CRANFIELD UNIVERSITY

Matthew William Pearce

An integrated approach to Microalgae biomass generation and processing

School of Energy, Environment & Agrifood

PhD
Academic Year: 1st March 2012 to 12th December 2014

Supervisor: Professor Feargal Brennan
March 2016

CRANFIELD UNIVERSITY

School of Energy, Environment & Agrifood

PhD

Academic Year 1st March 2012 to 12th December 2014

Matthew William Pearce

An integrated approach to Microalgae biomass generation and processing

Supervisor: Professor Feargal Brennan

March 2016

© Cranfield University 2016. All rights reserved. No part of this publication may be reproduced without the written permission of the copyright owner.

Abstract

Liquid combustible fossil fuel empowers global society, yet is a non-renewable entity with time-constrained limits to supply. Advanced generation biofuel derived from microalgae could feasibly yield more than conventional biofuel crops, utilise non-agricultural land or the sea and remediate atmospheric carbon dioxide and anthropogenic waste. However, technical and economical limits have so far prevented the successful implementation of microalgae biofuels.

This thesis exemplifies how apparently disconnected technologies are able to become united in their provision for the growth and processing of microalgae. In so doing, it employs unique experimental methodology which unites inter-disciplinary themes with the proposition to cultivate and process microalgae biomass in a manner which has never been done before. The novelty of this endeavour presents a unique set of challenges, reasoning and results with implications for future creative research and investigation. The philosophical approach to conception and achievement of the laboratory work intercedes with entirely new methodology. Selected examples of such methodology follow. In chapter 3, a newly developed bio-composite gel disk was processed aligning a new design of apparatus for a geotextile puncture resistance test. In chapter 3, a novel formulation for harvesting microalgae is described. In chapter 5, a modified methodology of the preceding chapter is used to investigate seawater ion remediation via ionic and density phase separation. Chapter 6 integrates waste components from 5 different industries, namely dairy farming, anaerobic digestion, brewing, steel slag aggregates and coal power combustion with no previously known unification of such technologies in scientific literature. Chapter 7 assesses the lipid quality of biomass harvested by the novel formulation of chapter 3, before and after exposure to hydrothermal liquefaction. Chapter 8 extrapolates findings from the thesis to define an economic appraisal and suggest a cost saving process.

Acknowledgements

I would like to express my sincere gratitude to my supervisor Professor Feargal Brennan for his guidance and this unique opportunity to investigate the practical culturing and processing of microalgae at Cranfield University.

Thank you to my wife & family for their endearing love, support and encouragement, and my parents for creating and nurturing inquisitiveness in my character.

My thanks also go to: Señor Réne Piantini, General Manager of Algas Prodalmar Ltda, for provision of dried Chilean seaweed samples; Señor Alberto Shamosh, General Manager of Solarium Biotechnology S.A. Chile for provision of Spirulina microalgae and both of their kind hospitality during my visit to Chile in December 2012.

Thank you to: Dr Andrew Spicer, Algenuity, UK for provision of growing algae biomass, and the loan of Algem®; Prof Hans-Ulrich Endreß, Herbstrieth & Fox KG, Germany for donation of commercial pectin samples; Ann Cornish, Salix River & Wetland Services Ltd, UK for donation of coir fibre; Jon Gantlett, Church Farm, Faringdon for an Organic Dairy Manure sample; Dr Nizar Ghazireh, LaFarge Tarmac for steel slag samples, White Park Brewery for a brewery waste sample and Fernbrook Bio for an anaerobic digestate sample.

I am grateful for scientific editorial advice and construct catalysis from Dr Rafaella Villa. Thanks to other technical colleagues and aspiring algae researchers' who have helped with elements of the practical within this thesis including Freda Onakpoya and Utsab Guharoy.

It has been of curious interest and pride to have been able to use four of the five senses during my research; a thousand shades of green, yellow and brown, the sounds of bubbles bursting out of algae culture, the smell of the seaside, fish, the sea and the textures of harvested microalgae in its' myriad of forms.

Table of Contents

List of Figures

List of Tables

List of Equations

List of Abbreviations

AAS	Atomic Absorption Spectroscopy
ABSIG	Algal Biofuels Special Interest Group
AD	Anaerobic Digestion
ANOVA	Analysis of Variance
BBSRC	Biotechnology and Biological Sciences Research Council
BOD	Biological Oxygen Demand
BIOMARA	The Sustainable Fuels from Marine Biomass Project
BTU	British Thermal Units
CCAP	Culture Collection for Algae and Protozoa
CCD	Central Composite Design
CCS	Carbon Capture and Storage
CSP	Concentrated Solar Power
COD	Chemical Oxygen Demand
CP	Citrus Pectin
DECC	Department of Energy & Climate Change
DHA	Docosahexaenoic acid
DOC	Dissolved Organic Carbon
DSG	Direct Steam Generation
DTC	Doctoral Training Centre
EABA	European Algae Biomass Association
EDX	Energy Dispersive X-ray spectroscopy
EEDA	East of England Development Agency
EFSA	European Food Safety Authority
EIA	Energy Information Administration
EU	European Union
FAME	Fatty Acid Methyl Ester
FCR	Feed Conversion Ratio
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GIF	Gel Induced Filtration
GC-FID	Gas Chromatograph Flame Ionisation Detector
GC-MS	Gas Chromatography–Mass Spectrometry

HTF	Heat Transfer Fluid
HTL	Hydrothermal Liquefaction
IC	Ion Chromatography
IEA	International Energy Association
IPCC	Intergovernmental Panel on Climate Change
KeV	Kilo Electron Volts
KTN	Knowledge Transfer Network
MEA	Monoethanolamine
MJ DE/kg	Mega Joules Digestible Energy per kilogram
MRI	Magnetic Resonance Imaging
MRL	Maximum Residue Limits
MSF	Multi-Stage Flash distillation
NASA	National Aeronautics and Space Administration
NERC	Natural Environment Research Council
NIBB	Networks in Industrial Biotechnology and Bioenergy
NNFCC	National Non Food Crops Centre
NREL	National Renewable Energy Laboratory
NTU	Nephelometric Turbidity Unit
OMEGA	Offshore Membrane Enclosure for Growing Algae
PBR	Photo Bioreactor
PCR	Polymerase Chain Reaction
PSI	Pounds per square inch
RED	Renewable Energy Directive
RSM	Response Surface Methodology
SAMS	Scottish Association for Marine Science
SBP	Sugar Beet Pectin
SEM	Scanning Electron Microscopy
SURF	Sustainable Use of Renewable Fuels
TAG	Triacylglyceride
TAP	Tris-Acetate-Phosphorus
TC	Total Carbon
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon

TE	Transpiration Efficiency
TEP	Transparent Exopolymer Particles
TOC	Total Organic Carbon
TN	Total Nitrogen
TP	Total Phosphorus
TSB	Technology Strategy Board
UK	United Kingdom
WUE	Water Use Efficiency

1.1 Algae bioenergy context

1.1.1 An overview of algae

Microalgae comprise diverse unicellular organisms such as prokaryotic cyanobacteria and eukaryotic protists that can quickly replicate in favourable growth conditions. Algae refer to both macroalgae and the highly diverse microalgae estimated to be between 30,000 to 1 million species (Guiry, 2012). Physical dimensions range between less than a micron for species of marine picoplankton such as *Synechococcus* and *Prochlorococcus* (Worden, Nolan and Palenik, 2004) to about 60 metres in length for giant kelp seaweed macroalgae. (Barsanti & Gaultieri 2006; Ross *et al.* 2008).

Ocean waters cover over 70% of the earth's surface and include more than 5000 species of planktonic microalgae which form the base of the food chain and produce 50% of atmospheric oxygen (Barsanti & Gaultieri 2006). Whilst microalgae produce life giving oxygen, they can also be of detriment to other life forms when excess nutrients induce high density plankton blooms that block light penetration and kill other photosynthetic life as well as shrimp via accumulation of bio-toxins (Alonso-Rodríguez, R. and Páez-Osuna, F. 2003), and penguins (Ho *et al.* 2003). The common colour classification of algae into brown, green and red is indicative of proportional inclusion of photosynthetic pigmentation which allows niche community habitats dependent on the intensity, spectrum and photoperiod of solar irradiation where algae live (Six *et al.* (2007). Lichens are a mutually beneficial synergy of microalgae and fungi whereby algae provide oxygen and complex nutrients to fungi and receive protection and simpler nutrients in return (Barsanti & Gaultieri 2006).

Algae obtain nutrient requirements mixotrophically or by a combination of both phototrophy and heterotrophy. Heterotrophic nutrition permits carbon acquisition where light levels are low and photosynthesis is not effective. Some species cannot synthesise vitamin B_{12} complexes or fatty acids and must obtain these by association with soil-borne bacteria such as *Mesorhizobium* (Kazamia *et al*, 2012) which are commonly associated with root nodules in nitrogen fixing legume crops. Microalgae can be motile (free swimming), non-motile, unicellular or

colonial. There is a vast future biotechnological potential for microalgae appropriated to human application, yet to date the largest algal production facilities have focussed on extremophile species (Day *et al.* 2012), tolerant on the basis of pH; *Arthrospira/Spirulina* (Benemann, 2003) for dietary supplements, or salinity for beta β-carotene production using *Dunaliella salina* (Ben-Amotz, 2004). Extremeophile species utilisation prevents invasion and competition from other potential contaminating microorganisms which cannot thrive in such conditions. Microalgae species producing high value end products can briefly be defined as those producing one or more of the following products: carotenoids, phycobilins, long-chain polyunsaturated fatty acids, sterols, polyhydroxyalkonates, polysaccharides, antibiotics, anti-hypotensives, drug-development compounds, cosmeceuticals, nutraceuticals or functional foods (Borowitzka, 2013). Microalgae are cultivated in either capital intensive low volume dense bioreactors for marine aquaculture larval diets (Ferreira *et al.* 2009), or expansive open ponds with lower productivity per unit volume. Recently microalgae have been genetically modified to produce a range of biochemicals including alkanes, isoprenes, glucose, fructose and polyhydroxyalkanoates (Ducat, Way & Silver, 2011). Genetic engineering also has the potential for expression of recombinant proteins and monoclonal antibodies, which are currently produced by high capital investment costs in transgenic mammalian cells and fermentation facilities (Mayfield and Franklin, 2005). Although, microalgae are eaten as a food in China and Chad and had been considered as a solution to the world's food shortage, their use on a global scale appears limited to health food and food supplements (Becker, 1994; Borowitzka, 2006; Spolaore *et al.* 2006). Table 1-1 highlights the current state of microalgal production and applications.

Table 1-1 – Current state of microalgal production and application (Spolaore et al. 2006)

Alga	Annual production	Producer country	Applications and products
Spirulina	3000 t dry wt	China, India, USA, Myanmar, Japan	Human and animal nutrition, phycobiliproteins, cosmetics
Chlorella	2000 t dry wt	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetics
Dunaliella	1200 t dry wt	Australia, Israel, USA, China	Human nutrition, aquaculture, β carotene
Aphanizomenon	500 t dry wt	USA	Human nutrition
Haematococcus	300 t dry wt	USA, India, New Zealand, Israel	Aquaculture, Astaxanthin
Crypthecodinium	240 t DHA oil	USA	DHA oil
Schizochytrium	10 t DHA oil	USA	DHA oil

Of research scientists who are promoting microalgae biofuel technology, there are two branches of thought. Firstly, there exist scientists who advocate genetic modification via synthetic and transgenic pathways. Secondly engineers who aspire to re-configure containment vessels for growing microalgae, or alternatively to manipulate the downstream processing technology which is used for conversion of microalgae into biofuel. This research avidly avoids any attempt to delve within the realm of synthetic biology and its derivatives whilst simultaneously accepting that these technologies have an important role to play in advancement of progress. Instead, consideration is given to the novel manipulation of growth, harvesting and processing with an innovative engineering perspective.

1.1.2 **Conventional oil, biofuels and advanced generation biofuels**

The IEA forecasts global crude oil production will peak in the year 2030, however Sorrell *et al.* (2010) conclude from their contemporary analysis of 14 forecasts that a peak of conventional oil production before 2030 appears likely. There is a need for humanity to conserve precious energy and nutrient resources in the present day and into the near future.

Challenges for societal progression within the context of historical and future development depend on implementation of innovation into practical answers to real world issues and problems. Arguably, the greatest problem humanity is faced with is the continuation of the provision of energy in the way it has been accustomed to over the last half a decade. The Intergovernmental Panel on Climate Change (IPCC) 4th Assessment report (AR4 2007) states that there is an urgent need for interdisciplinary collaboration and engagement with a new generation of scientists and engineers in order to confront the significant issues of population growth, greenhouse gas accumulation and global temperature rise. It is this new generation of technically minded people who will have to be involved in this process as it is these people who will be living with the consequences of the world's major issues. Both conventional fossil/mineral oil and biofuel are liquid forms of energy storage. This has pertinent applications to be able to provide energy for modern day transportation vehicles, an energy source quite distinct from coal, nuclear, solar, wind and tidal. Unknowingly, liquid biofuels are often classified in conjunction with their electrical generating counterparts, however their distinction is important. Although gas transportation vehicles are now widely distributed for smaller transportation systems such as motorboats and motorcycles in countries such as China, the nature of compressed liquid gas limits the development of larger transportation fleets (Heywood, 2006). Liquid Propane Gas is now becoming more proliferated, but it is unlikely to replace the unique position of oil powered transportation vehicles. Hydrogen transport may seem like a non-polluting 'silver bullet', yet the energy cost to detach hydrogen from fossil derived methane is expensive and defeats the overall objective to be a renewable fuel (Turner, 2004; Simpson & Lutz, 2007). At the same time no suitable technological alternative for hydrogen generation has come to light to

provide the massive volumes of compressed storage gas feedstocks which would be required as an alternative to hydrogen derived from natural gas. The UK government is aware of technical developments and aspirations for developing technologies such as biofuels from algae (UK parliament briefing paper – biofuels from algae 2011).

Yet these new technologies need to be integrated into a national and European framework which already has established ‘red tape’ and governance mechanisms. For example, the EU Renewable Energy Directive (RED) mentions biofuel sustainability criteria, but less the provision for algae biofuels in terms of legislative sustainability criteria, with consideration that this is a future emerging technology with associated research and investigation challenges this brings. As a nation within the EU, the UK also complies with further EU legislation such as the Integrated Pollution Prevention Control Directive (2010/75/EU) and the Water Framework Directive (2000/60/EC). Future commercially developed algae biofuels will require to be cultivated with the use of cheap fertiliser using un-sterilised, non-pasteurised and non-axenic organic waste material. As algae can be grown in large water bodies, and the requirements for algae biofuels necessitate large masses of cultivation, it is possible that algae biofuel development could subsequently be hindered by legislative compliance and coordination with other water utilising industries including domestic water processing companies, sewage remediation, aquaculture, fisheries, water based recreation and both land, freshwater ecosystem and sea based logistic transportation networks. European legislation has yet to account for the coordinated provision of these integrated technologies when they become implemented on the required scale of production. The European Algae Biomass Association (EABA) is pro-actively negotiating to unite science, legislation, policy and public opinion to address some of these concerns for future technological implementation. Directive 2009/28/EC of the European Parliament stipulates a common framework for energy production from renewable sources, more specifically in addition to individual member states allocated target shares of renewable generation, EU law requires that shares of energy from renewable sources in the transport sector must contribute at least 10% of final energy

consumption in the sector by 2020. However, in 2015 liquid biofuels represented 3.3% of petrol and diesel consumed in road transport, down from 4.2% a year earlier (UK Gov DECC, 2016).

With a global perspective, the International Energy Agency (IEA) describes a slowing trend of growth in first generation liquid biofuels (IEA, 2014). A 'first generation' biofuel (i.e. biodiesel (bio-esters), bio-ethanol, and biogas) is characterized either by its ability to be blended with petroleum-based fuels, combusted in existing internal combustion engines, and distributed through existing infrastructure. Second-generation biofuels produced from 'plant biomass' refers largely to lignocellulosic materials, as this makes up the majority of the cheap and abundant non-food materials available from plants (Naik *et al.* 2010). The US is reviewing past policies regarding corn biofuels. In Brazil the economics and viability of sugar cane ethanol and associated subsidies relative to fossil fuel prices is in a state of flux. In Europe the sustainability of biofuels with a proposed cap on conventional first generation biofuel production. In 2013 the global production of biofuels was 115 billion litres of which 2 billion litres were from advanced biofuels from biomass conversion to gasification, bio-butanol and Fischer-Tropsch biomass process refinement (IEA, 2014). Algae biofuel is presently a small contributor to liquid biofuels both in the UK, EU and worldwide.

1.2 Constraints to commercial exploitation

This research has taken the standpoint that the provision of liquid combustible fuel from either conventional fossil or biological biofuel derived origin is of concern in order to continue to sustain or increase present day consumption habits. Increasing the contribution of biofuels to the energy mix can be achieved by one or a combination of four methods, namely 1) increase in overall production of biomass, 2) increase in system efficiency or resilience, 3) increase in yield per unit area or per unit volume 4) exploitation of waste products (Woods, 2013). Market economic forces rather than government incentives control commercial industrial development. An increase in microalgae biomass production and associated system efficiency or resilience is therefore unlikely to be acted upon by businesses until there is a significant positive profit margin and commercial

benefit in producing biofuels from microalgae. This has yet to happen, or yet to be demonstrated sufficiently to financial investors in order for a biofuel market transition revolution to occur. The increase in unit yield per unit area or per unit volume is already being done by genetic modification and strain selection studies at laboratory scale. The mass culture of microalgae for biofuel production is not an engineering or biological challenge, but is a combination of both (Richmond, 1999). It is the energetic and economic cost of commercial microalgae biofuel which is prohibitive to its development, yet this can potentially be remediated by a combination of using waste products and reduced energy harvesting techniques. This is the main way of minimising the cost because nutrient production inputs and harvesting are known to be the most expensive components of the production system. Consequently, this research has coupled nutrient and industrial wastes with microalgae biofuel production.

This research studies microalgae from a practical perspective and pilot scale production process. Unlike much biological research work on small culture volumes of up to 200 ml microalgae flasks, this research is not overtly concerned with axenic culture systems. As microalgae cultures are scaled in production volume, sterilisation of containment vessels becomes impossible. Though disinfection is a viable alternative, microalgae cultures are never axenic and various contaminants are always present in the local environment. The benefit of culturing phototrophic microalgae in comparison with heterotrophic or mixotrophic microalgae is that phototrophic microalgae photosynthesise carbon from dissolved carbon dioxide rather than obtaining energy from dissolved sugars. In this way, there isn't a readily available carbon based sugar source for bacteria to go into competition with microalgae. Despite the presence of bacteria and other contaminants in non-sterile culture systems the microalgae still flourish.

Many laboratories investigate enhanced lipid generation or lipid recovery in laboratory conditions, but it is not laboratory conditions which are required to produce biofuels, it is macro-scale. Replacing all the transport fuel consumed in the United States with biodiesel will require 0.53 billion m^3 of biodiesel annually at the current rate of consumption (Chisti, 2007). Microalgae have quick growth

rates and superior photosynthetic conversion compared to terrestrial plants, but to provide sufficient volumes of bio-diesel or bio-oil, they are going to have to be grown in huge volumes and be grown phototrophically in order to avoid nutrient energy inputs from synthetic fossil fuel derived agricultural fertilisers, hence searching for energy from a positive energy return on energy invested. Huge volumes of microalgae growth cannot be axenic due to an open environmental system with nutrient acquisition from non-synthetic organic nutrients and their associated contaminants. However heterotrophic microalgae can be grown in very large volumes. Solazyme Inc., San Francisco, USA has successfully partnered with the US Navy to produce commercial quantities of algal fuel. Solazyme's fuel feedstock has already been demonstrated and approved as a commercial aviation fuel blend. This microalgae strain was grown in large fermenting vessels which used sugars and organic sources to produce the algae oil and thereafter the biofuel (Chang *et al.*, 2015). From an energy balance perspective sugar crops require fertilisation whose production is energetically and fossil fuel expensive. Fermentation facilities are less scalable than microalgae photobioreactors or open ponds due to the high capital expense and the requirement for sterility. However, heterotrophic microalgae production recycles wastes or unused sources of carbohydrate converting them into more valuable lipid sources. In the longer term future, a more resilient energy efficient microalgae biofuel production system should be appropriated for an era of potentially imminent fossil fuel depletion. Heterotrophic microalgae production could be considered to be merely an intermediary step in the final quest for phototrophic derived microalgae biofuel which should be both economically and energetically viable. The research challenges remain however, considerable.

1.3 Algae, and the importance of algae in the new economy

1.3.1 **Introduction**

The United States National Renewable Energy Laboratory (NREL) began an Aquatic Species Program from 1978 to 1996 with a focus on fuel from microalgae. 3000 strains of microalgae were collected and screened with a 1000 m^2 outdoor test facility in Roswell, New Mexico (Sheehan, Dunahay, Benemann and Roessler, 1998). Research considered lipid extraction and conversion to biodiesel with genetic manipulation in the final years of the project. It was abandoned in favour of corn based ethanol production, despite the energy output/energy input of US ethanol being 1.01-1.13, comparing less favourably to Brazilian sugar cane ethanol energy ratio of 2.5-3.5, i.e. 30-40% of fuel energy used in production. Consequently, in non-tropical countries biomass for liquid biofuel production offsets less carbon than biomass used for electricity generation (Cannell, 2003). The US diesel demand is more than 60 billion gallons per year (Pienkos, 2007). Resource requirements for the theoretical achievement of 60 billion gallons per year microalgae biofuels could theoretically feasibly be achieved with between 6 and 48 million acres of agriculturally un-productive desert land mass (Pienkos, 2007). Agricultural productivity from crops such as soybean and corn cannot be diverted from food to first generation biofuel crops to sustain these levels of biofuel production because food based commodity production is matched to market demand and with a rising population, any reduction in food commodity supply would result in insufficient food for human consumption. Conversely, the net effect of biofuel production via clearing of rainforest, peatlands, savannas or grassland habitats is to increase CO_2 emissions by 17 to 420 times more than the annual greenhouse gas (GHG) reductions that these biofuels would provide by displacing fossil fuels (Fargione *et al.* 2008). In principle, diverting a small fraction of total plant growth into biomass energy could satisfy the majority of global energy needs. However, the potential for producing biomass energy without negative climate or food security impacts lies mainly in the use of abandoned agricultural lands. The total above ground net primary production on these lands represents just 5% of global energy demand (Field, Campbell and Lobell, 2007).

Algae have greater productivity than terrestrial crops and are a non-food resource (Pienkos, 2007). Algae use seawater, waste carbon dioxide and are able to combine with waste water treatment. The U.S has vast underground reserves of saline aquifers which could be used for microalgae cultivation, and according the NREL, industrial carbon dioxide supply could theoretically more than provide sufficient requirements, Figure 1-1. The economy of growing microalgae is significantly more efficient in water consumption in comparison to corn and other terrestrial crops due to the comparative difference between water loss to the atmosphere via pond evaporation and crop transpiration (Smith *et al.* 2010).

Resource Requirement: CO_2 and Water
(Basis: algal oil needed for 60 billion gal/yr biodiesel)

	10@15 Productivity	50@50 Productivity
CO_2		
▪ Usage (ton/year)	1.4 billion	0.9 billion
▪ % of US Power Plant Emissions	56%	36%
Water		
▪ Usage (trillion gallons/yr)*	120	16

*Compare to ~22 trillion gal/yr saline water extracted in 2000 in U.S. (primarily for power plant cooling) (USGS), and to >4000 trillion gal/yr of water used to irrigate U.S. corn crop (USDA).

Figure 1-1 CO_2 and water provision to theoretically fulfil the US present diesel equivalent demands from biodiesel derived from microalgae shown at two levels of biomass productivities - 10% oil content at 15 g/m^2/day productivity and 50% oil content at 50 g/m^2/day productivity (Pienkos, 2007).

In order to theoretically fulfil the US present diesel equivalent from microalgae derived biofuel, between 6-48 million acres of open ponds would be needed, in comparison to the practical reality of 74 million acres presently used for US soybean agriculture (Pienkos, 2007). If this theory were ever to become real, then the sheer mass of moving such water volumes for nutrient mixing and harvesting biomass would be vast. Practical considerations of the conversion of this theoretical production would require pumps, pipes and pond liners as well as many other engineering challenges.

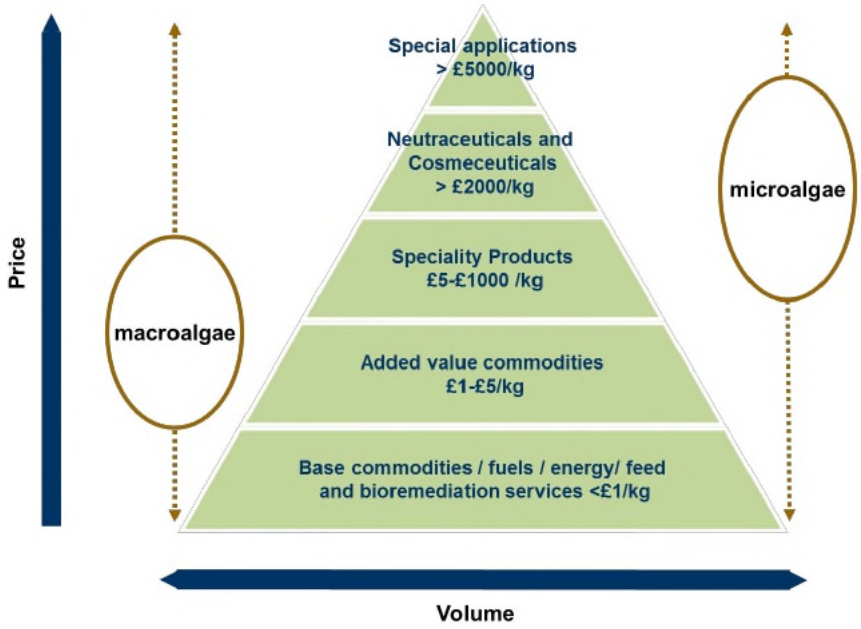


Figure 1-2 Current volumetric and costing of marketed products derived from microalgae and macroalgae (Schlarb-Ridley & Parker 2013).

Microalgae are probably the only source of renewable biomass which could potentially meet global demand for transport diesel (Chisti, 2007, Amin 2009). According to Schlarb & Ridley 2013, Figure 1-2, more pertinence is given to the viability of macroalgae rather than microalgae breaking into the value added commodities and later base commodity future markets. Macroalgae do not need land and freshwater for their cultivation (Lobban et al., 1985). Macroalgae can convert solar energy into chemical energy with higher photosynthetic efficiency (6–8%) than terrestrial biomass (1.8–2.2%) (FAO, 1997). Also, macroalgae have a lower risk for the competition for food and energy than other energy crops like corn and wheat as seaweed markets are mainly in a few East Asia countries where seaweed is used for food, hydrocolloids, fertilizer, and animal feed (Bixler and Porse, 2011; McHugh, 2003). Despite the environmental and economic merits of macroalgae, many challenges exist as macroalgae have unique carbohydrates, which are distinctively different from those of terrestrial biomass (Roesijadi et al., 2010; Sze, 1993). This thesis considers and incorporates the values and traits of these unique carbohydrates into industrial phycological applications by combining extracts of both macroalgae and whole microalgae biomass into an integrated production and biomass processing system.

1.3.2 **Growth & waste**

Chemical and biological energy are not only two distinct entities; they are also fundamentally united. Geological petroleum based chemical energy is merely historic biological energy, most of which has formed from ancient marine microalgae, entrapped and processed under favourable geological conditions. Modern farming is powered by equipment and fertilisers produced using petroleum energy. Recycling nutrients therefore equates to recycling energy. Biofuel technologies intertwine disciplines of agriculture, biotechnology, engineering and energy use.

There are several nutrient formulations used for the cultivation of microalgae in laboratory conditions. Macronutrient concentrations in formulated media are in excess in comparison with natural concentrations. For example, nitrate in Walne's medium was in excess of 40 times the concentration found in coastal waters (Andersen, 2006). A popular growth media for saltwater species is Guillard's f/2 medium which has isolated and cultured a wide range of marine microalgae (Stein 1979). Researchers do not uniformly comply with prescribed media for specific strains of microalgae, as different formulated media will culture many species. For example in experiments cultivating *Dunaliella salina* (Prieto *et al.* 2011) used Guillard's f/2, 8.8 x 10^{-4} M $NaNO_3$; (Çelekli & Dönmez, 2006) used Johnson's medium, 10^{-2} M KNO_3; (Mendoza *et al.* 2008) used Walne's medium 1.2 x 10^{-3} M $NaNO_3$; and (Tammam *et al.* 2013) used MH medium 10^{-2} M KNO_3 (Loeblich, 1982). Variation in nutrient supplies between researchers' experiments, demonstrates microalgae's adaptability to a range of different compositional formulations from the nitrogen salts of both potassium and sodium. This also gives rise to the justification for the use of organic based nutrient formulations. Such organic formulations have variable nutrient compositions of both their sources and the individual samples from these sources. According to the Redfield ratio, the chemical composition of the average phytoplankton is 106C:16N:1P, or 6.7C:1N (Andersen, 2006).

Microalgae has been cultured in swine manure (Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J. and Ruan, R. (2010). Microalgae has also been cultivated

in nutrients obtained from anaerobically digested dairy manure (Wang *et al.* 2010), the supernatant of filtered organic compost (Lam and Lee, 2012), poultry manure supplemented with ammonia oxidising archaea bacteria (Xie *et al.* 2012), sewage sludge (Cheung and Wong, 1981) and chicken manure & blood waste (Wong, 1981). These nutrient organic nutrient formulations are compositionally diverse.

1.3.3 Production systems

Growth in photobioreactors (PBRs), ponds, or ocean pods means containment or separation of the microalgae dense aqueous culture media from the surrounding environment – which is either land, sea or air depending on which system of production is practiced. Commercial microalgae culture systems are described in Table 1-2.

Table 1-2 Commercial microalgae culture systems currently in use and the algal species cultured (Borowitzka, 1999).

Culture system	Algae	Approximate maximum volume (l)[a]	Location
Tanks	Many species (for aquaculture)	1×10^4	World wide
Extensive open ponds	*Dunaliella salina*	1×10^9	Australia
Circular ponds with rotating arm	*Chlorella* spp.	1.5×10^4	Taiwan, Japan
Raceway ponds	*Chlorella* spp. *Spirulina* spp. *Dunaliella salina*	3×10^4	Taiwan, Japan, USA, Thailand, China, India, Vietnam, Chile, USA, Israel, China,
Cascade system with baffles	*Chlorella* spp.	3×10^4	Czech Republic, Bulgaria,
Large bags	Many species (for aquaculture)	1×10^3	Worldwide
Fermenters (heterotrophic)	*Chlorella* spp. *Crypthecodinium cohnii*	$>10^3$	Japan, Taiwan, Indonesia, USA
Two-stage sytem (indoors in closed reactor and then out-doors in paddlewheel ponds)	*Haematococcus pluvialis*	?	USA

[a] These are order of magnitude estimates only.

For the construction of open ponds, land containment and mixing costs are expensive comprising site preparation, grading, compaction, pond levees, geotextiles and paddle wheels at a 1996 cost estimate of $11,000/ hectare based on a 400 hectare production plant proposition (Benemann & Oswald, 1996). The reason being that it should be resistant to the suns' ultraviolet radiation, wind, rain and sand. Although PVC is cheap, the chemical structure is not highly UV resistant, a chemical characteristic required for microalgae pond liners. Geo-membrane pond lining surfaces act to separate microalgae culture media from the bare earth in open pond cultivation systems. These systems can be used in terrestrial environments to convert water-permeable surface soils into impermeable ponds for functions such as sediment ponds, wastewater treatment and manmade lagoons (Benemann & Oswald, 1996).

The commercial application of PBR technology remains limited mainly to the production of two Chlorophyte algae: *Chlorella* and *Haematococcus* (Pulz, 2001; Olaizola, 2003). Scale up of research PBRs to commercial scale is not trivial. Two

commercial failures due to improper scale up have been outlined (Tredici, 1999). PBR scale up needs to take into consideration changes in illumination, gas transfer and temperature (all three affected by the turbulence in the reactor) and their control. Indeed, scale up is an engineering problem, not a biological one. Much work has been done to describe the light field inside PBRs and general recommendations as to possible maximum scales have been made (Molina Grima, 2000). From a business point of view, a PBR must have as many of the following characteristics as possible:

- high area productivity (g m^{-2} per day), since many costs scale with plant size;
- high volumetric productivity (g l^{-2} per day), since some costs scale with the amount of water needed for culture;
- large volume (l PBR^{-1}), since some costs scale with the number of reactors needed;
- inexpensive to build and maintain (\$ PBR^{-1});
- easy to control culture parameters (temperature, pH, O_2, turbulence); and
- reliability

A flexible geo-membrane pond lining surface has been proposed for use in the ocean to culture microalgae at sea (Sea Green, EEDA, 2010). Sea Green was an acting branch of the international SURF consortium (Sustainable Use of Renewable Fuels) developed to investigate advanced biofuels from algae for aviation including the advisory and steering group members including Airbus, Gatwick Airport, Rolls-Royce and IATA. SURF aimed to address five major considerations for the successful use of renewable fuels from microalgae, including environmental impact; processing, capacity and distribution; commercial; and legislation and regulation. Such a system would be a shallow mobile floating pod, with buoyant supporting sides similar to the design of salmon aquaculture cages with the exception of the net being replaced with a flexible geo-membrane liner rather than a net. The cost of the membrane component of such a structure has been estimated in the region of £25 per square metre, with an estimated lifespan in the region of 10-20 years (Sea Green, EEDA, 2010). This considerably increases the production costs of producing marine microalgae

biofuel compared to land based production systems where harvesting and processing can be centralised and controlled more easily. NASA's Offshore Membrane Enclosure for Growing Algae (OMEGA) project is designing a horizontal floating elongated membrane bag structure visually but not functionally similar to polyethylene bag cultivation systems of freshwater microalgae at sea in proximity to the shoreline for waste nutrient remediation to produce microalgae biofuel. This system integrates energetic pumping costs of water.

Oceans of the world have a natural capacity for extensive biomass production. The annual gross production for all seas is between 55-70 g C/m^2 which with a 40% loss through respiration and a sea area of 361 x 10^8 square kilometres, this equates to 1.2 – 1.5 x 10^{10} tons of carbon (Nielsen & Jensen, 1957). Assuming a maximum algal growth rate of two divisions a day (and most algae attain half this because their cell cycle is linked to the diel day–night cycle), using a typical physiological configuration for cellular photosystem operation, yields year-averaged areal production rates around 5 g $C/m^2/d^{-1}$ over a wide range of optical depths (0.01–1 m). The output of the most productive oceanic waters is around 100 mol $C/m^2/y^{-1}$ (Sarmiento & Gruber 2006). This equates to a daily rate of 3.29 g $C/m^2/d^{-1}$, a value quite similar predicted from PBRs making use of naturally occurring high growth rate phytoplankton. In comparison, rates that have sometimes been informally advertised for microalgal productivity in bioreactors have been as high as in the region of 100 $C/m^2/d^{-1}$, only considered attainable from simulations of GM organisms (Greenwell *et al.* 2010). Oceanographic and meteorological events can combine with nutrient loading to produce massive coastal algae explosions as has happened in Qingdao, China most recently in 2008 and 2013 (Figure 1-3).

Figure 1-3 Bloom of *Ulva spp.* Qingdao, China, 2013 (New York Times, 5.7.13).

More unusually, in Australia an outbreak of sea foam encroached on the shores in the austral summer of 2013. The foam was caused by exo-polymeric secretions from organic matter and nutrients, saline salts and the churning action of waves which rendered the sea surface into a natural bio-surfactant creating the foam (Figure 1-4).

Figure 1-4 Sydney, Australia oceanic foam outbreak, 23.1.2013

The two naturally occurring events in Fig 1.3 and 1.4 were also likely to have been instigated by anthropogenic nutrient discharge into water bodies, either directly into the sea or via land surface run-off. The parallel between both of these phenomena was that they both had input from oceanic microalgae and macroalgae organic matter, they were uncontained open environment production

systems with no human intervention or control over the initiation of the event or the duration or the subsequent dispersion of the biomass. If such an event could be controlled and harvested to farm an oceanic source of algae biomass, then the advantages for this production system would be that utilisation of seawater and preservation of agricultural land as well as the production of a marine biomass with a different nutritional profile to that of terrestrial biomass. So doing would require, the bio-system to float and retain microalgae within or in close association with the supporting structure causing flotation. Adhesion of the growing biomass to the supporting matrix would also result in the biomass being difficult to thereafter separate, requiring sacrificial harvesting of both the microalgae and the supporting matrix simultaneously. The replication of the Australian foam bloom in a regulated aquaculture style biomass production system would be advantageous because of the air pockets which would aid flotation of the structure to help maintain the algae within the photic zone at the interface between the ocean and the atmosphere.

Considered strategies of a sacrificial harvestable organic substrate function in several ways. Multi-aims for this harvestable organic surface would be:

- To float
- To be flexible and resistant to wave action
- To be cheap & organic in composition
- To supply nutrients to microalgae for their growth as a batch culture system
- To provide a surface for microalgae to attach and grow on
- To provide an open structure matrix / foam with exposure to the air and sea
- To be harvestable and capable of being processed together with microalgae

This multi-criteria objective has the capacity of being achievable, successful and an interesting topic of innovative research. If such a production system could be achieved in the laboratory and feasibly scaled-up for commercial reality, then the elimination of the costs of a flexible containment membrane would significantly improve the production costs of microalgae biofuel. It is envisaged that the microalgae production systems would merely have a protective exterior containing surface floating barrier but no internal base. One area of growing

research is biomass for fluid bed pyrolysis to produce bio-oil and bio-char, which are resilient end products for integration into a variety of potential uses. Due to the high mass fraction of water in harvested microalgae (80-90%) (Patil, V., Tran, K-Q., Giselrød, H. R., 2008), traditional thermochemical processes like pyrolysis and gasification are economically not very interesting (Amin, 2009). Thermochemical processes for wet biomass, such as hydrothermal gasification, hydrothermal liquefaction or hydrothermal carbonisation appear to be more suitable for microalgae feedstock. Hydrothermal liquefaction (HTL) is an approach that requires no drying because the whole microalgae biomass is decomposed and converted in hot compressed water. A bio-crude oil is obtained as the main product, next to gaseous, aqueous and solid by-products (Barreiro, Prins, Ronsse & Brilman, 2013). As such, a sacrificial matrix would be advantageous for hydrothermal liquefaction downstream processing technologies which isn't scrupulous about the purity or the biomass.

The advantage of integrating such a sacrificial harvestable organic surface for cultivation with thermochemical conversion is that the entire end product of cultivation can be used for energy production and that the microalgae component of the bio-matrix could increase the overall oil profile as some microalgae species are known to accumulate high amounts of oil in their biomass compared to a lower oil content in seaweeds. Post processing, this could positively affect the physical properties of the resulting biofuel, hence a potential advantage of bio-processing extracts of macroalgae and microalgae simultaneously. It has been reported that biofuel chemical properties from *Laminaria saccharina* is more closely correlated with bitumen than biofuel (Anastasakis, K., & Ross, A. B. (2011).

Many discoveries are inspired by the natural environment including the morphology engineered structure of transportation vehicles such as hydrodynamic and aerodynamic exterior surfaces. Macroalgae is a global commodity currently supplying the pharmaceutical, food additive and food processing industries. The company FMC BioPolymer is one of three multinational macroalgae buyers which obtain its produce from seaweed

producing nations including Chile, Japan and China. The flexible gelatinous, nutrient trapping hydro-colloid properties of alginates extracted from seaweed are used widely already in scientific research. Purified agar gel media for bacteriology and microalgae cultivation either within the gel itself or on the surface is a well-known technique. There are other low-cost raw plant based materials which have gelling properties such as sugar extracted sugar beet pulp. A combination of such cheap organic nutrients “by-products” synthesised in the correct manner has the capability of achieving a sacrificial harvestable organic surface for the cultivation of microalgae.

