also create electro-static interactions between the gas bubbles and the harvest particles improving flotation (Liu et al., 1999). Figure 5.3 is a visual representation of this suggested mechanism describing how algal growth phase affects the harvesting efficiency of foam fractionation.

Day 15 is thought to represent the peak of the linear growth phase, however the cell count was taken every 3 days, and therefore the peak may have occurred during days 13-14. This may explain why day 15 shows a low concentration factor of 111.3 ± 6.5, suggesting that cells at day 15 are more likely to be in the stationary growth phase.

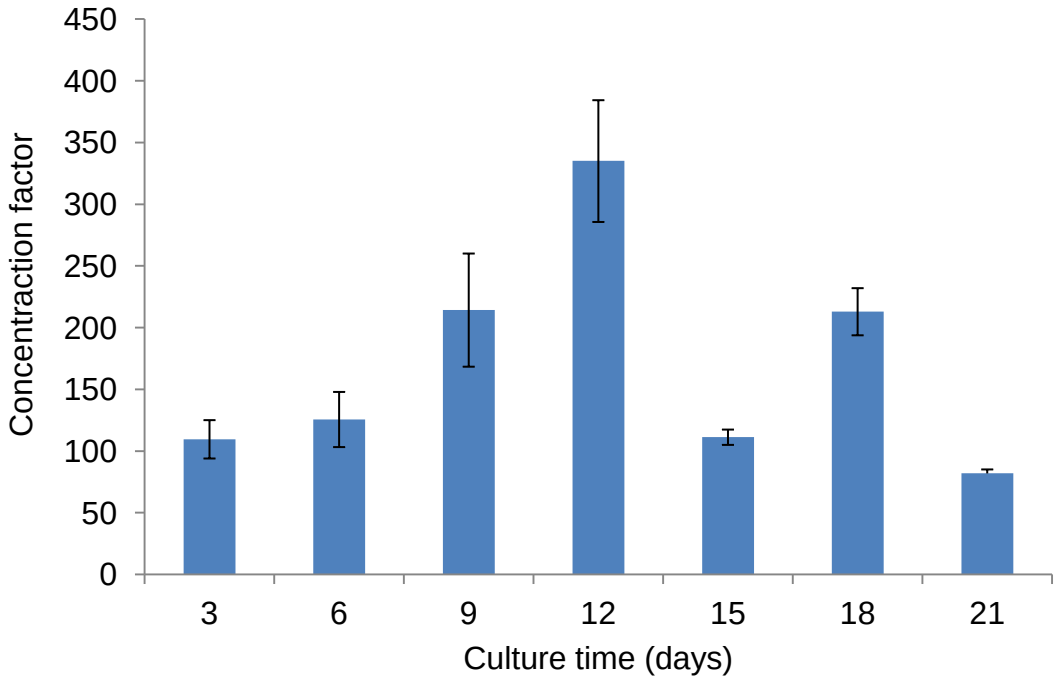

Figure 5.2 A comparison of the concentration factors gained from the microalgae harvested via foam fractionation between 3-21 days after the start of culture.

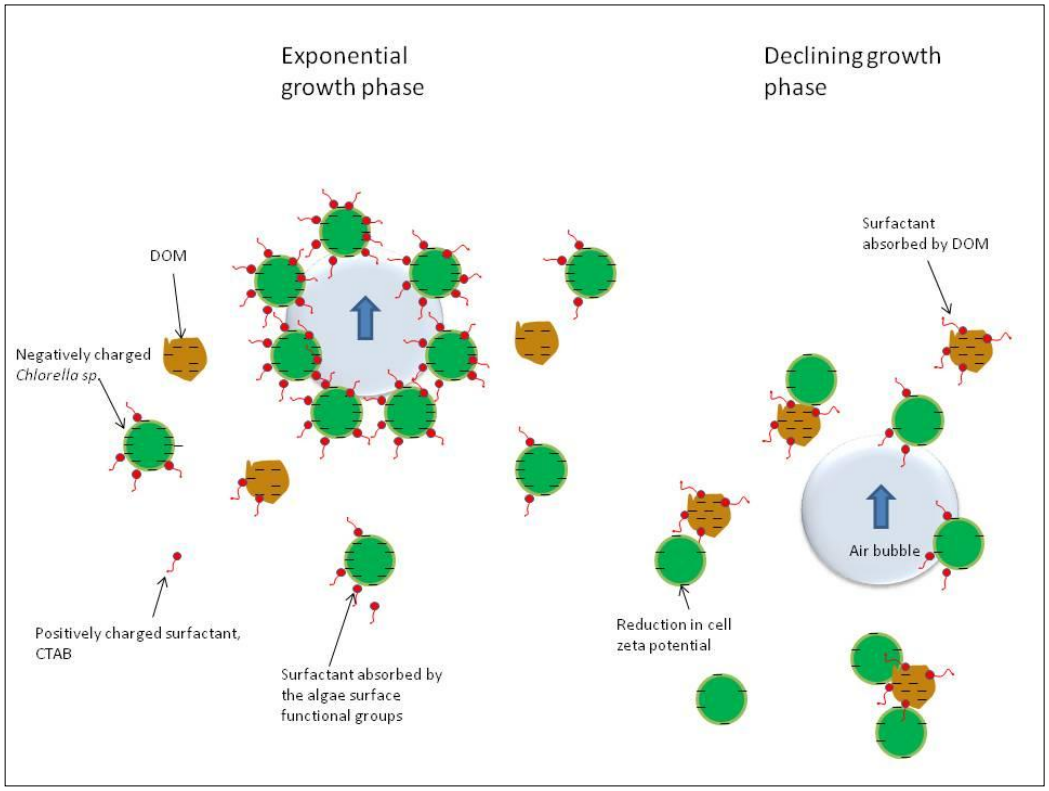

Figure 5.3 The suggested mechanism for the effect of algal growth phase on the harvesting efficiency of foam fractionation, with the use of CTAB. (Diagram based on the works of (Phoochinda et al., (2004) and Zhang et al., (2012)).

5.3.3 Biomass yield

The influence of growth phase on the harvested biomass yield of *Chlorella sp.* is shown in figure 5.4. Microalgae harvested during the linear growth phase gained significantly more biomass yield when compared to cultures harvested during lag and declining phases (p=<0.001), gaining up to 22.3 ± 4.7 g L^{-1} (based on cellular dry weight). However, the biomass yield gained from cultures harvested during the linear growth phase was not significantly different to the biomass yield gained from cultures harvested during the stationary growth phase (p=0.028).

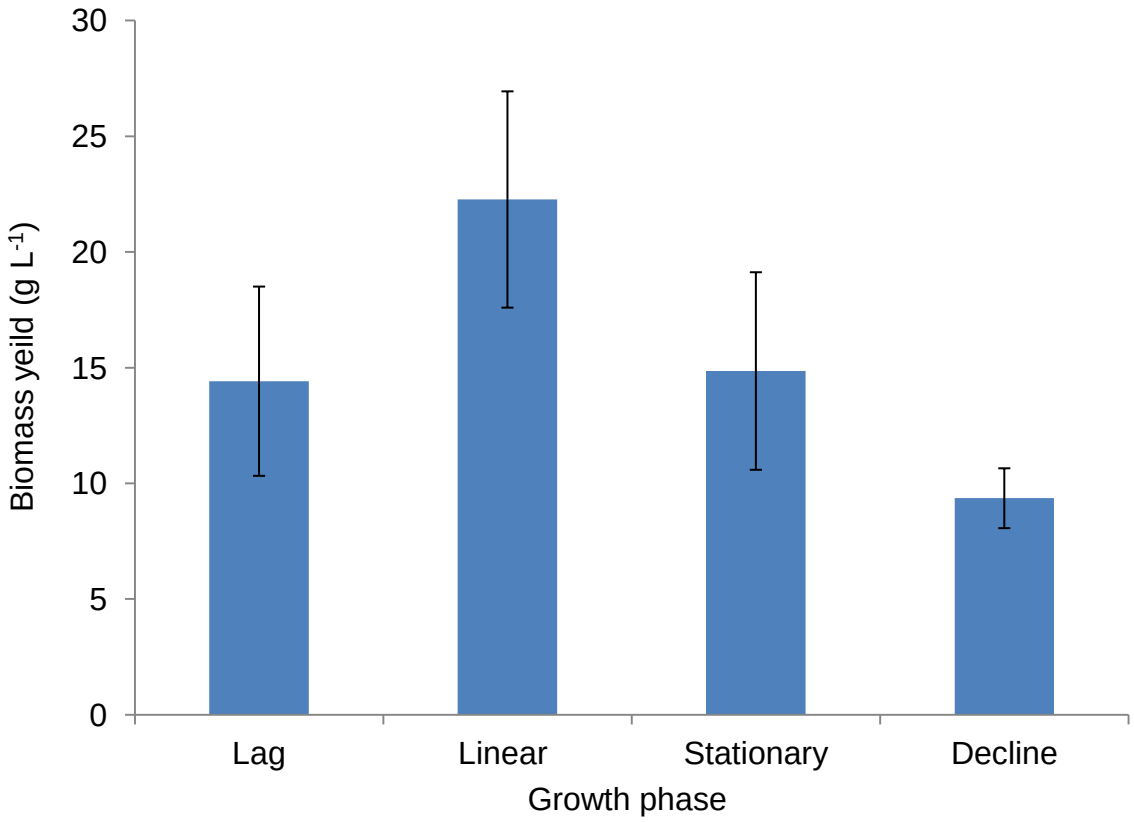

Figure 5.4 The influence of growth phase on the biomass yield of *Chlorella sp.* harvested via foam fractionation.

DOM is composed of cellular secretions and cellular decay (Pivokonsky et al., 2006). Work conducted by Zhang et al. (2012) noted that DOM concentration increased in an approximately linear pattern with culture time,

however there was no significant difference in the levels of DOM gained between the end of the exponential phase and the start of the stationary phase, due to an increase in the standard error with culture time. This may explain why there is no significant difference in biomass yield. Further work must be conducted, examining the levels of DOM and its effect on harvesting via foam fractionation.

5.3.4 Total lipid

The total lipid content of *Chlorella sp.* harvested via foam fractionation and centrifugal recovery throughout all the growth phases was determined. The total lipid extracted from the dry biomass harvested via foam fractionation varied between a minimum of 14.5 ± 0.47% on day 3, to a maximum of 17.63 ± 0.53% on day 6. For the cells harvested via centrifugal recovery a minimum of 8.3 ± 0.16% was gained on day 3 and a maximum of 11.4 ± 0.47% was gained on day 21 (Figure 5.5). The average total lipid content gained from all harvests of cells harvested via foam fractionation was 15.4 ± 0.77% on a dry weight basis, which was significantly higher than that gained from cells harvested via centrifugal recovery, which was 9.9 ± 0.56% ($p=<0.001$). No significant relationship was observed between total lipid content and growth stage for algae harvested via centrifugal recovery ($p=0.066$) or foam fractionation ($p=0.106$). The lipid contents gained were of similar scope to those gained in other studies (Yoo et al., 2010; Renaud et al., 1994). The results indicate that harvesting via foam fractionation has a significant influence on the lipid content of *Chlorella sp.*

Lee et al. (1998) tested the effect of three different flocculating methods; pH adjustment, treatment with aluminium sulphate and treatment with Pestan, on the lipid content of *Botryococcus braunii*. It was found that the lipid content was unaffected by the harvest method, however no investigation into the fatty acid profile was carried out. Borges et al. (2011) also found no significant difference for the total lipid content with respect to harvest method when comparing anionic and cationic polyacrylamide flocculants. However, it was found that the fatty acid profile significantly differed when the microalgal cells

were treated with the different flocculants. Therefore the choice of harvest technique can greatly affect the potential product to be made.

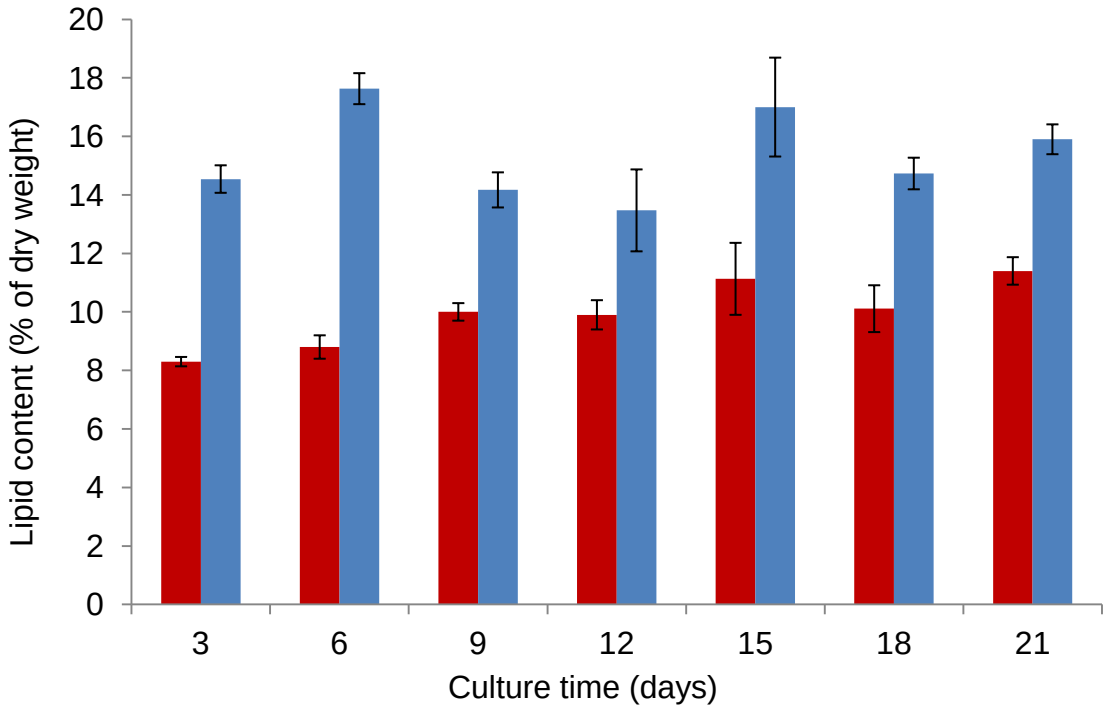

Figure 5.5 The percentage of lipid extracted from cells harvested via centrifugal recovery (red) and foam fractionation (blue).