Coal is an energy resource in nations such as China and Poland that will continue to provide a substantial component of electricity energy generation. At present coal fired power stations have not been coupled with carbon remediation mechanisms. Carbon capture storage is a potential sequestration method. Another viable alternative is to take advantage of the need microalgae has to absorb and utilise carbon dioxide for photosynthesis. Flue gas from coal fired power stations emits vast volumes of atmospheric carbon dioxide which contributes to global increases in carbon dioxide gas and global warming. It also contains small amounts of NO_x and SO_x gases which in combination with water produce nitric and sulphuric acids with a very low pH. For uninterrupted logistic links between global markets, many coal fired power stations are sited on estuaries or ports in close proximity to seawater such as Mejillones in Chile. Such proximity would help the mutually beneficial co-location of biomass production with gross emitting carbon dioxide industries. Microalgae are good sequesters of CO_2, however some species, but not all, require a neutral pH range of between 6 to 8 for optimal growth. Coal fired power station flue gas which is dissolved into water can have a pH of between 2 and 5. This could be a potential constraint in using microalgae as a vehicle for carbon dioxide sequestration. A pH closer to the preferable tolerance limits of microalgae is also favourable for nutrient bio-availability. Steel slag is a major by-product of commercial steel production in the UK. It contains about 30% Calcium oxide, a strong alkali agent. This research proposes to use two waste by-products namely Steel slag as a pH neutralising agent and anaerobic digestate as a source of fertiliser from commercial sources

as ingredient compositions to improve the growth performance of microalgae in simulated flue gas.

1.3.4 **Attached microalgae growth systems**

The properties of surfaces and the division interface between solids, liquids & gases play a significant role in the behaviour of colloidal systems (Ward, 1945). Agents acting upon the surfaces of colloids such as surfactants affect the state of flocculation within the system. Biological important colloids include cellulose, lignin, starch, glycogen, pectin and proteins. A range of surface binding and repelling forces are present in such colloids including, Van der Waals forces, hydrogen bonding and polar linking peptide bonds. According to Johnson and Wen, (2010), there has been no reported research into the growth of mobile single microalgal cells on solid supporting surfaces. In many laboratories, microalgal cultures are widely grown on agar plates. Johnson and Wen found encouraging results in cultivation the interfacial solid-liquid surface for the growth of microalgae using dairy manure organic fertiliser as a nutrient source harvesting freshwater *Chlorella* strain microalgae species which preferentially attached to a flat polystyrene surface rather than freely mobile suspended cells in the culture media. There has been little research into this area of freely suspended microalgae attaching to a liquid/solid surface, despite being a promising option for harvesting microalgae. Most conventional microalgae species considered as agents for biofuel production are cultured within the liquid aqueous media phase, rather than attachment to surfaces. The only exceptions are benthic diatoms which have a silica “glasshouse” style exterior protection system which can attach and colonise surfaces. The disadvantages of such diatom adhesion, is that they are susceptible to fouling of bioreactor solar intake surfaces and reduce photosynthetic capacity by shading other cells, consequently preventing the desired high volumetric density of biomass. Of the predominant cultivation systems using aqueous media, some companies such as Varicon Aqua have developed self-cleaning mechanisms to resolve the issue of attachment of microalgae onto the internal receiving light surface. Studies have demonstrated rapid light attenuation in high density closed photo-bioreactors within just several millimetres of the vessel wall, due to a threefold combination of mutual shading,

light scattering and light absorption by microalgae pigments (Greenwell *et al.* 2010). A German company IGV Biotech has revealed a novel construct of a mesh aerosol-style microalgae photobioreactor with a very low water usage based on high humidity using an attached cultivation mechanism, though a commercial shroud of secrecy in the finer detail resides in a difficult interpretation of the details.

1.3.5 **Harvesting**

1.3.5.1 Filtration

Harvesting microalgae can typically constitute 20-30% of the total production costs (Johnson and Wen, 2010; Sharma *et al.* 2013). The small size of microalgae and diversity of their anatomy presents a problem with conventional separation technologies such as filtration, even using filters with a very small pore size. Membrane filtration and ultra-filtration are costly for large-scale operations due to high operating costs for membrane replacement, clogging and pumping (Pittman *et al.* 2011; Molina Grima *et al.* 2003; Mata *et al.* 2010; Wang *et al.* 2008; Brennan *et al.* 2010; Schenk, *et al.* 2008; Amaro *et al.* 2011; Rawat *et al.* 2011). Membrane-based microalgae de-watering microfiltration systems consumed between 1.44 and 2.68 kWh/m^3 (Gerado, Oatle-Radcliffe and Lovitt, 2014). Tangential filtration has been found to concentrate *Tetraselmis suecica* algae from an initial to final concentration of 0.06% to 8.88 (w/v) using 2.06 kWh/m^3. By contrast the same authors' found that polymer flocculation could concentrate from 0.04% to 15% using $Al_2(SO_4)_3$ of 15mg l^{-1} with an energy consumption of 14.8 kWh/m^3 to on account of the high energy costs for mixing (Danquah *et al.* 2009). *Tetraselmis* sp. is a flagellated marine microalgae between 11-20 % lipid (Reitan *et al.* 1997).

1.3.5.2 Centrifugation

Centrifugal de-watering separates the bulk volume of water from the culture, 2-8% solids (w/v). Secondary de-watering is typically more energy intensive than the primary process and concentrates the microalgae culture to between 75-80% solids by either centrifugation or ultrasonic aggregation (Sharma, 2013). Massive variation exists in cell morphology of microalgae. Some cells form chains (e.g.

Thalassiosira, *Chaetoceros spp.* and some diatoms), others species are more spherical in form (*Chlorella spp., Emiliana huxleyi* and cyst stages of reproduction of many species) whereas others are motile (Marine dinoflagellates, *Tetraselmis spp., Dunaliella spp.*). Generally, the difference in specific gravity between water and some species of microalgae cells results in the requirement for very high spinning revolutions typically over 10,000 revolutions per minute in order to separate microalgae from their aqueous environment. Only microalgae with a specific gravity greater than 1 are able to be centrifuged, however lipid accumulation can sometimes result in neutral or positive buoyancy. Microalgae lipids are a desired for biofuel production, though species with a lower percentage lipid composition are also able to convert into biofuels via thermochemical conversion processes such as hydrothermal liquefaction. Separation efficacy of highly oleaginous microalgae from water can be compromised by a density: volume reduction and a reduced separation efficiency. Centrifugation can also disrupt microalgae cells (Sharma, 2013). In some respects, this may be an advantage for simultaneous disaggregation of biochemical components prior to downstream processing; contrarily, it may be that a fraction of the unharvested culture is needed as an inoculant for re-growth of the successive biomass batch to convert an otherwise semi-continuous production system into a continuous one. Vortex separation is a lower energy intensive process than centrifugation but still requires moving high volumes of water. Reviews of the different techniques available (including flocculation, filtration, centrifugation and air flotation) have concluded that centrifugation is possibly the most reliable technique and only slightly more expensive than other techniques (Molina Grima *et al.*, 2003; Becker, 1994). High centrifugal force requires powerful motors resulting in centrifugation being an uneconomical separation technology in terms of energy return on investment (EROI). Decanter centrifuges have been found to be as effective as solid-bowl centrifuges for separating microalgae, but the energy consumption of decanter centrifuges is higher than that of disc bowl centrifuges at 8 kWh/m^3 (Molina Grima *et al.*, 2003). Solid bowl centrifuges were found not to be superior to disc-bowl centrifuges in the recovery of micro-algae grown on pig waste with an energy consumption of 1.4 kWh m^{-3} reported (Goh, 1984).

Higher performance commercially available centrifuges designed for harvesting microalgae are able to achieve 0.55 KWh/m^3 using single step centrifugation (Sharma, 2013).

1.3.5.3 Dissolved Air flotation

A bubble reactor for microalgae separation has been developed by researchers at Sheffield University (Zimmerman *et al.* 2011). A columnar bubble reactor for sparging microalgae with surfactants has been developed at Newcastle University (Coward *et al*. 2014). Technologies using bubbles as harvesting devices such as suspended air flotation, dissolved air flotation, electro-flotation, surfactant and flocculation methodologies function by minor changes in surface tension and electrostatic bonding forces. Microalgae cells have negative surface sites such as carboxylate anions of poly-galacturonic acid (Gardea-Torresdey *et al.*, 1990). Dissolved air flotation is used in waste water treatment plants in the UK. Bubbles are formed by physical changes in the dispersion environment between the gaseous and aqueous environment. Technology such as Venturi jets (no direct energy), bubble generation and diffusion equipment with various apertures can initiate bubble formation. Energetic costs of de-watering processes depend on system operational energy expenditure and costs have been prohibitive to the development of large scale commercial microalgae de-watering processes for lower value products such as biofuels.

1.3.5.4 Flocculation

Flocculation of cells into clusters increases their relative mass to volume ratio and can increase sedimentation. Sedimentation is a very slow process 0.1-2.6 cm/hr, and biomass is liable to deteriorate during the process, especially in higher temperature environments (Choi *et al.* 2006). An improved energy efficiency method by means of bio-flocculation has been developed by (Salim *et al.* 2012), who investigated mixed species compositions of auto-flocculating microalgae with non-flocculating fast growing oleaginous microalga *Chlorella vulgaris*. When the auto-flocculating microalgae *Ettlia texensis*, *Ankistrodesmus falcatus* and *Scenedesmus obliquus* were added to *Chlorella vulgaris* at a ratio of 0.25, the recovery of *Chlorella vulgaris* increased from 25% to, respectively, 40%, 36% and

31%. Their research claims to reduce the energy required for centrifugation from 13.8 $MJDW^{-1}$ to 1.83 $MJDW^{-1}$ when using bio-flocculants as a method of harvesting microalgae. In the natural environment, there exist several other factors which could influence rates of microalgae sedimentation such as the rate of bio-decomposition of microalgae into humic matter, temperature, pH, salinity, Ca^{2+} aqueous ionic composition. It has been proposed that such humic-ionic flocculation in estuaries followed by sedimentation is a major elemental transport chain of carbon from the terrestrial to marine environment (Sholkovitz, 1976, Tipping, 2002).

There is a complex pH mediated aqueous interaction between divalent metal ions, the cell walls of microalgae (as sites of potential flocculation) and water soluble polymeric carbohydrate molecules. These interactions become manifested in various ways. It is well documented by several authors that high pH stimulates flocculation of microalgae. High alkalinity flocculation by NaOH addition occurs above pH 10.5. Divalent calcium and magnesium ions chelated out of solution by EDTA reduced the flocculation efficiency of *Chlorella vulgaris* at pH 11 (Wu *et al.*, 2012; Choi *et al.* 1998). Also, divalent magnesium ions preferentially over divalent calcium ions were responsible for the chemical flocculation (Vandamme *et al.*, 2012). The coagulant properties of aluminium and ferric salts induce polyhydroxo- aluminium or ferric precipitates with dissolved organic matter (Rebhun and Lurie, 1993). Increased antimicrobial properties against *E. coli* has been demonstrated by the presence of alginate bound Cu than by dendritic Cu alone (Thomas *et al.* 2014). In a controlled diatom bloom, exopolysaccharide exudates (EPS) associated with marine diatoms prior to a flocculation event (Passow & Alldredge, 1995). EPS extra-cellular secretion into the growth medium is common to many species of microalgae both in the natural environment and the laboratory (Passow, 2002).

1.3.6 **Drying**

At 15-20% solids (w/v) microalgae is no longer a fluid and not able to be pumped making handling difficult (Greenwell *et al.* 2010). Harvesting microalgae to ambient dryness is a multi-step process. De-watering separates the bulk volume of water from the culture, 2-8% solids (w/v). Secondary de-watering is typically more energy intensive than the primary process and concentrates the microalgae culture to between 75-80% solids using centrifugation or ultrasonic aggregation (Sharma, 2013). The final stage of drying depends on thermal processes as interstitial water with high affinity for cell surfaces connected via hydrogen bonding cannot be removed by further mechanical compression or pressure squeezing. The enthalpy of vapourisation of water is 2260 kJ/kg^{-1} (Varadarajan, Philip & Ramamoorthy, 2002). Thermal driers use either heat or sublimation freeze drying processes both of which are highly energy intensive. This tertiary drying stage removes all interstitial water from cell surfaces reducing to less than 5% moisture content for long-term storage in ambient atmospheric conditions. Microalgae paste which has been exposed to primary and secondary de-watering, but not drying cannot be stored at ambient atmospheric conditions due to airborne contaminants such as bacteria, mould and fungi. Such contaminants on microalgae paste rapidly degrade nutritional composition to humic substances with corresponding losses of methane and carbon dioxide. There is not yet any implemented commercial process for simultaneously harvesting, de-watering and drying microalgae biomass.

1.3.7 **Conversion to Biofuel**

There exist various options for conversion of microalgae biomass to fuel including fermentation, anaerobic digestion, pyrolysis, hydrothermal liquefaction or esterification to produce biodiesel FAME's. Choice of these conversion processes would depend on water content of the final biomass prior to downstream bio-processing. This thesis advocates hydrothermal liquefaction as an integral component within the processing chain of production.

1.4 Aims & Objectives

The main aims of the project were to reduce the cost of cultivation, harvesting and processing of microalgae biomass in biofuel. It was hypothesised that the process costs could be reduced by inclusion of extracellular polysaccharides. Objectives included:

1) Establishment of a novel attached microalgae growth mechanism using hydrogels

2) Establishment of a novel microalgae harvesting mechanism

3) Investigation of seawater ionic modification by alginate ionotropic gelation

4) Integration of industrial waste technologies for microalgae cultivation

5) Evaluate the hydrothermal liquefaction conversion of microalgae biomass into bio-oil

6) Estimate a holistic and economic commercial integration process of energetically favourable growth, harvesting and processing

A conceptual diagram of integrated processing is shown in Figure 1-5.

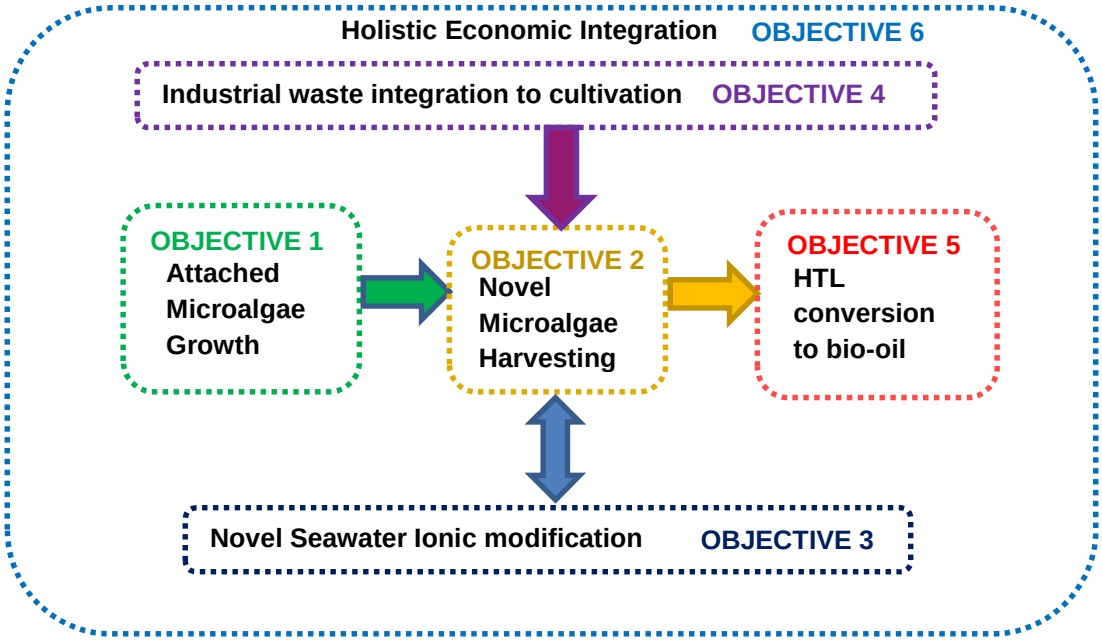


Figure 1-5 Objective of Integrated Process Engineering System for Biofuels derived from Microalgae

2 General Methodology

This chapter highlights the generic operational methodologies used prior to many of the experimental procedures. Specific methodologies are described in more detail for each chapter, where appropriate.

2.1 Microalgae culture maintenance

Freshwater and salt water media were prepared from Millipore™ de-ionised water. f/2 freshwater media was used from Varicon, Cell-Hi NC, or alternatively made from stock solutions (Table 2-1). Marine salt from Instant Ocean™ was used to formulate artificial seawater dissolved in 18.2 MΩ de-ionised water, Merck Millipore Millipore™ to a final concentration of 35 g/l. Laboratory reagent grade chemicals and vitamin stock solutions were used from commercial suppliers (VWR, Sigma Aldrich, Thermo Fisher Scientific). Micronutrients were made according to Kropat, 2011.

Table 2-1 f/2 freshwater media

Component	Stock solution (g/l)	Quantity used (ml)	[Final medium]
$NaNO_3$	75	1	8.82×10^{-4}
NaH_2PO_4.	5	1	3.62×10^{-5}
Na_2SiO_3.	30	1	1.06×10^{-4}
Thiamin	-	0.2	2.96×10^{-7}
Biotin	1	1	2.05×10^{-9}
Cyanocobalamin	1	1	3.69×10^{-10}

2.2 Equipment, sterilisation and disinfection

Axenic cultures of microalgae were purchased from the culture collection for algae and protozoa (CCAP), Scottish Association of Marine Science, Oban, UK. Cultures were maintained in axenic culture using 250 ml, 1 l and 2 l Erlenmeyer flasks. Equipment was autoclaved prior to utilisation. Erlenmeyer flasks were sealed with non-absorbent cotton wool to allow for gas exchange and stored at room temperature on a flask shaker. A temperature controlled incubator shaker (Brunswick Scientific) was also used for 250 ml Erlenmeyer flasks, which had

replaceable flask holders for flasks of different volumetric capacities. Long term preservation of stock algal cultures was at 18°C, continuous 150 µmol.m^{-2}.s^{-1} cool-white fluorescent light. CCAP cultures were inoculated at 10% (v/v) into 250 ml Erlenmeyer flasks containing 100 ml liquid medium and incubated at 22°C with 150 µmol.m^{-2}.s^{-1} continuous cool-white fluorescent light with 120 rpm on an orbital shaker. Following exponential growth and prior to development of stationary phase, cultures were batch scaled at 10% (v/v) via 1 and 2 l autoclaved Erlenmeyer flasks at room temperature and 150 µmol m^{-2}.s^{-1} continuous cool-white fluorescent light. 20 l carbouy polycarbonate vessels, (Nalgene) were used for transportation and growth of microalgae indoors and within the glasshouse facility. 20 l carbouys were not autoclaved but were disinfected using Virkon Aqua™ scrubbed, and thoroughly rinsed with de-ionised water prior to use. Innoculation and scaled growth occurred via 1:10 (v/v) dilution into fresh culture media. All cultures were grown by batch cultivation methods. Re-innoculation occurred in exponential phase of microalgae growth as assessed by microscopic cell counts using a haemocytometer. In the event of cultures becoming contaminated or excessive cell death, additional cultures were purchased from CCAP. Cell counts were measured by light microscopy and haemocytometer (Andersen, 2006). Tri-phasic conventional harvesting and drying of microalgae biomass was achieved by using a GEA Westfalia disk-stacked centrifuge, 15,000 rpm, pumped via a peristaltic pump a Thermo-Scientific Sorvall desktop laboratory centrifuge, 12,000 rpm and a laboratory lyophiliser (Christ). Freeze dried microalgae biomass was stored at -80˚C until analysis, or processed immediately after recovery.

3 Immobilised microalgae culture using pectin-alginate hydrogel bio-composite disks

3.1 Introduction

Alginates can entrap, immobilise and support growth of both animal and plant cells including bacteria, yeast, fungi and algae (Gombotz & Wee, 2012). The recovery of alginates from dried seaweed has been quoted as being 30-40% of total dry weight (Piantini, 2012, Vauchel *et al.* 2008). They form strong gels without heat treatment in the presence of divalent calcium cations. Many different species of microalgae have successfully been grown via immobilisation (primarily alginate) including *Chlorella vulgaris, Anabaena doliolum, Chlamydomonas reinhardtii, Chlorella emersonii, Chlorella kessleri, Chlorella pyrenoidosa, Scenedesmus obliquus, Dunaliella salina, Phormidium laminosum, Spirulina maxima* (Hameed & Ebrahim, 2007). Alginate vectored microalgae immobilisation has been proposed for heavy metal biosorption via chelating complexes and waste water remediation for nitrate and phosphate (Hameed & Ebrahim, 2007). Brown seaweed species commercially exploited for alginate production include: *Laminaria hyperborea, Macrocystis pyrifera and Ascophyllum nodosum*. Alginates are mainly manufactured in the USA, Japan, China, France, and Norway (Gomez *et al.* 2009).

Pectins from sugar beet and citrus peel agricultural by-products also form gels in the presence of calcium ions with gels of clear to opaque transparency. Rheological properties of both alginate and pectin gels can be improved by the addition of a plasticising agent such as glycerol. The inclusion of a plasticiser decreases intermolecular forces along polymer chains, improving polymer flexibility and decreasing brittleness (Silva *et al.* 2009). Coir fibres from coconut provide longitudinal strength in a gel and prevent tearing. Coir fibres contain porous lumen and lacuna internal spaces with a density of 0.8 g/cm^3 (Yao, 2012). Due to the high affinity of gel material with microalgae growth in natural ecosystems, this research investigates the physical tensile and compression strength properties of gelatinous microalgae culture with a view for exploitation of hydrogel biomimetics for mass cultivation of microalgae. If the gel bio-composite

disk cultivation system demonstrates economic feasibility for scalability, it would be necessary to ensure that the structure holds together during the cultivation cycle in order to withstand environmental and biological physical forces such as oceanic winds, waves, birds and potentially fish. Although algae immobilisation has been proved extensively, methods for potential batch cultivation using this technique have not yet been exploited. Scientific literature on immobilisation refers to microspheres of approximately 2 mm in diameter formed when drops or droplets of alginate gel form within a calcium chloride solution (Wee and Gombotz, 1994; Austin, Bower and Muldoon, 1996; Elqin, 1995; Gray and Dowsett, 1988; Bodemeier, Chen and Paeratakul, 1989; Martinsen, Skjak-Braek and Smidsrod, 1989). This is a micro-gelation principle with application to pharmaceutical and water remediation technologies rather than macro-gelation for scaled biomass production as proposed and investigated in this study. Other applications of alginate beads include drug delivery and time release gastric activity (Iannuccelli *et al.* 1998). Other studies have investigated attached microalgae culture, which is again dissimilar to the approach taken in this research. A productivity comparison of *Scenedesmus obliquus* grown attached was 70.9 g/m^2 verses 8.9-14 g/m^2 in liquid media and *Botryococcus braunii* attached was 5.5-5.7 g/m^2 verses 2.4 g/m^2 in liquid media (Liu *et al.* 2013; Cheng *et al.* 2013). Advantages of immobilised cultivation systems however include nutrient entrapment and the ease of harvesting by means of a sacrificial organic matrix containing embedded microalgae. Entrapment by alginate is a mild, safe, and simple method which is generally suitable for immobilizing any type of cell (i.e., bacteria, yeast, fungi, higher plant cells, animal cells, and even embryos) while retaining maximal bio-catalytic flexibility (Nussinovitch, 2010).

A possible option for scaled biomass culture would implement a wide and flat floating bed of immobilised gel composite for algae cultivation, rather than individual gel disks. Such a system could feasibly be enacted via the utilisation of a working marine vessel with mixing and holding tanks of alginate extraction chemicals incorporating low-grade recycled heat from the engine emissions for the 50°C aqueous Na_2CO_3 alginate extraction reaction from dried seaweed or pectin, and a reconfigured engine design for oil-powered hydraulic fluid

separation and filtration systems for the alginate and water separation. Enhanced modern engineering designs of fluid processing and food extraction technology used in protein and water separation such as fishmeal processing and milk protein separation are both powerful and compact in their physical size, making them compatible with application on-board seagoing vessels. Working marine vessels already contain seawater desalination equipment within the lower decks for conversion of saltwater into drinking water. Mixing of seawater with alginate and $CaCl_2$ in mixing/holding tanks and on-site gel placement into the sea via a "floating surface sheet-delivery" approach would be advocated. Such a theoretical system would be able to deliver thin yet structurally stable applied sheets of gel composite up to 10 m wide which could be relayed in parallel to provide a wider surface area of application in a similar way sports pitches are mowed or farmers' fields are harvested. The challenge and caveat of this process and the technological constraint for scale-up is the continued provision of flotation of the matrix which has a final density just below seawater resulting in slow sinking. This theoretical scale-up approach contrasts to the scaled experimental volumetric application of 20 cm diameter scaled disks which in increased numbers on the sea surface would overlay and stack up upon one another with prolonged wave motion, as well as being difficult to contain and viably harvest.

3.2 Methods

3.2.1 Materials

Alginic acid sodium salt, from brown algae, calcium chloride and glycerol were purchased from Sigma-Aldrich (UK). Pectins were kindly donated by Herbstreith & Fox (Germany). Coir coconut fibres were kindly donated by Salix River & Wetland Services Ltd (UK). A Clarke AP500B Arbor press was purchased from Machine Mart (UK). An LCM101-10 "S" Beam load cell was purchased from OMEGA Engineering Ltd (UK). 20 cm diameter plant pot trays, waterproof film and plaster of paris were purchased from a local retailer. A UK wild strain of *Chlorella vulgaris* 4TC 3/16 was isolated in Bedfordshire from an open environmental bird bath and confirmed as *Chlorella vulgaris* by PCR at Algenuity. This UK business is working on genetic transformation platforms for

transformational molecular modification enhancement of both microalgae wild type (via bio-prospecting) and laboratory collections including the Scottish Association of Marine Science (SAMS) Culture Collection of Algae and Protozoa (CCAP). Density and longevity growth trials in seawater used the saltwater species *Chlorella salina* (CCAP, 211/25.)

3.2.2 **Gel selection**

Gelation characteristics were determined by pilot trials using three variants of commercially available pectins and a medium viscosity refined alginate. Table 3-1 shows the superior properties, lower cost and higher solution pH prior to gelling of the amidated pectin latterly used for structural integrity, growth and ionic retention trials.

Table 3-1 Gelation characteristics to determine the rheological characteristics of the commercially obtained pectin in comparison to alginate as defined by the solution pH prior to gelation, cost and rheological characteristics using gel and $CaCl_2$ concentrations of 2%.

Gel type	Solution pH prior to gelation	Cost (£/kg)	Gel Characteristics
Amidated Pectin	5	28	Strong
Sugar Beet Pectin	3	33	No
Classic Pectin	4.1	29	Crumbly
Refined Alginate	7.6	84	Strong

3.2.3 **Gel disk structural integrity**

Structural integrity tests were investigated to find out the response of the bio-composite to differing inclusions of coir fibre as a means for changing the density and potentially flotation characteristics of the disks. A base of 20 cm diameter fabricated from plaster of paris was formed to a thickness of 2 cm in the plant pot trays and allowed to set, then overlayed with waterproof film. Coir fibre was determined gravimetrically and layered evenly in the base of the plant pot trays. Treatment 1, 2 and 4 had 3 g, 0 g and 6 g coir inclusion respectively Coir fibre

inclusion in treatment 3 was included at 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7 and 7.5 g. Batches of 2% alginate solution (w/v) of alginic acid sodium salt, 2% solution (w/v) of amidated pectin, 2% calcium chloride (w/v) and 5% glycerol (v/v) in de-ionised water were prepared prior to usage and stored in separate 2 l autoclaved and sealed Erlenmeyer flasks. 150 ml of alginate, 150 ml of pectin and 15 ml of glycerol were mixed in a magnetic stirrer, and poured evenly into the trays containing coconut fibre for each disk. There were 10 repetitions per treatment. Treatment 4 was agitated by means of 5 min vigorous shaking in an enclosed container prior to gelation to induce bubble formation.

200 ml of 2% calcium chloride (w/v) was poured over the gel blend and gently agitated to fully permeate and induce gelatinisation and was rinsed in 33 ppt saline after 2 hours. Two hours post-incubation, disks were removed for structural integrity trials. An arbor press and a “S” Beam load cell were modified to align with the criteria for the British Standard Methods of test for Geotextiles puncture resistance test BS6906:1989 (Figure 3-1). The difference between the standard and the test rig used was that the standard specified a displacement of 50 +/- 10 mm per minute which depends on a constant force application. As this test rig was a hand crank mechanism, a regular and continual force was applied, but it could not be a constant displacement as specified within the standard.

Figure 3-1 Structural Integrity geotextile puncture resistance trials

Upper: load cell and modified Arbor press visible with raised plunger. Middle: clamp for holding the gel disks prior to placement of disks. Below: rupture event during testing. On all figures the limited clearance (<1 mm) between the outside diameter of the plunger and the internal diameter of the plunger penetration hole in the circular base plate clamps is evident.

Fine adjustment of the disk holding base plate was necessary in order to position the plunger directly above the hole so that it would not impede the smooth transit when penetrating the disks and falsify the readings on the load cell. Load cell signals were converted into a mV output (OMEGA RS485 & LASCAR display unit). Calibration was achieved using metallic weights and a gravimetric analysis (Bibby Scientific Limited). Kitchen scale weights were used to calibrate the load cell. Firstly, the mV reading was read without mass added which gave a baseline reading of 31 mV via the software. Actual known forces comprised various items of known masses including kitchen scale weights and heavier items ranging between 62 g and 7.7 kg. These forces were recorded in Newtons, calculated against gravity at 9.81 m/s^2 and compared with the mass readings to verify the validity of the actual forces derived from the load cell using the calculations of the load cell results. The overlaid points on the graph show that the two data sets are closely aligned to validate the authenticity of the testing procedure (Figure 3-2 & 3-3).

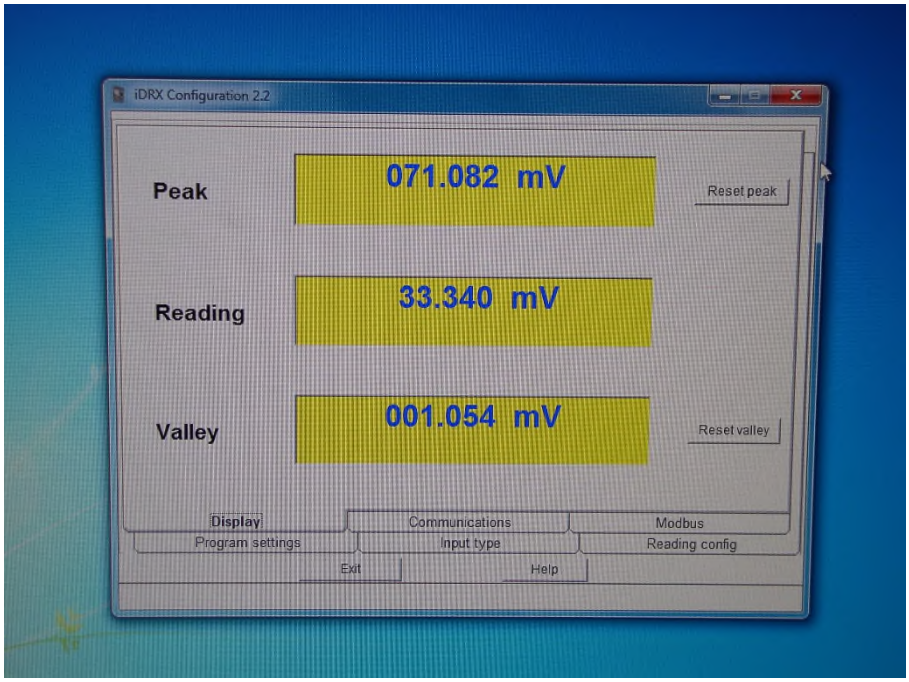
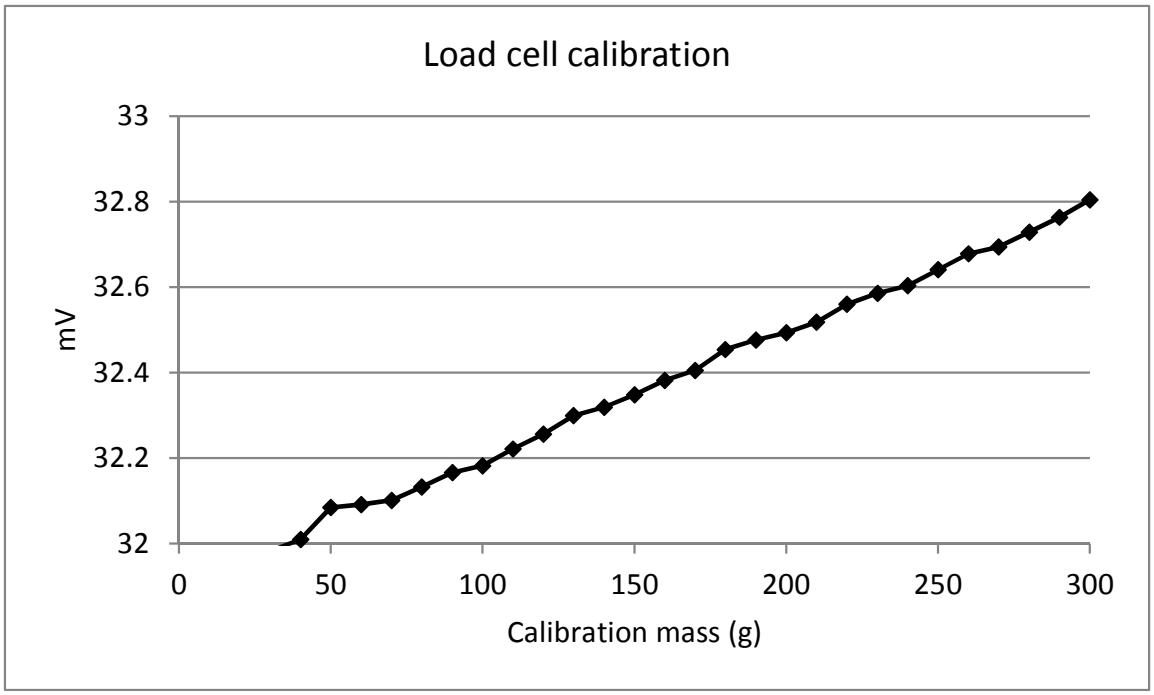


Figure 3-2 mV display from the load cell
showing the mV reading which was subsequently converted into force.



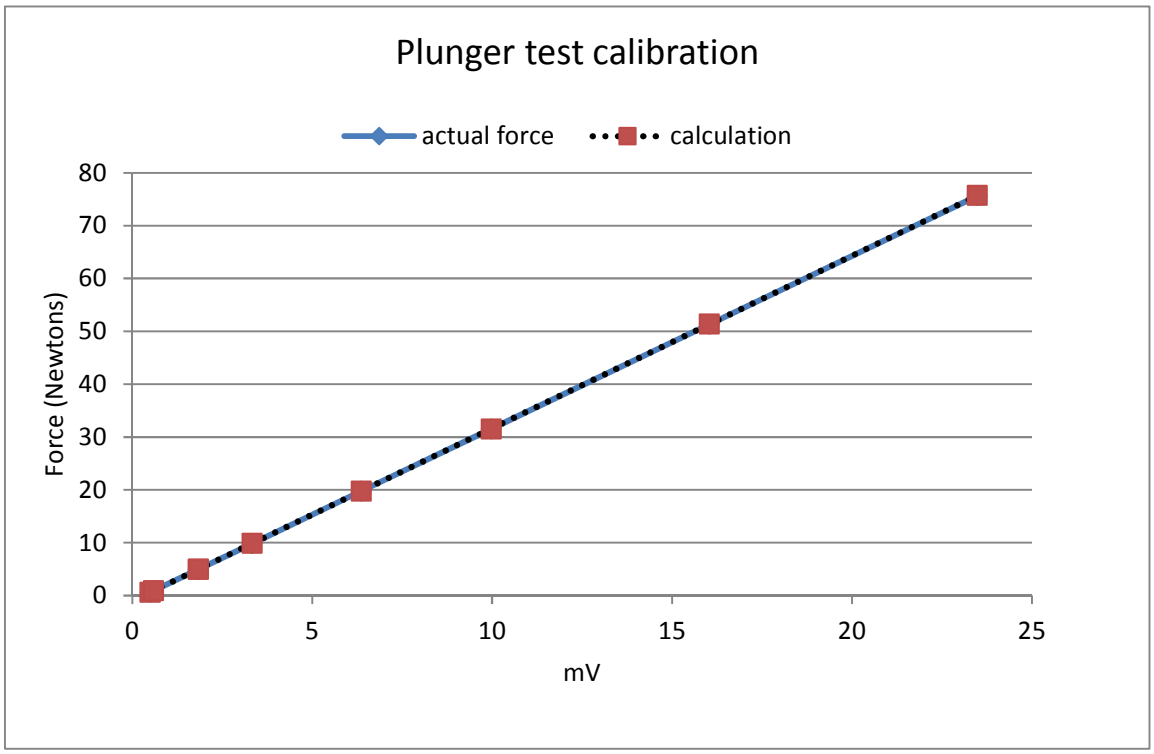


Figure 3-3 Load cell and plunger test calibration graphs showing relationship between mV recordings and incremental mass on the load cell (above) and relationship between the forces of a range of objects between 60 g and 7.7 kg below and the load cell mV.

3.2.4 **Gel disk flotation trials**

Flotation potential was tested using a 120 l plastic capacity container 85 cm long by 40 cm wide filled to a depth of approximately 30 cm with 35 ppt artificial seawater (Instant Ocean). 32 Alginate and glycerol composite disks were constructed using concentrations of 0.5%, 1%, 1.5% and 2% alginate and 1%, 2%, 5% and 10% of glycerol at each concentration of alginate. 2 replicate disks were tested for each varying treatment. To test for displacement flotation, disks were carefully placed by hand onto the surface of the testing tank and allowed to either float or sink. The process was performed with due diligence in order to avoid water surface disruption and artificially causing the disks to sink. Each disk was left for up to 2 hours and visually analysed for flotation status. After the test time period, if disks remained on the surface they were classified as being able to float by means of displacement. If disks sunk soon after placement, they did not float via displacement. To test for buoyancy flotation, disks were placed 5cm below the surface of the testing tank. They were visually observed for a period of up to 5 minutes to determine if they floated as a result of positive buoyancy. In order to avoid water movement distortion and potential error from adjacent disks, each disk was tested on its own.

3.2.5 **Induced flotation trials**

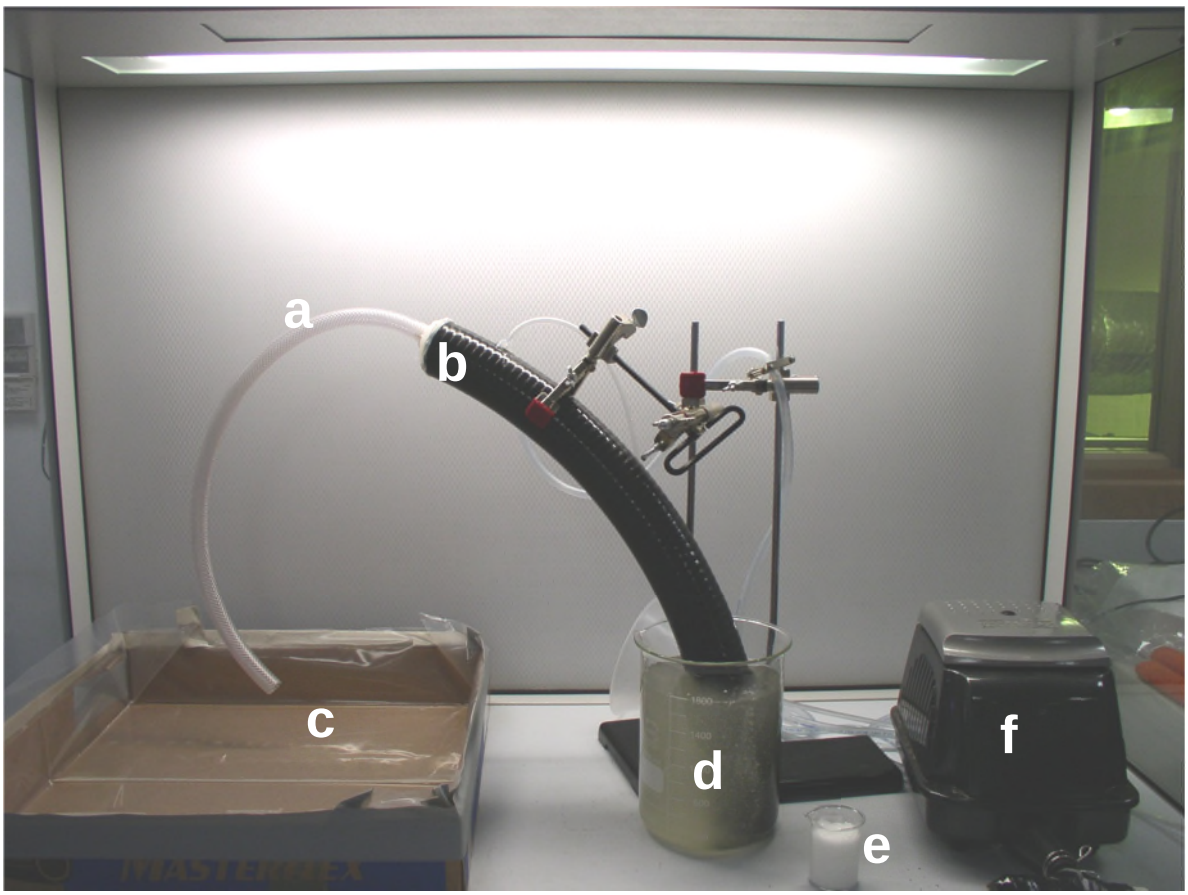


Figure 3-4 Foam fractionation experiment
a. 10 mm ID hose, b. 50 mm ID hose airtight connection with silicon cement, c. waterproof lined collection tray, d. 2% Alginate gel in a 5 l beaker with internal airline and airstone (not visible), e. Coconut soap surfactant, f. 2400 l per hour air pump.

An experiment was devised using alginate gel to evaluate the gelling properties in association with surfactants. Figure 3-4 shows the experimental apparatus used to test for alginate gel foam fractionation. This investigated the potential for reducing the inclusion of alginate concentration in the gel by mechanically inducing bubble formation. Alginate was tested between 0.5% to 2% with the addition of surfactants, commercially available and manufactured soaps and detergents to modify bubble size and numbers. Variable parameters included the height of the internal air stone within the tubing, length of tubes and airflow (Figure 3-4).

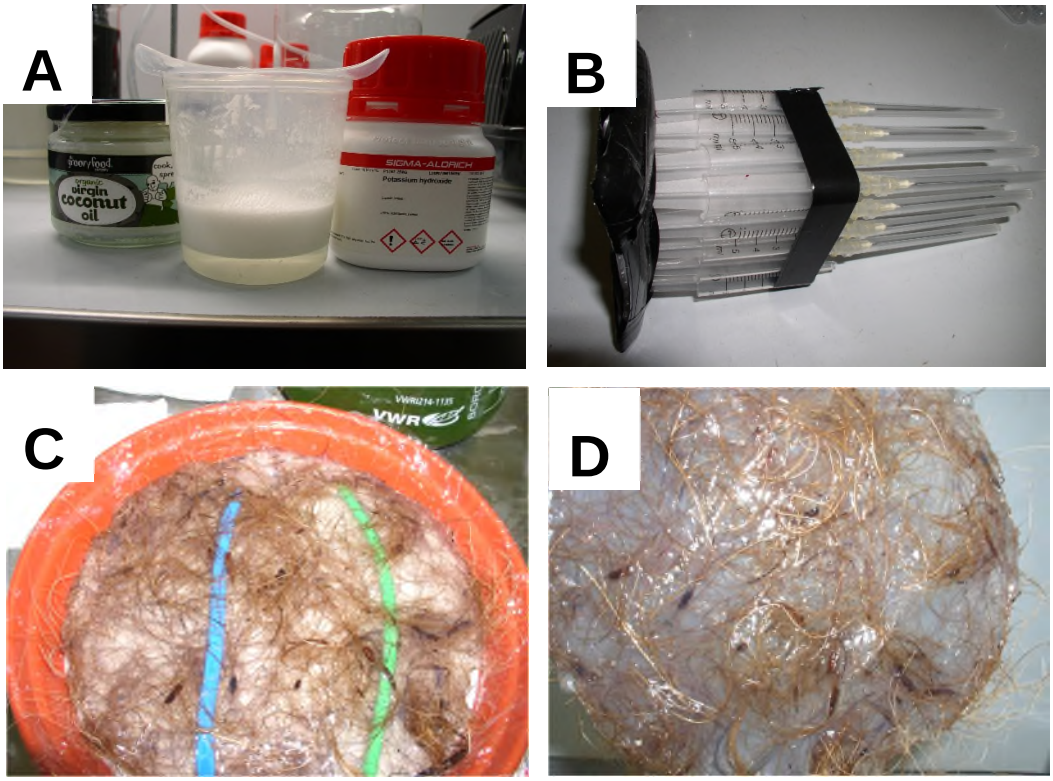


Figure 3-5 Tested mechanisms for the modification of disk density in order to induce flotation

A – saponification of rapeseed, corn and coconut oils to create bubbles, B – Multi-injection to inject air into partially gelled disks, C – air spaces of various volumes, D – coir fibre inclusion.

3.2.6 **Effects of microorganism contamination**

Procedures were conducted for the testing of atmospheric surface contamination onto, and within microalgae immobilised alginate and pectin composites. 20 ml of gels previously sub-cultured with *Chlorella vulgaris* were formed with varying ratios of pectin:alginate (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) in duplicate and placed into weighing boats. 2 ml of de-ionised water was added daily to compensate for disk dehydration with exposure to the open environment. After 14 days, the gels were photographed for visual analysis of organic contaminants.

3.2.7 **Gel composition**

Pectin and Alginate crude powder extracts were analysed for atomic composition by Energy-dispersive X-ray Spectroscopy (EDX), (Oxford instruments Scanning Electron Microscopy) in order to determine the differences in elemental composition between the algae, and embedded algae flotation matrix.

3.2.8 **Algae growth in liquid**

Chlorella vulgaris 4TC 3/16 was maintained in Tris-Acetate-Phosphorus (TAP) media (Harris, 1989) and cultures were preserved long-term by cryopreservation in liquid nitrogen (Algenuity Ltd). Algae were inoculated at 10% (v/v) into 250 ml Erlenmeyer flasks containing 100ml liquid medium and incubated at 22°C with 150 µmol m^{-2} s^{-1} continuous cool-white fluorescent light with 120 rpm on an orbital shaker. Previous *Beta* test research of Algenuity's ALGEM laboratory microalgae bioreactor has demonstrated that *Dunaliella salina* growth responses under similar nutrient, mixing, light spectral composition and inoculating culture densities had similar growth profiles for both continuous and periodic light. Differences in the respective ALGEM growth responses were accounted for by an oscillating rather than smooth growth curve because of the diel day-night cycle. Following growth to exponential phase, assessed by microscope cell counts using a haemocytometer (Andersen, 2006), and prior to development of stationary phase, cultures were batch scaled at 10% (v/v) via 1 and 2 l autoclaved Erlenmeyer flasks at room temperature and 150 µmol m^{-2} s^{-1} continuous cool-white fluorescent light.

3.2.9 **Nutrient media ionic composition analysis**

Nutrient media was supplied in order with manufacturer's specifications at 1 ml Cell-hi NC media (Varicon Aqua). Ion chromatography analysis of major ions was measured on a Dionex ICS-900 integrated single channel ion chromatography system with an AS-DV autosampler and Chromeleon software. Anion and cation multi-component standards (Chloride, Bromide, Nitrate, Phosphate, Lithium, Sodium, Potassium and Calcium) were prepared to provide a linear concentration calibration against unknown samples using integrated software. 5 ml samples

were diluted 1/50 to reduce overloading to the column and preserve the instrument and multiple injections were performed for average values. As a single column instrument, two samples were retained for both anion and cation analysis using two separate columns and suppressors. The IonPac AS12 anion column used a 0.8 mM carbonate and 0.1 mM bicarbonate eluent with an AMMS suppressor and the cation IonPac CS12 column used a 20 mM methane sulphonic acid eluent with a CMMS suppressor. Between anion and cation column exchanges, the instrument was thoroughly rinsed with high purity de-ionised water followed by the required eluent until a constant baseline was obtained prior to subsequent analysis.

3.2.10 **Growth analysis of alginate gel disks**

Gel disks were prepared using 2% sodium alginate excluding coir fibre. 15 ml of stationary phase *Chlorella vulgaris* 4TC 3/16 with a cell density of 10^7 cells/ml determined by microscope and haemocytometer (Andresen, 2006) and 330 µl of Cell-hi NC nutrient media (Varicon Aqua), which is specially formulated nutrient media for *Nannochloropsis* and *Chlorella* algae species, were inoculated into the gel mixture comprising 300 ml of 2% alginate and 15 ml glycerol. After stirring, and pouring into the reaction container, 200 ml of 2% $CaCl_2$ was added and left to react for 2 hours. Two hours post-incubation, disks were removed, rinsed in de-ionised water and retained in a hydrated state by immersion in 200 ml de-ionised water, covered with waterproof film to prevent ambient evaporation. A second treatment of disks used gel pH prior to gelation balanced at 7.5 with drops of 0.1 M NaOH and HCl. Disks were incubated at 22°C with 150 $\mu mol.m^{-2}.s^{-1}$ continuous cool-white fluorescent light. The characteristic reversibility of alginate gelation in the presence of a calcium chelation agent was used for digestion of daily samples from alginate disks. Typically 0.55 mM sodium citrate has been used for the digestion of alginate microspheres from a 0.22-gauge needle (Masuda, Sah, Hejna and Thornar, 2002), however due to the larger samples of 1 g used, a higher concentration of 0.1 M sodium citrate was used for alginate digestion. 1 g gel daily samples were removed from the gel disk and dissolved in 10 ml 0.1 M sodium citrate for analysis of total inorganic, total organic, total

carbon, total nitrogen, (Shimadzu) and cell counts. 5 ml samples of the hydrating de-ionised water were removed daily and analysed by ion chromatography (Thermo Fisher Scientific). The ratio of gel to the hydration solution remained the same throughout the duration of the experiment due to prevention of evaporative losses, however daily samples of 5 ml of hydrating liquid and 1 g of gel were extracted for the daily analysis. Experiments were duplicated and average values recorded. Higher nutrient growth trials were simultaneously conducted using similar gel preparation parameters with pH 6 and 7.5 and 1 ml of Cell-hi NC nutrient media per disk. The higher nutrient inclusion was used to ensure batch cultured nutrient limitation was avoided. Disks were inoculated at pH 6 and 7.5 was taken because the pectin gel prior to gelation was pH 5, the alginate was pH 7.5 and the disks were a composite blend of the two un-gelled solutions, consequently this range provided a reasonable level of expected frequency distribution for pH variation between composite treatments.

3.2.11 **Scanning Electron Microscopy**

Samples were mounted onto aluminium stubs using double sided carbon tape and coated with a 2 nm layer of gold palladium using a Polaron E5100 sputter coater. The samples were imaged in an FEI XL 30 ESEM and analysed using Energy Dispersive Analysis Aztec software (Oxford Instruments). The acquisition time was 60 seconds and predominantly using 20 KeV (Kilo electron volts). The scale was shown on the images.

3.3 Results & Discussion

3.3.1 **Structural integrity**

The structural integrity of gel alginate and pectin disks was assessed using an Arbor press, as described in 3.2.3. A higher breaking force indicated a likely greater longevity of the immobilising matrix material for the duration of the 8-day growth cycle and resistance against natural physical forces.

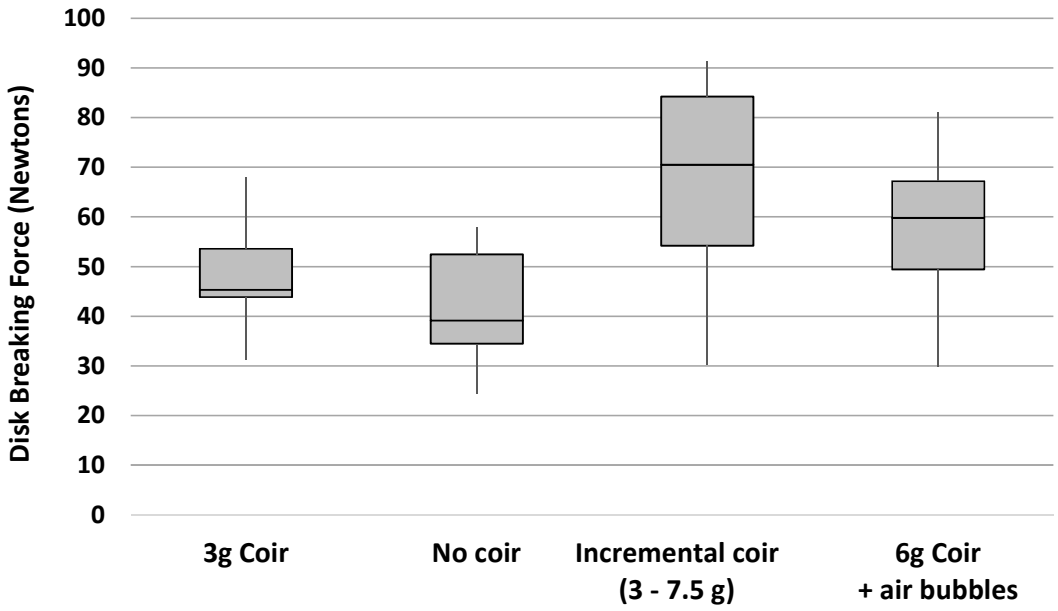


Figure 3-6 Box plot of forces required for the 50 mm diameter plunger to puncture the alginate-pectin gel disks

Disk composition comprised 150 ml alginate, 150 ml pectin, 15 ml glycerol, and variable inclusion of coir fibre. 3g of coir, no coir, incremental coir between 3 g and 7.5 g at 0.5 g intervals, and 6 g of coir plus air bubbles. The BS6906:1989 geotextile puncture resistance test was followed with for the exception that the displacement was delivered via a hand cranked mechanism rather than the specified 50 +/- 10 mm per minute.

Figure 3-6 shows no difference between treatments. The inclusion of air bubbles did not compromise structural resistance for disks containing coir fibre resulting in a breaking force of between 30 and 80 N, compared to 30 and 90 for incremental inclusion of coir fibre without air bubbles. Disks without coir had a

mean breaking strain 5 N less than groups with 3 g coir. The coconut fibre and gel combination acted to spread the duration of rupture (elongation to failure) and hence the change in breaking force was not a sudden rupture event. This was due to the elongated lateral embedment of the coir fibres within the gel which were laterally exposed to pulling torsion forces. Sample variability in disk breaking forces for both those with and without coir fibre was apparent. Being a natural material, unequal uniformity of fibre orientation and fibre diameter was apparent and this would have contributed to sample breaking force variation. Coir fibres did not rupture during the puncture resistance test. Either spaces between coir fibres opened as the plunger penetrated the disk, or the plunger pulled individual coir fibres out of the gel as the rupture event occurred. Individual coir fibre longitudinal tensile breaking strain was not assessed as part of this study, because there was more interest in the complete composite structure rather than that of the individual components, with the exception of the gel disks without coconut. Coir fibres were exposed to a humid gelatinous environment for the duration of the trial. Physical observational examination showed that coir fibres were not significantly damaged post-embedment in the alginate-pectin gels after 8 days exposure to this environment. Though the individual breaking strain of the fibres was not investigated, it was noted that they maintained structural integrity in excess of 100 N upon application of hand-held longitudinal tensile pulling forces. Mechanical properties of potential synthetic and natural fibres including coir were investigated by Cheung *et al.*, 2009 who found a tensile strength of between 106-175 MPa (106-175 N per mm^2) and an elongation at break of between 14 and 49%. Cheung's reported elongation at break appears high, considering the strength and durability of the coir fibre found in this study. As a natural product, coir fibre exists with a wide ranging natural distribution of fibre cross-sectional widths depending on the position on the longitudinal fibre, however due to the high durability of the coir structure used in this study, it is questionable if there is any tensile elongation at break at all. An immediate rupture is more likely.

Equivalent conversion of 50 N breaking strain to MPa at a plunger surface area of 1964 mm (50 mm diameter) as reported in this study equates to 25 KPa, much

less than that reported by Cheung. As this is such a novel and specific individual study, no other direct comparisons for similar bio-composite pectin-alginate disks tests were found in the scientific literature. However comparative forces for exposure in the natural environment are known. For theoretical weight load bearing comparative purposes, one of the physically largest birds and widely distributed in the Southern Ocean are the albatrosses. The average adult weight of Black-browed Albatrosses is 3.9 kg for males and 3.7 kg for females (Prince, Ricketts & Thomas, 1981). Such masses are distributed over webbed feet which have a surface area of approximately 100 cm^2 per foot (personal experience aboard commercial longline *Dissostichus eleginoides* fishing vessels, South Atlantic Ocean, 53°20 S, 57°47 W). Therefore, in theory, the compressional force of the mass of a flat-footed albatross on a composite gel structure with a surface area large enough to support it, would be insufficient to rupture the gel composite. Contrarily, physical morphology such as bird talons, beaks and an inquisitive disposition could render the structure to be ruptured by way of a piercing. Puncturing the sheet surface would result in seawater below the structure leaking above the gel air-water interface, potentially compromising displacement buoyancy and risking overall lack of flotation.

The distribution of four sample groups showed considerable individual sample variation in breaking force between 30 to more than 70 N. One major difference between this study and an authentically verified and audited BS6906:1989 sample test is the lack of constant velocity of the plunger during processing. This test used electronically calculated real time mV recordings as the plunger was constantly drawn down through the gel composite manually. The trial necessitated two workers due to the specific set-up of the equipment. BS6906:1989 states in the method statement that a constant rate of travel of 50 +/- 5 mm/minute should be maintained. In this trial the rate of travel of the plunger through the bio-composite gel disk was not recorded as there was not the facility to do so on the modified arbor press using the hand crank. This could be a factor for the sample variation distribution between treatments. Another reason could be the lack of consistent duplication in the formulation of the disks, specifically relating to the variable inclusion in coir fibre. Though coir fibre included on a mass

basis per disk sample was constant and precisely weighed prior to formulation, from the perspective of distribution of orientation of fibres, and dimension of fibres there was sample variation inconsistency. There are also many other options for flotation ingredients instead of coir such as air compartments (Iannuccelli *et al.* 1998) and CO_2 gas forming agents (Choi *et al.* 2002). For the purposes of mass scale biofuel production via a gelled flotation sacrificial structure, cost, large scale availability and manufacturing capability of incipient ingredients is paramount to viability. As such, flotation devices for 2 mm alginate beads with associated high cost margins cannot be scaled in mass production system for final application in biofuel energy from biomass.

3.3.2 **Gel disk composition**

The atomic composition of pectins were measured to determine any differences in elemental composition between the algae, and embedded algae flotation matrix. Analysis by EDX showed that alginate gels contained approximately 5% less carbon, 5% more sodium and fractionally less sulphur than pectin gels (Table 3-2).

Table 3-2 Energy dispersive x-ray atomic composition analysis of pectin & alginate

Atomic Composition (%)	**Amidated Pectin ($C_6H_{10}O_7$)**	**Alginate ($C_6H_8O_6$)**
Carbon	50.92	46.36
Oxygen	47.87	47.7
Sodium	0.68	5.78
Sulphur	0.44	0.16

A 1% gel disk contained 3 g of dried pectin/alginate or 10 mg/ml. By comparison, the chemical structure of alginate β-d-mannuronic acid block and α-l-guluronic acid block repeating units are both $C_6H_8O_6$. The differential between these values for alginate in terms of measured experimental EDX atomic composition and chemical formula structure of alginate atomic mass can be attributed to the detection of both sodium and sulphur in the EDX samples, EDX cannot detect

chemically bound hydrogen. Higher detected sodium in alginate samples could either be due to residual external dehydrated saline deposits, or an ingredient used within the manufacturer's alginate extraction and phenolic removal process. Amidation process involves the replacement of hydrogen with an acyl (Nitrogen) group. Amidated pectins had a less friable physical consistency with superior gel strength rheology, as defined subjectively by means of a non-numerical manual, tactile assessment. No nitrogen was detected using EDX. The main elemental percentage composition distinction between C, H and O in the samples by means of organic molecular mass determination in comparison with EDX is mutually representative between the two methods of sample analysis.

Table 3-3 Basal nutrient (1 ml/l) Cell Hi-NC analysis for major anions and cations by ion chromatography

Cell hi-NC nutrient profile		**mg /l**
Anions	Chloride	4.05
	Bromide	0.67
	Nitrate	334.5
	Phosphate	18.47
Cations	Lithium	0.02
	Sodium	155.5
	Potassium	10.1
	Calcium	1.12

A high nitrate growth media from a commercial supplier was used for this experiment and ionic composition is shown in Table 3-3. The growth media was designed for microalgae cultivation of fast growing green algae such as *Chlorella* and *Nannochloropsis spp.* The inclusion concentration of pre-formulated nutrient media was factored as recommended by the commercial supplier. The gel volume equated to the culture volume as if the microalgae were cultured in a conventional non-attached liquid growth system i.e. 200 ml disks = 200 µl media at 1 ml/l inclusion.

3.3.3 **Hydrogel pectin-alginate-glycerol matrix enclosed *Chlorella vulgaris* media growth trials**

Microalgae growth trials (Figure 3-7) demonstrated highest total organic carbon accumulation after 150 hours with the HNC7.5 (3 ml Cell-hi NC media /l, pH 7.5). 0.1 M sodium citrate contained high levels of organic carbon measured at 5716 mg/l. Digestion of alginate/pectin hydrogels with sodium citrate diluted the carbon content of the resulting solution. 1 g of gel contains approximately 5 mg alginate or pectin derived carbon. This was factored into the volumetric productive calculation and the corresponding carbon flux ratio presented in Figure 3-7. Organic carbon rather than inorganic carbon is the major constituent of both the gel matrix and the growing microalgae. Inorganic carbon constitutes less than 50mg/L in all treatments over the duration of the experiment. Total nitrogen also showed a constant range of between 20-40 mg/l with depletion in the HNC7.5 treatment from over 40 to approximately 25 mg/l over the 150 hours. Cell counts were taken using a haemocytometer and light microscopy. Sodium citrate gel digestion rendered some cells in a mild agglomeration physical state which could be disrupted via agitation, however as cells were small and uniformly spherical in shape, counting with a haemocytometer was feasible.

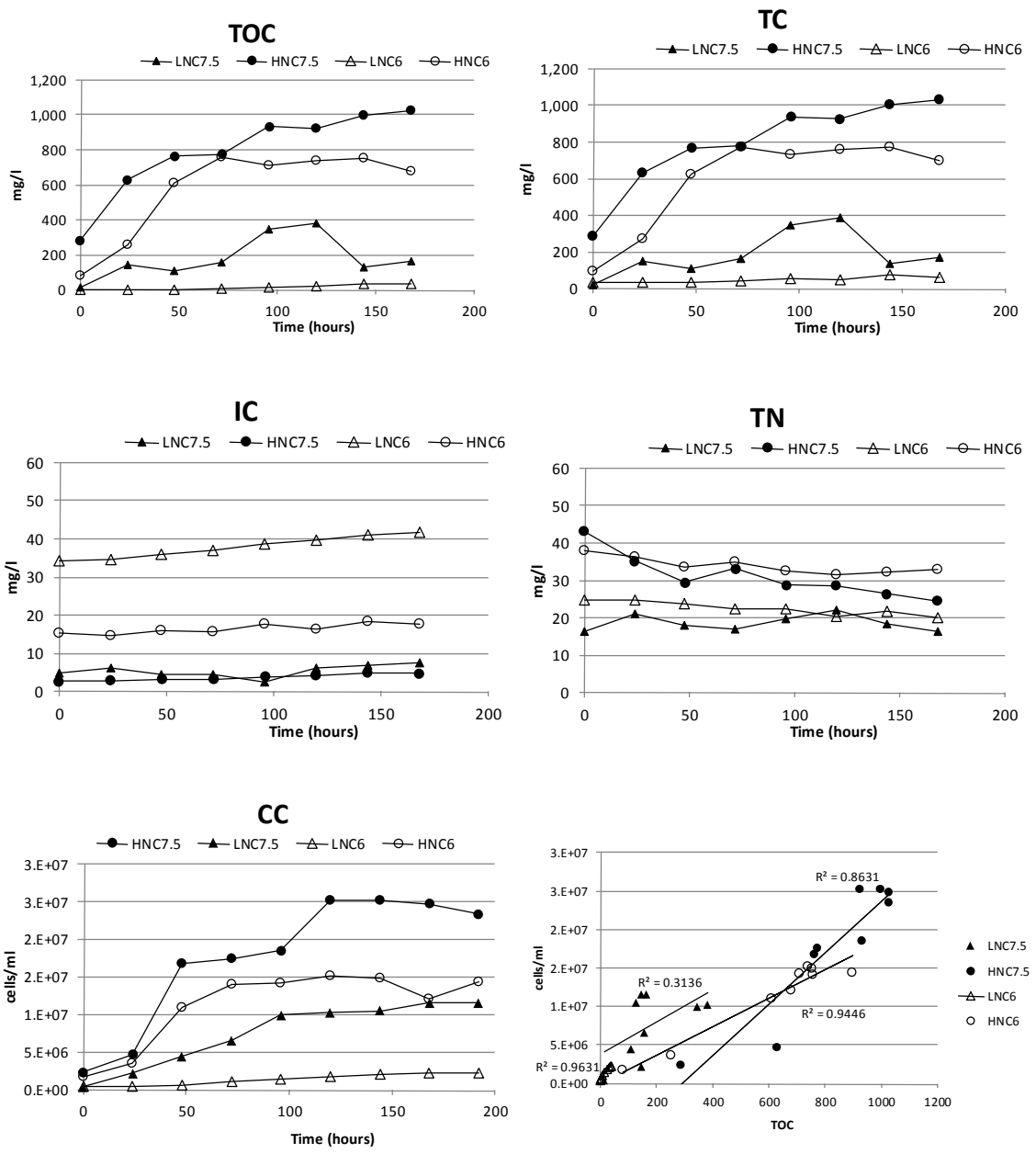


Figure 3-7 Total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), total nitrogen (TN), cell count (CC) and regression analysis of daily disk gel samples

growing Chlorella vulgaris 4TC 3/16 in treatments of 1 ml/l and 3 ml/l Cell-hi NC media (pH 6 and 7.5).

Cell counts and total carbon mainly composed of organic carbon exponentially increased within the first 3 days. Immobilised cultures were continually illuminated under artificial light. Growth curves of *Chlorella vulgaris* and *Pseudokirchneriella*

subcapitata showed that the maximum value for specific growth rate was achieved with *Chlorella vulgaris* with a constant supply of light (L:D ratio of 24:0), with irradiance of 96 µmol/m/s (0.738 d^{-1}) (Pires, *et al.* 2013); At higher light intensities, continuous light can potentially cause photo-oxidation (Chisti, 2008). However, photo-oxidation at the low light intensity of 150 µmol/m/s continuous cool-white fluorescent light, and an open culture system was not considered to contribute to growth limiting conditions in this trial.

It has been found that nitrate removal using *Chlorella kessleri* in wastewater within 3 days was 31.6 mg/l NO_3^{2-} under continuous lighting and 14 mg/l NO_3^{2-} under 12:12 light/dark cycles (Lee & Lee, 2001). By contrast in an immobilised cultivation system, this study showed an equivalent reduction in N of 13 mg/l in the first 2 days of the high nutrient 3 ml/l group and thereafter no significant variation. Provision of carbon for growth was made available from various sources. Carbon sources could have been provided from dissolved atmospheric carbonates from the incubation water. Carbon could also be provided from heterotrophic nutrition of glycerol (Liang, 2009). It has also been reported that inclusion of synthetic glycerol at 2 g/l improves microalgae growth rate by 18% relative to BG11 medium alone (Skorupskaite *et al.* 2015). However, in this trial glycerol was thoroughly mixed with un-gelled alginate and pectin blends as an immobilised composite, rather than a more conventionally studied non-immobilised culture system. This is significant because there is likely to be ionic cross linking and chemical bonding between glycerol molecules and hydroxyl groups on alginate and pectin long chain polysaccharides, effectively thus rendering such otherwise heterotrophically available carbon sources as unavailable for microalgae nutrient acquisition. The extent of chemical bonding between organic molecules of glycerol, pectin and alginate was not investigated in this trial. It can be hypothesised that the gelled composite mixture provided a source of available carbon for microalgae growth in addition to inorganic carbon dioxide and aqueous dissolved carbonate as a wholly or partially mixotrophic growth system, however further work to confirm this hypothesis would likely involve a traceability study such as an isotope-labelled carbon uptake trial.

Of interest from an engineering design perspective was an understanding of whether microalgae growth rate was comparable or better than an aqueous non-attached cultivation system. Most of the studies on immobilised algae have been performed using more regularly formed alginate beads rather than larger alginate structures as reported here. Alginate bead formation has been used because of the ease of bead formation and the higher surface area to volume ratio in a spherical small volume bead. As such, there is not the potential for a direct comparison with the gel disk techniques presented in this study. Within alginate bead immobilised formations, it has been detected that *Scenedesmus obliquus* shows a higher N & P uptake in real wastewater than *Chlorella vulgaris* (Ruiz-Marin, 2010). In this trial, the saline species *Chlorella salina* was utilised in immobilised bio-composite culture in 33 ppt saline. *Chlorella salina* has been reported in immobilised cultivation systems previously for investigations into Co, Mn and Zn ionic metal remediation of nuclear contaminated waste (Garnham, 1992). Garnham did not measure growth rate of *Chlorella salina* in the course of his experimental work as the investigation was more concerned with contaminated metal ion remediation, however it was reported that it was difficult to formulate a *Chlorella salina* immobilised alginate bead with a cell density greater than 10^8 cells/ml. During this study, the maximum attained cell density within the bio-composite matrix was 2.5×10^7 cells/ml in the higher nutrient group. This is comparable to the similar cell density on day 8 in the visual photographic representation in Figure 3-8. Daily 1 g of gels extracted from growing disks were digested in sodium citrate and then analysed using light microscopy for cell counts with a tally counter and haemocytometer. Garnham inoculated the alginate beads with a cell concentration of 2×10^6 cells/ml, however in this trial cells grew within the bio-composite. Analysis of growth rates of microalgae within immobilised gels is similar to rates of growth in aqueous media except that the lag phase was prolonged in immobilised culture using *Chlorella vulgaris* immobilisation in carrageenan (Lau, 1998) and *Scenedesmus obliquus* immobilisation in carrageenan (Mallick, 2002). Lau calculated a maximal cell density of 2.5×10^7 cells/ml in suspension culture and 2×10^6 cells/ml in

immobilised culture which equates to a similar cell density at maximal growth in this study.

An advantage of the microalgae immobilisation mechanism is the ease of separation of beads, disks or if scaled beyond laboratory culture, the mass sheets of bio-composite gels from the surrounding incubating water. However, this advantage is offset by the additional procedure for dissolving the alginate or pectin into solution prior to microalgae inoculation, and the requirement simultaneous need for calcium chloride.

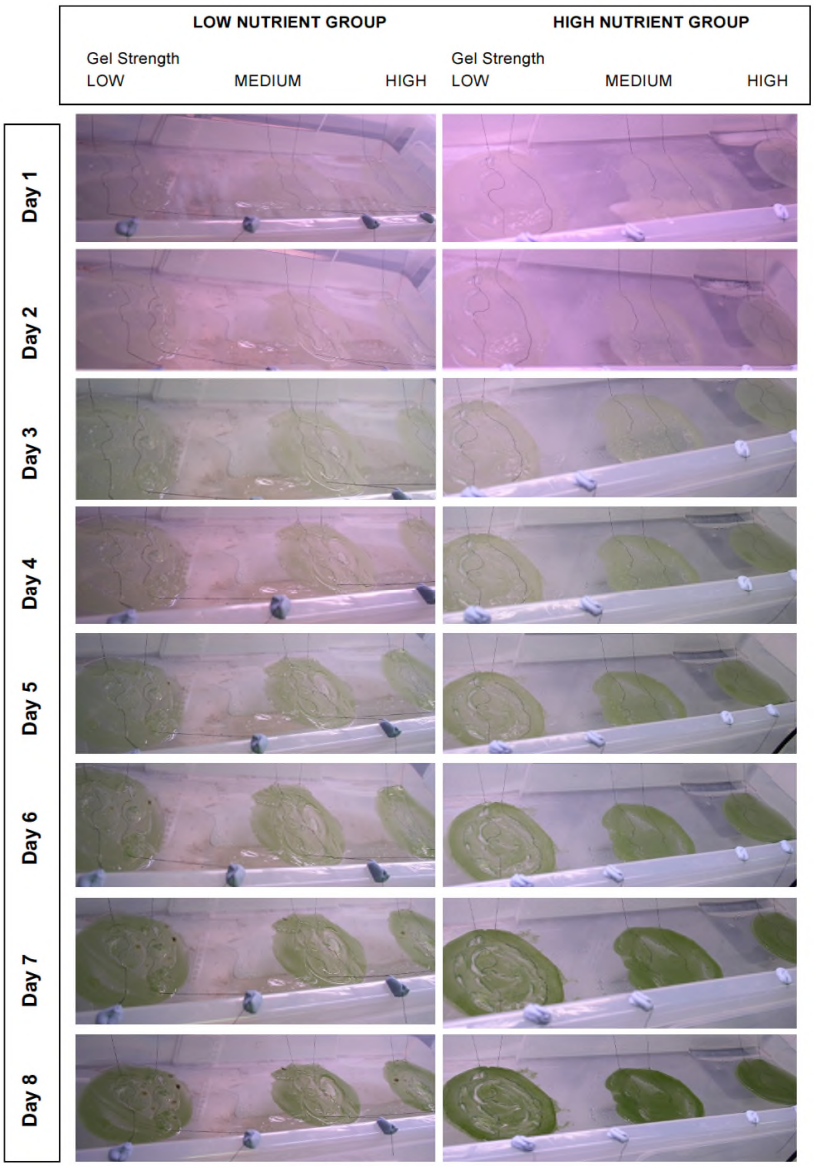


Figure 3-8 Daily photographs of microalgae growth

Figure 3-8 shows the growth of *Chlorella salina* gel disks in seawater suspended on the water surface with embedded cotton thread. A typical weekly batch growth cycle is shown with varying nutrient treatment variations not apparent by visual appearance alone.

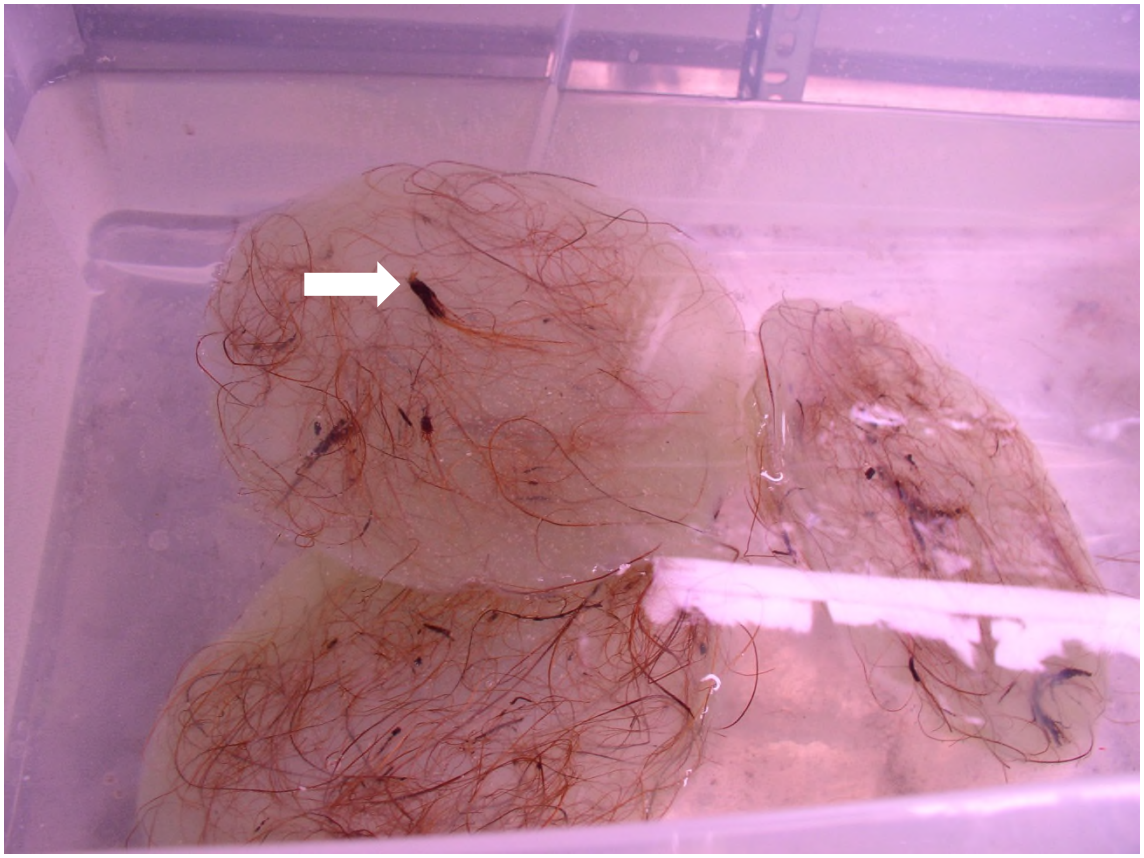


Figure 3-9 Partially floating attached microalgae growth (Day 2) bio-composite disks.
The arrow shows a dense core of adjoining coir fibres which would have originated in close proximity to the coconut shell.