Microalgae are well known for altering the amount of lipid produced according to the environmental conditions. Further investigation into the effect of CTAB and lipid content is needed. It has however, been found that the electro-static interactions that aid cell flotation, also create stress in the cell wall which can lead to cell lysis (Simões et al., 2006). It has also been suggested that CTAB causes gross membrane damage by protein denaturation, which, causes the cell membrane to rupture (Simões et al., 2006; Gilbert et al., 2002). It may be that the increase in total lipid from cells harvested via foam fractionation occurs due to an increase in phospholipids that have been released due to the cell membrane break down.

Recently research focus has turned to the extraction of microalgal lipids from wet biomass. Traditional lipid recovery methods generally use organic solvents to extract the lipids from dried biomass. The drying process required is

extremely energy intensive which reduces the economic viability of the feedstock (Teixeira, 2012; Patil et al., 2011; Wahlen et al., 2011; Zheng et al., 2011). A harvesting method such as foam fractionation using CTAB could combine dewatering with cell disruption, removing the need for grinding, ultrasonication, bead milling or enzymatic lysis, thus reducing the number of stages between harvesting and transesterification, however, some quaternary ammonium compounds, such as CTAB, have the ability to render fatty materials soluble. It is therefore important to determine if CTAB affects the FAME series of algae harvested via foam fractionation. If it is concluded that CTAB has a negative effect of the fatty acid profile then this would make it an inadequate harvesting technique for feedstock biomass. Further work in this area must be conducted.

5.4 Conclusion

Foam fractionation could provide a potentially economical way to harvest microalgae biomass for biodiesel production. The results indicate that harvesting during linear growth phase can greatly increase the concentration factor of a harvest, suggesting foam fractionation may be an advantageous continuous harvesting option. A significantly higher total lipid was gained when biomass was harvested via foam fractionation, indicating that harvesting via foam fractionation has a significant influence on the lipid content of *Chlorella sp.* when compared to centrifugal recovery. This study has confirmed that significant work needs to be conducted in order to determine the effect of CTAB on the fatty acid profile of microalgal cells harvested via foam fractionation, and therefore the suitability of this harvesting technology for feedstocks to be used in biodiesel production.

Chapter 6

Effects of harvesting *Chlorella sp.* biomass via foam flotation using CTAB surfactant on the lipid content and fatty acid profile

Abstract

The aim of this study was to establish the reason for the increase in total lipid as a result of harvesting via foam fractionation. Four theories were developed to explain and explore the reason for the lipid increase: Theory one: Surfactant entering extraction method, theory two: Lipid increase as a function of cell stress, theory three: Selective harvesting of high lipid cells, theory four: Cell lysis, and solubilisation of the phospholipid bilayer. The results demonstrated that cells containing higher lipid contents are more likely to be harvested when compared to cells with lower lipid contents; however the amount of extractable lipid of the lower lipid content cells was increased due to being exposed to Cetyl trimethylammonium bromide (CTAB). CTAB caused cell lysis and the solubilisation of the phospholipid bilayer, increasing the amount of total extractable lipid available. Therefore, theories three and four were accepted as explanations for the lipid increase. The effect of harvesting method on the fatty acid profile of *Chlorella sp.* was determined to assess the effect of harvesting method on biodiesel characteristics. Harvesting biomass via foam fractionation significantly increased the levels of saturated and monounsaturated fatty acids within the fatty acid profile, resulting in favourable biodiesel characteristics. The results indicate that foam fractionation is an advantageous harvesting technique for microalgal feedstocks to be used in the production of biodiesel and value-added co-products.

6.1 Introduction

Flotation is a separation process that originated in the mineral industry, and has since become established as an efficient technique for the removal of algae from solution, being explored from both environmental and aquaculture perspectives (Csordas and Wang, 2004; Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998). Microalgal blooms in water treatment plants are commonplace and can be an extremely detrimental contaminant to water quality. Flotation has emerged has as the favoured technique for the removal of algae, with dissolved air flotation (DAF) proving to be the most efficient and cost-effective method to harvest the unwanted waste algae (Wiley et al., 2009). However, over the past decades numerous products produced from microalgae have attracted increased investment and interest from both research and industry, proving microalgae to be a valuable raw material as opposed to a waste product. Markets for health foods from *Spirulina*, animal feed produced from *Chlorella* and pigments for food colouring produced from *Haematococcus* have become established and proven to be commercially successful (Pulz and Gross, 2004). As yet biofuels from microalgal lipids has not become a commercially viable product when compared to traditional petroleum based fuels. The need to harvest and concentrate algal biomass products for transport, storage, and lipid extraction processes has been highlighted as one of the major obstacles for the economic production of microalgal feedstocks (Borges et al., 2011; Borowitzka, 1999).

During DAF an inorganic flocculant such as alum is added to aggregate the microalgal cells, small bubbles are formed via energy intensive superstuartation of the water. These bubbles adhere to the suspended matter carrying it to the surface (Wiley et al., 2011; Uduman et al., 2010; Wiley et al., 2009; Chung et al., 2000; Liu et al., 1999). Foam fractionation uses a technique similar to DAF to harvest biomass; however, it eliminates the need for an expensive energy intensive compressor by generating bubbles and foam with the addition of a surfactant and a low pressure sparger or agitator (Wiley et al., 2011; Wiley et al., 2009). The use of a surfactant also removes the need for a flocculant, as the surfactant improves electrostatic interactions between the bubble and microalgae and acts as a collector (Henderson et al., 2008b).

Pervious chapters have demonstrated that foam fractionation could provide a promising tool for harvesting high volume-low value biomass such as microalgal feedstock due to the extremely low energy requirements, high concentration factors gained, and compared to DAF, filtration, and centrifugal technologies foam fractionation has relatively few mechanical parts which require little maintenance or replacement (Csordas and Wang, 2004; Phoochinda and White, 2003).

Flotation is often listed as a harvesting technology option for microalgae biofuel feedstocks in many review papers (Christenson and Sims, 2011; Wiley et al., 2011; Brennan and Owende, 2010; Uduman et al., 2010; Schenk et al., 2008). However, there is very limited evidence of its use for this type of low value product, where lipid quality is critical. Evidence of using foam fractionation to remove microalgae is all linked to wastewater treatment systems (Wiley et al., 2009; Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998), and therefore the algae is a waste product and the condition of the lipid is of no consequence. In many of these studies cationic Cetyl trimethylammonium bromide (CTAB,$CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$) has been shown as the most advantageous surfactant to use for algal removal (Henderson et al., 2010; DeSousa et al., 2006; Phoochinda et al., 2004; Phoochinda and White, 2003; Liu et al., 1999; Chen et al., 1998) . This is because most microalgae have a negative charge and therefore cationic surfactants such as CTAB may create electro-static interactions between the gas bubbles and the harvest particles, thus improving flotation (Liu et al., 1999). This leads to extremely efficient cell removal of over 90%.

Results from *Chapter 4* demonstrated that significantly higher total lipid extraction was achieved when *Chlorella sp.* biomass was harvested via foam fractionation, when compared to centrifugal recovery. This study indicated that harvesting via foam fractionation has a significant influence on the lipid content of *Chlorella sp.* when compared to centrifugal recovery. To my knowledge only one study has been conducted into the effects of flocculants on the fatty acid composition of microalgae (Borges et al., 2011). The work of Borges et al. (2011), noted higher percentages of C16:0, C16:1 and C20:5, and a decrease in C18:0 and C18:1n9c when a cationic flocculant was used to harvest

Thalassiosira weissflogii. This combined with the results gained in chapter 4 highlights the significant work needing to be conducted, in order to determine the effect of CTAB on the fatty acid profile of microalgal cells harvested via foam fractionation, and therefore the suitability of this harvesting technology for feedstocks to be used in biodiesel production.

For this study the increase in total lipid has been labelled the lipid paradox. Four theories were developed in order to explore the reason for the occurrence of the lipid paradox, and ensure that all potential causes are investigated. The four theories are as follows:

• Theory one: Surfactant entering extraction method.

- CTAB (Figure 6.1) has a carbon backbone similar to many lipids. Any surfactant retained upon the microalgae may be extracted and inadvertently contribute to the total crude lipid.

• Theory two: Lipid increase as a function of cell stress.

- Physiological stress increases lipid production in algae. Exposure to CTAB may potentially induce stress, and therefore the algae may increase lipid weight during the harvesting and processing phases.

• Theory three: Selective harvesting of high lipid cells.

- Cells with higher lipid content may be more hydrophobic and therefore have increased buoyancy. Foam fractionation may selectively harvesting higher lipid cells.

• Theory four: Cell lysis, and solubilisation of the phospholipid bilayer.

- CTAB is often used in used for cell disruption to aid DNA extraction techniques (Porebski et al., 1997). CTAB may be weakening the cell walls during the harvesting process and this may enable the extraction of harder to obtain lipids such as phospholids.

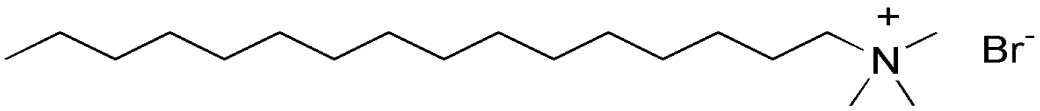

Figure 6.1 The carbon backbone of CTAB has a configuration similar to that of many lipids.

The aim of this study was to establish the reason for the lipid paradox and to determine the effect of CTAB on the lipid profile of the harvested *Chlorella sp.*

6.2 Materials and methods

6.2.1 Cultivation of algae

A mixed *Chlorella sp.* was obtained from Blades Biological Ltd, Kent, UK. For the majority of the experimental runs the microalgae were grown using a proteose peptone medium as described in section 3.3.5. However, in order to determine whether the foam fractionation harvesting technique results in the selective harvesting of microalgal cells containing higher lipid contents, a batch of microalgae was grown under nitrogen deprivation for 14 days, in order to induce oil accumulation (Sharma et al., 2012). The low nitrogen medium contained the following (per litre): 0.2 g $MgSO_4.7H_2O$, 0.2 g K_2HPO_4, 1g KNO_3 and 0.25 g proteose peptone (oxoid L85) (Sigma Aldrich). The low nitrogen medium reduced the available nitrogen sources by 58.4% when compared to the proteose peptone medium used throughout the rest of the study.

6.2.2 Harvesting experiments

The algal harvesting conditions have previously been described in section 5.2.3. Harvests from the same cultures were also conducted via centrifugal recovery,

also described in section 5.2.3, in order to compare the effect of foam fractionation on fatty acid composition, lipid fraction percentages and the presence of surfactant on the outer cell. In order to determine whether foam fractionation selectively harvests microalgal cells containing higher lipid contents, biomass was collected as described in section 5.2.3 from the collection cup (A in Figure 3.1), the residual biomass that that had been exposed to the CTAB but failed to be harvested, remaining in the culture chamber (E in Figure 3.1) was then harvested via centrifugal recovery at 10733 g for 20 minutes, using a Sigma 3K18C centrifuge. Four replicates were conducted.

6.2.3 Electron microscopy

Samples of freeze dried microalgal cells harvested via centrifugal recovery and foam fractionation were examined using Scanning Electron Microscopy (XL30 ESEM-FEG (ESEM)), (Electron Microscopy Services, Newcastle University) which was equipped with an EDAX®, energy-dispersive X-ray spectrometer, (RONTEC systems with Quantax software) with a liquid nitrogen-cooled anti-contamination device in place at all times. The electron microscope was operated at 20 KV in low vacuum mode.

6.3.4 Exploring CTAB's potential to induce lipid accumulation

The maximum duration between harvesting via foam fractionation and freeze drying the biomass was 6 hours. Microalgal cells were exposed to 10 mg L^{-1} of CTAB between 0-24 hours. Cells were removed every hour for 6 hours; a final removal at 24 hours was then conducted. After removal cells were concentrated using a Sigma 3K18C centrifuge for 20 minutes at 10733 g, the supernatant was carefully removed; the pellet was then washed in deionised water and centrifuged again at 10733 g for 20 minutes. The residual liquid from the wet pellet was removed with a pipette. The total lipid was then extracted from an

average pellet weight of 0.34 ± 0.17g, using the liquid-liquid method described in section 5.2.4, four replicates were conducted for the trial. The total lipid as a percentage of the wet pellet weight was then calculated.

6.2.5 Solid phase extraction

The total lipid extract from the microalgae was further separated into different lipid classes by solid phase extraction following Kaluzny et al. (1985) method: total lipid mixtures in chloroform were applied to the EASY® cartridges (Chromabond 3 mL, 200 mg, Macherey-Nagel, Germany), which had previously been conditioned with hexane. Solvent mixtures of increasing polarity were use to eluate the individual lipid classes: All neutral lipids were isolated with 4 mL Chloroform-2-propanol (2:1), free fatty acid were eluted with 4 mL of

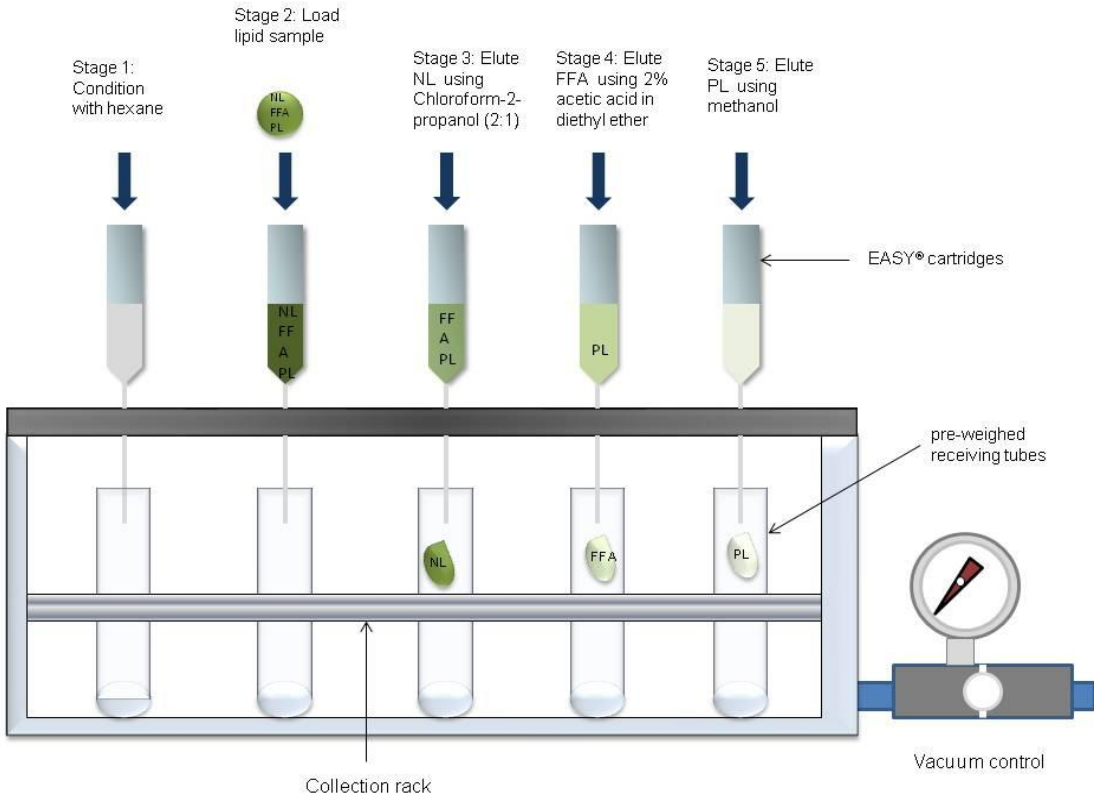

Figure 6.2 Diagrammatic representation apparatus and the elution steps used for the isolation of lipid classes for a total lipid mixture.

2% acetic acid in diethyl ether and the phospholipids fraction were isolated with 4mL of methanol (Figure 6.2). Each lipid fraction was caught in pre-weighed receiving tubes and was evaporated to dryness under nitrogen. The weight for each lipid class obtained was then determined gravimetrically (Peplow et al., 1990).

6.2.6 Fatty acid composition analysis

Fatty acid composition analysis was performed using a Carlo Erba Model Mega 5160 gas chromatograph (Carlo Erba, Milan, Italy). Ten milligram of freeze dried algae samples were placed into capped test tubes with heptadecanoic acid (C17:0) as the internal standard. Fatty acid methyl esters (FAMEs) were extracted with a one-step method of Gracés and Mancha (1993) (methanol: toluene: 2,2 dimethoxypropane (DMP): sulphuric acid; 39:20:5:2, by volume). An injection volume of 1 µl was loaded onto a Supelco column (sigma Aldrich) at 240°C (30m length x 0.25 mm ID, 0.25µm Film) with helium as the carrier gas. The temperature was programmed to ramp from 50°C to 240°C at 7°C per minute. Fatty acids were identified by comparing the obtained retention times with that of known standards (37 component FAME mix (SuplecoTM)). Fatty acid composition analysis was conducted in triplicate for each harvest method.

6.2.7 Statistical analysis

The extracted total lipid and FAME contents from the microalgal harvested via foam fractionation and centrifugal recovery were compared using an analysis of variance (ANOVA) test. An alpha level of 0.05 was used to determine the significance of the data.

6.3 Results and discussion

6.3.1 Theory one: Surfactant entering extraction method

It was initially hypothesised that CTAB residues may have become adsorbed onto the microalgal cell surface and could therefore be washed into the total lipid product, adding to the total weight, and therefore increasing the calculated percentage lipid for dry weight biomass. To confirm that CTAB could tolerate the lipid extraction process 0.15 g of pure CTAB was exposed to the same extraction process as the microalgal cells, 3 replicates were performed. It was found that 71.02 ± 6.17% of the initial starting weight was able to withstand the extraction process. Therefore if a CTAB residue were present it would add to the total weight of the lipid content. Bromine can be easily be detected via SEM-EDX, therefore the presence of CTAB residues after harvesting via foam fractionation, preparation, and freeze drying of the microalgal cells can be ascertained by the presence of Br (Xu et al., 2009). Figure 6.3 demonstrates that there is no significant difference between the Br levels of microalgal cells harvested via foam fractionation when compared to cells harvested via centrifugal recovery. Therefore it is unlikely that a CTAB residue was present on the cell upon at the start of the lipid extraction procedure. It is likely that the CTAB was removed during the centrifugation and washing procedure that occurred prior to freeze drying the biomass harvested via foam fractionation. However, due to a lack of reference for Br levels present in a CTAB sample that had gone through the fractionation process this theory cannot be rejected based on this evidence (see section 6.3.5 for further evidence).

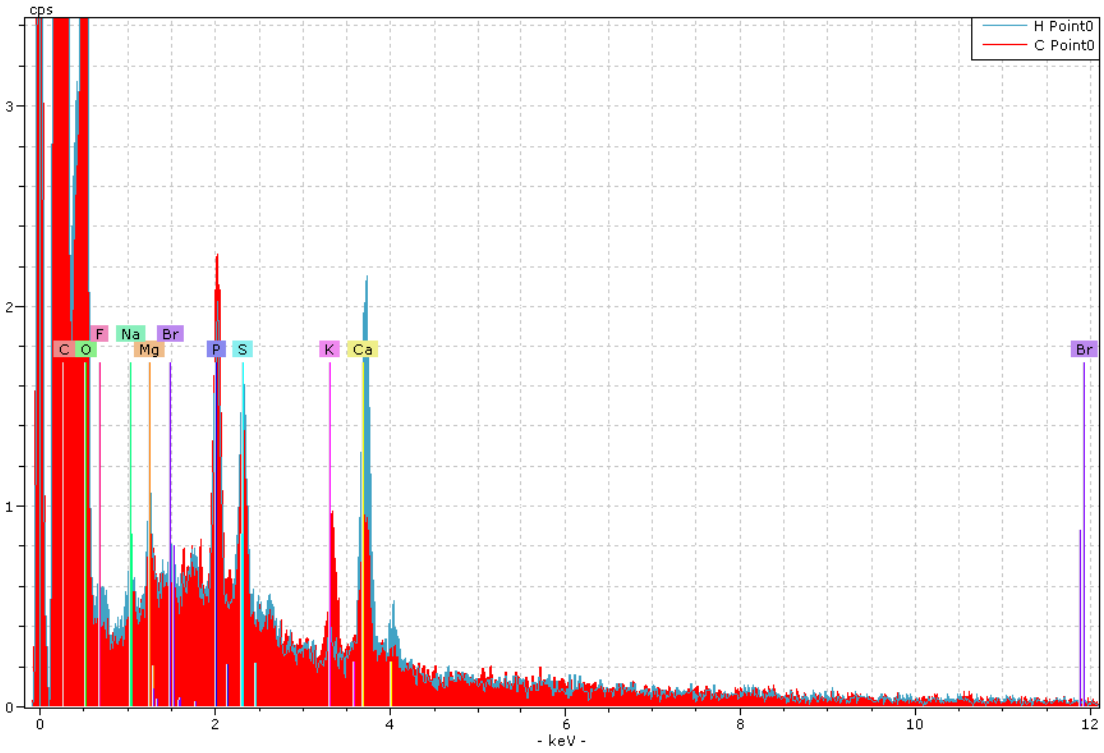

Figure 6.3 EDX spectrum of microalgal cells harvested via centrifugal recovery (represented in red) and foam fractionation (represented in blue). The main elements that occurred in both samples are labelled.

6.3.2 Theory two: Lipid increase as a function of cell stress

Microalgae have the ability to modify their lipid metabolism efficiency in response to environmental and physiological changes, commonly due to the limitation of nutrients such as phosphate and nitrogen (Mandal and Mallick, 2009), however lipid accumulation can also be a function of additional nutrients rather than to limitation (Liu et al., 2008). *Chlorella sp.* were exposed to CTAB between 0-24 hours to determine whether lipid accumulation was occurring in the time that would be taken between harvesting via foam fractionation and freeze drying. The total lipid extracted, in percentage of wet weight is shown in figure 6.4. Exposure to CTAB increases the average lipid content of the cell almost instantly, from 0.49 ± 0.02% to 1.92 ± 0.57%, representing nearly a fourfold increase within an hour. However, after initial exposure, no significant change occurs to the percentage lipid extract between 1-24 hours of exposure to CTAB ($p=0.395$). The average lipid content was 1.6 ± 0.4%. Lipid

accumulation due to nitrogen deprivation may take 2-5 days (Sharma et al., 2012), due to the significant speed at which the lipid content increased due to exposure of CTAB, and the lack of significant lipid accumulation within the time it would take from harvest to freeze drying, the second theory can also be rejected.

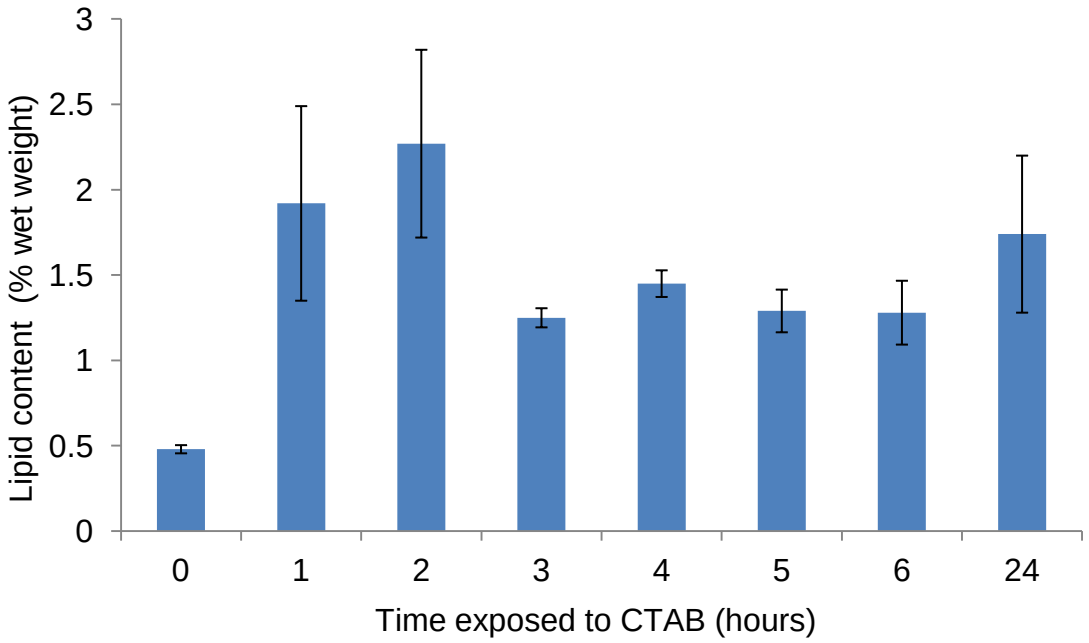

Figure 6.4 Total lipid as a percentage of wet weight as a function of exposure time to CTAB.

6.3.3 Theory three: Selective harvesting of high lipid cells

In order to determine whether foam fractionation selectively harvests microalgal cells containing higher lipid content, two batches of *Chlorella sp.* culture were grown in conjunction; the first grown under nitrogen limitation, and the second grown under non-limiting conditions using a standard proteose peptone medium. The batch grown under nitrogen limitation gained a significantly higher average total lipid content of 1.52 ± 0.13% compared to 0.96 ± 0.05% (based on wet weight samples) than the culture grown in a non-limiting environment. For this study cultures grown under nitrogen limitation will be referred to as high lipid content and those grown under standard conditions will be referred to as low lipid content. Figure 6.5 shows that high lipid content cultures gain significantly

higher concentration factors and biomass yields when compared to low lipid cultures when harvested via foam fractionation (p=<0.001), this may be due to the increase in buoyancy that would occur with the increase in lipid content.

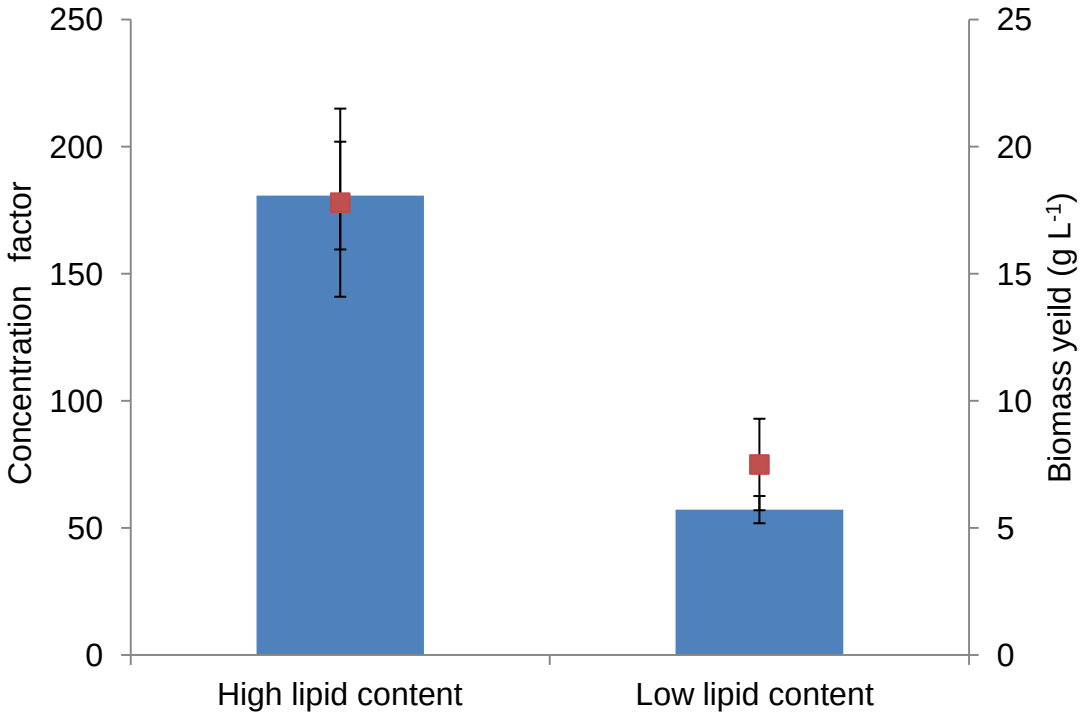

Figure 6.5 The concentration factor (represented in blue) and the biomass yield (represented in red) of *Chlorella sp.* harvested via foam fractionation as a function of cell lipid content.