Figure 3-9 visually shows the inconsistency in cross-sectional coir fibre morphology and the resulting distribution and orientation of coir fibre network within the formation of alginate disks. Despite a uniform depth of 1 cm, natural variation in composition and distribution of the coir fibre was present depending on the distance from the coconut from which the fibres were extracted, and method of formulation. The arrow in Figure 3-9 points towards a fused, dense terminal section of merging coconut fibres which in the raw product would have been attached in close proximity to the coconut shell. Some coir fibres are

profoundly visibly thick whilst others are inconspicuous. A mechanical product formulation strategy of bio-composite formation for even distribution of the coir fibre within the gel disks would be a desired trait. In the laboratory this was conducted using coir flotation trials and induced flotation trials. None of the disks had a positive buoyancy in material density compared to the surrounding artificial seawater as they all sunk when partially submerged 5 cm below the water surface. The 1% and 2% glycerol inclusion disks with 1%, 1.5% and 2% of alginate respectively showed positive buoyancy by displacement (Table 3-4). In response to tank surface agitation and manually induced artificial seawater movement, with water above the disk surface, these disks too sunk. Although displacement buoyancy was a desired physical characteristic in the structure, this alone was not sufficient to maintain flotation status. Disks which appeared to be floating for a period of 1 - 2 hours when left for 12 hours were later found to have sunk (Figure 3-9). This could have been to an uneven edge allowing gradual seepage of water onto the disk surface altering the disk displacement profile. Alternative flotation mechanisms attempted included bubble formation, multi-syringe injection for air bubble formation in partially set gels, macro air pocket inclusions of differing air volumes, and coir fibre (Figure 3-5). Of these, the only success for flotation was found to be the air pocket inclusions in the form of partially inflated balloons. The optimal desired characteristic of flotation would be small, regularly occurring natural air pockets in the form of a foaming agent. Equally effective, larger artificial periphery air pockets in the form of large buoys or bolsters would suffice for maintaining flotation even though they would be practically more difficult to deploy at sea, probably resulting in non-commercial sense to do so. The reaction of a mild acid with calcium carbonate also produces carbon dioxide bubbles within alginate gels. This approach has been considered in drug delivery alginate microspheres (Choi *et al.* 2002), though less practical as well as being counter-effective for large scale microalgae growth and atmospheric CO_2 remediation.

Table 3-4 Alginate gel disk displacement long term flotation status in artificial seawater

(+) positive displacement buoyancy flotation (-) sinks.

		Alginate inclusion (%)			
		0.5%	1 %	1.5%	2%
Glycerol inclusion (%)	1%	-	+	-	-
	2%	-	-	+	+
	5%	-	-	-	-
	10%	-	-	-	-

Pectin and alginate gels require direct contact with either seawater or freshwater in order to maintain a status of hydration. They cannot be exposed to a terrestrial environment without drying unless they are drip fed with water to prevent desiccation via ambient evaporation from gel surfaces. Similarly to evaporative losses from water body surfaces, increases in temperature and air flow will increase the rate of evaporative loss from hydrated gels. A significant advantage of obtaining flotation is that gels would get both access to the full environmental light spectrum, intensity as well as hydration.

The periphery of the disks shows a marked green colouration of early exponential phase growth of *Chlorella salina.* The disk with the arrow Figure 3-9 was in a state of surface floatation, the other two disks were in a state of partial flotation which justifies their oval depiction more clearly visible in Figure 3-10A. This did not have any effect on cell growth. Changes in density could be attributed to bubble formation which was noted on disk surfaces Figure 3-10A. These gas bubbles could either be loss of dissolved air from solution due to the temperature change from piped tap water used to mix with marine salt to manufacture the artificial seawater, or alternatively excretion of oxygen from microalgae photosynthesis. Experimentally, this was difficult to test because any mild vibrational energy to either the incubating cabinet within the laboratory, water tank, water surface or disks released the gas bubbles. Future work could test this gas by collecting it using a submerged inverted bowl-shaped collecting apparatus which would require in the absence of atmospheric air and with placement at the same time as sunken incubating disks in order to cumulatively collect released

gases from disk surfaces. Collected gas could then be analysed by means of gas detection equipment.

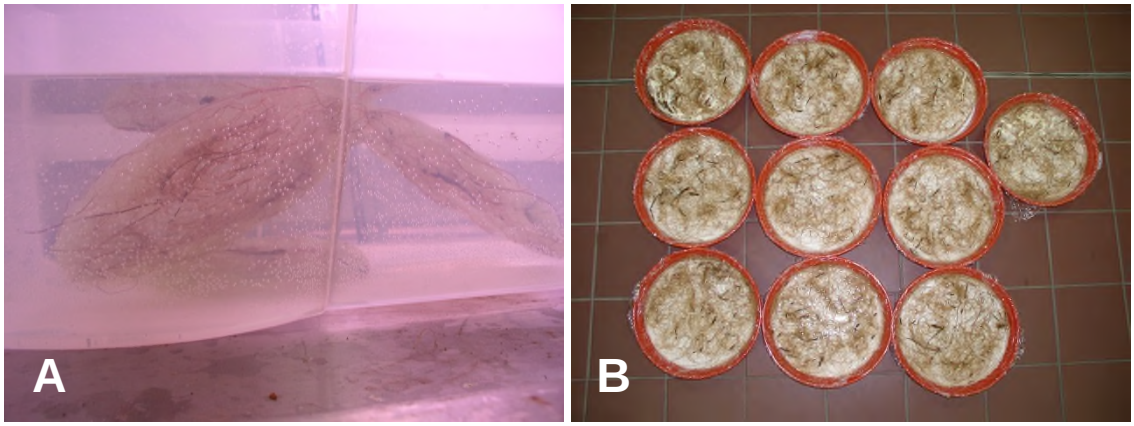


Figure 3-10 – (A) Side view of Figure 3-8. (B) Group of disks prior to growth trial

Figure 3-10 (A) shows bubbles attached to the inner surface of the polyethylene testing tank containment vessel. These bubbles formed up to two days after artificial seawater preparation. Some of these bubbles attached to the alginate-pectin gel and induced periodic inconsistent positive and negative density changes from surrounding 33 ppt saline in the flotation of the hydrogel bio-composite disks. (B) Arrangement of a batch of gel disks prior to experimentation.

3.3.4 **Ionic retention trials**

The presence or absence of leaching major ions from gel disks into the surrounding was investigated using ion chromatography as reported in section 3.2.10. This was investigated to find out if the gel matrix entrapped nutrients for utilisation by batch cultured immobilised microalgae over the duration of the culture cycle (between 5-10 days). If incrementally higher nutrient concentrations were detected in incubating de-ionised water over the test period, it would suggest that the alginate or pectin gels were not binding or bonding nutrient ions. Conversely, if detected ions remained constant within the incubating water, it suggests affinity between the gelling materials and the algae nutrients. This would be the most desired finding as optimal macro and micronutrient application delivered at the start of the experiment would be bio-available for the duration of microalgae culture. Indeed, this is what was detected in Table 3-5.

Table 3-5 Detection of major anions and cations in hydrating de-ionised water surrounding gel disks (*brackets St Dev.*)

Day	Chloride	Calcium	Sodium	Ammonium	Sulphate
0	497.1 (10.7)	580.0 (4.2)	44.8 (1.5)	12.8 (2.4)	10.2 (0.6)
1	1057.8 (8.2)	1336.0 (9.9)	148.0 (7.8)	31.8 (1.6)	30.2 (6.8)
2	1103.5 (16.3)	487.5 (12.0)	150.5 (6.0)	31.3 (1.1)	37.0 (8.5)
3	1089.5 (19.1)	562.0 (2.8)	161.5 (4.6)	33.5 (0.7)	26.2 (5.9)
4	1112.5 (24.7)	485.5 (13.4)	142.0 (6.4)	21.0 (4.2)	26.4 (5.2)
5	1111.8 (36.5)	442.0 (15.6)	149.0 (8.5)	23.5 (4.9)	27.0 (4.2)
6	1194.0 (24.0)	498.5 (17.7)	146.0 (2.8)	24.0 (8.5)	26.2 (2.3)
7	1209.0 (35.4)	492.0 (29.7)	148.0 (1.4)	24.0 (7.1)	27.3 (1.1)
8	1146.0 (14.1)	500.5 (31.8)	143.0 (2.8)	25.5 (1.4)	26.6 (0.8)

Table 3-5 reports the presence or absence of leaching major ions from gel disks into the surrounding 200 ml of incubating (disk dehydration prevention) de-ionised water over the course of 8 days. It was found that despite several initial rinses of the gel disks in de-ionised water, high ionic levels of both calcium and chloride were detected with ion chromatography as the residual gel induction agent. The calcium anomaly on day 1 cannot be accounted for but suspected to

be error. A control with constantly replenished incubating solution would be a beneficial experimental improvement for future investigation as this would help to confirm if ions originated from initial inoculation or leaching. Sodium ions were present in the microalgae nutrient formulation, and also detected surrounding the gel disks. Ammonium and sulphate were detected in the low range of between 10-40 mg/l. Nitrate and phosphate were not detected despite being present in the 1mg/l media at 335 mg/l and 19 mg/l. The ionic retention trials demonstrate that over the duration of a complete batch culture, ammonium is not leached into the surrounding de-ionised incubating water. This finding follows in accordance with the ability of alginate immobilised balls to remediate N and P (Ruiz-Marin, 2010).

3.3.5 **Airborne contamination trials**

Composite pectin and alginate co-gels tested for contamination showed that the 100% alginate and 80% alginate treatments did not grow mould, whereas the high pectin concentration treatments were contaminated. The surface growth of mould grew sufficiently well to block light to immobilised algae beneath in the core of the gel composite (Figure 3-11). This suggests that although pectin can be used to grow microalgae, relative inclusion of this commodity ingredient should be limited to less than 20% in order to prevent undesired contamination from other microorganisms.

Figure 3-11 Microorganism duplicated contamination 14 days post-incubation at room temperature on hydrated gel composites. Left to right 2% [Pectin/Alginate] (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100).

3.4 Discussion

Although immobilised microalgae culture is structurally feasible for scaled growth systems, a mixture of selected bio-composite gelling agents is not price competitive with conventional microalgae culture systems in water. Results show that the costs of production are almost threefold greater than the production incomes per cycle, using an alginate concentration of 2%. Conversely however, an immobilised production system could have merits as a higher value biochemical production platform. This research has demonstrated the ability of the pectin and alginate hydrogels to both capture and retain microalgae nutrients, as evaluated by lack of incremental increase in ion detection within the hydrating de-ionised water over the duration of the experiment. It has also been demonstrated that lack of buoyancy is a functional constraint to enteric system development.

Typically biomimetics has been successfully applied to structural and applied mechanical engineered products, as well as pharmaceutical and biotechnology products. Examples include the hydrodynamic and aerodynamic exterior surfaces of transportation vehicles. Biomimetics has been less effectively applied to engineer the living environment of microorganisms destined for biofuel technologies. A controlled replication event of a marine foam or an algae bloom

which could be simultaneously be harvested for an end product for human appropriation could possibly be a way to grow algae on a massive scale. Mimicking the natural world is not the only way to facilitate this process though it is an effective delivery strategy. This could have a profound impact on the scalable development of this technology from laboratory to commercialisation. Coupling fast growth rates of microalgae with a mechanism for optimisation of containment is necessary to ensure the viability of scalable development of microalgae for biofuels. The close association of microalgae with gels is of particular interest for investigation, considering the natural exopolysaccharide secretions from both microalgae and macroalgae into their surrounding environment. In order to achieve a scalable production process of microalgae using gelation technology, it is necessary to ensure that the structure can withstand torsion and tension forces. The proposition for implementation of this technology would be to grow the structure in a marine environment where there would be limiting conflicting business activities, land or sea utilisation. This investigation has demonstrated an objective test methodology for quantitative measurement of structural forces in biopolymer pectin-alginate hydrogel disks. This could align with the geotextile puncture resistance test if the displacement delivery is 50 +/- 10 mm per minute rather than a hand cranked delivery action. This research has demonstrated the ability of 300 ml gel disks to support between 30 and 60 N breaking strain force. This is considered to be in the range sufficient to tolerate physical forces from the natural environment including wind, waves and birds. The plasticity of the structure will allow further deformation and memory stresses which would otherwise cause shear tearing within the structure. The use of natural fibres such as coconut increases the composite nature of the gel matrix material to further increase multi-directional strength (Yao, Hu and Lu, 2012). Air bubbles were primarily included as an investigative potential means for increased structural flotation, but they could also be used as a strategy for increased gas delivery at higher cell densities and light intensities, if the structure can achieve cost-effective flotation. In terms of the application for usage of coir fibre together with microalgae, marine diatoms have an obligatory requirement for silica (Anderson, 2006), and therefore association with the silica nodules on the

longitudinal surface of coir fibres could have implications for commensal association in conjunction with alginate. However, growth of microalgae irrespective of their type was considerably less of a problem than achievement of flotation. The algae grew, but the structure did not float. As such, more pertinence was given to try to make the structure float than to try to grow the algae within the composite which was already achieved with ease.

The chronological daily detection of ions in the hydrating “de-ionised” water surrounding the gel disks evaluated the potential for the gels to adequately encapsulate the nutrient media. This is of interest because of the batch cultivation process which provides the entire nutrient requirements for the remainder of the growth and harvest cycle up until 14 days. Alternatively, if the growth priority was for lower value smaller scaled high value biochemical production, the algae could be drip fed with nutrients rather than batch culture. Varying nutrient growth media compositions could feasibly have been used as there was demonstrably no leakage of nutrient ions into the incubating de-ionised water. This research has demonstrated the ability of the pectin and alginate hydrogels to both capture and retain microalgae nutrients, as evaluated by lack of incremental increase in ion detection within the hydrating de-ionised water over the duration of the experiment. Nitrate and phosphate, both present in the nutrient media, were not even detected in the incubating de-ionised water which suggests that the gel has good capability for nutrient capture and retention. Optimisation of nutrient resource acquisition during the growth lifecycle will allow effective nutrient delivery without incurring nutrient wastage once microalgae cells have grown to the maximum cell densities.

Engineering products by aligning their construct with design structures of the natural environment is an effective strategy to novel product development. Advanced generation biofuels utilise micro-organisms as scalable converting platforms of waste nutrients into valued outputs. Industrial development of third generation biofuel technologies will rely on a versatile re-enactment of traditional engineering practices on account of the fluid characteristics of the habitat of these microorganisms. In particular, microalgae are more efficient than terrestrial plants

in converting sunlight to biochemical energy (Stephenson *et al.,* 2011). This facet, together with microalgae being able to be grown on land or water bodies unsuitable for conventional agricultural production deems microalgae pertinent for third generation biofuel research.

Culturing microalgae in liquid water as opposed to gelled water has inherent issues with downstream processing including harvesting, drying and the associated high energetic and economic costs of such procedures. It may be that control over containment of microalgae is one of the most critical factors in successful amplification of scale from the laboratory to commercial production. Attached microalgae cultivation can either happen as a biofilm or within an enclosed matrix. When grown as a biofilm, a significant production constraint is the shading of high density cells in close proximity to each other which acts to alter spectral composition, reduce gas exchange and nutrient acquisition. Unlike liquid water, the addition of ionotropic gelation agents to water at ambient conditions can increase the viscosity of water to form a gel, an intermediary state between solid and liquid matter. Such gels have been demonstrated to allow the successful growth of microalgae and entrapment of the required nutrients for batch culture (Hameed & Ebrahim, 2007). Enclosed matrix immobilised microalgae growth is also advantageous because cellular growth occurs in uniform proximity, therefore avoiding issues of light attenuation and nutrient acquisition aforementioned. Ionotropic gelation agents are refined to various levels of purity for commercial processes, typically reducing the phenolic content, therefore improving transparency and light transmission. These processes increase commercial production costs as described in the final chapter, yet the raw materials for gelation are of comparable value with other tradable raw commodities such as sugar beet and citrus fruit pectin and seaweed alginates. Food grade standard product refinement is not a pre-requisite for the manufacture of a gel required to culture microalgae. Gel composite structure costs can further be reduced using bio-composites of other lower value gelling products such as terrestrial pectins from agriculture and by-products from fast growing tropical traded commodities including coir fibre.

3.5 Experimental constraints

The manual crank of the arbour press could not entirely replicate the specifications of BS 6906:1989 geotextile puncture resistance test. The ideal scenario of recreating a microalgae biomass controlled version of the envisaged Chinese outbreak of *Ulva* sp. or the Australian oceanic foam was not achieved. Moreover, the density inclusion of alginate or pectin gels could not be reduced in order to re-create a gelatinised foam technology using natural or synthetic surfactants or detergents. Flotation was achieved only by the addition of high volumetric internal air spaces which were not considered to be easily scalable either numerically or volumetrically. A negative control variable would improve inter-comparison of ionic retention trials, and could impact on findings of the work by contextualising results. The high carbon content in sodium citrate for alginate digestion relative to the lower microalgae total carbon content resulted in a degree of partial inaccuracy due to the high baseline recording. However, despite the limitations of these experimental trials, a baseline for further experiments has been initiated which can provide successive investigators with a channel for further insight and development.

3.6 Conclusions

This paper outlines the development of a novel mode of growing microalgae using the enclosure method of microalgae immobilised within a semi-solid gel membrane disk which can be floated upon seawater.

Optimisation of nutrient delivery and ease of harvesting using immobilised microalgae cultivation methods are beneficial. Further research would need to develop flotation via foam based technologies to reduce density and increase buoyancy, as well as reduce incipient costs of the gelling agents. If a gelled immobilised sheet sank into the depths of the sea, not only would it be unrecoverable and therefore not able to be harvested, but it could cover the benthic ecosystem with unknown consequences. Control of flotation is ubiquitous to microalgae in their natural environment as it allows them to go nearer and remain in the presence of the source of sunlight. As light enters deeper into the water column, there is a gradual reduction in both the intensity and frequency of

photosynthetic active radiation which is able to penetrate such depths down to a maximum of 100 m in clear pelagic oceanic seawater known as the light extinction coefficient. Future research would also identify the nutrient profiling of harvested microalgae biomass processed simultaneously with dried pectin and alginate hydrogels. Compositions of hydrogel formulations are worth investigating further due to the relative cost fluctuations of the respective ingredients, some of which are low grade by-products of industrial food and feed processing. Though this mechanism of production has proven to be uneconomically viable in the present form for biofuels, development of foam technologies would simultaneously reduce inclusion of ingredients and improve flotation characteristics. An alternative strategy to achieve a profitable production system would be to increase the cost return per unit volume or mass on the microalgae product composition with high value biochemicals such as nutraceutical or feed products potentially in shallow ponds. Density control of floating disks is possible; though density derived flotation rather than displacement derived flotation is desired and not achieved during these trials. At current market prices, the intended gel substrate is too expensive as to be discussed further in the final chapter. However, seaweed farming is growing in interest and this could potentially affect the supply and demand as well as influencing market prices.

Continued research for the next chapter of this thesis considers the beneficial attributes of alginate with regard to microalgae gels in an alternative function, using a lower inclusion concentration and for another application; to investigate harvesting of microalgae biomass from growth media.

4 Low-cost microalgae harvesting

(This chapter has contributed to the formation of a new International PCT application PCT/GB2015/050298 priority claimed 11th February 2014).

4.1 Introduction

A technology to harvest microalgae simultaneously requires an appreciation of the aqueous liquid entity from which algal cells are separated. Microalgae live within liquids. Liquids flow; are amorphous, have a fixed volume and resist compression. The flow rate of a liquid depends on the magnitude of the intermolecular forces and the shapes of the molecules, the larger the molecule the slower they move (Toledo, 2008). Microalgae are themselves comparable particulate matter equally distributed within a liquid. Different species of microalgae vary in size from a few to a few hundred microns. Most of the commercially useful microalgae species including those used in marine algaculture and aquaculture are typically in the lower range of this size spectrum between 5 to 30 microns.

Molecular associations interact between water molecules and non-water particulate matter irrespective of size, to form coagulated mixtures of variable homogeneity and inter-particulate affinity. High turbidity values 150 Nephelometric Turbidity Units (NTU) and 80 NTU calcium alginate proved to be a very effective coagulant causing turbidity removals generally over 98% in drinking water (Devrimci *et al.* 2012). Pectin gelation requires a reduction in water activity, induced by co-solutes such as ethylene glycol or sucrose. Co-solutes play an active role in stabilisation of gels by formation of hydrogen bond links contributing to overall gel strength (Mitchell, 1976). A characteristic of sodium alginate solutions is the ability of gel formation in the presence of polyvalent cations, such as Ca^{2+} (Draget, Skjåk-Bræk & Smidsrod, 1997). Extraction and purification processes of alginates from seaweed are based on the conversion from the insoluble form in the algae to the soluble one, normally the sodium salt, followed by successive dissolutions and precipitations to eliminate impurities (Larsen, Salem, Sallam, Mishrikey and Beltagy, 2003; McHugh (2003).

Alginate extraction from seaweed is a multiple step process. The process can either be preceded by drying seaweed and re-hydration within solution or by the use of wet seaweed. In summary, 1) Size reduction of raw material by chopping 2) Dilute acid treatment whereby calcium alginate is converted into alginic acid which is more readily extracted with alkali than the original calcium alginate (Haug, 1964), 3) Formaldehyde treatment for additional phenolic compound removal, 4) Alkaline alginate extraction with 1.5% sodium carbonate to solubilise sodium alginate from insoluble calcium alginate, 5) Separation of insoluble seaweed residue by either flotation or filtration, 6) Precipitation of calcium alginate from calcium chloride, 7) Bleaching to reduce colour and odour with sodium hypochlorite, 8) Conversion of calcium alginate into alginic acid with acid filtration, 9) De-watering alginic acid with a screw press, 10) Conversion of alginic acid into sodium alginate with solid sodium carbonate for paste formation of pellet extrusion and drying on a fluid-bed dryer, 11) Milling sodium alginate into a powder form (McHugh, 2003).

In the early patents for the industrial production of alginate, the acid pre-extraction step was described as an essential step to make the alginate more readily soluble in the alkaline solution (Clark & Green, 1936; Bescond, 1948; Secconi, 1967). None of the above authors' gave any chemical explanation for the use of the acid treatment. The first scientific explanation was given by Haug (1964) who assumed that alginate occurs as an insoluble salt with calcium as the main cation, so the extraction of alginate could be regarded as a two-step process, a transformation of insoluble alginate into soluble alginate i.e. sodium alginate, followed by a diffusion of the soluble alginate into the solution. He proposed that this transformation could be conveniently carried out by converting the algal alginate into alginic acid, followed by neutralization of the alginic acid with an alkaline sodium salt (Hernández-Carmona, 1999). However, alginate extraction can occur by omission of the acid pre-treatment as with the patented boiling alkali method (Lukachyov & Pochkalov, 1965; Baranov *et al*., 1967). Acid pre-treatment for alginate extraction from *Sargasssum spp.* gave 13.8% alginic acid on extraction with sodium carbonate, while the untreated seaweed gave 13.7% alginic acid, suggesting that acid treatment of the seaweed is not essential for the

extraction of sodium alginate. These authors' also proposed that alginic acid is mostly present as free acid rather than calcium salts as reported by other authors (Shah, Mody and Rao, 1967). Polymeric chain degradation is high in the presence of HCl solution during the acid pre-treatment of the alginate extraction process, the acidity must be constantly kept at pH 4. Additionally, acidic pre-treatment produces hydrolysis of some linkages with a decrease in the molecular weight and a lower rheological performance for these samples. (Gomez *et al.* 2009).

Alginates are a widely abundant food commodity and vital pharmaceutical component (Gombotz & Wee, 2012). Experiments to test the mode of action of low concentrations of alginates as initiators of mild gelation, in proximity to microalgae cells, and as an agent of applied microalgae harvesting in culture were conducted using a non-motile freshwater species *Chlorella vulgaris,* flagellated marine species *Tetraselmis chui*, and non-flagellated marine species *Chlorella salina*. It was hypothesised that at very low concentrations of gelation, microalgae will bind together in a gel like manner and effectively increase their relative size enabling an improved filtration procedure. This process allows "free water" to pass through the filter but does not allow water in the gel to pass through. Upon filtration, as the gelatinised microalgae cells become concentrated within the filter, pressure forces more water out of the gel, thus concentrating filtered microalgae cells and concomitantly separating microalgae from culture media. This succinct hypothesis was tested in the laboratory.

The Bligh & Dyer 1959 lipid extraction method uses the miscibile and immiscible gravimetric chemical analysis properties with the ratio of 0.8:1:2 of citrate buffer:chloroform:methanol. It has been the widely applied benchmark for the extraction of lipids from microalgae (Mercer & Armenta, 2011).

4.2 Aims and Objectives

The aims were the development of a novel low-cost harvesting technology. Objectives were the evaluation of the harvesting and economic features of the process; identification of low-cost ingredient formulations and the optimisation of the harvesting technology using modifications to flocculation and filtration.

4.3 Methods

4.3.1 **Crude alginate and pectin extraction**

Experiences of the operations of the raw material process is taken from personal discussions and a visit with Don Réne Piantini, CEO of Algas Prodalmar in Chile, 2013 (Figure 4-1). Chilean brown seaweeds have a preferential higher guluronic to mannuronic acid ratio than the abundant *Laminaria japonica* species from China used for alginate production (Bixler & Porse, 2011). Seaweeds are harvested along the entire coast of Chile manually by both storm wash and by divers. Harvesters' aim to pick the entire frond i.e. the stipe, float and blade of macroalgae, but not the holdfast because extraction of the holdfast can prevent later subsequent re-growth and effectively destroys the algae. In practice, and due to lack of knowledge on the part of the rural workforce, this is not always realised, though as the business enforces sustainability and continuity of supply, great effort is made to educate local communities' whose livelihoods depend on the harvest of this marine commodity. Firstly, fresh seaweed from harvest is dried. Air drying in the coastal region of the Atacama Desert, exposes seaweed to regular daily sea breezes. The Atacama Desert is the oldest and driest desert in the world, owing its extreme aridity to the climatic regime dominated by a constant temperature inversion due to the cool north-flowing Humboldt Ocean Current and the presence of the strong Pacific anti-cyclone (McKay *et al.* 2003). Chilean manual labour daily turns over harvested seaweed until it is brittle and completely desiccated. Drying seaweed extends the lifetime of the commodity product for greater longevity during transportation to market and storage. Seaweed can be chopped either prior to or post-drying. In larger commercial volumes of production, chopping after drying is a more controllable mechanistic process where large crushing equipment is able to reduce dried seaweed for later grading with gauze separation, typically between the ranges of < 2 mm to > 20 mm grades. Size reduction of raw seaweed is beneficial because it allows higher surface area to volume ratio for chemical reaction with acid, alkali and formalin, but also because it allows the dried mixture to be pumped in pipes when mixed with water (McHugh, 2003).

Some of the alginate extraction processing steps exist in order to remove phenolic compounds and purify alginate to a dry powder. Achieving a dry powder requires a longer production process, more equipment and greater overall energy expenditure. Additionally, for the purposes of the proposed application in this chapter, a dry powder was not necessary because sodium alginate needed to be dissolved in solution prior to use, and extracted sodium alginate is already removed from seaweed as a solution. Therefore, rather than in accordance with McHugh, extracting alginate to form a powder and subsequently dissolving it into solution again, instead the extracted alginate solution was left in its unabridged form as a crude sodium alginate aqueous solution for direct subsequent experimental procedures.

Pilot experiments were used to verify the requirement for an acid pre-treatment as an inclusive component of the alginate extraction procedure. As described in 4.1 by other authors', an acid pre-treatment (step 2) is not an absolute requirement for alginate extraction from seaweed. This was tested in the laboratory using commercially obtained <5 mm grade dried *Macrocystis pyrifera* from Chile. The acid pre-treatment alginate extraction procedure summarised in 4.1 using the procedure of McHugh, 1987 was performed with the exception that de-watering and drying (steps 9 and 10) was done via lyophilisation. The resulting sodium alginate obtained was used to form ionotropic gelation alginate gel beads of 1% and 2% concentrations in accordance with the procedure of Bajpai and Sharma, 2004. Calcium alginate beads were analysed by eye and manually for physical rheological characteristics. Alginate extraction with the additional acid pre-treatment did not produce alginate beads but merely the liquid dissolution of the sodium alginate into the calcium chloride rather than a firm gel as would have been expected. Conversely, the same process excluding the acid pre-treatment and formalin treatments (steps 2 and 3), also de-watered and dried by means of lyophilisation produced ionotropic gel beads in the same way as gel beads obtained from using a commercially extracted laboratory consumable alginate powder from brown macroalgae species. Step 4 of McHugh, 2003 uses 1.5% sodium carbonate solution to solubilise sodium alginate into solution. It is this step alone which solubilises the alginate from the seaweed into solution. Step 5

separates the solid seaweed residue from the sodium alginate crude solution. Subsequent following steps (6-11) are refinement and purification of this crude sodium alginate solution by means of precipitation of the solution back into calcium alginate form, acid and formalin odour and colour removal, acid conversion into alginic acid, de-watering, drying and milling. For the purposes of this study, a crude extract of dissolved sodium alginate was required which could be obtained from 1.5% sodium carbonate incubation of seaweed and subsequent separation of particulate chopped seaweed from the gelatinous solution. Successive dissolutions and precipitations to eliminate impurities and obtain a pure powder form of sodium alginate were not of concern because only the dilute aqueous solution for later microalgae harvesting was needed. Colour and odour was also not of great concern because of the dilute application of the process; though the ionotropic gelation formation and gel resilience was important. Acid hydrolysis of branched polysaccharide chains thus rendering degradation and lower molecular weight alginate would act to compromise the rheological ionotropic gelation performance so the acid pre-treatment (step 2) was not a desired characteristic. For this reason, the previously described McHugh alginate extraction procedure was significantly simplified to obtain a viscous gel of crude sodium alginate. In summary it comprised steps 1, 4 and 5 of McHugh's alginate extraction procedure. A reduction in processing steps also simultaneously saved on consumable processing costs, time and energy.

The modified alginate extraction procedure (McHugh, 1987) was used to extract alginate from dried *Macrocystis pyrifera* obtained from René Piantini, Algas Prodalmar Ltda, Antofagasta in Chile (Figure 4-1). 5 x 100 g of dried macroalgae was re-hydrated in 5 x 1 l borosilicate glass beakers and mixed with 5 x 500 ml of 2% (w/v) Na_2CO_3 in de-ionised water, covered with aluminium foil and incubated at 50˚C for 2 h, stirring every 30 min. Post incubation, macroalgae was sieved and manually compressed to extract the filtrate which was covered with laboratory film and stored. Residual wet macroalgae from the sieve was oven dried at 50°C for 48 h, then weighed to determine the loss of alginate from the extraction procedure.

Figure 4-1 – The author's photographs of a commercial seaweed harvesting business in the Atacma desert, Chile located near to the Tropic of Capricorn. Drying operations of the marine macroalgae *Macrocystis pyrifera* in Antofagasta, Chile, Jan 2013. A) macroalgae are left in the desert sunshine and daily turned over for heat exposure to annual temperatures between 25 – 35°C. B) crushing machines for size grading dried macroalgae. C) close-up of a dried macroalgae showing dried holdfast on right hand side. D) bagged final product.

A UK horse feed manufacturer (Equibeet, Trident feeds) supplied processed de-molassed sugar beet pellets containing 89% dry matter, 9.1% crude protein, 11 MJ DE/kg and 16.5% crude fibre (Appendix Figure A-6). The above described process for alginate extraction from dried seaweed was used for pectin extraction from dried de-molassed sugar beet pellets. Procedures, masses and volumes were used as with alginate. Likewise, collected orange peel from UK supermarkets was frozen at -80°C. Upon attainment of 500g, peel was thawed, macerated and processed in the same way as both de-molassed sugar beet pellets and seaweed.

4.3.2 **Microalgal cultures**

Microalgae biomass cultures used for experiments were batch grown indoors and outdoors, transported between production sites in 20 l Nalgene carbuoys as required to maintain volumetric batches for experiments and keep cultures in serial dilution. A 1000 l horizontal axis custom made mixing tank and indoor holding were able to culture non-axenic cultures, and an outdoor glasshouse facility was used for the mass cultivation of microalgae in tubular polyethylene bags (Transpack) (Figure 4-2).

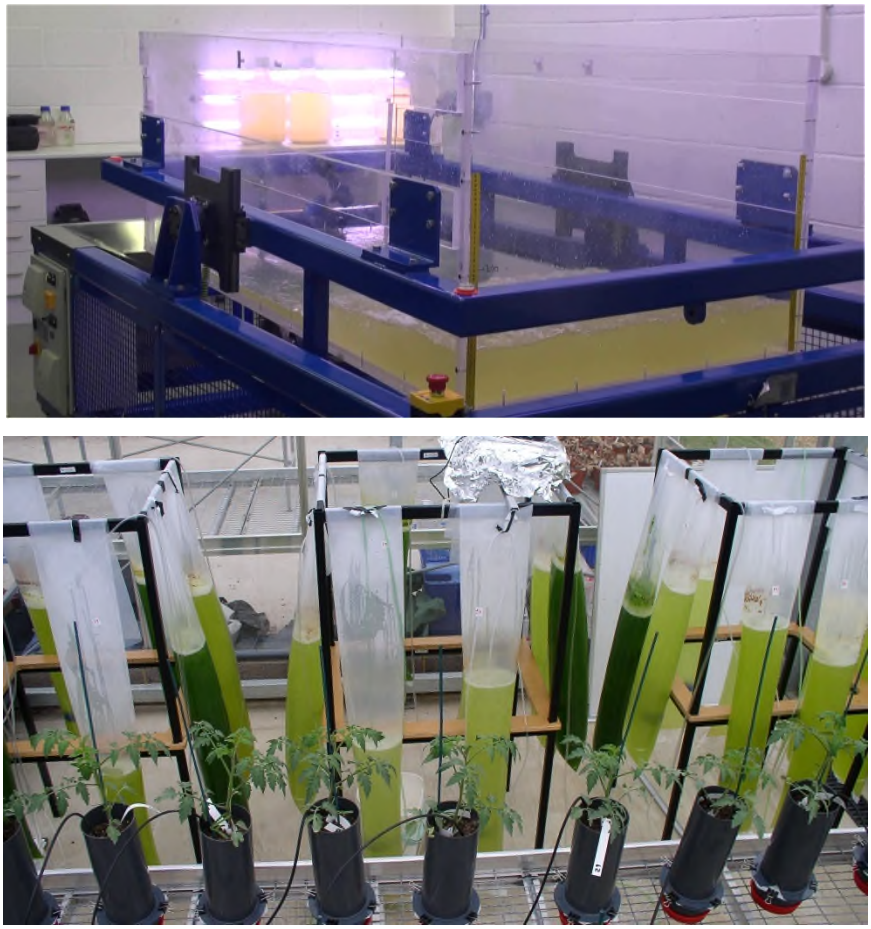


Figure 4-2 – Outdoor and indoor *Chlorella vulgaris* & *Chlorella salina* production. (Above) 1000 l illuminated horizontal axis microalgal mixing and holding tank. (Below) 24 x extra thick grade polyethylene tubing hung from modified desk steel supports, aerated with 2400 l/h aquarium air pump and 50 mm airstones. A central temperature thermostat, localised light meter and waterproof temperature log probes were also used.

Outdoor microalgae cultivation occurred between the months of May and September, 2012 and 2013. Indoor cultures were maintained by serial dilution in f/2 (Varicon) for use as required throughout the year. Microalgal culture collections were obtained from CCAP, Oban. Cell counts were measured by light microscopy and haemocytometer (Andersen, 2006). Tri-phasic conventional harvesting and drying of microalgae biomass was achieved by using a GEA Westfalia disk-stacked centrifuge (15,000 rpm), pumped via a peristaltic pump a Thermo-Scientific Sorvall desktop laboratory centrifuge (12,000 rpm) and a Christ laboratory Lyophiliser. Freeze dried conventionally harvested microalgae biomass was stored at -80˚C until analysis.

4.3.3 **Gel induced filtration optimisation**

The low-cost harvesting process was conducted in accordance with the method described in the new patent application “Processing of microalgae”, UK Patent Application 1402298.2, filed 11th February 2014 invented by the author. The process is described as Gelation Induced Filtration (GIF) harvesting. Figure 4-3 describes the process. Volumes and concentrations used are outlined as follows:

- Gelling agent 0.5% (100 g/20 l water)
- Volume of gelling agent used 20% (i.e 4 l for 20 l of algae culture)
- Gel induction agent 1% (200 g/20 l water)
- Volume of gel induction agent used 20% (i.e 4 l for 20 l of algae culture)

For example, in 20 l of microalgae culture media for harvest, 4 l of 0.5% gelling agent is added followed by 4 l of 1% gel induction agent.

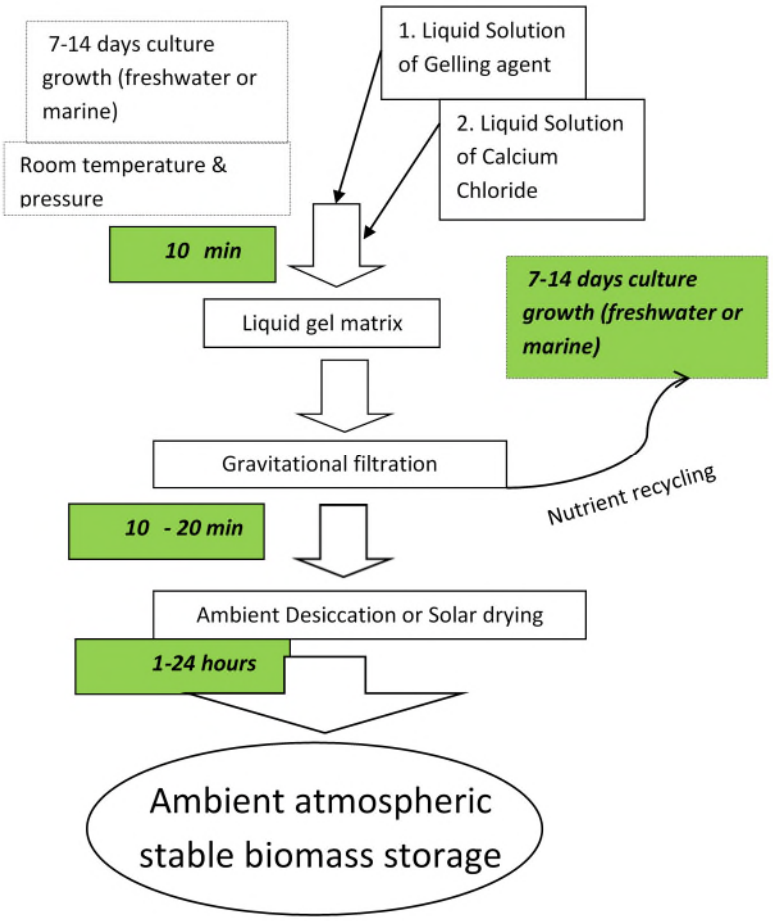


Figure 4-3 Process summary of low-cost microalgae harvesting & drying

The filters used for the optimisation experiments were snap ring collared filter bags (Lenntech). They were a multi-filament polypropylene mesh rated 1 µm pore size, 7 x 32 inches in size with a holding capacity of 15 l. Filters used for the pilot trials of alginate and pectin extraction were cleanroom wipes (VWR, Spec-Wipe® 3E). Specifications comprised 46% polyester, 54% cellulose. The fabrication was hydro-entangled without the use of chemicals to reduce ionic contamination. The filters were a white lightweight, thin, sorbent, creped fabric fibre, providing both softness and bulk. Specifications were: 5.43 ml/g intrinsic absorbency; 283 ml/m^2 extrinsic absorbency; 1 second absorbency rate; 58.2 particle generation (>=0.5 µm) (x 10^6 /m^2); 44 fibres (>100 µm) (x 10^3 /m^2), 52 g/m^2.

Filtered gel was removed from filters to dry at ambient conditions. Biomass and gel material was mildly squeezed to extract excess gel-retained water and reduce

ambient drying time. Ambient atmospheric biomass storage conditions comprised room temperature, pressure and humidity. Algae was equally distributed onto a polyethylene horizontal sheet surface to a uniform depth of 1 cm and left to desiccate. Tests for integrity of the potential alginate preservation properties on the nutritional profile, evaluated FAME's of the lipid fraction of *Chlorella vulgaris* analysed immediately after drying by GC-FID (4.3.5) and again following ambient storage conditions for 6 months and then re-tested by the same process.