To further confirm this concept the total lipid content of *Chlorella sp.* biomass harvested via foam fractionation was compared to that of the biomass that remained un-harvested in the harvesting unit, and to the biomass of algae that had not been exposed to CTAB and had been harvested via centrifugal recovery. Figure 6.6 shows that there is a significant difference between the total lipid content of the biomass harvested via foam fractionation (17.1 ± 1.2%) compared to the un-harvested residual biomass (13.8 ± 1.6%) (p=0.015). It should also be noted that the total lipid content of the un-harvested cells is significantly higher than those harvested via centrifugal recovery (9.5 ± 1.4%)

(p=0.001). This confirms that exposure to CTAB, even over short time periods and at low levels (10 mg L^{-1}) causes an increase in the total lipid extracted (Figure 6.4).

CTAB has previously been used to permeabilize red beet, *Beta vulgaris*, to recover and concentrate valuable betalaines pigments, which can be used as food colouring (Thimmaraju et al., 2003). Thimmaraju et al (2003) noted that CTAB caused pigment efflux from the red beet immediately after its addition; which was linked to the dissolution of the phospholipid layer.

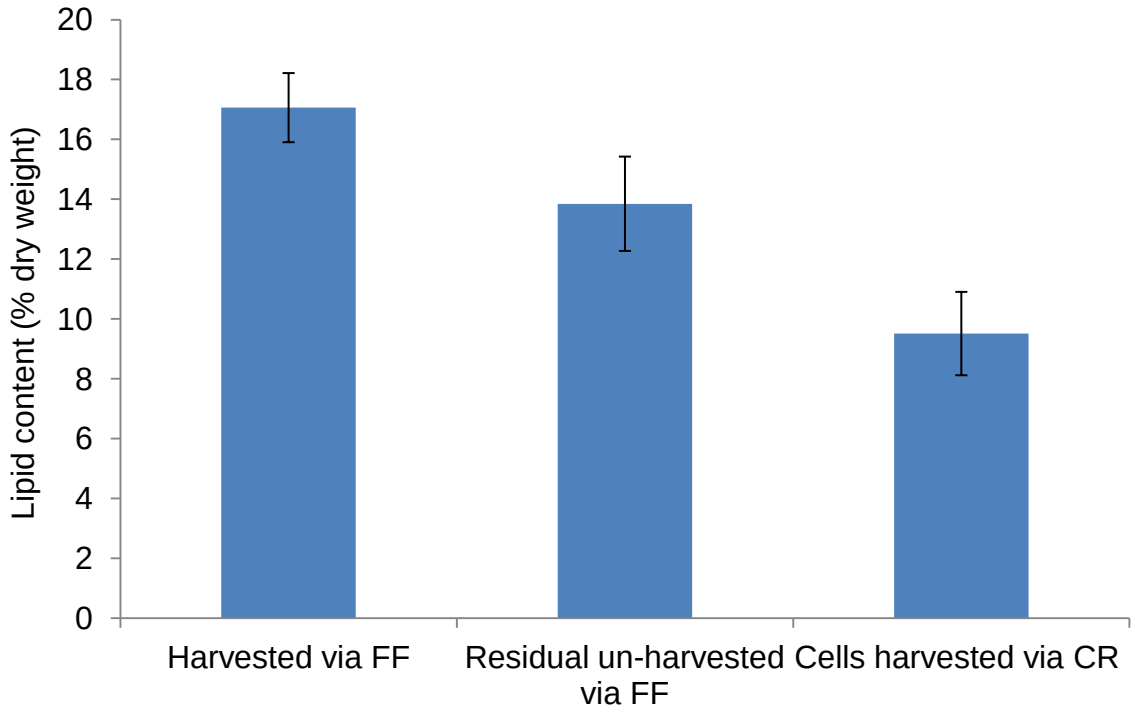

Figure 6.6 The lipid content of cells harvested via foam fractionation (FF), residual un-harvested cells after foam fractionation and cells harvested via centrifugal recovery (CR).

The results demonstrate that microalgal cells with high lipid contents are more likely to be concentrated and harvested via foam fractionation than microalgal cells containing lower lipid contents. However, the increase in total lipid cannot be totally explained by the selective harvesting of higher lipid content cells, due to the significant increases in extractable lipids when

comparing the un-harvested cells with the cells harvested via centrifugal recovery. The effect of CTAB lipid composition *Chlorella* sp. was examined using solid phase extraction (SPE).

6.3.4 Theory four: Cell lysis, and solubilisation of the phospholipid bilayer.

Total lipids are composed of neutral lipid in the form of energy storage bodies, as well as glyco- and phospholipids in the structural membranes. Within the literature the total lipid content of microalgae is commonly quoted as an indication of how appropriate it is as a biodiesel feedstock (Rösch et al., 2012). However, not all lipid fractionations can be easily esterified into biodiesel, and therefore a high total lipid quote can be misleading. Solid phase extraction (SPE) was used to determine the effect of CTAB on the composition of the total lipid extract, and to investigate the effect of harvesting technique on the production of biodiesel from *Chlorella sp.* SPE of the total extracted lipids from the microalgal cells harvested via foam fractionation and centrifugal recovery demonstrated that the majority of the lipid consisted of neutral lipids (Table 6.1). There was no significant difference between the neutral lipid fraction ($p=0.739$) and the free fatty acid fraction (FFA) ($p=0.085$) when comparing the lipids from cells harvested via both methods. However, there was a significantly higher phospholipid content in the total extracted lipid of the microalgae harvested via foam fractionation ($p=0.008$), when compared to cells harvested via centrifugal recovery. All harvested cultures were grown under the same environmental conditions, making it unlikely that cells harvested via foam fractionation would contain high levels of phospholipids, therefore CTAB must have increased the amount of phospholipids available for extraction due to the solubilisation of the phospholipid cell membrane (Chao and Lee, 2000).

Table 6.1 Percentage of lipid class fraction with respect to total extracted lipid. Data displayed is the average of 4 replicates and the standard error is indicated.

Lipid fraction	Centrifugal recovery	Foam fractionation
Neutral lipid	58.3 ± 6.1	60.1 ± 6.2
Free fatty acids	25.5 ± 2.3	16.1 ± 4.5
Phospholipids	16.14 ± 6.4	23.6 ± 2.7

The neutral lipids that are found within microalgal lipid extracts are mainly comprised of triacylglycerols (TAGs) (Hu et al., 2008). Although both polar and neutral lipids can be converted to biodiesel, neutral lipids are the desired fraction since TAGs are easily transesterified to biodiesel (Ma and Hanna, 1999). FFA can also be converted after esterification (Doan et al., 2011). However, Van Gerpen (2005) reported that phosphorus compounds, such as phospholipids, in the crude lipid oil did not convert into the methyl esters, this can cause problems during the conversion process and in engines using the biodiesel (Pruvost et al., 2009; Van Gerpen, 2005). However, while not necessarily valuable for biodiesel production, phospholipids can be added back to the residual algae biomass, post lipid extraction, increasing the value of the biomass for animal feed, and producing a by-product which can compensate the cost of production (Rösch et al., 2012). The phospholipids can also be recycled and used as a source of nitrogen and phosphorus nutrients for microalgae cultivation, which could significantly reduce the operational costs of algal production systems (Rösch et al., 2012). Phospholipid fractions could contain high-value products such as polyunsaturated fatty acids (PUFAs), which have gained growing interest in recent years as a health food supplement (Alonso et al., 1998). As described previously CTAB can be used as a food grade chemical for the extraction of pigments from red beet, therefore procedures to ensure that the product is fit for human/animal consumption are available (Thimmaraju et al., 2003).

CTAB causes cell lysis and solubilisation of the phospholipid bilayer, increasing the total extractable lipid compared to cells harvested via centrifugal recovery; therefore theory four was accepted. The use of foam fractionation for harvesting feedstocks for biodiesel production has no detrimental effect on the

desirable lipid fractions. Increasing the available phospholipid for extraction increases the number of potential value added co-products, which can help to off-set the cost of biodiesel production.

6.3.5 Determination of the fatty acid profile

The effect of foam fractionation on the fatty acid profile of *Chlorella sp.* was investigated using gas chromatography (Table 6.2). It was important to characterise the fatty acid profile as this can dramatically affect the quality of the biodiesel product (Gouveia and Oliveira, 2009). No significant difference was found between the amount of FAMEs gained from cells harvested via centrifugal recovery and foam fractionation (p=0.609). The total transesterifiable lipid for cells harvested via centrifugal recovery was 6.4 ± 1.3% cellular dry weight (CDW ± standard error) and 5.6 ± 0.3% CDW for cells harvested via foam fractionation. During esterification 30% of the original lipid mass can be lost to the polar phase. Nagle and Lemke (1990) demonstrated that as little as 65.1% of the phospholipid phosphatidyl chloride, commonly found in microalgae, was converted to FAME, this may explain the lack of significant difference between the total FAME of the harvested cells. However, discernible changes within the FAME can be noted, significantly higher amounts of the monounsaturated fatty acid oleic acid (C18:1n9c) occur in cells harvested via foam fractionation (5.1 ± 0.133% CDW) compared to cells harvested via centrifugal recovery (2.17 ± 0.08% CDW) (p= <0.001). Significantly higher total amounts of monounsaturated fatty acids (p=<0.001) and saturated fatty acids (p=0.006) were found in the lipids of cells harvested via foam fractionation (Table 6.3). Generally higher proportions of these FAMEs are desired as this increases energy yield, and cetane number, it also improves the oxidative stability (Doan et al., 2011). Surprisingly, cells harvested via centrifugal recovery showed higher levels of total PUFAs (p=0.001), although cells harvested via both methods had high levels of PUFAs. Cells harvested via centrifugal recovery have significantly higher amounts of linoleic acid (C18:2n6c) (p=0.001) and linolenic acids (C18:3n3) (p=0.001), however, there is no significant difference between the C18 series for each harvest type (p=0.084). A report by Knothe (2008) stated that palmitic, stearic, oleic, and

linolenic acid are the most common fatty acids found in biodiesel. The total of these desired fatty acids was 24.7 ± 0.46% FAME for cells harvested via centrifugal recovery and 23.3 ± 0.30% FAME for cells harvested via foam fractionation, with no significant difference between the harvesting methods (p=0.091). In the microalgae harvested by both methods linoleic acid (C18:2) was dominant, containing a higher portion than found in Caster, *Lesquerella*, and rapeseed FAMEs and a similar amount to that found in soybean FAMEs (Geller and Goodrum, 2004).

Table 6.2 Content of fatty acid methyl ester with respect to total fatty acids (% DW). Data displayed is the average of 3 replicates

Fatty acid methyl ester	Centrifuged	Foam fractionation harvested
Caprylic (C8:0)	0.04	0.05
Capric (C10:0)	0.03	0.05
Lauric (C12:0)	0.24	0.21
Myristic (C14:0)	1.27	1.77
Pentadecanoic (C15:0)	0.12	0.18
Palmitic (C16:0)	1.76	1.52
Stearic (C18:0)	0.30	0.69
Elaidic (C18:1n9t)	4.54	4.53
Oleic (C18:1n9c)	2.17	5.16
Linoleic acid (C18:2n6c)	22.30	17.48
Linolenic acid (C18:3n3)	10.20	8.14
Arachidic (C20:0)	2.23	1.68
cis-11-Eicosenoic (C20:1)	0.12	0.10
Behenic (C22:0)	0.10	0.24
Total C18 Series	41.12	38.85

Further evidence to support the rejection of theory one can also be found in the FAME series. Gas chromatography has been long used for the determination of long-chain cationic surfactants (Tsai and Ding, 2004; Metcalfe, 1984; Takano et al., 1977). CTAB is a 16-carbon quaternary ammonium polar surfactant (Figure 6.1). Polar solvents such as methanol used in the FAME

extraction technique (see section 6.2.6) would therefore remove any CTAB residue if present, resulting in a significant increase in the C16 series. However no significant difference was found in the C16 series of alga harvested via foam fractionation when compared to cells harvested via centrifugal recovery ($p=0.133$). Therefore it can be concluded that a CTAB residue was not present on the cell prior to lipid, and FAME extraction. Further work to support the rejection of theory one is planned see section 7.2.

Table 6.3 Composition and yield of FAME of *Chlorella sp.* harvested via centrifugal recovery and foam fractionation

Properties	Centrifugal Recovery	Foam Fractionation
Saturated fatty acids	6.0±0.08	6.4±0.1
Monounsaturated fatty acids	6.7±0.18	9.7±0.3
Polyunsaturated fatty acids	32.5±0.96	25.3±1.08
Dominant biodiesel components (% FAME)	24.7±1.84	23.3±0.60
Total fame content (%CDW)	6.4±2.54	5.6±0.52

Saturated fatty acids = C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C20:0, C22:0, Monounsaturated fatty acids = C18:1n9t, C18:1n9c, Polyunsaturated fatty acids = C18:2N6c, C18:3n3., Dominant biodiesel components = C18:0, C18:1n9c, C18:2n6c (Knothe, 2008) Data expressed as average ± SD (*n*=3).

The results of the fatty acid profile analysis indicate that harvesting microalgal cells via foam fractionation would contribute to the production of a biodiesel with many desirable features. The increased presence of monounsaturated fatty acids and saturated fatty acids would produce a biodiesel with a higher energy yield, superior oxidative stability and a higher cetane number.

6.4 Conclusion

This study was the first of its kind to investigate the effect of harvesting via foam fractionation on the lipid content and fatty acid profiles of microalgal cells. Four theories were explored in order to determine the reason for the lipid paradox. The results of this study indicate that the lipid paradox occurs in part due to the selective harvesting of higher lipid content cells. However, the majority of the lipid paradox is due to an increase in the total extractable lipid caused by the solubilisation of the phospholipid bilayer by the surfactant CTAB. Analysis of the fatty acid profile demonstrated that harvesting via foam fractionation also results in a predominance of saturated fatty acids and monounsaturated fatty acids within the fatty acid profile, which result in many favourable features for the production of biodiesel. The results indicate that foam fractionation is an advantageous harvesting technique for microalgal feedstocks to be used in the production of biodiesel and value-added co-products.

Chapter 7

Conclusions and recommendations for future work

7.1 Conclusions

In this research the feasibility of utilizing foam fractionation as a low cost harvesting technology was shown, specifically for harvesting microalgal feedstock to be used in biodiesel production. The major conclusion that can be drawn from this study is that harvesting microalgae via foam fractionation provides one of the most advantageous cost-benefit relationships when compared to the current commonly used harvesting technologies, gaining high concentration factors and biomass yields while using a fraction of the energy demand of other technologies.

A full literature review was conducted into biodiesel derived from microalgae in order to understand the cultivation stages pre-harvesting which could potentially affect harvesting efficiency and the potential effects of harvesting technique may have on downstream processing that occurs post-harvesting. Gaps within the research field were noted and from this a foam fractionation column was designed. The harvesting unit was designed and constructed with readily available low cost materials, with a configuration that could easily be changed and adapted to meet different experimental requirements. A design of experiments and a central composite design were used to screen key variables and to determine the optimal levels of major variables influencing the harvest of the freshwater microalgae *Chlorella sp*. The effects of bubble size within the liquid pool and foam phase of the harvesting unit were determined by altering the sparger set-up within the harvesting unit. The influence of microalgal growth phase on harvest efficiency was investigated to gain insight into the optimal time to harvest during the cell cultivation period; data from this study highlighted the need to investigate to what extent foam fractionation affects the quality of the end product. The effect of the surfactant, used to induce foaming, on lipid recovery was examined through methods including total lipid recovery, gas chromatography, energy dispersive x-ray and

solid phase extraction. The promising conclusions gained from this research are broken down into two main sections; variable selection and harvest optimisation and, determination of the how suitable foam fractionation is for harvesting biodiesel feedstocks.

7.1.1 Variable selection and harvest optimisation

A review of the literature highlighted that microalgae provide the only feedstock source able to meet biodiesel demand in a sustainable manner. One of the major obstacles to the production of economically viable biodiesel from algal feedstocks is the need to harvest and concentrate biomass products for transport, storage and lipid extraction. Comparing the current harvesting technologies has made clear that dispersed air flotation (DiAF) combined with foam fractionation could provide an economical way to harvest microalgae, offering significantly lower construction, maintenance and energy costs when compared to centrifugal recovery and reductions in the time and space required when compared to flocculation and sedimentation. Foam fractionation is able to physically separate off the biomass product reducing the need for energy intensive pumps, and could potentially improve water and chemical recycling. Foam fractionation has only been researched into as a removal technology for waste water treatment, therefore several gaps within the research regarding the harvesting and concentration of microalgae were noted including;

- Integrating commonly used theories from within the mineral industry
- The effect of key variables on the biomass yield and concentration factors gained from harvesting via foam fractionation
- Gaining a constant and predictable high harvest yield
- Harvesting on a larger scale
- Cost of harvesting biomass via foam fractionation

Initial work demonstrated that negatively charged latex beads provided a satisfactory substitute for microalgae during fractional factorial designed experiments. Latex beads allowed the influence of only known variables on the concentration factor of the harvest to be determined, creating an accurate understanding of the key variables. Verification work using *Chlorella sp.*

established that the cationic surfactant, Cetyl trimethylammonium bromide (CTAB) yielded high concentration factors and biomass. This was attributed to the electro-static interactions that occurred between the gas bubble and the microalgae, thus increasing the hydrophobicity of the once hydrophilic solid–liquid interface and improving harvest potential. The concept of using a cationic surfactant to improve the flotation of microalgae is not a new one; however the fractional factorial design also demonstrated theories that as yet have only been applied to protein and mineral recovery and harvesting. The use of tall column heights and low surfactant dosages allowed for long foam residence times, which is critical for the drainage of liquid and the concentration of biomass. Foam residence time was shown to be an important factor to consider when attempting to concentrate the microalgal biomass. This has thus far not been related to this field of activity. A central composite design conducted with only microalgae demonstrated that the microalgae added a large number of unknown variables and therefore data could not be predicted from the model. However the optimisations of known variables were demonstrated, signifying that column heights of 1.5 meters and CTAB concentrations of 10 mg L^{-1}, gave the highest concentration factors. This research demonstrates that for effective harvesting of microalgae for further processing foam residence time is an important factor, which thus far has rarely been considered.

Bubble size and distribution within a foam column is a critical variable and was therefore measured in isolation. Bubbles produced by three different sparger and air input set-ups were characterised, the set-ups were; lime wood sparger with linear air flow input, ceramic flat plate sparger with linear air flow input and ceramic plate sparger with an oscillatory air flow input. Bubble size and bubble rise velocity was greatly dependent on the sparger set-up. The data displayed in figure 4.5 and 4.7 do not conform to the conventional theory that smaller bubbles have a slower rise velocity, and a larger interfacial area. The ceramic spargers generate dense clouds of bubbles, which increased the surface area to volume ratio of the bubble phase within the liquid. Momentum was transferred due to shear stress that occurs across the surface area of the bubble, ambient liquid is dragged into the bubble cloud, reducing drag.

Fast bubble velocities produced by the ceramic spargers were associated with a reduction in the bubble’s residence times, reducing the gas

hold-up within the system and therefore resulting in a reduction of the interfacial area within the liquid pool. High concentration factors of 426.5 ± 34.7 were gained using the ceramic plate sparger with an oscillatory airflow input. The increased harvesting efficiency was related to an increase in collection efficiency due to a decrease in bubble size. Analysis of the energy consumption of a foam fractionation harvesting technique revealed that using a ceramic sparger with a linear flow gains an advantageous cost-benefit relationship for the bulk harvesting of microalgae. This operational set-up only required 0.019 $kWhm^3$ which is considerably lower than commonly used harvesting technologies, it also achieved harvesting efficiencies comparable to or better than, those achieved using tangential flow filtration, nozzle discharge centrifuge, hydrocyclone centrifuge, dissolved air flotation and dispersed air flotation.

Relationships were observed between the foam fractionation harvest efficiency and the growth phase of the microalgae. Harvesting during the exponential growth phase can greatly increase concentration factor, therefore, monitoring the cell growth phase can be an effective and economical tool to increasing harvesting efficiency. The study into the effect of growth phase on harvest efficiency revealed the need for further work in a number of areas, see *section 7.2*.

7.1.2 Determination of how suitable foam fractionation is for harvesting biodiesel feedstocks

Comparing the total lipid content of cells harvested via foam fractionation and centrifugal recovery it was noted that a significantly higher total lipid was gained when biomass was harvested via foam fractionation. The increases in lipid content occurred in part due to the selective harvesting of higher lipid content cells. However, CTAB had a significant influence on the lipid content of *Chlorella* sp. This was due to an increase in the total extractable lipid caused by the solubilisation of the phospholipid bilayer by CTAB. Provided the phospholipids are removed prior to the transesterification process, an increase in the phospholipid fraction in the total lipid should not affect the biodiesel quality. A number potential benefits could be gained from an increase in

phospholipids extraction, such as of value added animal feed, reduction of the operational cost of cultivation due to nitrogen and phosphorus nutrients recycling, and production of additions to health food supplement. Unlike many chemical additives CTAB can be used as a food grade chemical and therefore procedures to ensure that the product is fit for human/animal consumption are available. CTABs effect on the cell wall may also provide a useful tool for the wet extraction of lipids from microalgal biomass. A harvesting method such as foam fractionation using CTAB could combine dewatering with cell disruption, removing the need for grinding, ultrasonication, bead milling or enzymatic lysis, so reducing the number of stages between harvesting and processing.

Analysis of the effect of CTAB on the fatty acid profile of microalgae was the first of its kind. The results demonstrated that harvesting via foam fractionation also resulted in a predominance of saturated fatty acids and monounsaturated fatty acids within the fatty acid profile, which result in many favourable features for the production of biodiesel.

These encouraging results indicate that foam fractionation is an advantageous low cost harvesting technique for microalgal feedstocks to be used in the production of biodiesel, gaining high concentration factors, biomass yields and a number of potential value-added co-products.

7.2 Recommendations for future work

As a result of the promising data gained during this study a number of further research projects have been identified. Initial future work would have to focus on gaining zeta potential data. Although encouraging results were obtained with regard to the affect of growth phase on harvesting efficiency, specific data on the surface functional groups, dissolved organic matter and zeta potential throughout the growth phase need to be collected. Each of these cellular characteristics previously listed change throughout the microalgal growth cycle and greatly impact upon electro-static interactions that occur between the microalgal cell and the gas bubble, therefore affecting the harvesting efficiency (Zhang et al., 2012; Danquah et al., 2009b). Understanding specific species cell

characteristics may enable the correct selection of surfactant and surfactant dosing.

Further work to support the rejection of theory one (surfactant entering extraction method) will be conducted by reproducing figure 6.3 with the addition of a Br reference for a CTAB sample that had gone through the foam fractionation process.

In order to utilise foam fractionation on a large scale the unit would need to be adapted to harvest either semi-continuously or continuously. Gary Caldwell and Jonathan Lee recently secured funding to enable the fabrication of a continuous harvester and research into continuous harvesting via foam fractionation. Figure 7.1 shows the proposed continuous harvester, designed by Jonathan Lee. Once fabricated the harvesting unit would process 30 L of microalgae every 5 minutes, removing up to 80% of the biomass in the time period (Chen et al., 1998), and potentially concentrating the biomass to similar degrees as discussed throughout this thesis. The lid of the culture chamber and the entrance into the collection cup of the harvesting unit has been redesigned to reduce the potential build up of biomass in corners that was noted throughout this research (Figure 7.2).

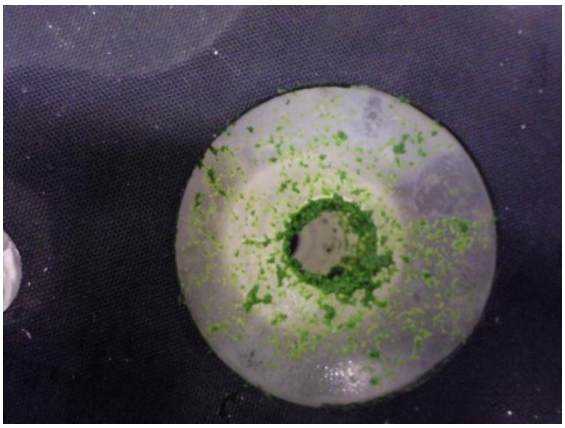

Figure 7.2 Biomass accumulation that occurred at the entrance to the collection cup **A)** and around the top of the culture chamber **B)**.

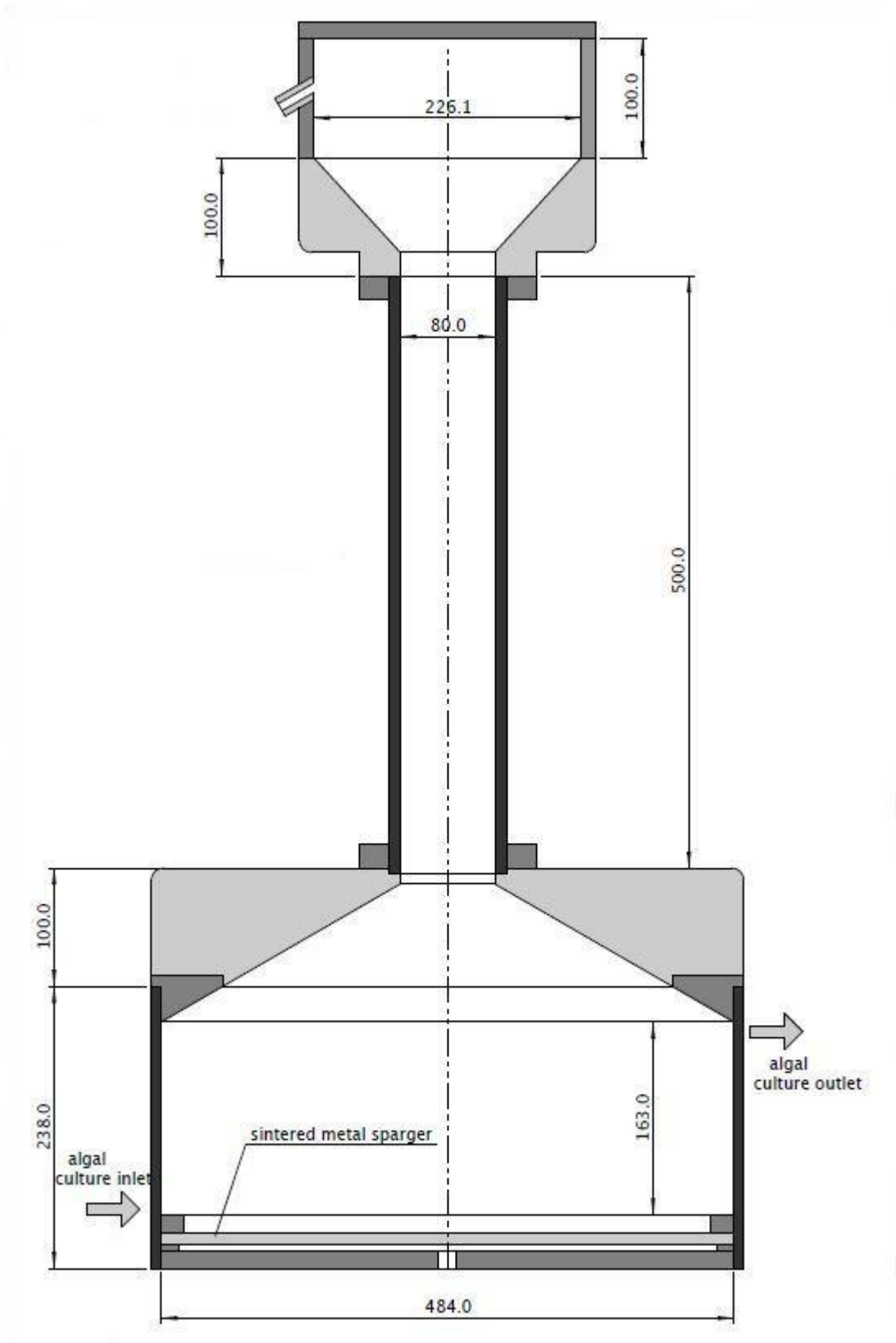

Figure 7.1 Proposed continuous foam fractionation harvesting unit as designed by Jonathan Lee.

Although not noted on figure 7.1 the continuous harvesting unit will also have a foam riser plate. As the foam rises up the column the foam is forced through a narrower tube section (foam riser plate), enhancing liquid drainage and biomass concentration factor (Li et al., 2011). During the time at which this thesis was being conducted Catherine Lloyd (Master's student) researched the use of foam riser plates to reduce the liquid content and increase the concentration factor of *Chlorella sp.* noting a significant reduction in the foamate liquid content and an increase in concentration factor using a 40mm inner diameter foam riser plate (Lloyd, 2012). Specific data regarding the gas hold-up must be collected to gain better understanding of the hydrodynamics and mass transfer within the new column design, as it may have a strong impact upon the scale up of future designs. A number of methods for measuring gas hold up have been proposed however, a standard method has not yet been determined (Meng et al., 2002). The most suitable method appears to be electrical resistivity probes, which allow the measurement of gas-hold up at various locations in a column (Meng et al., 2002; Kemoun et al., 2001).