In order to standardise uniform gelation properties between sample treatments, a commercially available medium viscosity gelling sodium alginate extract from brown seaweed (Sigma Aldrich) was used for low-cost harvest optimisation experiments. Pilot experiments determined the ranges of alginate and calcium chloride solution concentrations and their respective volumetric inclusion and these values were used to form the central point values of the optimisation described below. Optimisation experiments used homogenously mixed stock solutions of 0.5% alginate and 1% calcium chloride solutions prepared in de-ionised water. Variable volumetric inclusions of these stock solutions were used for gel induced filtration (GIF) harvesting determination.

Design-Expert® software version 8.0.7.1 from Stat-Ease, Inc. was used to design experimental set-up and analyse results. This software allows a multi-parametric 2^k factorial design with variable outputs to reduce confounding data and analytical isolation specificity, using targeted applications, statistics and visual representation. It is used across a range of scientific and engineering applications for commercial product development and performance assessment. In the context of this study, it was one tool to help determine the influence of two variable factors, namely $CaCl_2$ and alginate concentrations to attain the desired response in harvesting efficiency of *Chlorella vulgaris* using a new harvesting method. The first step was to estimate dependent variable factors which may be important and, if necessary, in which direction these factors should be adjusted to improve the expected response. Other variable dependent factors considered here were temperature, mixing rate, filtration pressure, residence time and filter specifications. Whilst these factors were relevant, they were not the primary

influence of the response and could be effectively standardised throughout the research. These were:

- Room temperature 24°C (thermostat controlled)
- Uniform mixing for each replicate by the same number of manual flask rotations prior to placement in the filter
- Gravitational filtration pressure delivered at uniform height from filter
- Uniform residence time of maximum 5 min filtration
- Filter specifications, Lenntech snap ring filter bags

After preliminary experimentation and factors reduction, Alginate concentration [X_1], and $CaCl_2$ concentration [X_2] were selected as chosen factors in the model. Quadratic behaviour of the responses was anticipated, so a two factor polynomial function was fitted:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i x_i + \sum_{i<j}^{2} \beta_{ij} x_i x_j + \sum_{i=1}^{2} \beta_{ii} x_i^2$$

Equation 4-1 Polynomial function

where Y is the predicted response, β_0 is the constant process effect, β_i is the linear effect of x_i , β_{ij} are the interactions of the first order and β_{ii} are the quadratic effects of x_i . Stat-Ease Design Expert 8.0 indicated that 14 experimental runs were necessary to calculate the regression coefficients for the full model. Analysis of variance tests the significance of the main effects and interactions between multiple variables simultaneously. Depending on the complexity of the variables, later refinement of the model can be applied to analytical approach and to check assumptions. The final step is graphic analysis by means of interaction plots, response surface and contour plots (Montgomery, 2006).

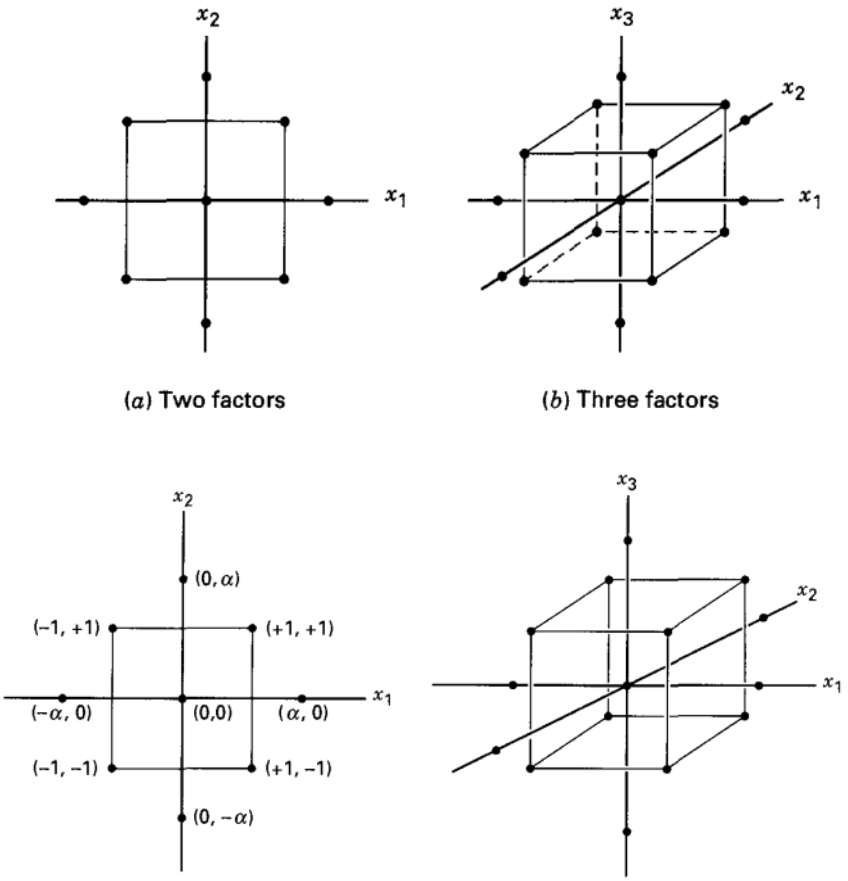


Figure 4-4 Augmented experimental design of 2k with four axial runs (central composite design) (a) k=2 (b) k=3 (Montgomery, 2006).

A central composite design (CCD), 2^2 full-factorial design with 2 factors, 3 responses, 8 central points, 4 face points and 4 star points was used. The distance from central to corner and central to star points was 1 and (α, 1.414) respectively to allow for design rotability. Volumetric inclusion ranges of 0.5% alginate and 1% calcium chloride solutions prepared in de-ionised water were 20-60 ml of alginate solution, with a central point (ml) 40 and 30-90 ml of $CaCl_2$ with a central point of 60. Corresponding star points of α 1.414 were 11.72 and 68.28 for alginate and 17.57 and 102.43 for $CaCl_2$. Response Surface Methodology (RSM) optimisation experiments analysed 200 ml of microalgae solution per experiment (*Chlorella vulgaris)* at a cell density of 1.8 x 10^8 cells /ml. Cells were counted using a haemocytometer under a light microscope (Andresen, 2006). 3 responses including optical density of filtrate (spectrophotometer), cell count of filtrate (cells/ml) and dry weight (g/ml) were recorded. Harvesting efficiency (%)

was quantified as the reduction of cell counts from the initial culture at 1.8 x 10^8 cells /ml to the filtrate.

Table 4-1 Factorial 2^2 central composite design optimisation methodology of alginate and $CaCl_2$ gel induced filtration harvesting of *Chlorella vulgaris*.

Experimental design variables use 8 replicate central points (0,0) of 40ml 0.5% alginate and 60ml 1% $CaCl_2$, 4 star points (0,α; 0,-α; α,0; -α,0), 4 corner points (-1,+1; +1,-1; -1,-1; +1,+1). Measured responses were optical density, cell count (cells /ml) of filtrate and extract dry weight per 200 ml (mg).

Factor 1	Factor 2	Factor 1 0.5% Alginate (ml)	Factor 2 1% $CaCl_2$ (ml)	*Chlorella vulgaris* (ml)	TOTAL VOLUME (ml)
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
0	0	40	60	200	300
+ α	0	68.28	60	200	328.28
- α	0	11.72	60	200	271.72
0	+ α	40	102.43	200	342.43
0	- α	40	17.57	200	257.57
+1	+1	60	90	200	350
-1	-1	20	30	200	250
+1	-1	60	30	200	290
-1	+1	20	90	200	310

4.3.4 **Energy Dispersive X-ray Electron microscopy**

Energy Dispersive X-ray Electron microscopy (Oxford instruments / AZtecEnergy analysis software) was used to both quantify and secondary validate elemental composition of dried microalgae biomass samples, alginate and pectins. Samples were mounted onto aluminium stubs using double sided carbon tape and coated with a 2 nanometre layer of gold palladium using a Polaron E5100 sputter coater. The samples were imaged in an FEI XL 30 ESEM and analysed using Energy Dispersive Analysis Aztec software (Oxford Instruments). The acquisition time was 60 seconds and predominantly using 20 KeV (Kilo electron volts). The scale was shown on the images.

4.3.5 **Carbon and Nitrogen analysis**

The SSM-5000A is a special accessory for the TOC-V series Total Organic Carbon Analyzer which combines with the TOC-V to create a TOC solid sample analysis system. Carbon content in organic matter can be expressed either as TOC, Biological Oxygen Demand, (BOD) or Chemical Oxygen Demand (COD). BOD is a slow analysis and ammonia can result in false positive results due to biological oxidation of ammonia to nitrate. COD is analysed by a strong oxidising agent, though some cyclical carbon compounds such as benzene may give a false low COD. TOC replaces COD as an analysis and measures the carbon content of organic matter in different matrices, though it doesn't define the nature of the carbon material. EU Standardisation has adopted TOC for water analysis (EU Standard EN1484, 1997). Total Carbon (TC) is defined as the sum of organically bound and inorganically bound carbon present in water, including elemental carbon. Total Inorganic Carbon (TIC) is defined as the sum of carbon present in water, consisting of elemental carbon, total carbon dioxide, carbon monoxide, cyanide, cyanate and thiocyanate. TOC is defined as the sum of organically bound carbon present in water, bounded to dissolved or suspended matter. Total Nitrogen (TN) is a measure of bounded nitrogen in water and waste water. TN replaces the determination of nitrogen measured by the Kjeldahl method (EU Standard EN 12260, 2003).

Carbon & Nitrogen analysis was undertaken using the Solid Sample Module (SSM) SSM-5000A of the Shimadzu Total Organic Carbon (TOC) Analyzer TOC-VCPH/TOC-VCPN. Ceramic furnace boats were used to contain solid samples and the instrument was calibrated with laboratory grade glucose (Sigma Aldrich). 20 mg samples of biomass were weighed gravimetrically and placed into the instruments' furnace at 720˚C. Released carbon dioxide from combustion was reacted chemically, detected by the instrument and recorded as mV peaks.

4.3.6 **Lipid extraction and analysis**

Direct transesterification of FAME's from wet lipid samples was achieved in a single vessel processing method (O'Fallon *et al.* 2007). This direct FAME analysis method combines cell digestion, saponification, extraction, and esterification of triacylglyceride's (TAG's) quantitatively to FAME in two steps within one vessel for direct analysis using Gas Chromatography. The process uses screw cap tubes, methanol, pottasium hydroxide, sulphuric acid and hexane.

1 g microalgae samples were placed into a 16 × 125 mm screw-cap Pyrex culture tube to which 0.7 ml of 10 N KOH, and 5.3 ml of MeOH were added. The tube was incubated in a 55°C water bath for 1.5 h with ultrasonification to permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 ml of 24N H_2SO_4 in water was added. The tube was mixed by inversion and with precipitated K_2SO_4 present was incubated again in a 55°C water bath for 1.5 h with vortexing for 5 sec every 20 min. After FAME synthesis, the tube was cooled in a cold tap water bath. 3 ml of hexane was added, and vortex-mixed for 5 min. The tube was centrifuged for 5 min in a tabletop centrifuge, and the upper hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and placed at −20°C until GC analysis.

GC analysis was performed on a Shimadzu GC-2010 Gas Chromatograph Flame Ionised Detector (GC-FID) with a Shimadzu shim-wax 60 m GC capillary column, 0.25 mm outside diameter, 0.25 µm inside diameter. Column specifications include a bonded phase shim-wax (polar) fused silica material with a maximum operating temperature of 260˚C. Samples were dissolved in hexane and 1 µl injections were taken from the auto-sampler using a pre and post rinse in heptane. A helium carrier gas was used with a pressure of between 23 and 46 psi, column flow of between 0.8 and 2.7 ml/min, and total flow between 60 and 85 ml/min. A split ratio of between 20 and 100 was used. The oven was ramped from between 100-140˚C to 240-250˚C at a rate of 4-5˚C/min and held for between 30 min or until the maximum cycle end (85 min). A 37 FAME standard with the range of C4-C24 saturated and unsaturated fatty acids was used (Supelco, Sigma Aldrich) as defined in the appendices. Program methods

including flow rates, split ratios and durations of oven ramping temperatures were developed to define peak quality, quantification and intensity, and compared against the 37 FAME standard to determine compositional analysis using the instrumental software.

4.4 Results

4.4.1 **Crude alginate and pectin extraction**

Extraction of alginate from seaweed and pectin from de-molassed sugar beet showed that the optimal mixing ratio was 20% (w/v) solid in 2% Na_2CO_3 solution. Due to the hygroscopic and water entrapment properties of dried seaweed, a reaction of 100 g of seaweed with 500 ml of 2% Na_2CO_3 solution yielded 250-300 ml of sodium alginate extract. A 9% dried mass yield of 45 g of sodium alginate (Table 3-2) was obtained from the above extraction procedure and subsequent lyophilisation. Sodium alginate produced a more viscous liquid extract than sodium pectinate, though rheological properties were not quantified numerically. Sodium pectinate extract produced a marginally higher volume yield of 300-350 ml from 100g. An analysis of pectin composition and alginate composition extracts from de-molassed sugar beet pellets and seaweed respectively is displayed in Table 4-2. 12% soluble fibre (pectin) from de-molassed sugar beet pellets was obtained by gravimetric analysis of lyophilsed material.

Table 4-2 Crude extract yields of Alginate & Pectin using alkaline extraction from *Macrocystis pyrifera* seaweed, *Beta vulgaris* sugar beet, and *Citrus sinensis* oranges measuring soluble fibre losses from biomass upon oven drying and gel extraction efficiency.

Crude Biomass	Seaweed	Sugar Beet	Orange peel
Processed biomass	Crude Alginate	Crude Pectin	Crude Pectin
Raw dried biomass start mass (g)	500	500	500
2% Sodium carbonate added (L)	2.5	2.5	2.5
Dried Biomass post extraction (g)	455	438	464
Extracted alginate or pectin (L)	1.3	1.4	1.4
Extracted alginate or pectin (g)	45	62	36
Total Alginate or Pectin extracted (%)	9	12	7

4.4.2 **Gel induced filtration optimisation**

The 2D contour plot in Figure 4-5 graphically represents the microalgae harvesting efficiency for varying concentrations of alginate and $CaCl_2$. >90% harvesting efficiency could be achieved with >58 ml 0.5% alginate and >80 ml $CaCl_2$. Contours lines show equal harvesting efficiencies. Blocking is used to reduce or eliminate variability transmitted from nuisance factors which may influence the experimental response but in which there is no direct interest (Montgomery, 2006). There are no model constraints in terms of concentration ranges applied, as such no blocking of regions within the ranges of the two concentrations was applied.

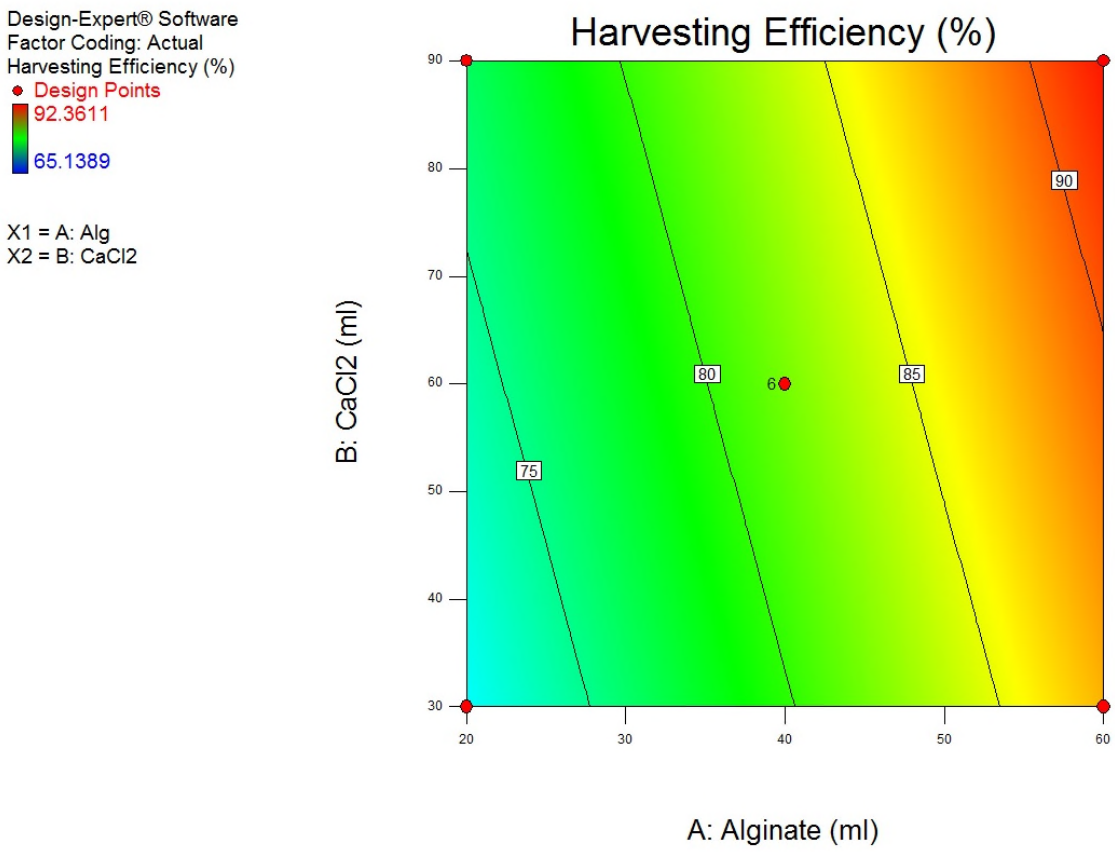


Figure 4-5 Factorial 22 design Response Surface Methodology contour plot of GIF harvesting efficiency 0.5% Alginate (ml) and 1% CaCl2 (ml).

Variability in responses used the Analysis of Variance (ANOVA), an algebraic relationship between the measured response and statistically significant variables. Assumptions required that observations are adequately described by the model and that the errors are normally and independently distributed with mean zero and constant but unknown variance. Validity of assumptions and σ^2 model adequacy is achieved by a normal probability plot of the residuals indicating model robustness as defined by normal distribution and lack of outlying points Figure 4-6.

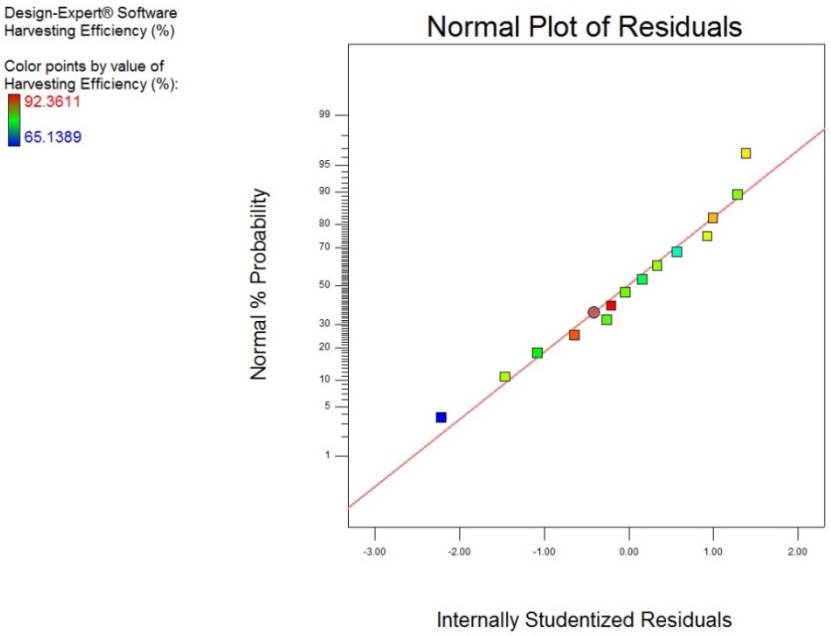


Figure 4-6 Harvesting efficiency plot of residuals

The *p* value Table 4-3 helps determine data significance and threshold 0.05; lack of fit is the sum of squares of factors. The assumption was taken that the significant lack of fit is given by random errors in the data because insignificant terms were excluded from the model. 'Non-significant lack of fit', means that the model is fit for analysis. *Predicted* R^2 values use the variability explained by the model for new data, and are in reasonable agreement with the *Adjusted* R^2 values and in all four cases the *Adequate Precision tests* which measure the signal to noise ratio, are greater than 4, which is the minimum value typically accepted for this term.

Table 4-3 Statistical tests showing factors, responses and significance values shown in bold (p<0.05).

Response	Factor	P value
Optical Density	Alginate	**<0.0001**
	$CaCl_2$	0.1428
Cell count filtrate	Alginate	**<0.0001**
	$CaCl_2$	0.0501
Dry weight filtrate	Alginate	**0.0017**
	$CaCl_2$	**0.0316**
Harvesting efficiency	Alginate	**<0.0001**
	$CaCl_2$	0.082

Table 4-4 identifies R^2 and *Adjusted* R^2. The R^2 coefficients measure the proportion of variability in the data, indicating that over 82% of the variability can be explained in the models. The exception to this is the dry weight response which scored 67%. The reduction in this response relative to the other calculated responses in Table 4-4 could be due to the physical recovery and gravimetric analysis of small quantities of microalgal biomass associated with alginate complexes and partial loss of recovery from mild bonding forces between the filter and the filtered sacrificial matrix of biomass. However, the adequate signal precision value of 10 for this response suggests measuring signal to noise ratio more than 4 which is the minimally accepted term.

Table 4-4 Statistical tests performed on the final models used for factor analysis

Response	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	Lack of Fit p-values	P-values
Harvesting Efficiency	0.8268	0.7953	0.6854	15.099	0.286	0.286
Optical Density Filtrate	0.8492	0.8039	0.5695	13.57	0.1551	0.1551
Cell count	0.8882	0.8385	0.6748	14.199	0.3688	0.3688
Dry weight	0.6755	0.6165	0.5292	10.029	0.82	0.82

The alginate response *p-value* was <0.0001 using a linear model ANOVA implying significance of this response, with an F value of 26.26, there is only a 0.01% chance that a Model F-value this large could be due to noise. The mean diameter of *C. vulgaris* cells was found to be 5.7 µm (Powell, Mapiour, Evitts and Hill, 2009). Filter specifications outline 44,000 fibres >100 µm (46% polyester, 54% cellulose) per m^2. Filter pore size was not identified in company specifications though unlikely to be uniformly distributed as a microfiber lattice network. Microalgae culture media untreated with application of $CaCl_2$ and alginate, demonstrated that cells passed through the filter without any retention in the matrix core of the filter, suggesting that a significant proportion of the pore sizes were > 5.7 µm.

Cells treated with lower concentrations of $CaCl_2$ and alginate which passed through the filter were an indication of a less effective harvesting procedure. Therefore, harvesting success could be defined as the lowest count of cells in the filtrate. Statistical interpretation for all 4 responses show that the chosen model was significant with the alginate factor more significant than $CaCl_2$ (Table 4-3).

Table 4-5 Central Composite Design (CCD), 2^2 factorial design factors & responses

Chlorella vulgaris (ml) 1.8×10^8	0.5% Alginate (ml)	1% $CaCl_2$ (ml)	OD Filtrate	Cell count filtrate (cells/ml)	Extract dry weight per 200ml (mg)	Dry weight extract equivalent (mg/l)	Alginate used per 200ml sample (g)	Dry weight of microalgae per 200ml (mg)	Dry weight of microalgae per Litre (g)	Alginate used per litre (g)	Alginate used as a % of filtered microalgae
200	40	60	0.854	3.280E+07	525.8	2629	0.2	325.8	1.629	1	61.4
200	40	60	0.898	3.075E+07	508.7	2543.5	0.2	308.7	1.5435	1	64.8
200	20	30	1.559	4.775E+07	313.2	1566	0.1	213.2	1.066	0.5	46.9
200	40	60	0.914	2.750E+07	487.2	2436	0.2	287.2	1.436	1	69.6
200	68.28	60	0.475	1.375E+07	700.9	3504.5	0.3414	359.5	1.7975	1.707	95.0
200	40	17.57	0.669	3.200E+07	457.3	2286.5	0.2	257.3	1.2865	1	77.7
200	60	90	0.508	1.775E+07	685	3425	0.3	385	1.925	1.5	77.9
200	60	30	0.748	2.925E+07	655.5	3277.5	0.3	355.5	1.7775	1.5	84.4
200	40	60	0.813	2.500E+07	591.8	2959	0.2	391.8	1.959	1	51.0
200	20	90	1.205	4.200E+07	412.3	2061.5	0.1	312.3	1.5615	0.5	32.0
200	11.72	60	1.357	6.275E+07	307.5	1537.5	0.0586	248.9	1.2445	0.293	23.5
200	40	60	1.073	3.850E+07	497.6	2488	0.2	297.6	1.488	1	67.2
200	40	102.43	0.651	2.250E+07	523.9	2619.5	0.2	323.9	1.6195	1	61.7
200	40	60	0.756	3.400E+07	545.2	2726	0.2	345.2	1.726	1	57.9
200	40	60	0.854	3.280E+07	525.8	2629	0.2	325.8	1.629	1	61.4
200	40	60	0.898	3.075E+07	508.7	2543.5	0.2	308.7	1.5435	1	64.8

4.4.3 **EDX SEM analysis**

SEM EDX analysis was used to identify the elemental difference between alginate and pectins, macroalgae biomass *Macrocystis pyrifera* and microalgae *Chlorella salina* and *Nannochloropsis sp*.

Table 4-6 Comparison of TC and EDX percentage compositional biomass

	Amidated Pectin		**Classic Pectin**		**Sugar beet pectin**	
	TC	**EDX**	**TC**	**EDX**	**TC**	**EDX**
C	N/A	43.35	N/A	37.15	N/A	44.94
O		54.29		53.54		51.24
Na		1.1		7.77		0
S		0.99		1.54		0.71
Ca		0		0		2.86
K		0.26		0		0.27
	Refined Alginate		***Macrocystis pyrifera***		**Crude sugar beet**	
	TC	**EDX**	**TC**	**EDX**	**TC**	**EDX**
C		38.19	19.26	36.7	28.06	53.9
O		52.34		36.91		39.39
Na		9.11		7.07		0.06
S		0.36		0.72		0.94
Ca		0		1.62		1.1
K		0		4.37		0.81
	Coconut fibre		***Chlorella salina***		***Nannochloropsis***	
	TC	**EDX**	**TC**	**EDX**	**TC**	**EDX**
C	30.53	55.2	28.4	19.74	30.69	65.98
O		43.76		46.88		17.22
Na		0		3.76		4.61
S		0		10.51		0.66
Ca		0		13.1		0.06
K		0.63		0.38		0.27

SEM EDX analysis showed variation in recorded elemental compositions between analysed samples. This was due to changes in sample surfaces, representative oxidation states and the subjective operator's decision on where to acquire the elemental capture analysis by EDX. EDX carbon values reported (Table 4-6) were above the mean averages of the same samples analysed by TC because TC obtained a more complete sample carbon content via combustion of whole samples using the mean of 3 replicate runs rather than analysis of a small surface area of the representative sample. Analysed biomasses showed a similar range of carbon compositions between 40-60% with the exception of *Chlorella salina* which showed a 50% increase in carbon when analysed by TC rather than EDX. Higher Ca and S present in *Chlorella salina* could be attributed to residual interstitial salt present within the encapsulating polymeric carbohydrate matrix surrounding the cells during the drying process.

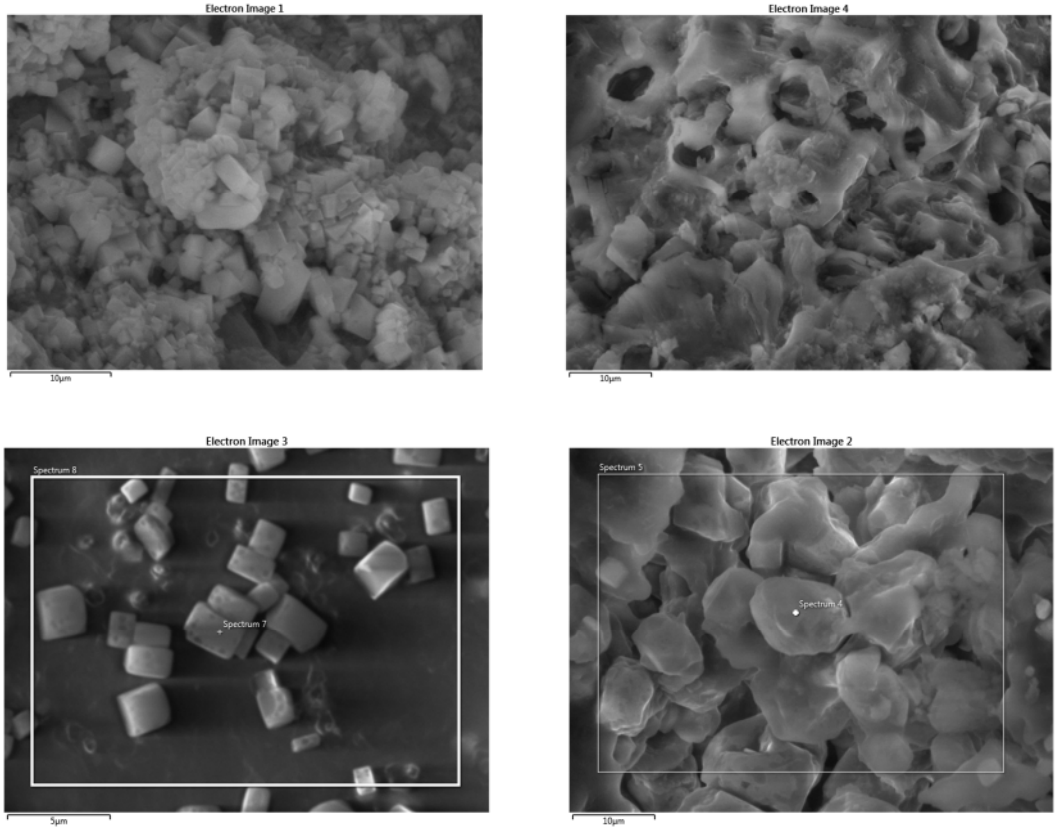


Figure 4-7 SEM freeze dried *Chlorella salina* (above) and *Nannochloropsis salina* (below)
cuboid salt crystals on left images and fused cellular material on right.

Figure 4-7 shows crystallised salt on the external surfaces of the dried saline microalgae samples obstructing the electron pathway to the biomass deeper into the sample, sodium and chlorine composition of the representative sample. In the same way, surface oxidation of biomass samples exposed to the air also resulted in higher analysis of oxygen by EDX. Cells were not washed because imaging software allowed the selection of a representative part of the biomass which was free of salt crystals, clearly detected by a brighter image resonance and characteristic uniform crystal lattice.

Following low-cost GIF harvesting of 10 l of *Chlorella vulgaris* with sugar beet pectin derived from 500 g of dried de-molassed sugar beet, the wet weight of harvested material was extracted from filter bags and weighed at 333 g. This gelatinous mass was spread uniformly over a waterproof surface and dried at ambient temperature over 48 h. The dry weight was measured at 31 g, with post-

harvest GIF biomass calculated at 9% solids. This value was re-confirmed by measuring 10 g of wet weight harvested material and oven drying at 60°C for 2 h, then re-weighing dry mass of 0.867 g or post-harvest GIF biomass of 8.7% solids (w/v).

4.4.4 **Carbon and Nitrogen analysis**

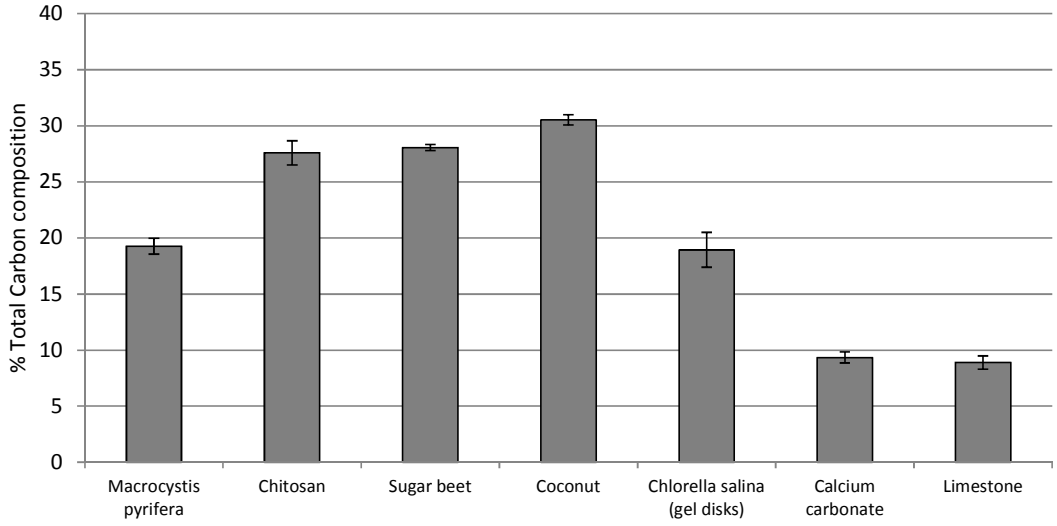


Figure 4-8 Total Carbon analysis of crude biomass & inorganics

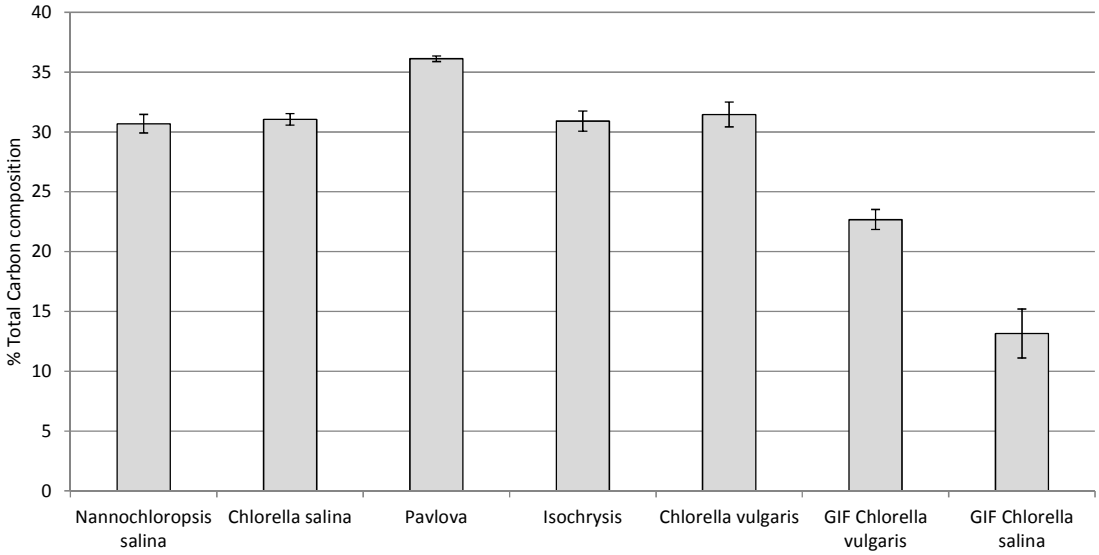


Figure 4-9 Total Carbon analysis of dried crude microalgae & GIF microalgae

Figure 4-8 shows total carbon content of crude materials analysed for experimental investigation. Figure 4-9 shows the total carbon analysis of identical cultures of *Chlorella salina* harvested both conventionally and via GIF low-cost harvesting. A reduced carbon content of GIF harvested microalgae compared to conventionally harvested microalgae was observed. This could be due to lower carbon component of the GIF alginate residue which surrounds each of the cells in a condensed embedding matrix. As a sacrificial non-toxic carbon based harvesting and trapping material, this was not considered to be an issue for the downstream processing application of microalgae into biofuel. Conventionally harvested marine microalgae species in Figure 4-9 also show a higher carbon composition compared to GIF harvested *Chlorella salina*, suggesting that salt water could be affecting the GIF harvesting or the gel-salt association.

The TOC analyser quantified Total and Inorganic Carbon in SSM and Inorganic Carbon, Total Organic Carbon and Total Nitrogen in liquid microalgae samples, results were expressed in either mg for SSM or mg/l for aqueous samples. Experimentally, solid samples of dried microalgae biomass from established laboratory cultures were harvested conventionally (*Nannochloropsis salina, Chlorella salina, Pavlova, Isochrysis & Chlorella vulgaris),* and by GIF harvesting (*Chlorella vulgaris and Chlorella salina).* GIF harvesting yield quantification required high culture volume for processing in excess of 10 litres. Higher biomass volumes were more readily extracted from filters for gravimetric analysis. Scaled production of all microalgae species for testing was not achieved and could be actioned for further work. Samples were chosen according to their logistical culture availability at the time of experimentation.

4.4.5 **Lipid extraction**

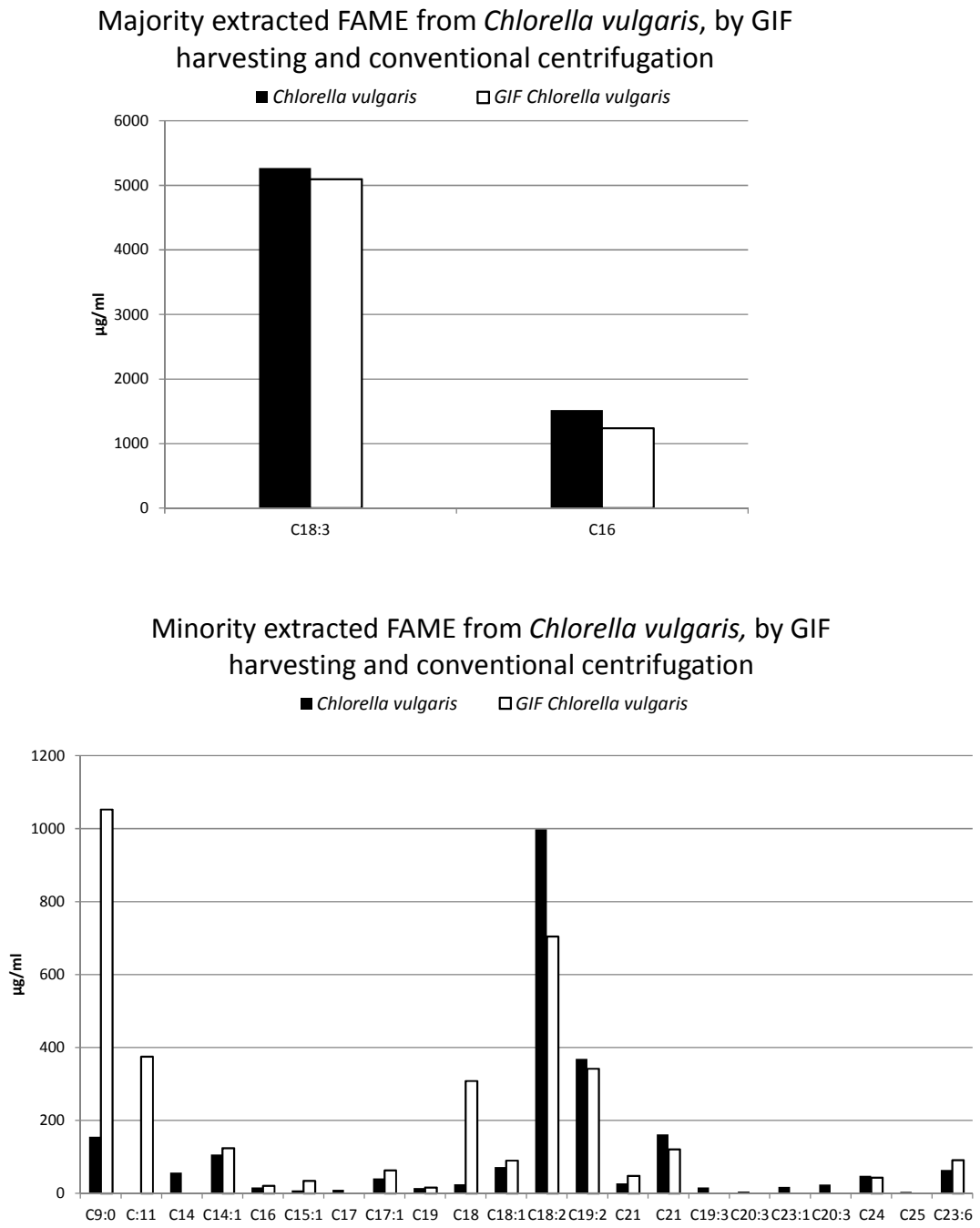


Figure 4-10 FAME yields from *Chlorella vulgaris*, conventional harvesting and GIF

Comparative quantitative and qualitative yields of lipids from *Chlorella vulgaris* are a positive interpretation of the viability of this harvesting technique to preserve biomass integrity and lipid profile. Figure 4-10 shows results from GC demonstrating that lipid composition has been preserved post-harvest. GIF lipid

fractions were detected via peak detection software from the FAME standard. There was no eye observed visual indication of degradation or decomposition by bacterial or fungal contamination prior to lipid extraction.