The separation and recycling of the CTAB post harvest needs to be trialled to determine the viability of this process. If possible this process could dramatically cut the cost of harvesting via foam fractionation. In this research it was identified that the destruction of the cell wall as a result CTAB may aid in the wet extraction of lipids from microalgal biomass, combining dewatering with cell disruption. Further investigations are required into the specifics of how CTAB affects the cell membrane, and the potential of integrating harvesting and wet extraction methods.

If freshwater microalgae are not cultivated in combination with wastewater treatment and nutrient removal, marine microalgae are commonly considered to be advantageous as biodiesel feedstocks, because they do not require freshwater during cultivation. The use of foam fractionation with marine species needs to be researched into.

Current domestic agricultural crops used for the production of terrestrial based biodiesel have been cultivated for thousands of years, with the desired traits being selected over time. Genetic engineering is likely to be the key to by-passing a lengthy selection process and producing a near term economically

viable fuel from microalgae (Radakovits et al., 2010). Microalgae are currently being selected to live in high salinity environments to reduce the risk of contamination (Schenk et al., 2008; Lee, 2001). Unfortunately, high ionic strengths greatly reduce the efficiency of flocculation and flotation (Liu et al., 1999; Chen et al., 1998), due to the sodium ions reducing the effectiveness of the electrostatic interactions between the chemical additive and the microalgal cell. The 'ideal' genetically modified microalgae would have the high levels of liquid hydrocarbons, like that of *Botrycoccus braunii*, be able to thrive in a high salinity environment, such as *Dunaliella salina*, have high growth rates like that of *Nannochloropsis sp.,* and produce the its own surfactant such as *Cryptomonas sp.* This would allow the feedstock to be grown on a large enough scale to meet biodiesel demand, to be economically produced using open culture raceway ponds and harvest economically via foam fractionation without the addition of chemicals.

The integration of microalgae derived biofuel and co-products production with wastewater treatment would provide major advantages for both industries. The integration of these industries would provide the important step for taking the foam fractionation harvesting unit from lab scale to the large scale, reducing capital costs and scalability issues, through the use of the wastewater treatment plants existing infrastructure (Christenson and Sims, 2011).

The harvesting of microalgae via foam fractionation should not be limited to biodiesel feedstocks as it may provide a particularly useful tool for the harvesting and improved removal of high-value pigments to be used in the health food and pharmaceutical industries (Pulz and Gross, 2004; Thimmaraju et al., 2003).

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Appendix 1

A1.1 Recycling of polystyrene latex beads

Due to the relative expense of the polystyrene latex beads (price as of 05/06/12 £254.50 for 10 mL), it was decided that the beads should be recycled. To avoid a time consuming and difficult washing procedure, in which all surfactant must be removed before the beads could be used for a new trial, a colorimetric method was utilised. The colorimetric method was used for analysing the quantity of detergent present within the harvested fraction, which contained the harvested concentrated latex beads and a surfactant concentrate. This harvest fraction could then be returned to the culture chamber and any additional required surfactant added for the next run within the fractional factorial design. To ensure a quick and simple determination of the quantity of surfactant a calibration curve was created. A modified methylene blue method (MMBM) was used for estimating the quantity of Ecover® (anionic surfactant) (Wang, 1975) and a methyl orange method (MOM) was used to determine the quantities of CTAB (cationic surfactant). Both colorimetric procedures are appropriate for producing a calibration curve since the intensity of the colour that is produced is proportional to the number of reagent (methylene blue or methyl orange) and surfactant complexes within the harvest water sample (Wang and Langley, 1975; Wang, 1975).

A1.2 Material and methods

A1.2.1 Standard anionic solutions

Standard anionic solutions of Ecover® of 0, 0.05, 0.1, 0.15, 0.2 and 0.3 mL L^{-1} were made by adding the appropriate amount of surfactant into distilled water and ensuring it is fully dissolved.

A1.2.2 Modified methylene blue method

Initially water samples ranging over six known surfactant quantities (anionic) were analysed and the absorbance at a wavelength of 652 nm was measured to create the calibration curve. The procedure was then carried out on the harvest water containing unknown surfactant levels. The surfactant levels were estimated using the equation produced from the line of the calibration curve. A methylene blue reagent and a buffer solution had to be made up before the MBBM procedure could be conducted; both solutions were made up in accordance to Wang (1975).

A1.2.3 Analytical procedure for MMBM

The following procedure was used for both the standard anionic solutions and the harvest water samples.

• A cuvette of clean chloroform was inserted into the UV spectrophotometer Cary 300-Bio (Varian, Palo Alto, CA), which was set at an absorbance of 625 nm, to create a blank.

• 30 mL of sample (either standard anionic solution or harvest water containing Ecover®) were then placed into a separatory funnel and a further 20 mL of distilled water added to dilute the whole sample to 50 mL.

• 1mL of methylene blue reagent, 5 mL of buffer solution and 25 mL of chloroform were added to the funnel.

• The glass stopper was put in place and the funnel was shaken vigorously for 30 seconds.

• The funnel was then left to stand for 5 minutes allowing the chloroform layer, containing the methylene blue-surfactant complexes, if present, to separate from the water fraction.

• The chloroform layer was then carefully removed into a clean glass flask, the flask was then swirled to remove any potential water contaminates.

• The dewatered chloroform was placed into a clean cuvette and absorbance measured at 625nm in an UV spectrophotometer Cary 300-Bio (Varian, Palo Alto, CA).

• The absorbance was then converted into mL L^{-1} with the calibration curve (Figure A1.1)

A1.2.4 Standard cationic solution

Standard cationic solutions of CTAB of 0, 10, 20, 30, 40 and 50 mg L^{-1} were made by adding the appropriate amount of surfactant into distilled water and ensuring it was fully dissolved.

A1.2.5 Methyl orange method

The methyl orange method is very similar to the MMBM procedure, again water samples ranging over six known surfactant quantities (cationic) were analysed, however a wavelength of 415 nm was used to measure the absorbance and to create the calibration curve. The methyl orange solution and buffer solution were both made up in accordance to Wang and Langley (1975).

A1.2.6 Analytical procedure for MOM

The following procedure was used for both the standard anionic solutions and the harvest water samples.

• A cuvette of clean chloroform was prepared and the absorbance measured at a wavelength of 415 nm using an UV spectrophotometer Cary 300-Bio (Varian, Palo Alto, CA) to create a blank.

• 10 ml of sample (standard cationic solution or harvest water containing CTAB) was then placed into a separatory funnel and a further 40 mL of distilled water added to dilute the whole sample to 50 mL.

• 5 mL of buffer solution, 0.5 mL of methyl orange, and 50 mL of chloroform were then added to the funnel.

• The glass stopper was put in place and the funnel was shaken vigorously for 30 seconds.

• The funnel was then left to stand for 20 minutes or until the chloroform layer no longer appeared cloudy, allowing the chloroform layer, containing the methyl orange-surfactant complexes, if present, to separate from the water fraction.

• The chloroform layer was then carefully removed into a clean glass flask, the flask was again swirled to remove any potential water contaminates.

• The dewatered chloroform was placed into a clean cuvette and absorbance measured at 415 nm in an UV spectrophotometer Cary 300-Bio (Varian, Palo Alto, CA).

• The absorbance was converted into mg L^{-1} with the calibration curve (Figure A1.2)

A1.3 Calibration curves

Figure A1.1 is the calibration curve achieved for standard anionic solutions of Ecover®. It shows the correlation between the anionic surfactant concentration and the absorbance of the methylene blue- surfactant complex. The six data points on the plot were fitted with a regression coefficient r^2 of 0.99, which indicates an extremely good correlation. The correlation can be described by equation (A1.1):

$$asc = \frac{MBab + 0.0391}{6.078} \qquad \text{(A1.1)}$$

In which *asc* represents the anionic surfactant concentration and *MBab* is the absorbance reading of the methylene blue- surfactant complex.

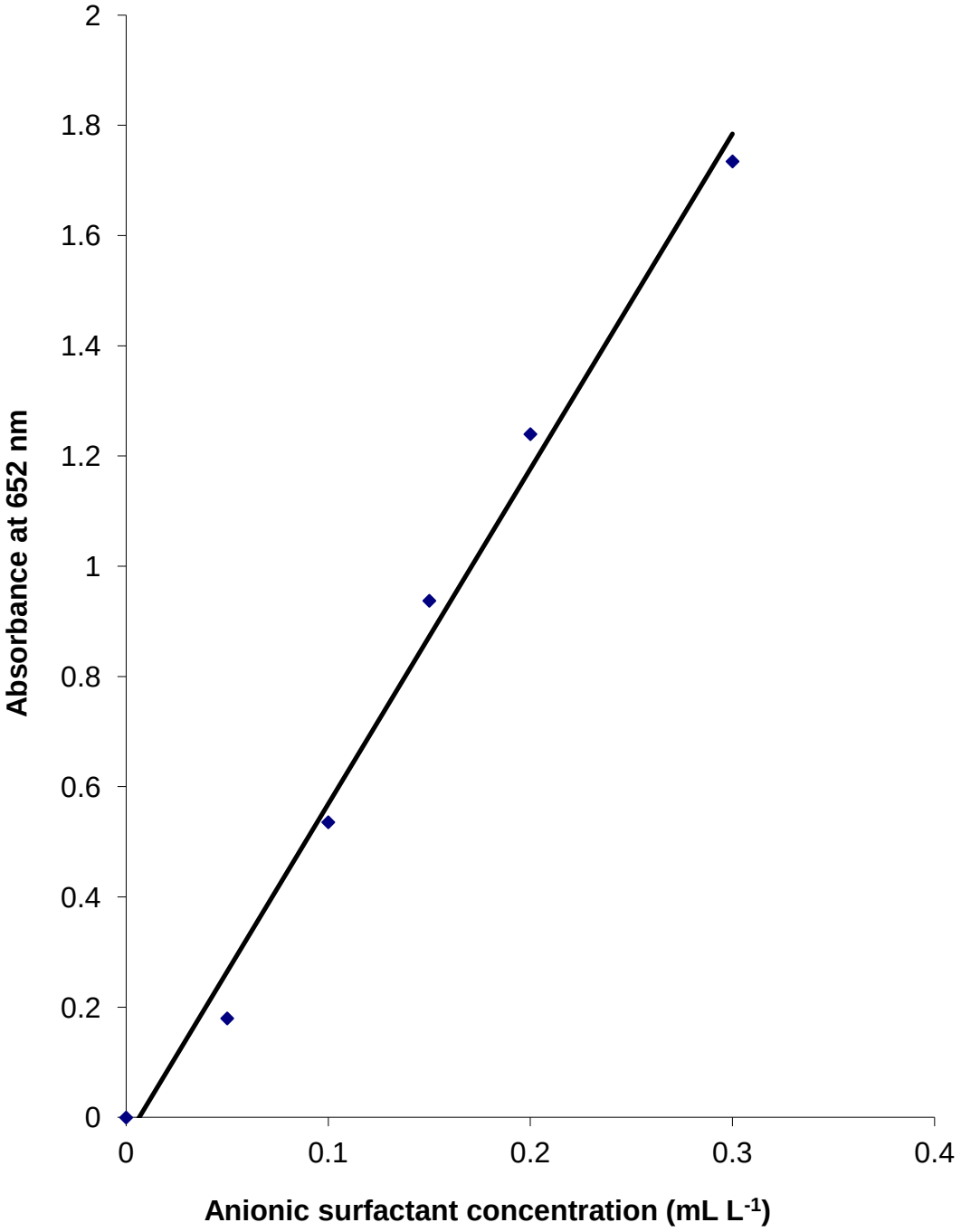

Figure A1.1 Correlation of the anionic surfactant concentration vs. the absorbance of the methylence blue-surfactant complexes at a 625 nm. The diamonds mark the six anionic standard solutions used to establish the correlation.

Figure A1.2 is the calibration cure achieved for standard cationic solutions of CTAB. It shows the correlation between the cationic surfactant concentration and the absorbance of the methyl orange- surfactant. The six data points on the plot were fitted with a regression coefficient, and again r^2 of 0.99 was achieved, showing an extremely good correlation. The correlation can be described by equation A1.2

$$csc = \frac{MOab + 0.006}{0.0035} \tag{A2.2}$$

In which csc represents the cationic surfactant concentration and $MOab$ is the absorbance reading of the methyl orange- surfactant complex.

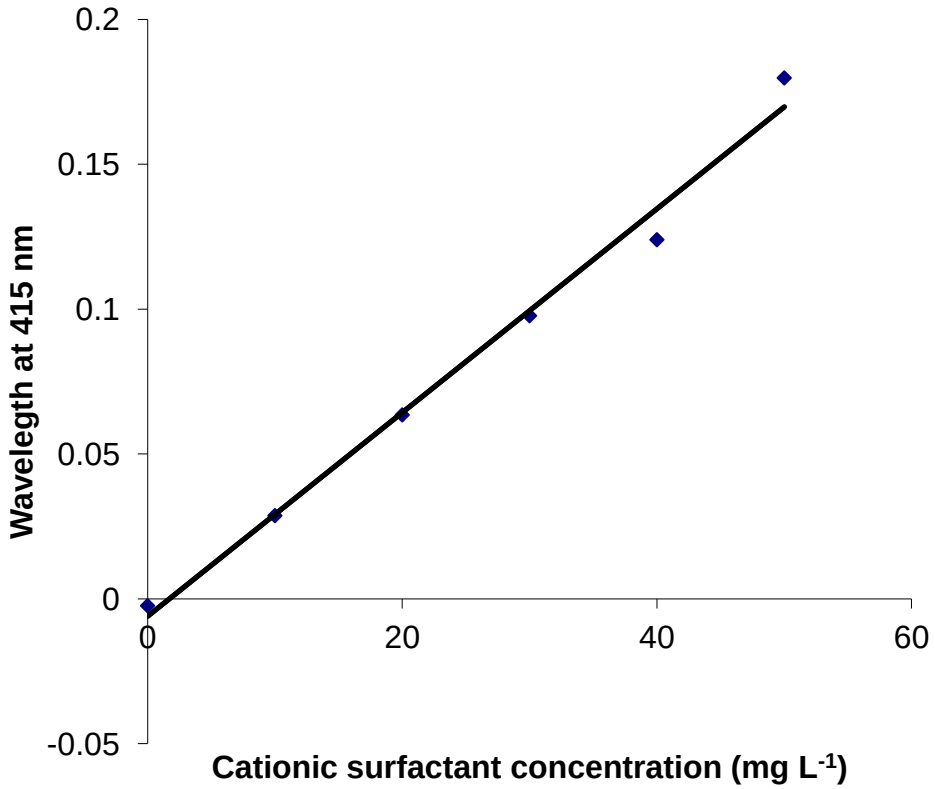

Figure A1.2 Correlation of the cationic surfactant concentration vs. the absorbance of the methyl orange-surfactant complexes at a 415 nm. The diamonds mark the six cationic standard solutions used to establish the correlation.

The use of the correlation curves allowed for a quick and accurate way to estimate the concentration of the two surfactants, enabling the recycling of a costly yet vital experimental tool.

Appendix 2

A2.1 Bubble size distribution within the foam phase

During the harvesting process the foam that travels up the column will yield a higher surfactant concentration than that of the liquid pool. This is due to the drainage of liquid as the foam travels up the column height resulting in enrichment of the surfactant at the gas-liquid interface (Rosa et al., 2007). All experiments conducted throughout this study were run as a batch process, in which the surfactant solution was added to the culture chamber at the beginning of the harvest and the foam produced continuously throughout the batch run-time. It was therefore predicted that the relative frequency of bubble size distribution would increase with time, due to the removal of surfactant resulting in a decrease in the liquid pool surfactant concentration. Figures A2.1-A2.3 display the effects of time, sparger type and air flow set-up on the bubble size distribution within the foam phase.

At an airflow rate of 100 L h^{-1} all sparger types and air flow set-ups peaked at a bubble size distribution between 500-1000 μm, 0 minutes into the batch run time. Within 10 minutes the foam produced by the LWLF and the CLF was dominated by bubbles of the size range 1000-1500 μm representing 43.6% and 27.16% respectively. This remained the dominant size range for both sparger types until the surfactant become depleted at 25 minutes for the ceramic sparger. Throughout the batch run time the dominant size range for the COF is between 500-1000 μm representing 33.9 ± 4.4% on average. The average bubble size produced by the LWLF significantly increased during the batch run time ($p=<0.001$) from 1413 μm to 2485 μm. There was a significant increase in the average bubble size for the CLF ($p=0.047$) and the COF ($p= 0.007$). When comparing the bubble sizes produced over the whole time period the COF generated significantly smaller bubbles, with an average of 1141 μm compared to the LWLF (1327 μm) and the CLF (1420 μm) ($p=<0.001$).

At the increased air flow rate of 150 L h^{-1} the bubble size distribution again peaked at 500-1000 μm for all sparger types and air flow set-ups at 0

minutes. However, within 10 minutes the bubble dominance has shifted to 1000-1500 μm for the LWLF representing 35.1%. The 3 bubble size distribution within the foam produced by the CLF and COF remained prominent between 500-1000 μm throughout the run-time, representing a frequency of 40 ± 2.5% and 37.2 ± 2.5% respectively. At 150 L h^{-1} there was a significant increase in the average bubble size produced by the LWLF (p=0.018), CLF (P=<0.001), and COF (p=<0.001) with the average bubble size produced between 10-30 minutes were significantly larger than the bubbles produced at 0 minutes into the batch run time. Across the whole time period bubbles produced by LWLF and COF where significantly larger when compared to bubbles produced by the CLF (p=<0.001). Bubbles within the foam phase produced at 150 L h^{-1} where significantly smaller than those in the foam phase produced at 100 L h^{-1} by the CLF (p=<0.001).

At 200 L h^{-1} bubble size within the foam was dominated by size ranges of 1000-1500 μm for all sparger types and air flow set-ups at 0 minutes into the batch run-time. COF and LWLF bubble sizes distribution peaks remained between 1000-1500 μm throughout the remaining batch trial time. The bubble size distribution within the foam phase produced by the CLF peaked as high as 2500-3000 μm at 20 minutes, representing 42.5%. The surfactant became exhausted in the batch using the CLF before 25 minutes run-time, and before 30 minutes run time for the LWLF. There was no significant difference in bubble sizes within the foam phase produced by LWLF (p=0.411) and COF (p=0.161). There was a significant difference between the bubble sizes within the foam phase produced at 0, 10, and 20 minutes by the CLF (p=<0.001). When analysing bubble size within the foam phase for the whole time period there was no significant difference between LWLF and CLF (p=0.935), however COF was significantly smaller (p=<0.001).. Bubbles within the foam phase produced at 200 L h^{-1} by CLF and LWLF were significantly larger than those produced at 150 L h^{-1} for all production methods (p=<0.001)...

The hypothesis proposed earlier is significant to the effect of bubble size within the foam phase with time. However, from the data it is evident that air flow rate and air input method has an influential effect on bubble size within the foam phase.

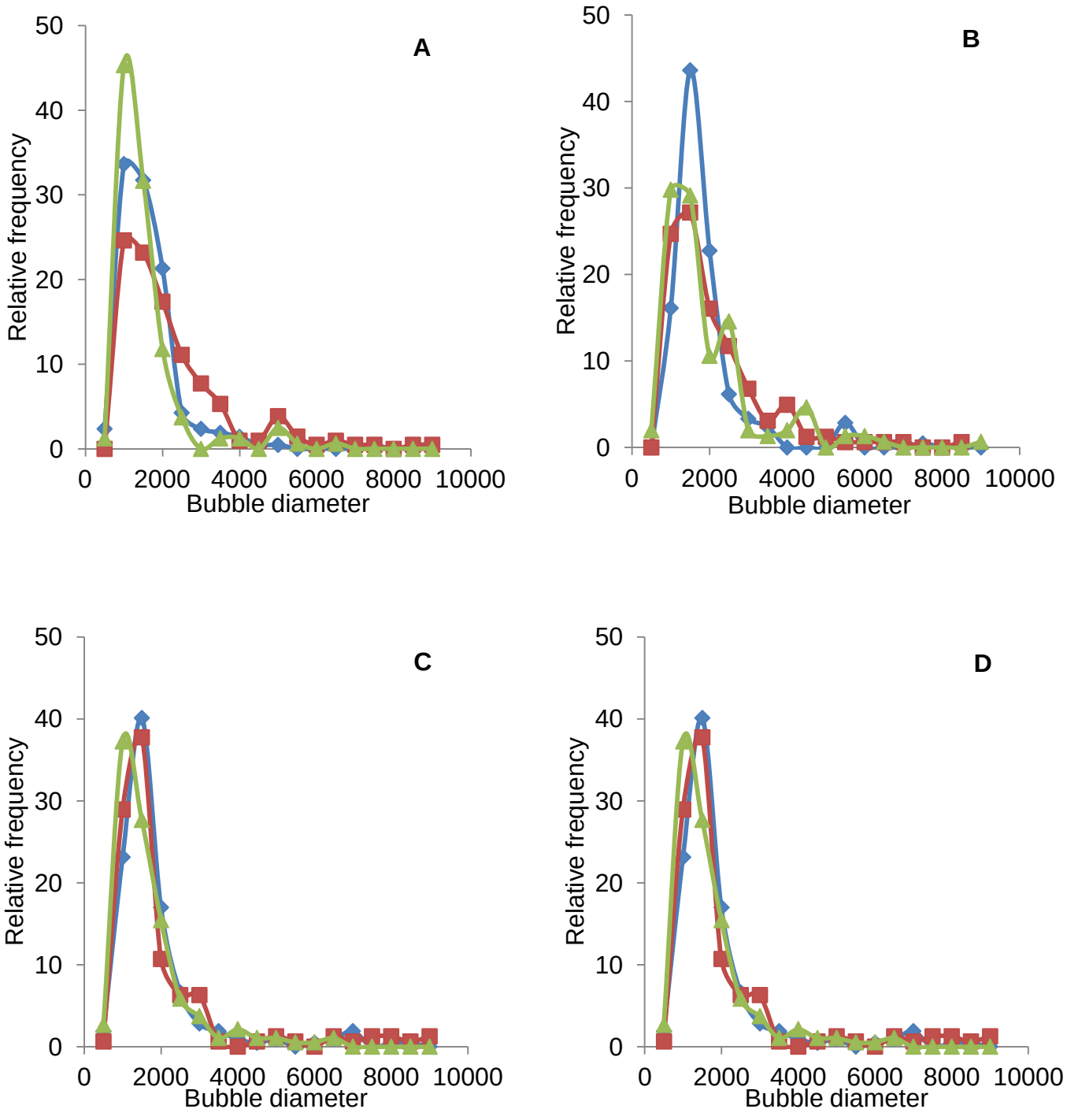

Figure A2.1 Bubble size distribution within the foam phase at 100 L h^{-1}. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatoryiallting flow by green. **A)** 0 minutes into batch run time **B)** 10 minutes into batch run time **C)** 20 minutes into batch run time and **D)** is 30 minutes into batch run time.

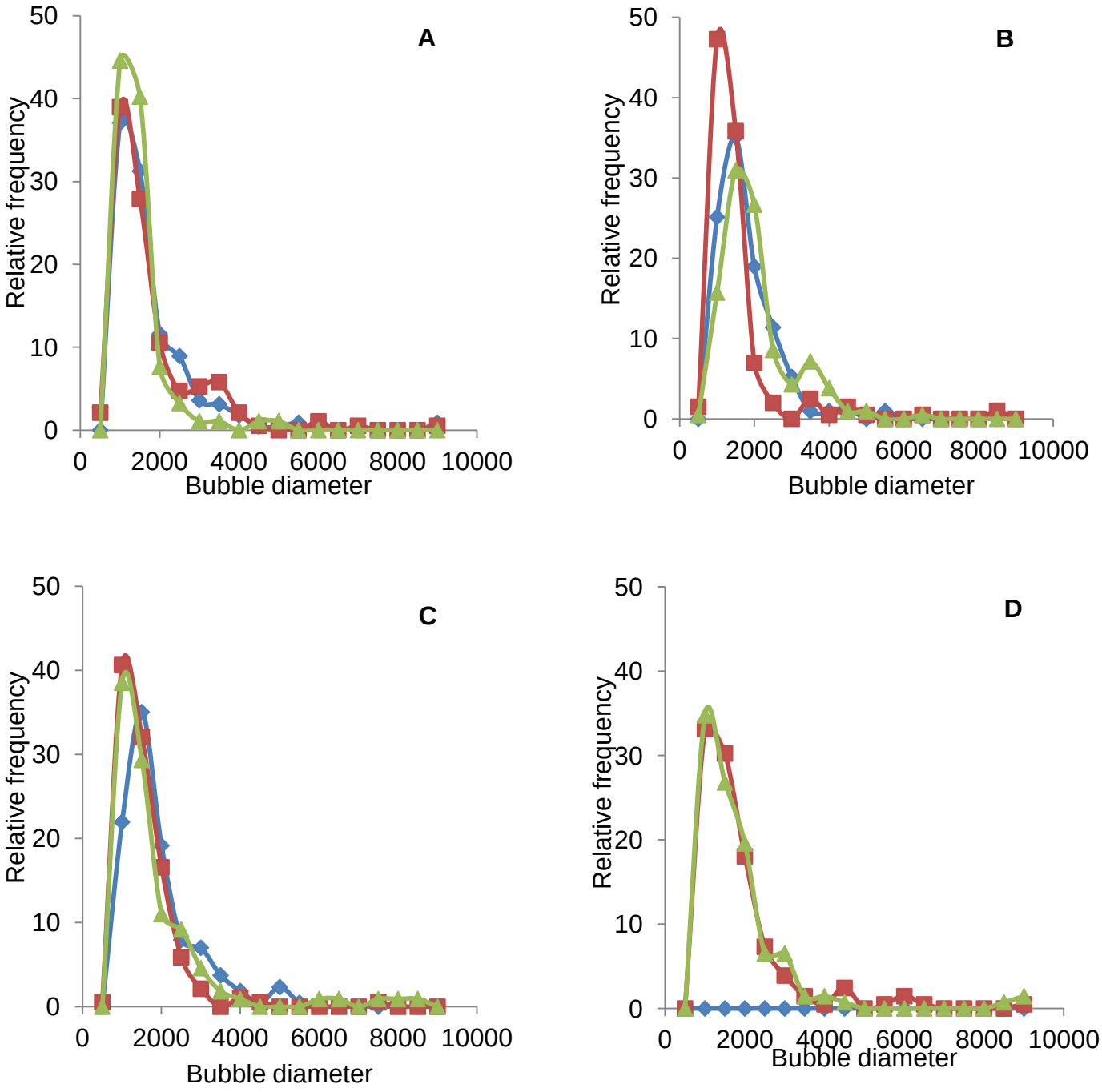

Figure A2.2 Bubble size distribution within the foam phase at 150 L h^{-1}. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatoryiallting flow by green. **A)** Is the bubble size distution at 0 minutes into batch run time **B)** 10 minutes into batch run time **C)** 20 minutes into batch run time and **D)** is 30 minutes into batch run time.