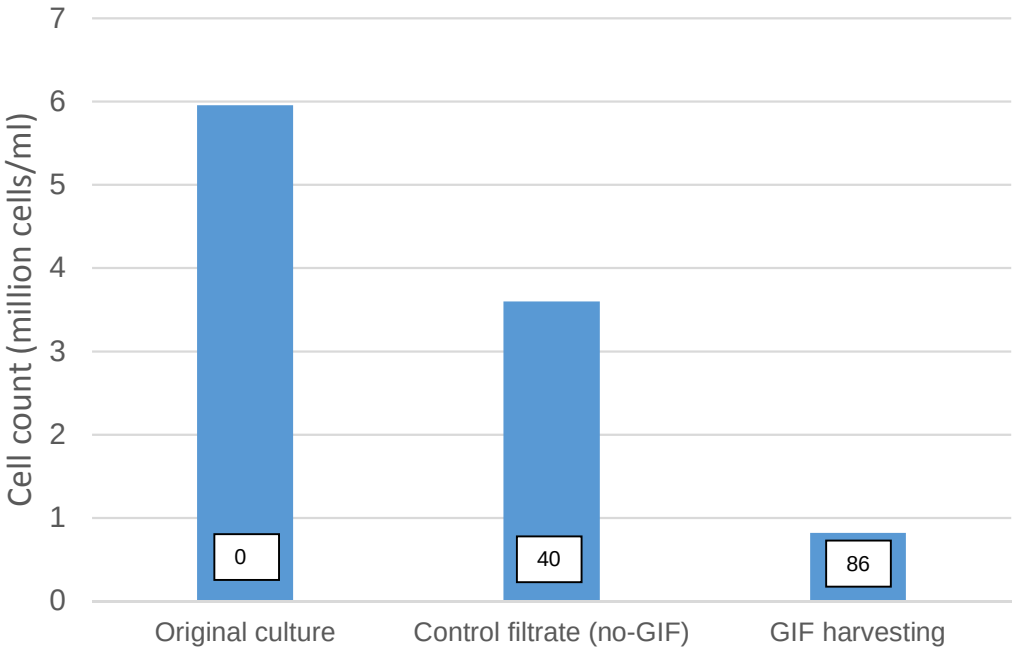


Figure 4-11 Filtrate cell counts per ml of original culture of *Tetraselmis chui*, with conventional filtration and GIF harvesting
Boxes indicate harvesting efficiency as percentage reduction of cell counts from the initial culture

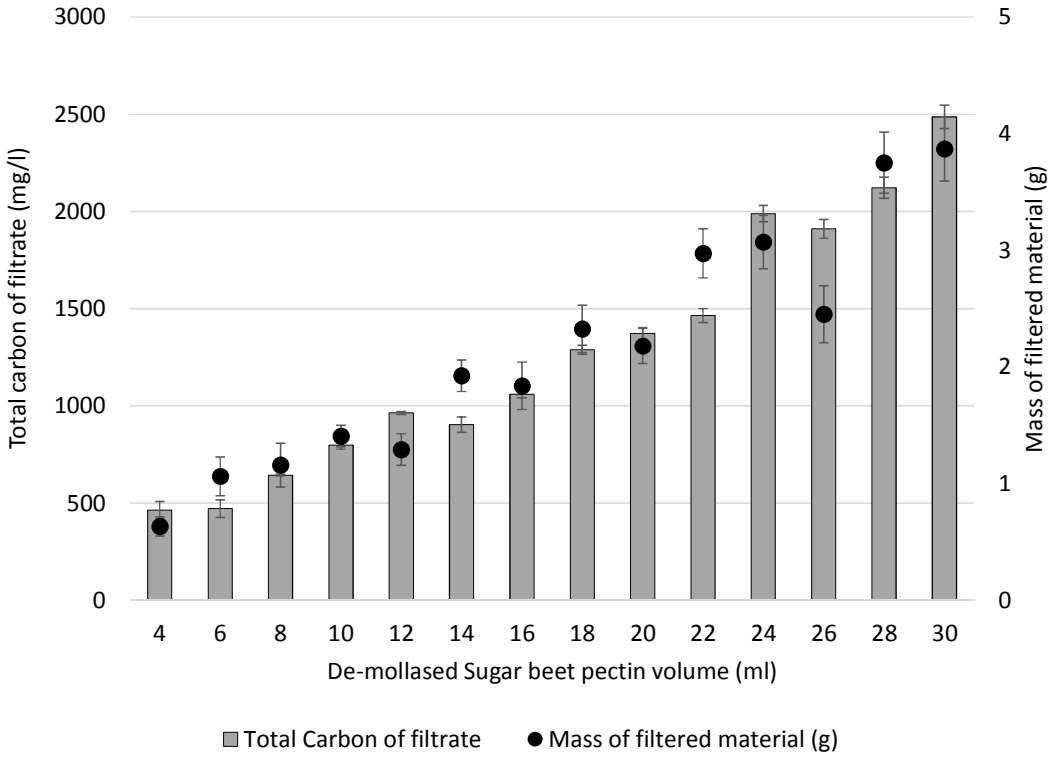


Figure 4-12 Comparison of mass of GIF harvested material and TC of filtrate using GIF harvested *Tetraselmis chui*

Tetraselmis chui is a flagellated microalgae which was not considered to be the best suited species for GIF harvesting as the flagella are thought to hinder the gelation entrapment process in comparison to non-flagellated species such as *Chlorella vulgaris.* The sacrificial component of the GIF harvesting process was apparent from the incremental mass of filtered material with increasing application of SBP (Figure 4-12). The TC content of the filtrate suggests that some of the pectin polymers are passing through the filter as shorter chained polymers or dissolved organic carbon (DOC) hence lost efficiency as a method of GIF harvesting. This is considered to be a function of efficacy of SBP extraction. However despite potential for further optimisation, Figure 4-11 shows that the GIF process using crude extracted SBP yields a lower cell count in the filtrate than both the original microalgae culture and non-GIF or conventionally filtered control. This equates to a GIF harvesting reduction in cell count from 2.66 million cells per ml to 600,000 cells per ml. Filters were new for each test and were not re-used during experimentation to ensure that any cells which were

retained within the filter mesh did not compromise the results from successive batches of filtrations. Filter bags were 3 mm in cross sectional thickness. Rated by the manufacturer at 1 µm pore size, *Dunaliella salina* cells were found to pass through the filter in pilot experiments. *Dunaliella primolecta* cells vary from spherical to ellipsoidal with a longest dimension of ~10 µm (Cunningham and Buonnacorsi, 1992). Given that both of these species belong to the same genus, therefore similar sizes, this raises the question of accuracy of claimed filter pore size as justified by means of experimentation. Similarly, *Chlorella vulgaris* with a cell diameter of 1.5 µm (Simon *et al.* 1995), and 4.4 µm (Babel and Takizawa, 2010) was filtered with the same filter bags which showed no retention of as the cells passed through the filter.

4.5 Discussion

Downstream process unification of macroalgae and microalgae with commercial implementation for market innovation, could produce novel feedstock materials. The extraction and utilisation of digestible fibre (pectin and alginate) for microalgae harvesting from the non-digestible fibre (non-starch polysaccharides) present in seaweeds could lead to new animal feed applications. Though molecular and compositional carbohydrate analysis was beyond the scope of this paper, it has been shown that non-starch polysaccharides constitute 38-42% of brown seaweeds including *Fucus vesiculosus*, *Laminaria digitata* and *Wakame* (Rupérez and Toledano, 2003). For the commercial implementation of alginate extraction, a screw press is used to build pressure with fine aperture dies to release dissolved sodium alginate (McHugh, 1987). In the lab, the crude seaweed extract solution excluded phenol extraction and consequently sodium alginate also yields polyphenols as detected by the optical darker pigments (McHugh, 1987). Commercially, phenol compounds are removed with formaldehyde (McHugh, 1987). The 9% yield of sodium alginate in this study could further be improved with an enhanced more efficient extraction process, similar to that used in industry. The reason that this improved alginate extraction process was not used in this study is that the additional gains in terms of percentage improved yield in total alginate extracted are at significant extra cost in processing

equipment, time and energy. Such additional capital outlay costs for their respective yield gains can be justified for businesses that operate machinery for high extraction and longevity, however for laboratory extraction approaches, yield gains were not as important as the effects of the utilisation of the alginate for GIF harvesting. With >90% mass of the original feed remaining, the viability of utilisation as a feedstock is considered to present a justified economic argument.

Microalgae generally have a negative surface charge which results in a stable cell suspension in the culture medium (Vandamme *et al*., 2015). A simple, low-cost solution would be to use flocculants that interact with the negative surface charge of the cells, followed by a separation step using simple sedimentation (Vandamme et al., 2013). However, the efficiency of these types of flocculants tends to be very sensitive to ionic strength and the presence of algal organic matter (AOM) (Vandamme et al., 2012). Chlorella cell walls contain chitin (poly-β-1,4-$_D$-*N*-acetyleglucosamin) and a plastic polymeric matrix containing uronic acids, rhamnose, arabinose, fucose, zylose, mannose, galactose and glucose with *N*-acetylglucosamine present as the predominant amino sugar (Takeda, 1991). The cell walls of *Chlorella vulgaris* are readily stained by Ruthenium red, which typically adheres to pectin-like substances or acidic sugars (Takeda, 1991).

Experimental design of gel induced filtration optimisation considered the following options:

a) Uniform volume with variation in the concentration of alginate and $CaCl_2$

b) Stock solution of equal concentration with varying volumetric inclusion

Option b) was used the chosen strategy due to the very low inclusion concentrations of both alginate and $CaCl_2$. Option b) is justified by determination of the experimental central composite design (CCD) model. CCD's consist of a $2k$ factorial with n_F runs, 2_K axial or star runs, and n_c centre runs; where k = number of factors, n_F = number of points used in the factorial portion of the design and n_c is the number of centre points. Axial runs are added to allow the quadratic terms to be incorporated into the model. The CCD is a very efficient design for

fitting the second order model. Two parameters in the design must be specified: the distance of α of the axial runs from the design centre and the number of centre points n_c. The model should provide good predictions throughout the region of interest. One way that this can be manifested is to ensure reasonable variance of predicted response at the points of interest. The model response surface design should be *rotatable* (Box and Hunter, 1957). CCD's are made rotatable by the choice of α, where $\alpha = (n_F)^{1/4}$.

Returning to the justification for selection of option b), the axial run from centre point to star point of α is a pre-requisite for efficacy of model design and performance of response surface methodology. Also, the concentration of alginate and $CaCl_2$ are both very low percentage inclusion. A lower star point using an alginate concentration of 0.5% as determined by pilot trials as being the optimal region of GIF harvesting efficacy, would have stipulated using a *negative* star point, which would be impossible. Conversely, if a positive lower star point was chosen, the central points would have been within higher inclusion limits, beyond the scope of optimal concentration for both harvesting and cost effective economics. Therefore, the alternative choice was to keep the concentration of both alginate and $CaCl_2$ constant, and change the exposure of microalgae to the effects of gelation by modification of the volumetric component. In so doing, it was possible to maintain the known (low) area of optimal response, whilst simultaneously abiding by the statistical rules of the CCD model. As with many experimental testing procedures, this had side-effect repercussions, namely a change in volume of total solution as stated in Table 3-1. Variability of the lowest test volume was 257 ml and highest of 350 ml. This also affected the optical density of filtrate, though less so the retention of microalgae by the GIF harvesting process.

Factorial designs and response surface optimisation methodology has been applied to applications including biological, environmental, pharmaceutical, industrial related processes and food technology. This approach is the opposite of the classical univariate methods, which is time consuming whereby the response is investigated for each factor while all other factors are held at a

constant level (Hanrahan and Lu, 2006). As noted by Spolaore *et al.* 2006, comparisons of works of RSM with other work can be risky since the optimum parameters of cultures depend strongly on microalgae species, reactor geometry, (bubble column, flat plate reactor, annular reactor), type of culture (batch, continuous). In the context of microalgae applications, response surface methodology has been applied to the measure growth responses in response to multi-variate variables of temperature, light, pH and aeration rate (Spolaore *et al.* 2006). Calcium chloride, pectin and chitosan gel bead formulation experiments using response surface methodology with application for pharmaceutical development (Mennini *et al.* 2008) are similar to the variables used within this study with the exception that chitosan was an additional factor and pectin replaced alginate for gel formation.

Filters or gauzes of variable dimensions could have resulted in comparable findings to this experiment which would have beneficially have added an additional factor (3*k* factorial) to experimental protocol. Degradation or contamination was not apparent with an ambient room temperature and pressure drying time of approximately 48 h. Alginate was found to have preservative properties considered to reduce incidence of contamination and degradation from airborne bacteria and fungi. Alginate has also been found to have preservation properties when used as an additive to increase the shelf life of bread for an industrial baker in the UK, (Chater, 2013). There was no distinction between FAME's of lipids analysed by GC from GIF *Chlorella vulgaris* immediately after methylation, and from GIF dried algae stored at room temperature for 6 months and subsequently re-tested for lipid profile, suggesting that the GIF process is protecting the lipid integrity.

An explanation of the functioning GIF mechanistic action can be due to the nature of the cell wall of *Chlorella vulgaris.* External secretions of transparent gelatinous coverings are present in both prokaryotic and eukaryotic algae, allowing coccoid-celled species to colonise either permanently or as a stress induced response (Barsanti & Gualtieri, 2006). Only a few cyanobacterial expolysaccharide secretions have been defined structurally. Of those which have, the sheath of

Nostoc commune contains cellulose-like glucan fibrils cross-linked with minor monosaccharides, and *Mycrocystis flos-aquae* consists mainly of galacturonic acid with a composition similar to that of pectin (Barsanti & Gualtieri, 2006). The carbohydrate polymer complexes in the cell wall and sheath of microalgae or collective terminology – theca, and the gelled polysaccharide alginates and pectins used for GIF harvesting are resoundingly similar in chemical characteristics and elemental composition. These similar biochemical morphologies are considered to be responsible for the close functional association between gelling processes used for this harvesting process and the biochemical structural formation processes by microalgae cells during growth and reproduction. It is therefore hypothesised that the GIF polymer network is a physiological synthetic chemical extension around the periphery of individual or colony cells, co-joined with chelating polymers and Ca^{2+} ion complexes. If this is so, the close lipid profiles reported between GIF and conventional harvesting as shown in Figure 4-10 are a confirmation of the non-invasive method of GIF harvesting, and lasting stable integrity of the cell wall during the drying phase.

Parallels exist between GIF harvesting and tangential filtration technology. Tangential filtration or microfiltration uses membranes which pass water over the membrane surface longitudinally multiple times. The retentate is recirculated across the membrane, keeping the cells in suspension and minimizing fouling (Uduman *et al.* 2010). In the same way that tangential filtration offers protection to the cells by keeping them recycled in suspension, GIF filtration offers the cells protection by an external low concentration gel polymer network. Microfiltration of *Scenedesmus* species using tangential filtration achieved a cell separation efficiency of 99.9%, and the energy requirement in the ideal case scenario was 0.87 kWh/kg of microalgae (7.6 m^2, 10 °C, 1.95 bar, and 2.0 g /l dry weight). This energy requirement reflects a 60 times concentration factor with a final biomass concentration of 116.0 g /l dry weight (Gerardo, Oatley-Radcliffe and Lovitt, 2014). By contrast in this study using GIF harvesting, a cell separation efficiency of >90% was attained with a gravimetric (and associated culture media lifting) fed energy requirement using only 1 pass through the filter. This reflects an initial 47 times concentration factor of post-harvested (retentate) biomass (9% w/v), which

subsequently upon ambient drying converts to a complete moisture-free state of microalgae and alginate composite, with additional lipid protection benefits.

Table 4-7 Percentage composition of lipid fractions from *Chlorella* species

Author	Cultivation	Species	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	C19	Other lipids	Total lipids (%)
Liu, 2011	Phototrophic	*Chlorella zofiniensis*	26.6	-	3.7	17.9	20.8	12.2	-	18.8	100
Liu, 2011	Heterotrophic	*Chlorella zofiniensis*	22.2	-	1.2	35.2	20.2	8.3	-	12.9	100
O'Grady, 2011	Heterotrophic	*Chlorella protothecoides*	24.4	-	2.4	69.1	3.6	-	-	0.5	100
Converti, 2009	Phototrophic	*Chlorella vulgaris*	63	-	9	3	11	13	-	1	100
Xiong, 2008	Heterotrophic	*Chlorella protothecoides*	-	9.6	1.4	-	-	-	87.7	1.4	100
Yeh, 2012	Phototrophic	*Chlorella vulgaris*	21.9	-	10	13.9	25.8	4.9	-	23.5	100
This study	**Phototrophic**	***Chlorella vulgaris***	**16.8**	**0.1**	**0.3**	**0.8**	**11.1**	**58.4**	**0.2**	**12.3**	**100**
This study	**Phototrophic**	***GIF Chlorella vulgaris***	**12.7**	**-**	**3.2**	**0.9**	**7.2**	**52.2**	**-**	**23.8**	**100**

Figure 4-10 is summarised in major lipid fractions in Table 4-7. C16 and C18 are the predominant lipid profiles extracted from *Chlorella* species. Contrary to the findings of other authors', higher proportions of C18:3 were detected in *Chlorella vulgaris* in this study. Though the lipid extraction technique of O'Fallon, 2007 was originally developed for application wet meat, oils and feedstuffs, it has concomitantly been used successfully for application to microalgae lipid extraction (Chakraborty *et al.* 2012 & Davey *et al*. 2012). The method determines total fatty acid classes including triacylglycerols, phospholipids, waxes and methyl esters using a simple, direct 1-step esterification procedure (O,Fallon *et al.* 2007). It eliminates the requirement for rotary drying of solvents under vacuum and nitrogen drying. The reported lipid extraction and methylation procedures by other authors' in Table 4-7 did not use the same O'Fallon, 2007 lipid extraction profile as in this study, which could be a contributing reason to the difference obtained in the high C18:3 between this and other trials. However, Yeh 2012, used a direct transesterification method similar to O'Fallon 2007, with 0.5 N KOH instead of 10 N KOH and the application of heat at 100˚C for 15 minutes, whereas O'Fallon used the exothermic chemical reaction heat from the KOH and HCl

reaction with 55˚C incubation. The high C:19 profile of Xiong, 2008 could be due to the heterotrophic lipid production from *Chlorella protothecoides* on a glucose substrate.

4.6 Experimental and Optimisation constraints

The analytical approach of central composite design experimental optimisation considers inputs into a black box with measured outputs (Montgomery, 2006). Numerical optimisation allows an understanding of component concentrations and respective measured responses, hence the potential provision of a predictive tool for quantification assessment. However, the constitutional relationship between physical components, comprising liquid rheology, homogeneity, liquid and solid affinity to surfaces such as the filter and vessel containment walls as well as mixing rate, flow rate and filter porous permeability is not accounted for using design of experiments and analysis of variance approaches. Scale up of this technology to microalgae batches of a greater volume than that investigated here in these reported trials pertains to changes in the relative proportions of fluid pressure, flow rate through the filter, surface area to volume ratios and fluid mixing dynamics which could change the ionic gelation affinity and hence harvesting efficiency. Because of this, an amplified scale of production would need to be investigated experimentally before drawing comparative conclusions for the application into a commercial scenario. By contrast, an amplified scale-up of experimental procedure creates logistical complications in replicate uniformity and experimental test runs for a RSM analysis. An alternative for future work could equally be to scale down volumetric size into microfiltration with and increase both replicates and experimental test runs. RSM is a useful tool for parametric multivariate analysis, however consideration should be given to the choice of model. A mixture experimental design with a tri-phasic ingredient choice of factors (water, alginate and $CaCl_2$) could give an alternative RSM and statistical approach worth considering.

Further limitations include the requirement to provide uniform volumetric multiple cultures from an original growing stock culture of equal cell density. Work processing time, thorough uniform mixing and a sufficiently suitable volumetric

original stock culture place limits on the number of experimental replicates without compromising authenticity of results. Moreover, the massive potential of species diversity, filter pore sizes and flow rates determined by liquid passage through the filter have not yet been determined, and would be worthy future research.

The extraction efficiency of alginate and pectin extraction from seaweed, de-molassed sugar beet and citrus peel was calculated pre and post analysis gravimetrically from crude materials. In order to check this extraction procedure, the same process could be run alongside commercial extracted alginate from brown algae and commercial pectin. It would be expected that no further alginate or pectin would be extracted from a commercial source because the products exist as a pure grade sodium alginate and sodium pectinate respectively.

4.7 Conclusions

A low-cost microalgae harvesting and effective ambient drying process has been developed. This process uses the physical and chemical properties of low-cost gels to entrap, harvest and dry microalgae. The process has been applied to both raw crude extracts of seaweed, citrus pectin and sugar beet pectin as well as commercially prepared extracts of alginate and pectin. Results indicate a 12%, 10% and 8% extraction efficiency of alginate, sugar beet pectin and citrus pectin from their precursors of seaweed, de-molasssed sugar beet and citrus peel respectively using a simplified extraction protocol. This laboratory extraction efficiency is not comparable, nor designed to be comparable with industrial alginate extractions as previously discussed. Post-harvest GIF biomass of *Chlorella vulgaris* from crude extracted sugar beet pectin was calculated to be 9% solids or a concentration factor from approximately 1.9 g/l biomass (w/v) to 90 g/l (w/v). EDX and TC analysis attributed GIF harvesting to have a corresponding approximate halving of carbon content compared to the conventionally harvested microalgae, due to the entrapment matrix of the gelation agent. The GIF harvesting process has been proved to function with both marine and freshwater microalgae species and protect the stability and integrity of the microalgae lipids. This is functionally thought to be attributed to the chemical structural similarity between the gelation matrix and the cell wall.

5 **Novel findings in desalination**

This chapter has been published in the Journal Desalination, (2015) 360: 13-18.

5.1 Introduction

The salinity of the sea ranges between 33 ppt and 38 ppt. The major contributing ionic composition of seawater has been measured as 19.353 g/kg (Cl), 10.781 g/kg (Na), 1.284 g/kg (Mg), 2.712 g/kg (S), 0.4176 g/kg (Ca), 0.399 g/kg (K), (Quinby-Hunt, 1987). The oceans contain over 97% of global water, another 2% is locked away as ice with 1% estimated to be distributed between the atmospheric and hydrospheric cycles. Agriculture, industry and a rising global population are placing great pressure on availability and distribution of water in some regions of the world. For example Los Angeles in California is the second biggest city in the USA, which imports the vast quantity of water it consumes (Sze, 2009). Freshwater from the Sacramento-San Joaquin Delta supplies 25 million people and has supported 450 miles of intensive agriculture in the central valley. Continued sustainability of such water provision and utilisation depends on a manmade network of flood engineering systems and irrigation infrastructure such as dams which have transformed and controlled water supplies. With weather patterns and climate change affecting conventional storage systems and distribution of fresh water supplies, desalination offers both an ionic conversion process and supply solution to a significant technical challenge. Many arid nations such as Saudi Arabia depend on the energetic intensive approach of conventional desalination processes for their water provision.

The two common commercial desalination technologies used in arid climates are Reverse Osmosis (RO) and multi-stage flash distillation (MSF). Both technologies are energy intensive. MSF uses 4kW h/m^3, RO uses 6-8 kW h/m^3 without energy recovery and 4-5 kWh/m^3 with energy recovery (Khawaji *et al.* 2008). Enhanced process efficiency combining ultra-filtration with two-pass reverse osmosis at 47% efficiency can achieve 3kW/m^3 (McGovern and Lienhard, 2014). Energy expenditure for RO is directly proportional to the salt concentration of the saline water being desalinated. The pressure required is about 70 bar for seawater desalination and from between 44.8 bar for 18 ppt to 82.7 bar for 45 ppt

salinities respectively (Charcosset, 2009). The energetic expenditure and associated economic cost of desalination has resulted in its use being restricted to countries with access to energy, capital expenditure or financial investment. Desalination technologies use energy in the form of electricity, pressure or heat to separate seawater into freshwater and brine. Distillation technologies at elevated temperatures are susceptible to salt corrosion and require specialist materials to avoid limescale and salt deposition. Reverse osmosis functions at ambient temperatures and elevated pressures (3-8 MPa) using specialist filters with a pore size of 0.0001 – 0.0006 µm that are able to filter monovalent, divalent ions, virus particles and bacteria. Reverse osmosis is the final stage of desalination which is preceded by micro, ultra and nanofiltration membrane technology.

Fouling of reverse osmosis membranes occurs by dissolved organic matter present in seawater as by-products of living organisms including bacteria, macroalgae and microalgae. Long chain carbohydrate polysaccharides gels or alginates are known to severely foul the specialist membranes used in reverse osmosis desalination plants (Lee, 2006). This fouling limits the life of reverse osmosis membranes to about 2 years, requiring stock replenishment and increased overhead costs over and above the energetic process of reverse osmosis desalination. Prior research has investigated membrane technology, the functional chemical molecular groups on the surface of the membrane in close interaction with saltwater as it intercepts the membrane and the affinity for fouling alginates (Wu, 2014). However, to date there has been no investigation of the ionic interaction of saltwater and alginates before and after filtration using a microfiltration membrane with a pore size in the region of between 0.5 and 5 µm. An increased pore sized membrane is advantageous in desalination technology due to a longer membrane lifecycle, lower or even gravity fed seawater pressure displacement, lower energy use and reduced capital and operational expenditure. The development of a low-energy desalination process could have profound implications for societal progress including the provision of freshwater to low-income communities. With detrimental implications for reduced sustainable supply of both fossil fuels and water in many regions of the world, this research

advocates an alternative approach in desalination technology functioning via modifications to the pre-treated water at ambient temperatures and pressures rather than the membrane.

Alginates are a worldwide commercial commodity extracted from brown macroalgae. Alginate is the most abundant polysaccharide of brown seaweeds constituting 40% composition of cell walls (Draget, 2005). Brown algae are classified as Phaeophyceae under phylum Chrysophyta. It is estimated that there are between 1500 to 2000 species of brown macroalgae (Hoek, 1995). World production of brown algae was 6,784,179 t in 2010, mainly from four species in East Asian seas *Laminaria japonica, Undaria pinnatifidia, Sargassum fusiforme* and *Phaeophyceae* (Jung, 2013). In natural environments, alginates exist as calcium, potassium and sodium salts of alginic acid present in the cell wall of brown seaweeds (Gomez, 2009). Alginates are a biochemical group of linear unbranched polysaccharides derived from marine macroalgae which contain β-D-mannuronic acid and α-L-guluronic acid residues. The composition, sequence and molecular weight determine the physical and rheological properties of alginates. Over 100 species of macroalgae are presently used in food, medicine, fertiliser or for phycolloids and chemicals. Seaweeds are mainly used in the human food industry where they are processed for texturing agents and stabilisers such as propylene glycol alginate (PGA). They also have usage in paper processing and textile industries. There exists variation in the quality of alginates from different seaweeds based on the ratio of guluronic to mannuronic acids. Chilean seaweeds have unique alginate hydrocolloid properties from *Lessonia* species for food and pharmaceutical applications. In the last decade there have been changes in procurement of alginates in the global market place as *Laminaria* and *Lessonia* species with high guluronic acid species produce the strongest gels for commercial applications (Bixler, 2011). Abundance of macroalgae species in temperate coastal regions is an attractive source of biomass for novel applications including energy sources from fermentation or anaerobic digestion. Allocation of such marine natural resources are of particular interest for local energy generation where terrestrial biofuel is unable to expand further due to high percentages of net primary productivity already being

appropriated for human use, such as the North Atlantic coast of North America and Europe and the western seaboard of South America (Hughes, Kelly, Black & Stanley, 2012). The novel application of alginates for use in desalination would complement current developing interest and market application potential of biofuel and food technologies.

5.2 Material & Methods

5.2.1 **Full salinity and freshwater desalination comparison**

A multifilament mesh polypropylene 1 µm rated 25 l capacity filter bag approximately 18 by 80 cm when flat packed (Lenntech BV) was used as the experimental microfilter. 18.2 meg ohm de-ionised water was used for freshwater (Merck Millipore) and 33 g/l aquarium salt (Instant Ocean®) was added to de-ionised water to make artificial seawater. Ion chromatography (ICS900 Dionex Thermo Fisher Scientific) analysed anions and cations for both seawater and freshwater at various stages of the desalination process. Energy Dispersive X-ray Electron microscopy (Oxford instruments / AZtecEnergy analysis software) was used to detect and quantify filtered material from both freshwater and seawater samples and secondary recycled alginate.

1% (w/v) refined sodium alginate powder derived from brown macroalgae with a medium viscosity composed of polyuronic, glucuronic and mannuronic acid residues (Sigma-Aldrich®) was dissolved into de-ionised water in a 2 l Erlenmeyer flask and stirred with a magnetic stirrer for 12 h until completely dissolved. 4 l of artificial seawater and 4 l of de-ionised water were prepared and stored in equal volumes in duplicate Erlenmeyer flasks. 10% (v/v) of a 1% (w/v) sodium alginate solution was mixed thoroughly into flasks containing both seawater and freshwater. During continued mixing 10% (v/v) of a 1% $CaCl_2$ (w/v) solution (Sigma-Aldrich®) was blended into the solution inducing a mild gelation in both of the seawater and freshwater. Solutions were poured into the filter bags. A sample of the filtrate was retained for analysis, the remainder was disposed. Filtered material was extracted from filter bags and dried at 60˚C for 48 h to avoid heat degradation and analysed by electron microscopy and gravimetrically. 5 g of the dry filtered seawater material and 2 g of the dry filtered freshwater material was

re-suspended in 500 ml of 1% sodium carbonate (Sigma-Aldrich®) and incubated at 50˚C for 2 h with regular stirring. The resulting solution was re-used for repeat filtration.

50 ml water samples were extracted from seawater and freshwater (SW/FW). Further 50 ml water samples were extracted at the following stages: after addition of sodium alginate solution (SAG/FAG), after addition of $CaCl_2$ solution (SBG/FBG) and from the final filtrate (SF/FF). A negative control analysed samples of artificial seawater with no additives before filtration (SB) and after filtration (SFNG). Water samples at each stage of processing were analysed for major anions and cations using ion chromatography. 100 µl samples were diluted with 4.9 ml de-ionised water and each analysed via ion chromatography for major ions (sodium, chloride, calcium) and minor ions (sulphate, potassium and magnesium).

5.2.2 **Variable salinity desalination**

To verify efficacy of variable salinity desalination, a secondary experiment was conducted with the same technique using 12 incremental salinities between 0 ppt and 33 ppt at 3 ppt intervals. 12 batches of 16 litre variable saline samples of artificial seawater prepared from tap water were analysed before and after gelling of seawater for major ions with ion chromatography. Identical unused filter bags and gelling process as described in 4.2.1 were used for each salinity concentration to avoid discrepancy of re-used nascent ions within used filter bags. Gelled seawater was pumped at constant velocity of 2.5 l/hr using a peristaltic pump (Watson Marlow) into 45˚ inclined filter bags and samples of filtered seawater were taken for ion chromatography analysis.

It was observed that during sample preparation, density separation of gelled seawater separated gravimetrically from un-gelled variable concentration seawater. This was apparent in only four concentrations of saline, namely 0, 3, 6 ppt and 33 ppt due to the respective resting time of these two concentrations left to separate gravimetrically post-gelled for at least 10 min, coincidentally, whilst other samples were being prepared or analysed. On observance of this event,

representative photographs were taken and non-gelled floating surface saline water was also sampled for ion chromatography analysis of ion composition.

5.2.3 **Recycled ionic and density gradient desalination**

Recovered dried gels from 5.2.2 were manually extracted with care to ensure full recovery from filters of 4 of the 12 samples at 0, 27, 30 & 33 ppt. These were measured gravimetrically and milled into a powder. Samples were then re-diluted in 1 l of 2% sodium carbonate (Sigma-Aldrich®) and incubated at 50˚C for 12 h using a shaker incubator at 200 rpm. Following incubation and 3 h resting in Erlenmeyer flasks, density induced solid-liquid phase separation between sodium alginate gel and salt-carbohydrate complex sedimentation were separated. The more viscous salt-carbohydrate complex mixture was dried at 60˚C for 48 h to avoid heat degradation and analysed by electron microscopy. The less dense gel phase was poured off and re-used for secondary recycled ionic and density gradient desalination. 16 l batches of saline water at 0, 27, 30 and 33 ppt were mixed with secondary recycled sodium alginate solutions from equal concentration salinities as described in 5.2.1. Density separation of gelled seawater separated gravimetrically. 50ml samples were taken in duplicate for ion chromatography analysis from the original saline prior to addition of sodium alginate and prior to gelling and then from the upper liquid phase of the post-gelled 20 l containers after density settlement separation of post-gelled seawater.

5.3 Results

5.3.1 Full salinity and freshwater desalination comparison (4 l)

Table 5-1 Major ions (mg/l) in seawater & freshwater (SW/FW) from 4 l trials before and after processing with alginate (SAG/FAG), $CaCl_2$ (SBG/FBG), and the final filtrate (SF/FF).

Treatment	Sea Water			Fresh Water		
	Na^+	Cl^-	Ca^{2+}	Na^+	Cl^-	Ca^{2+}
SW/FW	7,767 ±117	15,828 ±29	329 ±6	22 ±21	0	37 ±0
SAG/FAG	6,916 ±5	20,162 ±114	270 ± 7	96 ±2	0	12 ±8
SBG/FBG	6,269 ±40	14,417 ±956	528 ±9	66 ±1	420 ±27	305 ±8
SF/FF	6,466 ±4	12,568 ±388	557 ±3	72 ±4	1,405 ±125	305 ±13
Mass (g) Filtered material	8.418 ±0.04			2.249 ±0.02		

Table 5-1 shows ion concentrations from seawater and freshwater (SW/FW), after sodium alginate solution (SAG/FAG), after $CaCl_2$ solution (SBG/FBG) and from the final filtrate (SF/FF). A reduction of sodium ions from 7,767 mg/l to 6,466 mg/l and chloride ions from 15,828 to 12,568 mg/l was recorded. Of all ions recorded, calcium ions show the only increase in concentration in the final filtrate at completion of the process. This is a consequence of the solute ions un-associated with calcium ions bound within the alginate gel. Being naturally present in seawater, it can be seen that addition of alginate solution captures some of these innate seawater calcium ions, evident by the reduction between SW/FW and SAG/FAG. The increase in calcium solute ions thereafter in SBG/FBG and SF/FF suggests that the 1% $CaCl_2$ solution provides adequate additional available calcium ions for ionotropic gelation to occur. Table 5-2 shows the minor ionic constituents of seawater. Interestingly, it is noted that both

monovalent potassium and divalent sulphate and magnesium ions are all reduced significantly by the ionotropic gelation process.

8.418 g of dry weight gel was recovered from the seawater alginate and 2.249 g from the freshwater alginate. Attributing the difference 6.169 g as salt ions in 4 l of seawater represents a 4.67% mass desalination.

Table 5-2 Minor ions (mg/l) ions in seawater & freshwater (SW/FW) from 4 l trials before and after processing with alginate (SAG/FAG), CaCl2 (SBG/FBG), and the final filtrate (SF/FF).

MINOR	Sea Water			Fresh Water		
	SO_4^{2-}	K^+	Mg^{2+}	SO_4^{2-}	K^+	Mg^{2+}
SW/FW	1,607.0 ±53	360.6 ±8	965.9 ±10	0.0	0.2 ±0.03	24.2 ±0.07
SAG/FAG	2,001.6 ±68	300.1 ±12	865.9 ±22	0.0	0.7 ±0.02	6.4 ±0.01
SBG/FBG	1,365.5 ±35	256.4 ±4	760.8 ±19	0.0	0.6 ±0.04	1.2 ±0.01
SF/FF	1,271.8 ±17	264.3 ±7	799.1 ±16	0.0	1.6 ±0.01	1.1 ±0.01
Mass (g) Filtered material	8.418 ±0.04			2.249 ±0.02		

Table 5-3 Control non-gelled seawater ions (mg/l) from 4 l trials before and after filtration

CONTROL	Na^+	Cl^-	Ca^{2+}	SO_4^{2-}	K^+	Mg^{2+}
Seawater before filtration	7,448.7 ±23	15,539.4 ±88	306.0 ±5	1,350.6 ±55	481.6 ±39	951.0 ±37
Non- gelled Seawater after filtration	8,042.1 ±32	16,612.7 ±63	320.3 ±11	1,434.9 ±38	497.0 ±28	989.7 ±40

Table 5-3 shows the effect of filtration alone on ionic composition before and after without the additional process of ionotropic gelation. Increase in ion concentrations post-filtration indicates that the filter itself has a range of freely

leachable ions which are not retained by the filter and lost into the filtrate post-filtration.