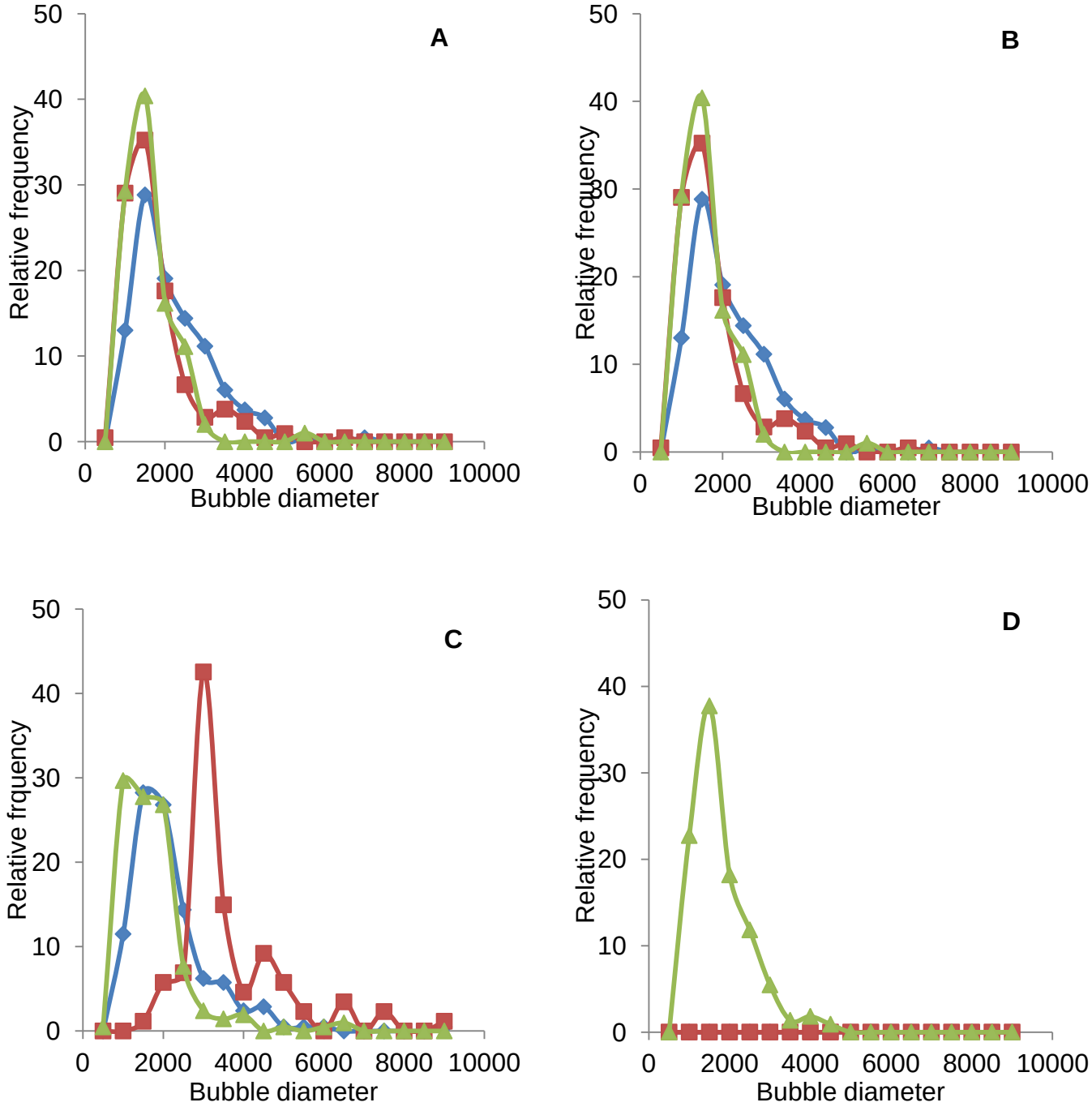

Figure A2.3 Bubble size distribution within the foam phase at 200 L h^{-1}. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatoryiallting flow by green. **A)** 0 minutes into batch run time **B)** 10 minutes into batch run time **C)** 20 minutes into batch run time and **D)** is 30 minutes into batch run time.

A2.2 Bubble rise velocity within the foam phase

Bubble rise velocity within the foam phase greatly affects the residence time of foam within the column. Foam residence time is influential upon bubble coalescence, foam draining and therefore concentration factor (Yan et al., 2011). The bubble rise velocity within the foam phase was calculated throughout the batch run time period for all air flow rates and sparger set-ups. At 100 L h^{-1} (Figure A2.4 (A)), 5 minutes into the batch run time, the bubble rise velocity produced by the LWLF was significantly faster than all the sparger set-ups across the whole batch run time period (p=<0.001), peaking at 0.04 m/s^{-1}, 2.7 times faster than the average rise velocity occurring throughout the rest of the batch run time of the LWLF. The bubble rise velocity within the foam produced by the CLF and the COF remained at a constant slow rate of 0.014 ± 0.0014 m/s^{-1} and 0.012 ± 0.0007 m/s^{-1} respectively. There was no significant difference between the bubble rise velocity over the batch run time for CLF (p=0.958) and COF (p=0.891).

At 150 L h^{-1} (Figure A2.4 (B)), the bubble rise velocity at 0 minutes into the batch run time for the LWLF was significantly faster when compared the rest of the batch run time (p=<0.001). There was no significant difference between the bubble rise velocity over the whole batch run time period for CLF (p =0.379). At 10 minutes into the batch run time, the COF produced a bubble rise velocity that was significantly faster than all the sparger set-ups across the whole batch run time period (p=<0.001), peaking at 0.08 m/s^{-1}, the fastest average bubble rise velocity measured throughout all trials.

At 200 L h^{-1} (Figure A2.4 (C)), 5 minutes into the batch run time, the bubble rise velocity produced by the LWL was significantly slower than the rise velocity created over the rest of the time period by the LWL (p=<0.001). The CLF bubble rise velocity peaked at 5 minutes and 15 minutes, at 0.062 m/s^{-1}, and 0.078 m/s^{-1} respectively. The bubble rise velocity created at 5 and 15 minutes by the CLF is significantly faster when compared to the rest of the sparger and air flow set-ups over the whole batch run period (p=<0.001). The bubble rise velocity within the foam created by the COF had no significant change in velocity over the whole run time (p=0.012).

The error bars for the bubble rise velocities throughout figure A2.4 can be seen to drop into negative figures. As surfactant levels started to become exhausted the foam no longer rises in solid columns, often creating void fraction (gas volume fraction) within the column. The section of foam within the column no longer had the pressure of the rising foam underneath it to push it up the column and it starts to slowly sink down the column, until anther foam section transferred its mass.

It is evident from the data that the combination of input air flow velocities, sparger type and air flow set-ups has a significant effect on the foam residence time within the column. At 100 L h^{-1} the CLF and COF a stable, slow velocity increasing foam residence time when compared to the LWLF.

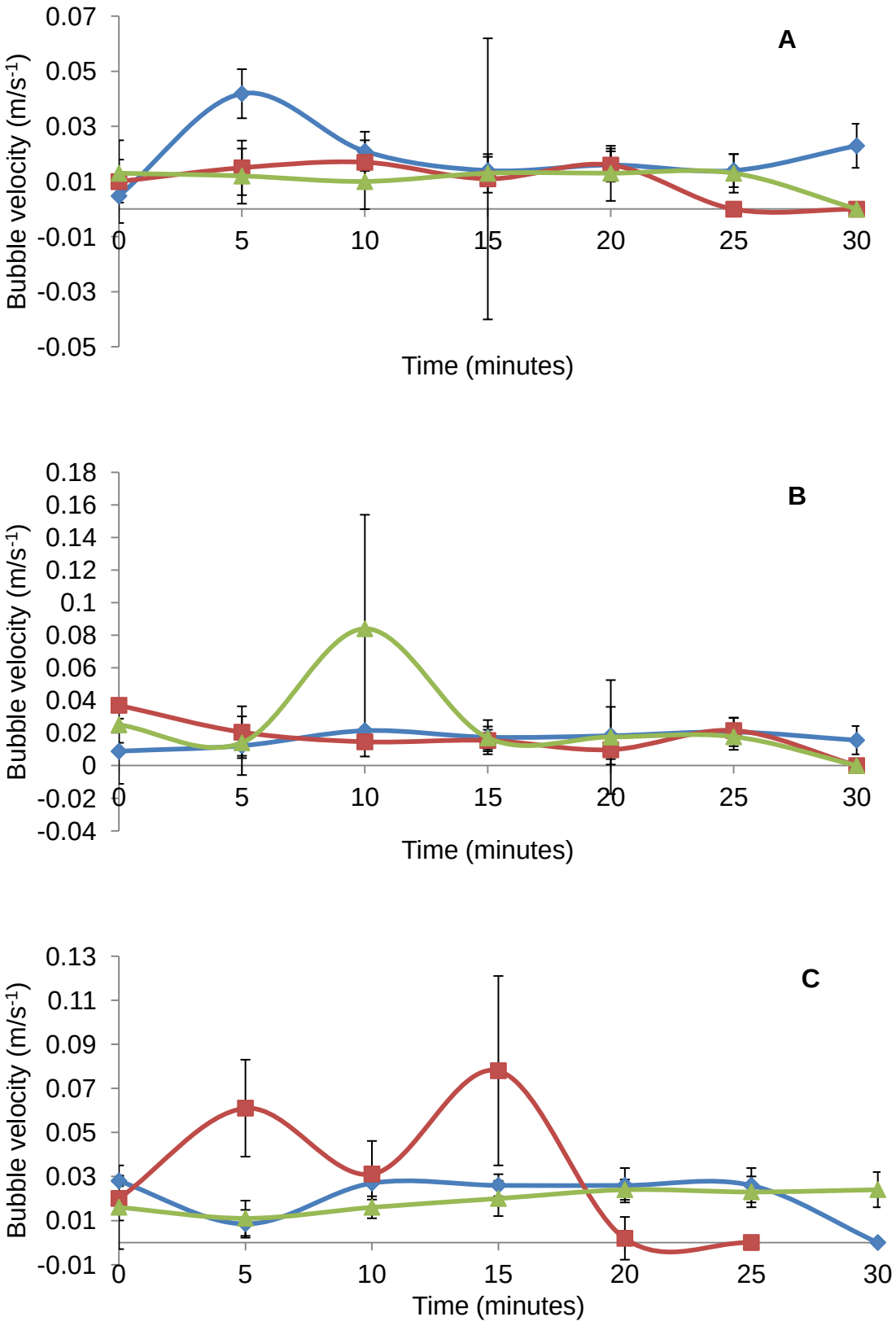

Figure A2.4 The bubble rise velocity within the foam phase between 0-30 minutes; **A)** 100 L h^{-1}, **B)** 150 L h^{-1}, **C)** 200 L h^{-1}. The lime wood sparger with linear flow is represented in blue, the ceramic flat plate sparger with linear flow by red and the ceramic flate plate sparger with oscillatory flow by green.

A2.3 Interfacial area calculations

Table A2.1 Interfacial area calculations for the lime wood sparger with linear flow air flow input, within the liquid pool

Q_{gas} (m^3/h^{-1})	$A_{sparger}$ (m^2)	$U_D = \frac{Q_{gas}}{A_{sparger}}$ (m/h^{-1})	V_B (m/h^{-1})	$\varepsilon = \frac{U_D}{V_B}$	d_{32} (m)	$a = \frac{6 \in}{d_{32}}$ (m^2m^{-3})	H_L (m^2)	$A = (aA_{sparger}H_L)$ (m^2)
0.1	0.001	100	144.72	0.69	0.001342	3084.94	0.167	0.52
0.15	0.001	150	161.4	0.93	0.001378	4049.35	0.167	0.68
0.2	0.001	200	156.6	1 (1.28)	0.00149	4026.84	0.167	0.67

Table A2.2 Interfacial area calculations for the ceramic sparger with linear flow air flow input, within the liquid pool

Q_{gas} (m^3/h^{-1})	$A_{sparger}$ (m^2)	$U_D = \frac{Q_{gas}}{A_{sparger}}$ (m/h^{-1})	V_B (m/h^{-1})	$\varepsilon = \frac{U_D}{V_B}$	d_{32} (m)	$a = \frac{6 \in}{d_{32}}$ (m^2m^{-3})	H_L (m^2)	$A = (aA_{sparger}H_L)$ (m^2)
0.1	0.0121	8.26	838.8	0.00985	0.000916	64.52	0.127	0.10
0.15	0.0121	12.4	723.6	0.0171	0.000905	113.37	0.127	0.17
0.2	0.0121	16.53	500.4	0.033	0.001127	175.69	0.127	0.27

Table A2.3 Interfacial area calculations for the ceramic sparger with oscillatoryillating flow air flow input, within the liquid pool

Q_{gas} (m^3/h^{-1})	$A_{sparger}$ (m^2)	$U_D = \frac{Q_{gas}}{A_{sparger}}$ (m/h^{-1})	V_B (m/h^{-1})	$\varepsilon = \frac{U_D}{V_B}$	d_{32} (m)	$a = \frac{6 \in}{d_{32}}$ (m^2m^{-3})	H_L (m^2)	$A = (aA_{sparger}H_L)$ (m^2)
0.1	0.0121	8.26	673.2	0.0123	0.000665	110.98	0.127	0.17
0.15	0.0121	12.4	694.8	0.0178	0.000694	153.89	0.127	0.24
0.2	0.0121	16.53	658.8	0.0251	0.000823	182.99	0.127	0.28

Table A2.4 Interfacial area calculations for the lime wood sparger with linear flow, within the foam phase, 5 minutes into run time

d_{32} (m)	$a = \frac{6.59}{d_{32}}$ (m^2m^{-3})	A_{column} (m^2)	C_L (m)	$A = (aA_{column}C_L)$ (m^2)
0.00416	1584.13	0.00166	0.5	1.31
0.0048	1372.92	0.00166	0.5	1.14
0.0028	2353.57	0.00166	0.5	1.95

Table A2.5 Interfacial area calculations for the ceramic sparger with linear flow, within the foam phase, 5 minutes into run time

d_{32} (m)	$a = \frac{6.59}{d_{32}}$ (m^2m^{-3})	A_{column} (m^2)	C_L (m)	$A=(aA_{column}C_L)$ (m^2)
0.003312	1989.73	0.00166	0.5	1.65
0.001696	3885.61	0.00166	0.5	3.23
0.001928	3418.05	0.00166	0.5	2.84

Table A2.6 Interfacial area calculations for the ceramic sparger with oscillatoryillating flow, within the foam phase, 5 minutes into run time

d_{32} (m)	$a = \frac{6.59}{d_{32}}$ (m^2m^{-3})	A_{column} (m^2)	C_L (m)	$A=(aA_{column}C_L)$ (m^2)
0.00644	1023.29	0.00166	0.5	0.85
0.001917	3437.66	0.00166	0.5	2.85
0.002158	3053.75	0.00166	0.5	2.54

A2.4 Reynolds number

The Reynolds number is defined as:

$$\mathrm{Re}_B = \frac{\rho_l V_B d_B}{\mu_l} \qquad \text{(A2.1)}$$

Where V_B is the bubble rise velocity, d_B is the bubble diameter, ρ_l is the liquid density, and μ_l is the liquid dynamic viscosity. The Reynolds number for each flow rate and sparger type and air set-up can be seen in table A2.7.

Table A2.7 Calculated Reynolds number for each sparger type and air flow in put set-up

Air flow (L h^{-1})	LWLF	CLF	COF
100	0.194	0.768	0.448
150	0.222	0.655	0.482
200	0.233	0.564	0.542