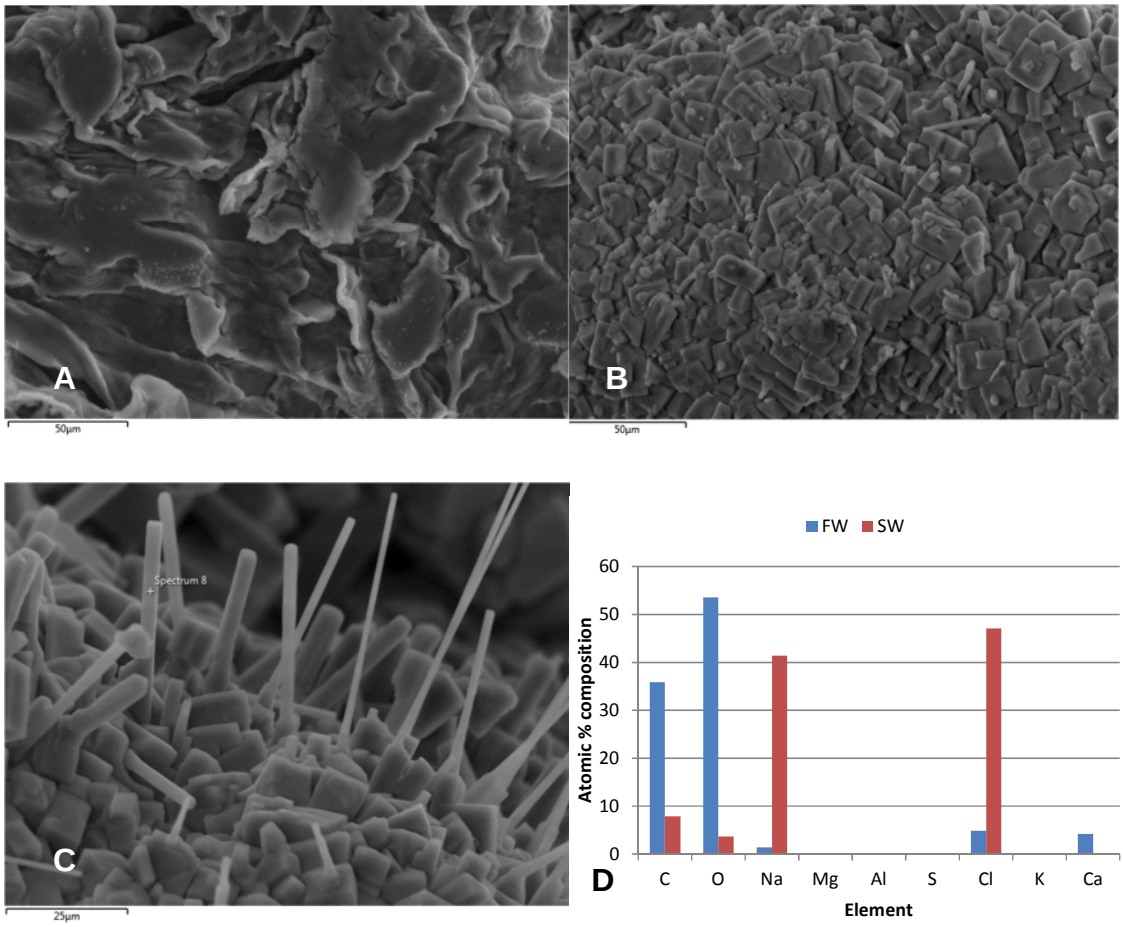


Figure 5-1 Scanning Electron Micrograph of dried recovered gel from primary desalination

A: De-ionised water. B: 33ppt seawater with cuboid salt srystals. C: 33 ppt seawater with pillar projection salt crystals. D: Freshwater (FW) & Seawater (SW) elemental comparison.

Figure 5-1 shows SEM images of dried recovered gels from filter bags. Image A shows the carbonaceous component of dried alginate gel in the absence of salt. Image B is predominated by an irregular latticed matrix of salt crystals. Image C was sourced from a different location of the same sample of image B. Here, it can be seen that cuboid salt crystals and pillared projections of pure salt surround the dried alginate gel, invisible in the image by overlaid salt deposition. Graph D shows the atomic percentage composition of elements recorded by EDX in the

surface layers of dried alginate samples. It can be seen that carbon and oxygen substantially represent the freshwater alginate gel whereas sodium and chlorine are the main atoms recorded in the saltwater alginate gel.

5.3.2 **Variable salinity desalination (16 l)**

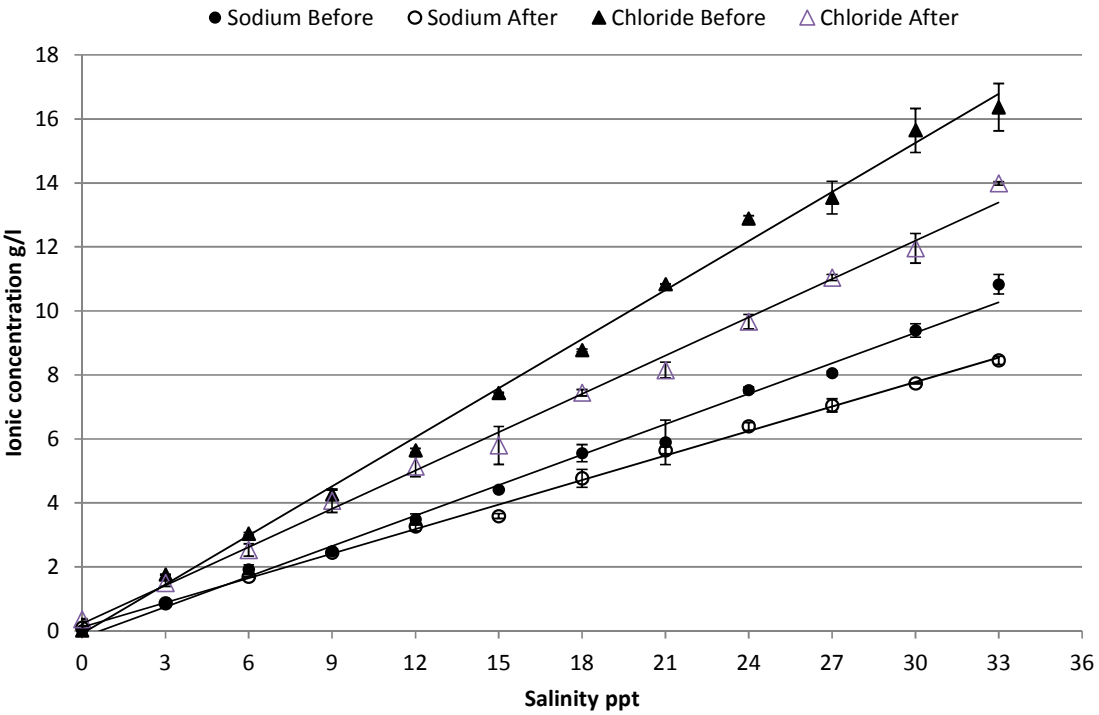


Figure 5-2 Sodium & Chloride ions from 16 l trials before and after filtration

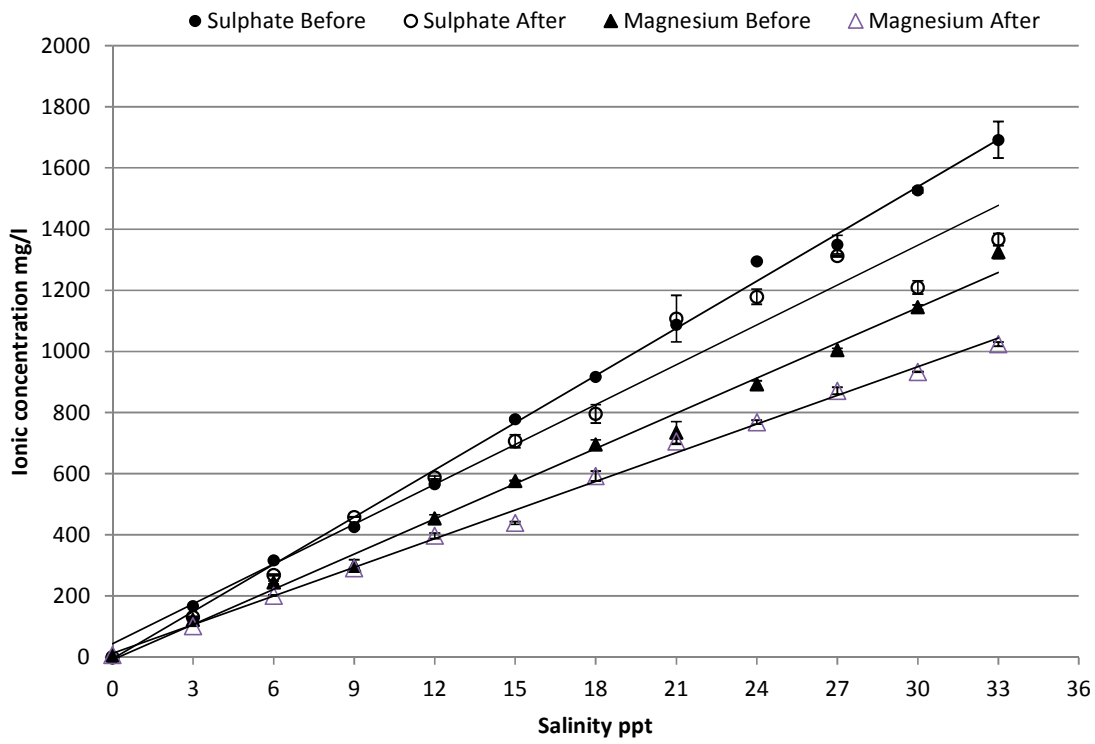


Figure 5-3 Sulphate & Magnesium ions from 16 l trials before and after filtration

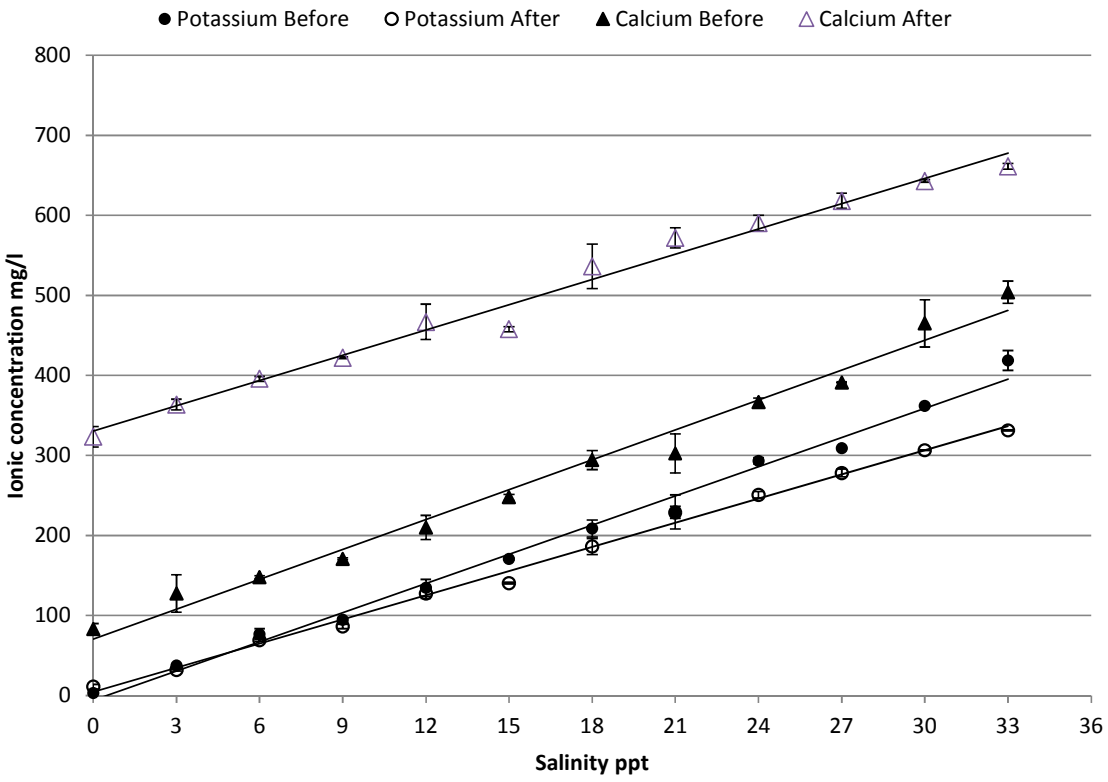


Figure 5-4 Potassium & Calcium ions from 16 l trials before and after filtration

Figures 5-2, 5-3 and 5-4 show the efficacy of a reduction in salinity and other seawater ion compositions at a range of original saline concentrations between 0 and 33 ppt. All ions except calcium are proportionally reduced over the range of treatments with best efficacy at maximum seawater salinity.

Figure 5-5 Density phase separation of gelled alginate in 33 ppt saline

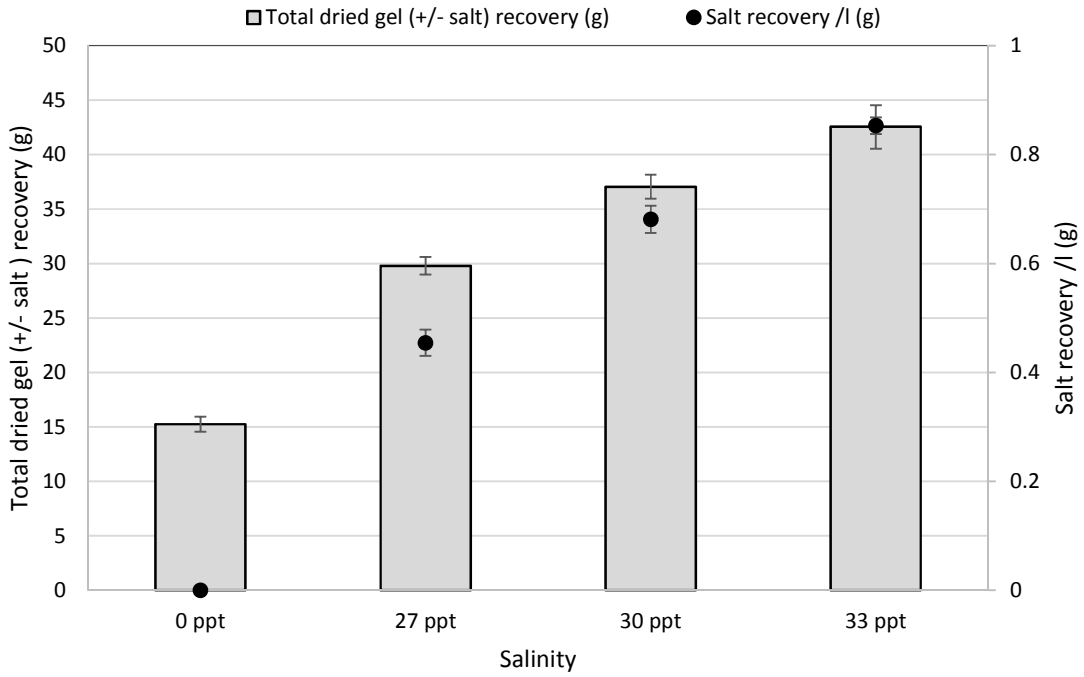


Figure 5-6 Gravimetric Alginate/Salt Composite Recovery (g) from 16 l batches of artificial seawater

Figure 5-6 shows the variation in compositions of recovery of alginate and salt from ranges of saline represented as both salt recovered per litre and per 16 l batch samples.

5.3.3 **Recycled ionic and density gradient desalination (16 l)**

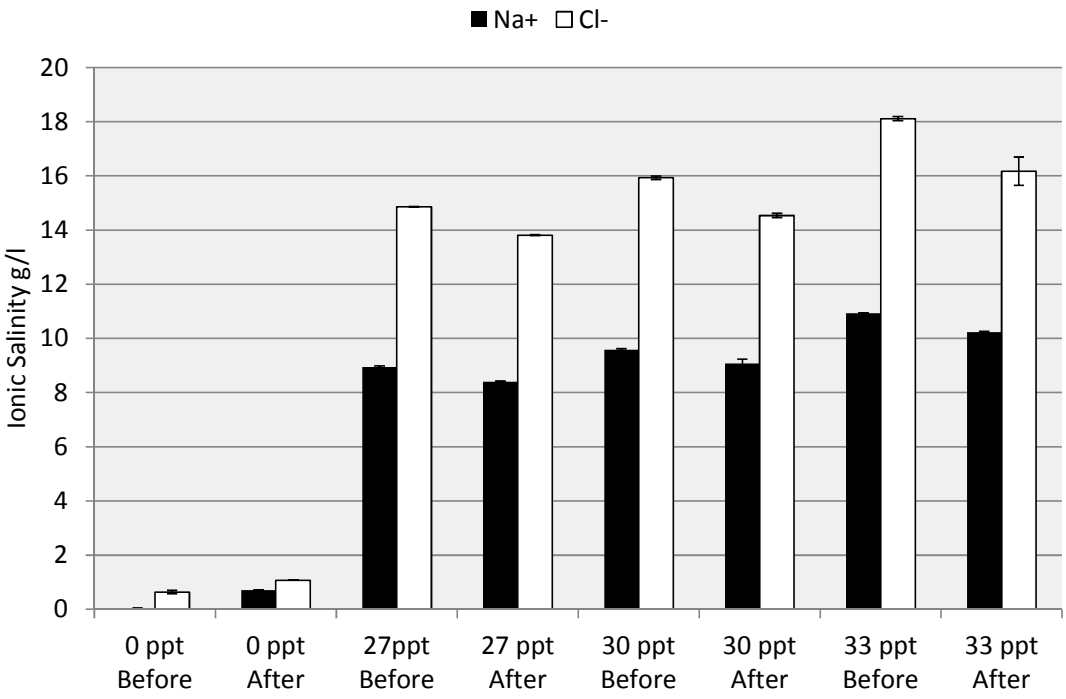


Figure 5-7 Sodium & Chloride density recycled desalination

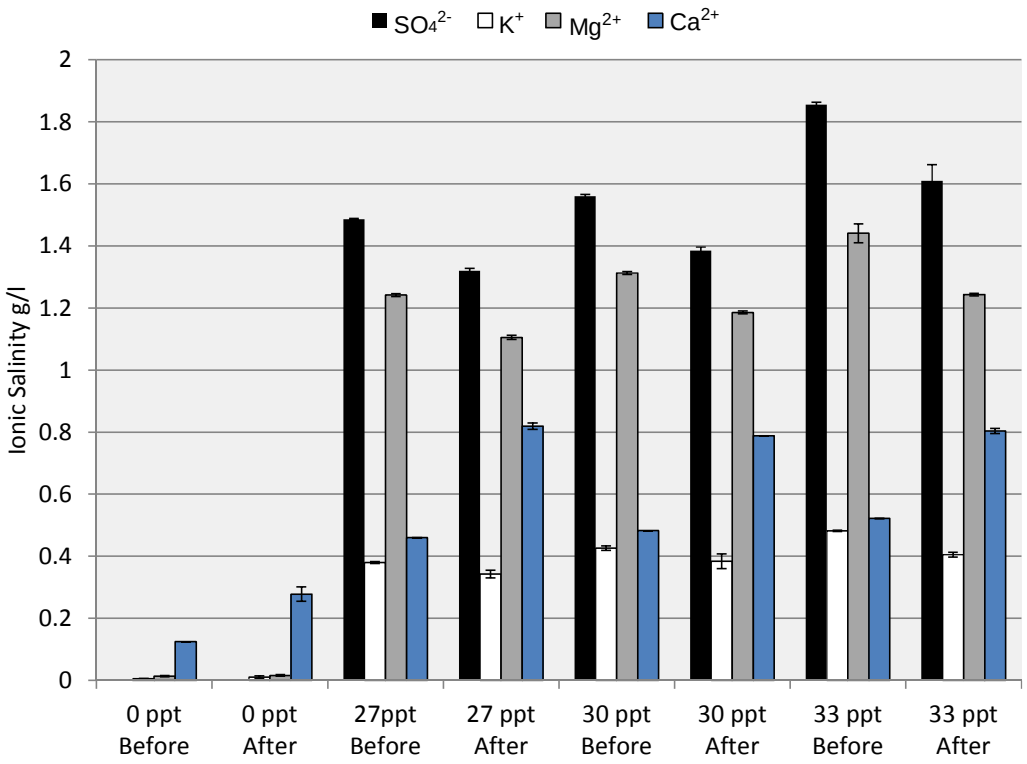


Figure 5-8 Minor ion density recycled desalination

Figure 5-7 and 5-8 demonstrates seawater ion reduction by secondary re-use of recovered dried alginate gel. Primary recovered alginate gels were recycled by reaction with sodium carbonate, density phase separation and re-exposure to saline solutions. Ionic entrapment by the alginate gel is again manifested in a secondary ionotropic gelation process, demonstrating the functioning recyclable nature of this bio-resource.

5.4 Discussion

This research has identified the ability of a low concentration of alginate to bind and capture saltwater ions. There was a 16.8% reduction in Na^+ and a 20.6% reduction in Cl^- ions following the gel desalination process. 4.7% desalination or 6.17 g dried salt from 132 g (4 l) of 0.033% seawater was recovered. The microfiltration process was completed in less than 60 seconds. However the flow rate of the peristaltic pump was 2.5 l/min, so 16 l samples took 6.5 min, of actual processing time. In addition to this, time was required to lift, move and mix the

heavy samples, so whilst the filtration process was relatively quick, the combined effect of multi-tasking and coordinating the work delivery protocol resulted in some of the samples taking longer than others and being left unattended once gelation had already occurred, and prior to filtration. The results of this added an extra and unexpected observation component to the research, whereby it was observed that there was gravitational separation of the alginate within solution Figure 5-5. Prior to this, samples were analysed for filtrate alone post-filtration, however the samples which separated by passive (density-related) sedimentation had their un-agitated surface water (*free water*) extracted and analysed for ionic composition also as well as their filtrate. Thereafter, although some of the samples (0, 27, 30 and 33 ppt) were left for up to 10 min, the actual filtration processing time for filtration was no different than for the other samples, only their passive settling time. The difference in observed ionic composition of major and minor ions in the *free water* of these ionotropic gelled solutions before and after the 10 min passive sedimentation period can be observed in Figures 5-7 and 5-8. It can be seen that as with the filtered treatments, there is a reduction in ionic composition in all differing treatment concentrations except for increased Ca^{2+} concentration with application of $CaCl_2$ prior to gelation. The benefits of this unexpected finding were the discovery that ionic affinity of salt with alginate and their subsequent density separation, did not just occur by means of filtration alone, but also the same process could be achieved by passive sedimentation, or merely by leaving the higher density gel to fall out of suspension Figure 5-5. The implications of this discovery could have potential applications for waste saline waters, either of anthropogenic or natural occurrence with remediation technology either by means of alginate or maybe pectin ionotropic gelation. Moreover, these potential benefits would not have to be associated with higher capital pumping and filtration technology but could be as a result of passive sedimentation with the natural vector of alginate and chemical affinity functioning in a ‘partial-desalination’ process which could recycle some of the calcium alginate with sodium carbonate as discussed previously.

Most of the artificial seawater passed immediately through the filter. The hydrated gel was immediately recovered and the saltwater gel was noticeably over double

the volume and a different colour to the freshwater gel. The mass transfer lifecycle include inputs into 1 l of seawater were 1g of alginate and 1g of calcium chloride to recover 1.54 g of salt. This recovered salt was analysed using electron microscopy and energy dispersive x-ray electron microscopy to determine its composition as being variably distributed on the external gel surface forming crystallising salt deposits.

In pure seawater, ions are not visible. However, on application of the low concentration alginate solution, a visible opaqueness and refraction to the water was observed. The ionic reaction of divalent cations with alginates was first described as the “egg box” process by means of calcium ion cationic bridges (Morris, 1978). Physicochemical research has investigated the mechanisms of the structural features involved in the gelation process. With the development of x-ray diffraction Li, 2007 proposed an alternative helical structure to Morris’s egg box model of gelation. This research has identified that it is not just divalent cations but also monovalent and divalent anions and cations which also have an affinity and are able to be retained physically within or in close proximity to alginate gels when passed through a microfiltration process.

In aqueous solution small solutes diffuse more rapidly than larger ones. This was proposed by Einstein who reasoned that high solute mobility is related to low friction. Einstein applied the Stoke’s law of friction and came up with the now famous Stokes-Einstein relation which predicts that a solute’s diffusion coefficient should vary inversely with its hydrodynamic radius (Einstein, 1905). Non-Newtonian fluids include fluid mixtures or suspension which display thixotropic or rheopectic properties as apparent viscosity changes in response to shear rate. Such non-Newtonian fluids do not abide by the Stokes-Einstein relation. Viscosity increase is the consequence of the formation of jamming clusters bound together by hydrodynamic lubrication forces, often denoted by the term hydroclusters (Bender 1996, Lee 2003). The rheological behaviour is controlled by a variety of parameters, the most important of which are the particle size distribution, particle shape, volume fraction, particle–particle interactions and the viscosity of the suspending phase (Barnes, 1989). Of particular interest is that the presence of a

magnetic field is also able to produce shear thickening behaviour (Galindo-Rosales, 2011). This magnetic response is likely to be linked to the change in orientation of ions within a non-Newtonian liquid and advocates the high affinity of ions with relation to viscosity or gels as observed in this research.

The association-induction hypothesis (Ling, G. 1963) describes association between water molecules and carbonyl (CO^-), amino (NH^+) and carboxyl ($COOH^-$) groups on protein chains. The induction component of the hypothesis explains how certain molecules induce a change in charge which is propagated by organic and water molecules. This is known as “structured water”. Polyamide reverse osmosis membranes surface functional groups contain COOH, NH_2 and $CONH_2$. Hydrophobic and electrostatic interactions as well as ionic bridging and hydrogen bonding between specific functional groups show appreciable influence on alginate adsorption kinetics (Wu, 2014). At low concentrations of gelation, ions associate and build concentric three dimensional structured water layers around the founding ion, reinforced by alginate gelation complexes and resulting in significant increases of their volumetric diameter. This prevents ions passing through a filter with a pore size much larger than the size of the respective ion and enables an improved filtration procedure at much lower energy expenditure. Unstructured water with low-affinity to ions can pass through the filter. Gelled ions become concentrated in the filter, their accumulation acts to compress structured water from the external perimeter of adjacent gelled ions, thus concentrating the filtered composition of gelled ions and separating gelled brine from freshwater. This process is a hypothesised explanation which is complex to test experimentally without the use of analytical equipment capable of monitoring changes in ionic charges of liquid solutions at molecular level, such as nuclear magnetic resonance spectroscopy.

Industrially utilised sodium carbonate is a widely used commodity for paper and glass production. 1 million t of sodium carbonate production via the Solvay process method yields liquid waste containing 432,000 t of NaCl and 1,047,000 t of $CaCl_2$ (Trypuc 2011). Calcium chloride is widely used in the food processing industry as a low-cost industrial by-product. Commercially available sources of

alginates fetch high prices in markets such as the pharmaceutical industry utilising highly purified and refined alginates. Pectins extracted from citrus peels or de-molassed sugar beet are also able to be used for gelation agents in this process (Chapters 3 & 4). Soluble pectin fibre is extracted as the minor component (<15%) with the majority being composed of insoluble cellulose and hemi-cellulose fibre. Rendered soluble pectin extracted feedstuffs are able to be re-integrated into the animal feed chain as the mild salts used for extraction do not compromise feed quality or impose on EU restrictions for feed additives.

This process is also not completely efficient as noted by a 4.7% salt recovery detected gravimetrically. However, given that this was only one quick pass through a microfilter, a 4.7% recovery was considered to be a valuable desalination procedure. It is possible that a further refinement in process engineering could improve on the percentage of salt recovery.

Secondary research on this desalination technique considered the requirement for a filter, flow rate and recyclability of the alginate gel, the most expensive component of the process. Filter porosity, gelation and divalent calcium ion inclusion and liquid volumes were identical in all experiments, with variable seawater ion concentrations. It was discovered that the ionic sodium and chloride content of gravity separated saline from gelled seawater was a more effective mechanism of desalination than using gel filtration separation. There was a 2.38 g reduction of sodium ions in 16 l of full strength seawater from 10.83 g to 8.45 g and a 2.38 g reduction in chloride ions from 16.37 g to 13.99 g using gel filtration. This compared to a 2.63 g reduction of sodium ions in 16 l of the same batch of full strength seawater from 10.83 g to 8.2 g and a 3.45 g reduction in chloride ions from 16.37 g to 12.92 g using gravity gel separation. These results could be due to the physical movement of the salt-gel complex through the peristaltic pump, pipes and filter where ionic bound sodium and chloride ions become un-bound and returned back into solution. These findings reduces further the complexity of the desalination process as a partial desalination process has been developed entirely using alginate as a complete replacement for any filter technology.

The following research focus considers the recyclable properties of the alginate. Alginate salt complexes can exist both as a sodium (water soluble) and calcium (water insoluble). Exploitation of the reversible interchangeable facet of alginate uses the same chemistry process to extract alginate from macroalgae, namely sodium carbonate and calcium chloride which are both inexpensive products of the solvay process. Spent water insoluble calcium alginate which has partially extracted saltwater ions from saline solution can be captured by density gradient desalination. Practically this occurs by flocculation and removal as syphoning the surface saline layer from the reciprocal vessel. The remaining gel is able to be filtered and dried at ambient temperature, pressure and humidity. Calcium ions in recovered alginate are then able to be interchanged via low temperature incubation with sodium carbonate. The resulting water soluble gel renders higher density insoluble salt-carbohydrate complexes which quickly separate out of solution also via flocculation. This final process removes salt and leaves a purified sodium alginate gel for secondary use as a vehicle for desalination. Recycled alginate also has the capacity to act in the same desalination process. The research has identified a dual factor approach:

- Functions at different salinities between full strength seawater and freshwater
- Is able to recycle the functioning vector of alginate

5.5 Conclusions

An experimental approach to modify the ionic structure of seawater for increased ionic filtration efficacy has been developed. This research has defined a 4.7% salt recovery of 33 ppt seawater demonstrated gravimetrically in comparison to de-ionised water. A 16.8% reduction in Na^+ and a 20.6% reduction in Cl^- ions following the gel desalination process has been achieved. Ionotropic gelation of sodium alginate with calcium chloride in artificial seawater at a range of salinities between 0 and 33 ppt has been demonstrated in conjunction with analysis of seawater ions prior and post gelation. Salt removal from artificial seawater at a range of salinities has been achieved. Desalination efficacy is increased at higher rather than lower salinities.

6 Integrated waste technologies

6.1 Introduction

In addition to light and CO_2, algae require water and nutrients. All of these components are generally found in treated or untreated livestock wastewater, and they can be recycled to conserve resources as well as reduce algal culture medium cost (Ayala and Vargas, 1987). Microalgae have been cultured on several waste materials. Capture and removal of both nitrogen and phosphorus from industrial or manure wastes' converts them into proteins within microalgae cells, with simultaneous synthesis of other carbohydrates and lipids.

6.1.1 Nutrients from waste material

Dairy manure effluent has been successfully used as microalgae nutrients by several authors' (Mulbry *et al.* 2005 & 2008; Wang *et al.* 2010; Wilkie and Mulbry, 2002; Kebede-Westhead *et al.* 2006).

Brewery effluent waste comprises organic compounds such as proteins, phosphates, ammonia and nitrate (Raposo *et al.* 2010). Removal of organic load from brewery effluent by autochthonous flora isolated from the same effluent, showed a reduction of between 13-15% COD, 18-27% BOD and *Chlorella vulgaris* cultured in 1:2 diluted effluent could remove 139 g nitrogen^{-1}/kg biomass^{-1}/day and 32 g phosphate^{-1}/kg biomass^{-1}/day (Raposo, 2010). The co-culture of microalgae and yeast has been studied for aquaculture feed (Cai *et al.* 2007) and fine chemical production (Dong and Zhao 2004). Authors' have reported the application of culturing microalgae on spent brewery yeast for enhanced DHA production (Ryu *et al.* 2013), and simulated brewery effluent for brewery wastewater treatment and biomass production (Mata *et al.* 2012). The symbiosis between a culture of the oleaginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* was shown to enhance lipid production from industrial wastes (Cheirsilp *et al.* 2011).

Similarly, Anaerobic Digestate (AD), is a rich source of nitrogen and phosphate which has been identified as a potential feedstock for microalgae growth. Nitrogen is predominantly present as ammoniacal nitrogen rather than nitrate in

anaerobic digestate, consequently can be utilised by microalgae, as evident by significant reduction in TN and TP (Wang *et al.* 2010; Wilkie and Mulbry, 2002). *Chlorella vulgaris* is partially resistant to ammoniacal nitrogen, where as some other species have reported toxicity (Wang *et al.* 2010). NH_4^+ is a preferential ammonia source and free ammonia NH_3 is toxic at higher concentrations. The rate at which photosynthesis is limited by 50% due to free ammonia toxicity (IC_{50}) is 0.6 mg/l in *Chlorella ellopsoide* (Mosier, 1978), and 20 mg/l in *Dunaliella tertiolecta* and *Scenedesmus obliquus* (Azov & Goldman, 1982).

6.1.2 **CO_2 from waste coal flue gases, and steel slag**

Phototrophic microalgae *Chlorella spp.* can convert solar energy to chemical energy with efficiency of 10–50 times greater than terrestrial plants, and CO_2 is utilised as a carbon source by microalgae during photosynthesis (Khan *et al.* 2009; Richmond, 2000). Several authors' have reported that microalgae can grow faster with increased concentrations of CO_2 in comparison to delivery of atmospheric concentrations of CO_2 (Lam *et al.* 2012). Levels of CO_2 between 5% and 20% have been found to be beneficial to microalgae growth and produce more biomass with 10% being the optimal concentration (Tang *et al.* 2011). However, with CO_2 levels in excess of 20% carbonic acid formation lowers pH and inhibits microalgae growth (Tang *et al.* 2011).

The biggest fossil energy resource available is coal, reserves of which are estimated to be sufficient for over 200 years at present rates of consumption (Khan *et al.* 2009). Coal flue combustion gases for non oxy-fired combustion power plants range between: 70-82% (N_2); 10-25% (CO_2); 4-10% (O_2); 50-1000 ppm (NO_x) and 50-500 ppm (SO_x) (Maeda, 1995; Brown, 1996; Nagase, 2001; Doucha, 2005; Hutson, 2008; Kaštánek, *et al* 2010; Chiu, 2011; Morais, 2011; Moheimani, 2013). Efficacy of allocation of coal combustion flue gas to microalgae culture and resulting growth is hypothesised to be dose related. Though the variable constitution of coal flue combustion gases including NO_X and SO_X are known, the effect of dissolution of coal flue gases into water, and microalgae tolerance to modified pH in solutions thereof is less understood. Despite being good sequesters of CO_2, many microalgae species, but not all,

need a neutral pH range of between 6 and 9 for optimal growth. Deviation outside of this pH range could be potentially detrimental for their survival, as too can be extremes of temperature which could occur from injection of hot flue gases. In the marine environment, the diatom *Phaeodactylum tricornutum* demonstrated an alkaline competitive advantage over *Dunaliella tertiolecta* which was unable to grow when the pH exceeded 9.3 (Goldman, Riley and Dennett, 1982). *Chlorella vulgaris* was found to be able to tolerate a pH increase from between 5 to 6.4 with a compromised slower growth rate, but tolerance and survivability under conditions of 100% CO_2 delivery (Widjaja, Chien, and Ju, 2009). Damage to photo system II via acidification of the stroma and inhibition of the enzymes responsible for CO_2 assimilation has been proposed as a potential mechanism for reduced growth under conditions of higher CO_2. This is manifested by experiments with constant pH of 5.5, measuring the internal cellular pH of *Chlorococcum littorale* with phosphorus-31 nuclear magnetic resonance spectroscopy (Iwasaki, Kurano and Miyachi, 1996). Coal fired power station flue gases dissolved into water can have a pH of between 2 and 4. This is a major constraint to using microalgae as a vehicle for carbon dioxide sequestration from coal flue gas.

To remediate this concern, whilst still utilising waste carbon emissions in flue gas, reduced integration of coal flue combustion gases can occur with lowered inclusion into a microalgae culture by dilution with air or nitrogen prior to injection. Alternatively, a higher emission concentration of flue gas can be used, injected at periodic intervals and potentially adjusting the pH of the culture solution by chemical neutralisation. As flue gases are emitted constantly during the course of a combustion cycle, any reduction in their total volumetric inclusion into a microalgae culture or periodic interval dosing both have the effect of reducing the total integration capacity (unless gases are stored for later use). For the purposes of biological sequestration of carbon, this reduces the efficacy of using microalgae as a biological remediation agent; however, adequate provision of nutrients and CO_2 ensures microalgae propagation and culture continuity. In summary, the CO_2 requirement of microalgae should be matched by the supply of CO_2 from waste gases, rather than the totality of flue combustion gases

delivered to a microalgae culture. The implications of this are that it is important to complement culture volume with gas delivery. Ultimately, this integration is complexed further by the acidic chemical characteristics of NOx and SOx gases, gaseous diffusion and bubble size, and the time taken for bubbles to pass from the diffuser into solution (gas to liquid assimilation) and finally gaseous release losses at the liquid-gas culture surface.

Steel slag, a residual waste product (or by-product) of steel production, contains a complex mixture of alkali-earth silicates and alumina-silicates formed at high temperatures. Source dependent, steel slag contains between 30-45% calcium oxide. The steel making industry in the United States generates 9-16 million metric tons of steel slag every year. Civil engineering applications utilise this resource, but 15-40% is never reutilised, stockpiled in steel plants and eventually landfilled at slag disposal sites (Yildirim and Prezzi, 2015). *Chlorella sp.* grew significantly better under enrichment with steel slag solution at 80% (v/v) than under synthetic seawater alone (Nakamura, Taniguchi, Okada, and Tokuda, 1998). Additionally, marine diatoms grew better under enrichment conditions than Chlorophyte species (Nakamura *et al.* 1998). The alkali inducing nature of steel slag has particular merit as a neutralisation strategy in combination with coal flue combustion gases and microalgae growth on waste nutrients.

Chemical composition of steel slag depends both on geological rock sources and the industrial process conditions. Consequently, there are variable composition of the major compounds, namely, oxides of silica, calcium and other metals. One of the major components of steel slag is calcium oxide which is highly soluble in water, forming calcium hydroxide. Calcium hydroxide and other dissolved metal ions from steel slag, are able to react with nitrogen and sulphur derivatives from post-combustion impurities in coal flue gases in a plethora of aqueous chemical reactions as flue gases dissolve into water. However, because steel slag has such a high composition of calcium oxide, two predominant chemical reactions are highlighted as being a significant component of the NO_X and SO_X de-acidification process.

Equation 6-1: SO_x neutralisation $Ca(OH)_2 + H_2SO_4 \longrightarrow 2H_2O + CaSO_4$

Equation 6-2: NO_x neutralisation $Ca(OH)_2 + 2HNO_3 \longrightarrow Ca(NO_3)_2 + 2H_2O$

The two products of equations 6-1 and 6-2 calcium sulphate, otherwise known as plaster of paris or gypsum and calcium nitrate, otherwise known as fertiliser; both have implications in batch culture of microalgae. $CaSO_4$ is not readily soluble in water and the reaction will form a precipitate denser than the solution. $Ca(NO_3)_2$ is water soluble and will provide an additional soluble source of nitrogen macronutrient for microalgae. Another reaction which occurs is the formation of calcium carbonate from gaseous carbon dioxide reacting with calcium hydroxide. However, carbon dioxide also dissolves as intermediary bicarbonate ions in solution which microalgae are able to assimilate via photosynthesis. Of the NOx components within flue gas, nitric oxide forms a high volume. Upon dissolution, NO forms nitrate or nitrite in water which can be utilised by microalgae. By contrast SOx converts to bisulphate, sulphite and sulphates, dependent on pH. The formation of highly oxidative intermediary chemical reactions can occur via the production hydroxyl radicals, superoxide anions and hydrogen peroxide. Such compounds are detrimental for the survival of microalgae and can cause lipid peroxidation, chlorophyll and pigmentation damage.

6.2 Aims and Objectives

The aim of this research work was to assess the growth of microalgae on the waste nutrients of anaerobic digestate, brewery waste and organic dairy manure. Aims also evaluated the synergistic effects of washed steel slag as a neutralisation agent with dissolved simulated coal flue combustion gases and their contribution to the growth profile of *Chlorella vulgaris*. Specifically, the objectives of this research were to investigate how animal manure, spent brewery yeast waste and anaerobic digestate nutrients in autoclaved and non-sterile growth media affected the growth of *Chlorella*. Microalgae growth and biomass production were determined quantitatively by microscopy, total carbon analysis and gravimetrically. Additionally, growth characteristics with coal flue gas CO_2

(which would otherwise be categorised as an additional atmospheric CO_2 emission burden), were offset against the detrimental effects of NO_X and SO_X acidification via pH neutralisation using washed steel slag solution. Hence, the research objectives determined the potential of a waste steel slag industrial product to remediate supply of acidic coal flue combustion gases to microalgae culture, simultaneously augmenting their provision of CO_2.

6.3 Methods

A UK wild type strain 4TC 3/16 was isolated from the natural environment in Bedfordshire and identified as *Chlorella vulgaris* by PCR at Algenuity. The species was maintained in Tris-Acetate-Phosphorus media (TAP) (Harris, 1989) and banked cultures were preserved long-term by cryopreservation. Experiments were conducted in sterilised transparent 20 l carboys (Thermo Scientific, Nalgene) with unfiltered aeration at 2 l/min using 4 mm airline and spherical aquarium 50 mm airstones. All nutrient treatments included an additional mineral micronutrient supplement (Kropat *et al.* 2011). Organic nutrient medium were diluted to 0.5% (w/v). The liquid phase of anaerobic digestate is often characterised by high turbidity and ammonia content, which is not reduced during digestion (Noike *et al.* 2004). Due to increased turbidity and corresponding reduced light penetration into the culture, dilution of the medium was a pre-requisite to inoculation within the microalgae culture. The anaerobic digestate dilution concentration used here was 0.5%. The 0.5% dilution concentration reported in this study is equally comparable with the work of Marcilhac, Sialvec, Pourchera, Ziebala and Bernet, 2014, whom used four dilutions of anaerobic digestate 1.0, 0.7, 0.3 and 0.17% (v/v), corresponding to an optical density at 680 nm (O.D.) of 1.3, 0.9, 0.5 and 0.2 mixed with trace nutrients to grow *Scenedesmus sp.*

Dark colouration of diluted dairy manure also presented similar considerations of turbidity and light attenuation as the anaerobic digestate samples, whilst brewery waste media was clearer with more suspended solid particulates in the form of yeast cells. The clarity of this media fluctuated depending on the extent of mixed suspended yeast cells within the solution. Basal nutrient formulations were

analysed by ion chromatography (Dionex, Thermo Scientific). After acclimatisation within the experimental facility, experiments were maintained at 25°C and 200 µmol/m^{-2}/s^{-1} continuous cool-white fluorescent light for 9 consecutive days. Daily samples were extracted for analysis of total inorganic, total organic, total carbon, total nitrogen, (Shimadzu TOC/N) pH, & cell counts, using sterile apparatus and due diligence to avoid cross contamination. On day 9 cultures were harvested by centrifugation (Sorvall) and lyophilisation (Christ) and measured gravimetrically. Duplicated and averaged values of all experiments were used.

6.3.1 **Media preparation and microalgae inoculation**

Anaerobic digestate, brewery waste and organic dairy manure were obtained from different sources, but prepared as media using similar methodologies. 15 l of liquid anaerobic digestate from commercial food waste was kindly donated by Fernbrook Bio Ltd, Kettering, Northamptonshire, UK. 15 l of liquid brewery waste (*Saccharomyces cerevisiae* and malted beer waste) was kindly donated by White Park Brewery, Cranfield, Bedfordshire, UK. 15 l of liquid organic dairy manure was kindly donated by an EU organically certified dairy farm, Church Farm, Shellingford, Oxfordshire, UK. Manure was collected from an open earth slurry pit receiving liquid manure from the organically fed cattle sheds. F/2 complete media preparation (Varicon Aqua) was used at a concentration of 1ml/l in de-ionised water, according to manufacturer's specifications.

In all nutrient formulations, suspended solids were removed using coarse filtration. Organic nutrient formulations replaced synthetic fertilisers at a final media concentration of 0.5% (v/v) diluted un-sterilised or 0.5% (v/v) diluted sterilised in de-ionised water (Merck Millipore). Axenic microalgae at exponential phase, approximately 10^7 cells/ml was inoculated 1:20 (v/v) into autoclaved or crude media dilutions using 20 l carbouys. Nutrient composition derived from a PAS110 Certificate of Analysis of anaerobic digestate is shown in Table 6-1.

Table 6-1 Supermarket food waste derived Anaerobic Digestate (AD) composition PAS110 Certificate of Analysis

Parameter	Units	Result	Method of test
pH	-	8.7	BS EN 13037
Dry matter	kg/DM/t	42.2	BS EN 14346
Loss on Ignition	kg/OM/t	26.5	BS EN 15169
Total Nitrogen (N)	kg/N/t	5.9	BS EN 13654-2 (Dumas)
Ammoniacal Nitrogen	mg/kg	5226	SOP Z/004 (soluble in KCl)
Total Phosphorus (P)	mg/kg	462	BS EN 13650 (soluble in water)
Total Potassium (K)	mg/kg	2167	BS EN 13650 (soluble in water)
Total Magnesium	mg/kg	58.2	BS EN 13650 (soluble in water)
Total Sulphur	mg/kg	214	BS EN 13650 (soluble in water)
Water soluble Cl	mg/kg	2595	BS EN 13652 (soluble in water)
Water soluble Na	mg/kg	1503	BS EN 13650 (soluble in water)

6.3.2 **Steel slag and simulated coal flue combustion gases**

Health and safety was a prime consideration in the experimental design as sulphur dioxide and nitrous oxide gases have severe toxicology and death risks. Mitigation therefore implemented a simulated flue gas experimental protocol rather than genuine coal flue captured gases. Experiments were conducted in a separate research facility which housed fuel combustion operations. In accordance with health and safety legislation compliance, experimental conduct within this facility was a pre-requisite to the fruition of experimental endeavours. The air exchange differential between inside and outside the facility was more liberal than conventional buildings, as too were associated daily temperature fluctuations. To remediate this, and provide the microalgal cultures with a known constant temperature for the duration of the experiments, pilot trials were conducted to provide uniform temperatures. The cultures were contained within 2 l Erlenmeyer flasks. These were housed within a 120 litre plastic capacity container approximately 90 cm long by 40 cm wide and 30 cm deep. On the base of this container a 10 cm deep glass wool with an aluminium foil lining was fitted

to prevent thermal conductivity losses from the floor. Above this a reptile mat was used to ensure delivery of constant temperature by application of continuous low-grade conductive and convective heat. The reptile heat mat was enclosed in a laminated waterproof plastic. The plastic container holding the 12 Erlenmeyer flasks acted as a bund in the event of water loss during sample collections at periodic intervals. Figure 6-1 shows the attained constant temperature of 27°C $_{\pm}$1°C which was marinated for 3 days in a fluctuating external ambient temperature by the apparatus. The plastic box housing, insulation, reptile mat and thermal retention capacity of the microalgae culture media acted to maintain a local temperature despite external ambient fluctuations due to an exposed environment. Light provision used reflective aluminium foil surrounding the experimental facility and 30W, 90 cm long fluorescent tube lights which emitted plant growth light spectrum in order to simulate daylight. Continuous light of 230 $\mu mol.m^{-2}.s^{-1}$ and diluted anaerobic digestate batch nutrient (Table 6-1) at 0.5% (v/v) was supplied by Fernbrook Bio Ltd. Nutrients included an additional mineral micronutrient supplement (Kropat *et al.* 2011).

Gases from 3 tanks were mixed in proportions to simulate coal flue combustion gas via a mass flow controller. A representative simulated flue gas was achieved for microalgae growth trials. Gas tanks included 100% CO_2, 1.3% SO_2 (air balance) & 1000 ppm NO_2, 1000 ppm NO (N_2 balance), as shown in Table 6-2.

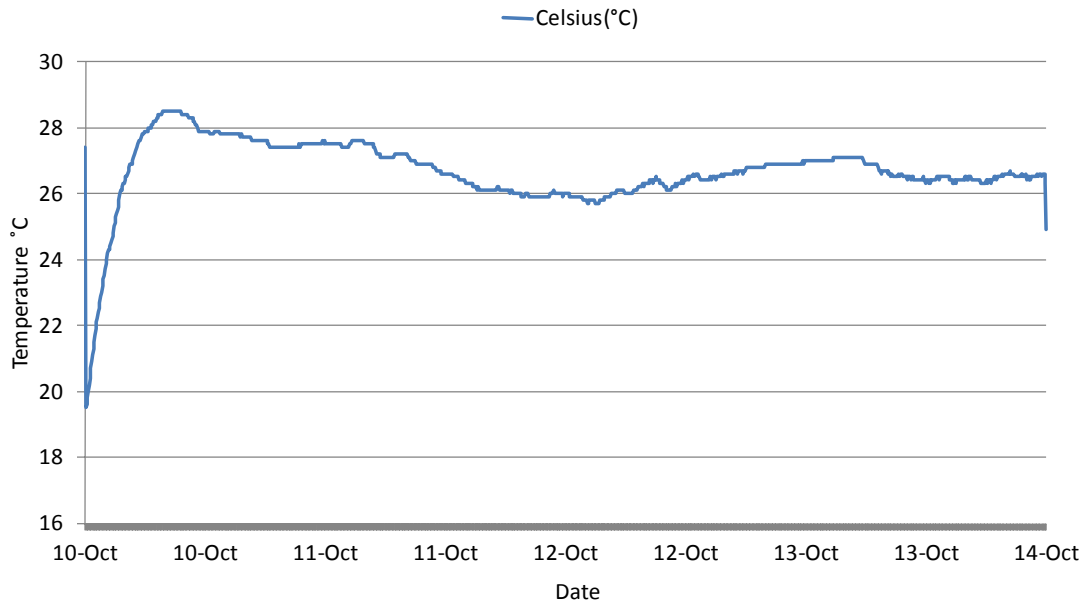


Figure 6-1 Temperature log of 1.5 l of water in an Erlenmeyer flask over a 96 h test period heated by a reptile mat at 20˚C ambient temperature

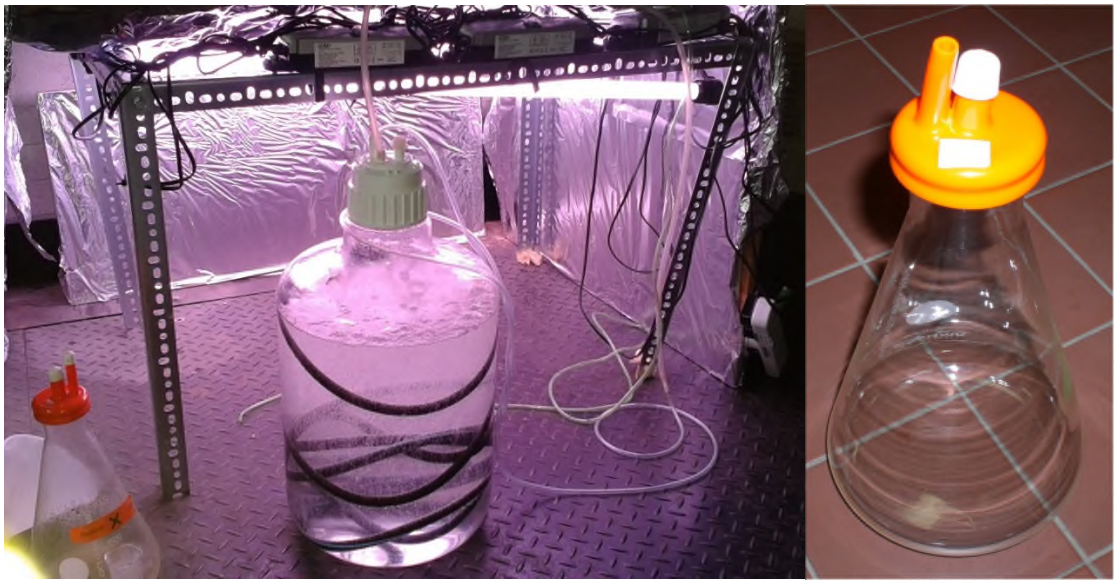


Figure 6-2 Experimental test facility of coal flue combustion gas trials.
Left: lighting arrangement and 20 l carbouy with *Aero tube* delivered gases. Right: top view of 2 l Erlenmeyer flask with silicon rubber dual port airtight assembly.

Table 6-2 Simulated coal flue combustion gas mixtures and composition

		Final constituent	**Final composition**
Gas Utilised	**Percentage (%)**	**NO_X**	700 ppm
100% CO_2 gas	15	**SO_X**	309 ppm
1.3% SO_2, Air bal.	50	**CO_2**	17.63 %
NO_2 1000ppm, NO 1000ppm, N_2 bal.	35	**O_2**	5.25 %
		N_2	77.08 %

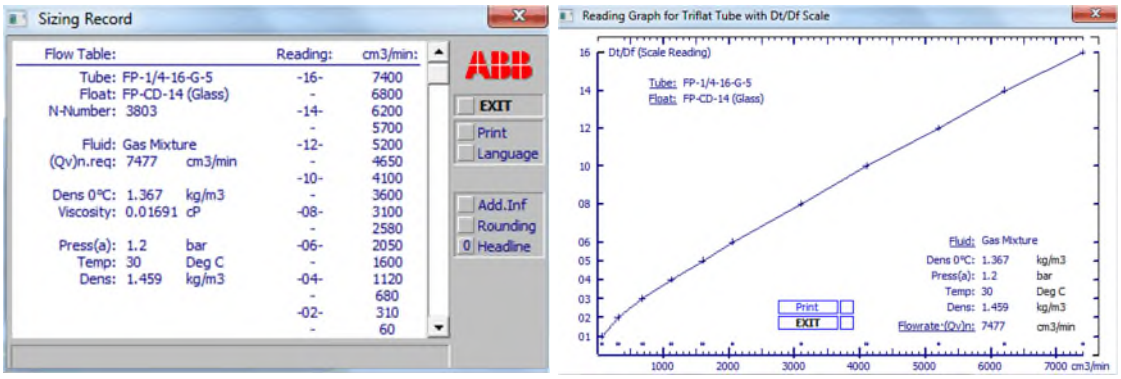


Figure 6-3 - Rotometer calibration according to tubular specifications, float material and float size, gas composition, viscosity and temperature.

Gases could be monitored both by computer controlled software for the mass flow controller and also physically by the use of a rotometer. Calibration of the rotometer was according to manufacturer's specifications of the float mass and physical dimensions of the tube and float at operational temperatures, gas densities and corresponding viscosities, Figure 6-3. Following mass flow control mixing of simulated flue gas, NOx and SOx gases were supplied to *Chlorella vulgaris* microalgae culture at a rate of 1.2 l^{-1}/min or 0.1 l^{-1}/min via 12 x 2 l conical flasks, at 20°C. A carbouy (Figure 6-2) together with an *aero tube* designed for dissolved oxygen delivery to aquaculture fish was used for the production of small bubbles < 1 mm diameter to test for acidification of de-ionised water with exposure of flue gas. Gaseous diffusion to 12 x 2 l Erlenmeyer flasks (Figure 6-

2) occurred via 3 cm x 0.8 cm sized aquarium spargers submerged at the base of flasks. Gas flow rates were measured with a rotometer and determined at point of delivery to each of the 12 Erlenmeyer flasks to ensure equal flow. Air flows were arranged in series with silicon glued dual port inlets and outlets to flasks to prevent systemic gaseous losses. Gases were delivered into microalgae cultures at equidistant time intervals for 3 h per day in order to comply with occupational health, safety and environmental legislation, and work schedules of trained technical staff. Additionally, this time period resulted in a diurnal pH reduction which was able to be neutralised by means periodic inoculation with 1% steel slag wash. Post -delivery, gases were vented to the atmosphere in accordance with safety procedures.

Steel slag was kindly donated by LaFarge Tarmac limited. Fine grade <1 mm particulate size was obtained by sieving <10 mm grade (Figure 6-4). 20 l of a fine particulate sized 1% (w/v) steel slag was dissolved in de-ionised water to obtain a concentrated stock culture of pH 11.7. Understanding the elemental composition dissolution of steel slag into de-ionised water was a pre-requisite to being able to comment on the subsequent chemistry of the reaction of washed steel slag solution with microalgae culture media injected with simulated coal flue combustion gases. Therefore, an experiment was conducted to test for the presence of metal oxide ions known to be present in steel slag, which elemental ions and at what concentration they dissolved into de-ionised water. Serial dosing and washing of 1% (w/v) mixture of steel slag in de-ionised water for 1 h determined the chronological ion dissolution from steel slag particles. After one hour, the supernatant was extracted, filtered, and the subsequent 1% slag solution was re-formed from the previously hydrated slag, this process was repeated 10 times. Post sedimentation of micro-particulate matter, ionic solutions were analysed by atomic absorption spectroscopy (AAS), testing for the 7 metal ion constituents known to occur in steel slag namely calcium, magnesium, iron, chromium, aluminium, zinc and manganese.

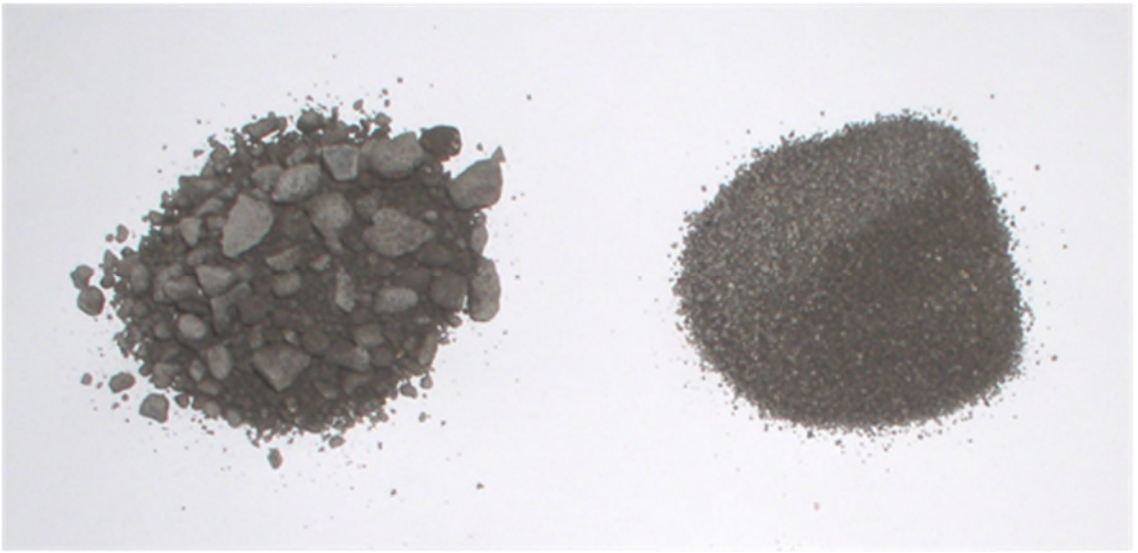


Figure 6-4 Crushed (left) and Sieved (right) Steel slag courtesy of LaFarge Tarmac

1% (w/v) of <1 mm diameter particulate steel slag was mixed into 20 l of de-ionised water and left to sediment for 48 h. Extracted particulate free surface water was used to periodically neutralise 1.5 l per flask batch cultures of *Chlorella vulgaris* at 10^4 cells/ml^{-1} sparged with simulated flue gases to pH 7. 50 ml samples were analysed at semidiurnal intervals for pH and cell counts. EDX analysed elemental composition of crude steel slag prior to washing. The trials were repeated in a 20 l carbouys, initially with a 5 l volume of *Chlorella vulgaris* at 10^4 $cells^{-1}$/ml sparged with simulated flue gases.

6.4 Results

6.4.1 Nutrient Media and growth trials

The nutrient composition of waste-based media and synthetic growth media was analysed using ion-chromatography.

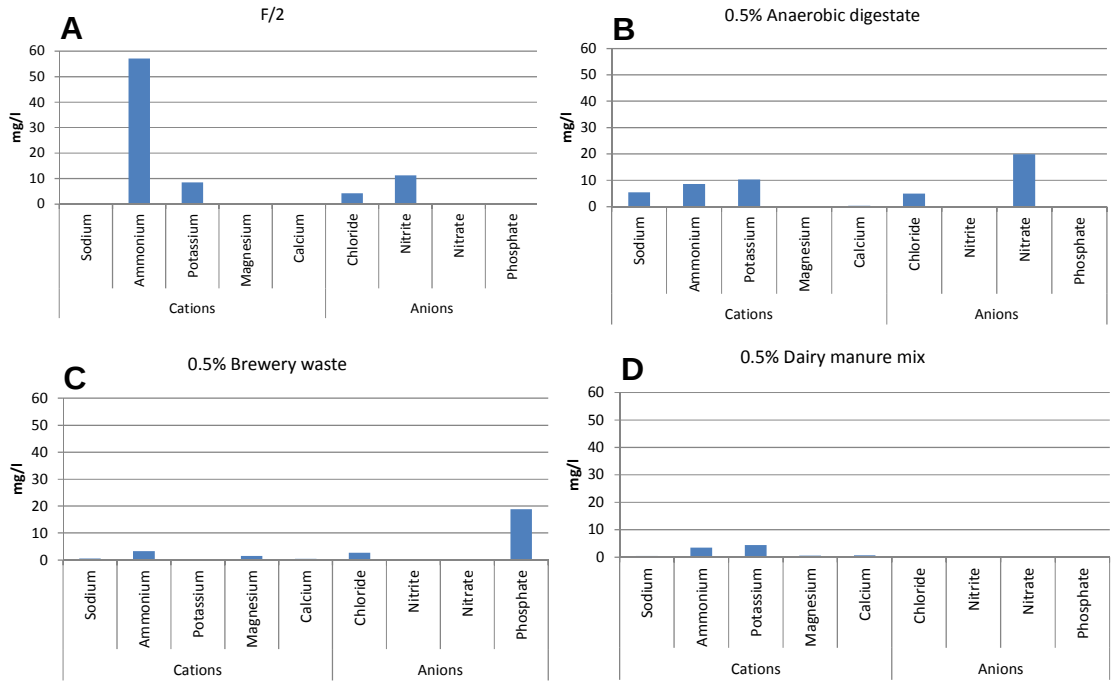


Figure 6-5 Ion chromatography nutrient composition of basal algae nutrients. A F/2 synthetic, B 0.5% Anaerobic digestate, C 0.5% Brewery waste, D 0.5% Dairy manure.

Anaerobic digestate and brewery waste at 0.5% (v/v) contain between 15-20 mg/l nitrogen and phosphorus respectively (Figure 6-5). Anaerobic digestate was the only source of nitrogen present in higher concentrations as nitrate rather than ammoniacal nitrogen (Figure 6-5). Dairy manure recorded the most dilute nutrient composition, with the synthetic media containing the highest concentration of ammoniacal nitrogen. Anaerobic digestate and brewery waste synergistically contained all of the major (N,P,K) macronutrients although with lower concentrations of nitrogen than synthetic media.

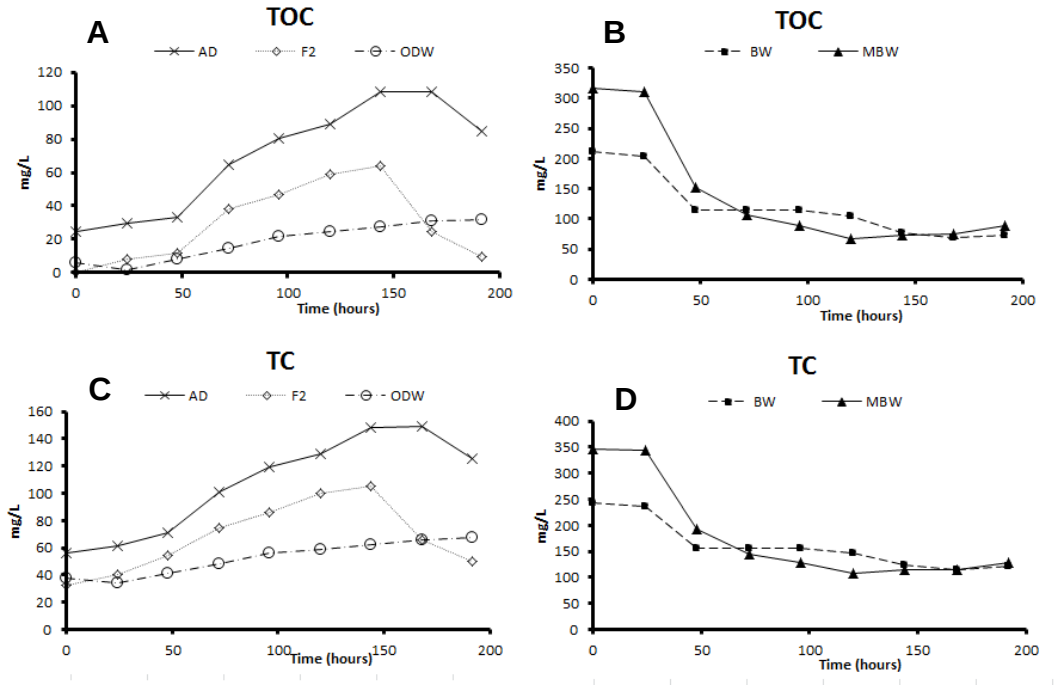


Figure 6-6 Autoclaved media growth trials (TOC, TC)

(A, B) Total organic carbon (TOC); (C, D) Total carbon (TC) of Chlorella vulgaris 4TC 3/16 using Anaerobic Digestate (AD), f/2 synthetic media (F2), Organic Dairy Waste (ODW), Brewery Waste (BW) and Mixed Brewery Waste (MBW).

Increased growth in autoclaved AD media was observed reaching in the region of 110 mg/l TOC (Figures 6-6 A). Maximum exponential growth rates were observed with sterilised AD media between days 2 and 3 post inoculation (Figure 6-6 A). Brewery waste yeast cells and associated dissolved alcohol had a high organic carbon composition which reduced from the range of 250-300 mg/l to 100-150 mg/l within 2 days post inoculation.

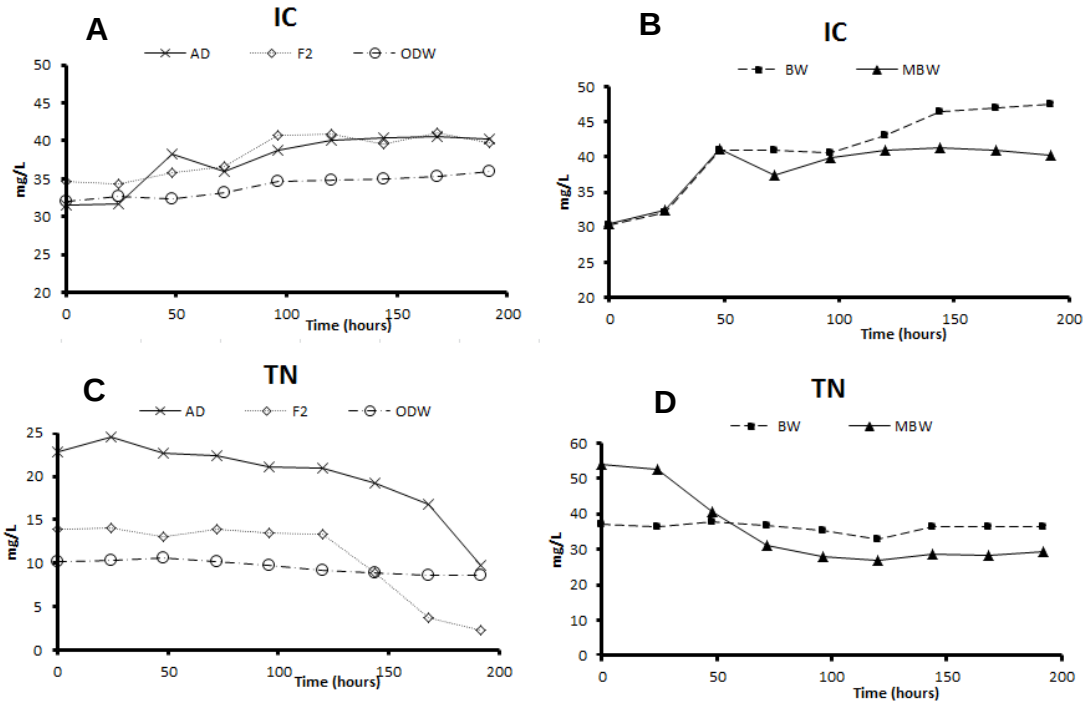


Figure 6-7 Autoclaved media growth trials (TN, IC)
(A, B) Inorganic carbon (IC), (C, D) Total Nitrogen (TN) of Chlorella vulgaris 4TC 3/16 using Anaerobic Digestate (AD), f/2 synthetic media (F2), Organic Dairy Waste (ODW), Brewery Waste (BW) and Mixed Brewery Waste (MBW).

Inorganic carbon, calculated by instrument analytical processes was determined by deduction of organic carbon from total carbon, measured between the ranges of 30-50 mg/l for all treatments over the 8 day sampling period. Batch nutrients were supplied at the beginning of the experiment during microalgae inoculation into the culture media. Therefore, any subsequent gains in total carbon could either be attributed to either cellular growth of microalgae, yeast or potentially bacteria (TOC); or inorganic (IC) atmospheric carbon acquisition from CO_2 during aeration. Sterile mixed brewery waste (MBW) nutrient media and brewery waste media (BW) were classified by higher and lower concentrations of mixed yeast cells respectively, analysed as both 100 mg/l higher compositions of carbon (Figure 6-6 B & D) and 15 mg/l nitrogen (Figure 6-7 D).

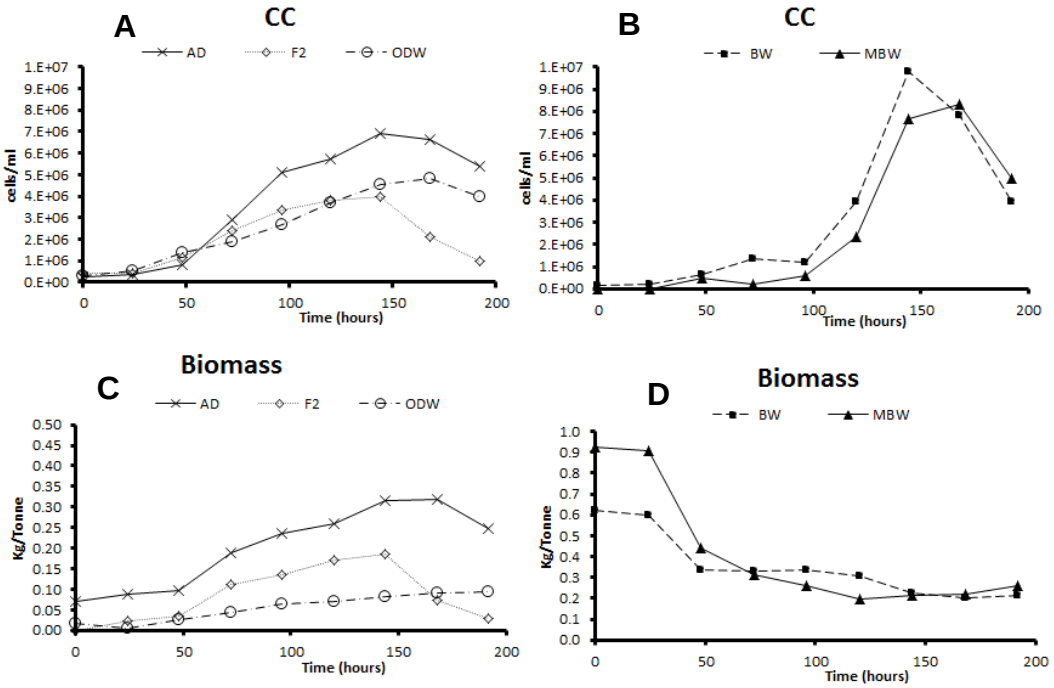


Figure 6-8 Autoclaved media growth trials (Cell count, Biomass)

(A, B) Cell count (CC), (C, D) Biomass autoclaved media growth trials of Chlorella vulgaris 4TC 3/16 using Anaerobic Digestate (AD), f/2 synthetic media (F2), Organic Dairy Waste (ODW), Brewery Waste (BW) and Mixed Brewery Waste (MBW).

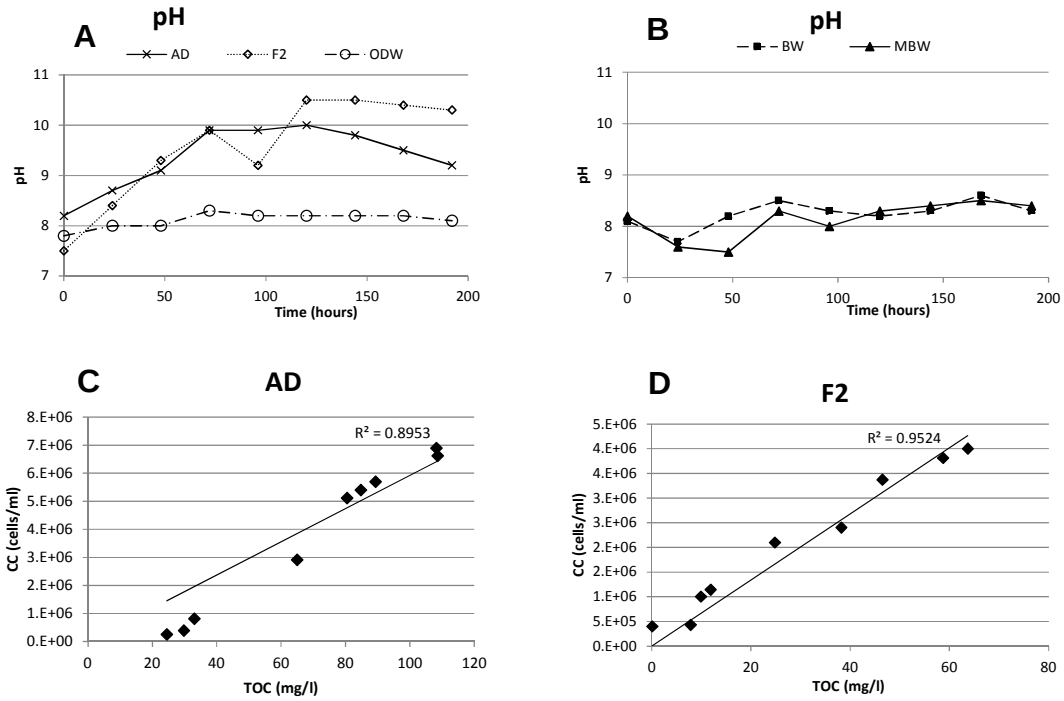


Figure 6-9 TOC & Cell count regression, pH of autoclaved media growth trials of *Chlorella vulgaris*
4TC 3/16 using Anaerobic Digestate (AD), f/2 synthetic media (F2), Organic Dairy Waste (ODW), Brewery Waste (BW) and Mixed Brewery Waste (MBW).

Figures 6-8 shows cell counts and biomass of samples. Light microscopy and total carbon analysis are a quantitative method of growth analysis in microalgae cultures which have been correlated by linear regression for sterilised AD and f/2 media (Figure 6-9 C&D) (r^2 > 0.89 & 0.95).

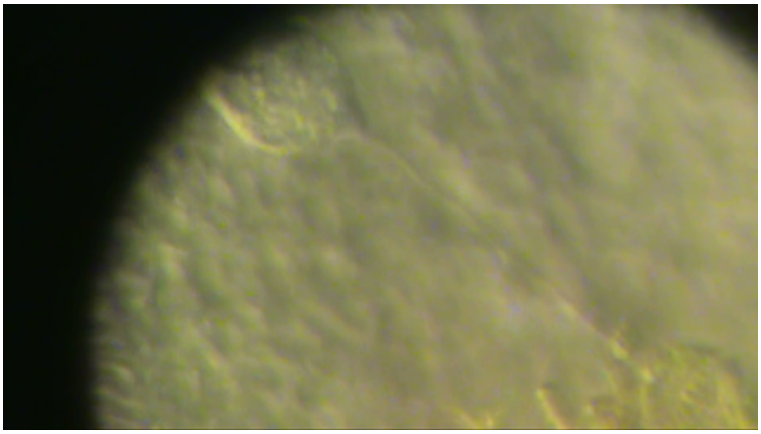


Figure 6-10 microscope image (x400) of Vorticella spp.
Zooplankton feeding on *Chlorella vulgaris* microalgae in non-sterile organic dairy manure media. Proboscis extending 'due south east' to flocculated microalgae in bottom right corner.

Non-sterile brewery waste and organic dairy manure media were contaminated by coccoid zooplankton protozoan parasites. This was evident 48 h post-inoculation of microalgal cultures (Figure 6-10). Zooplankton and parasitic contaminants actively preyed upon *Chlorella vulgaris* causing bioflocculation, and preventing viable cell counts. *Didinium spp*. have two rows of cilia and swim rapidly feeding exclusively on *Paramecium* unicellular ciliated protozoa. Rows of cilia were not visible to the naked eye via microscopy, but their motility was evident. Together with *Vorticella* protozoa (Figure 6-10), a consortium of living micro-life was encountered within unsterilized organic dairy manure which detrimentally fed on the microalgae causing colony collapse. The rapid contractile movement of the proboscis of *Vorticella* was viewed at approximately 60 second intervals with x400 magnification microscopy. This acted to dislodge the flocculated microalgae consortium which may act as a parasitic feeding strategy. In between contractile motions, the proboscis acted as a holdfast anchor point within the flocculated microalgae cells.

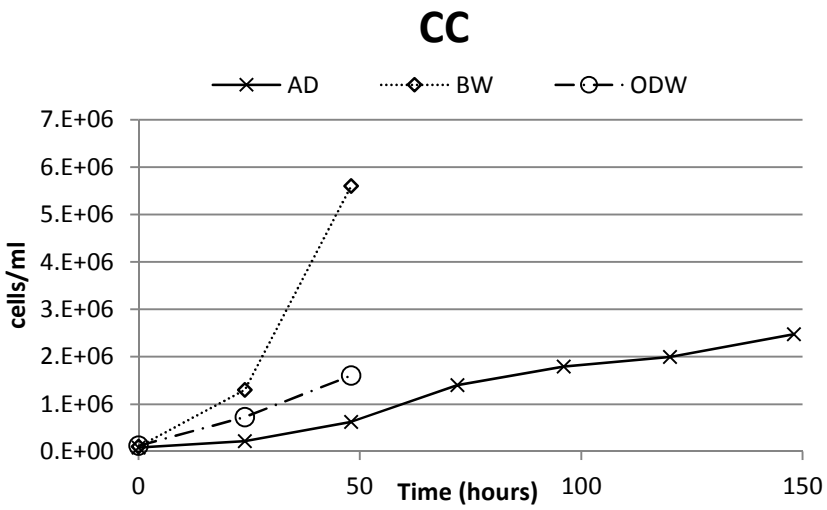


Figure 6-11 Cell counts of non-sterile brewery waste and organic dairy manure media 48 hours post inoculation, cell flocculation and death was evident due to microorganism competition.

6.4.2 **Industrial waste and simulated flue gases**

Steel slag and flue gases composition was evaluated by atomic absorption spectrophotometry and SEM-EDX analysis.

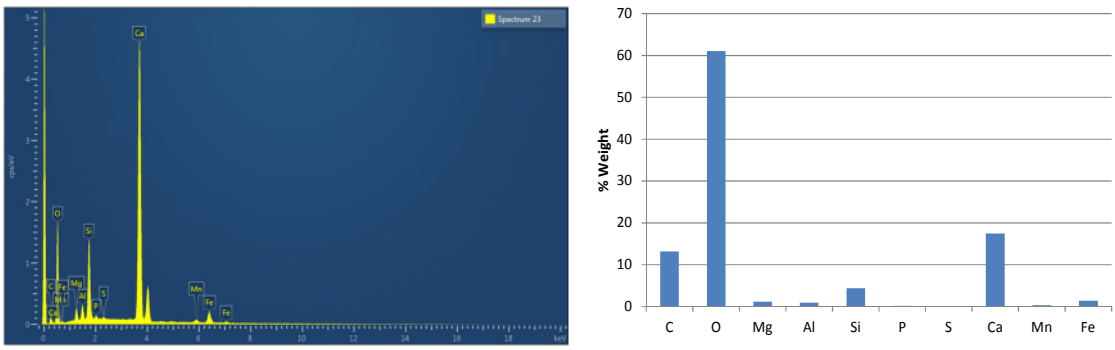


Figure 6-12 EDX analysed composition of steel slag, elemental composition % Atomic Weight

Steel slag contains calcium oxide and other metal oxides including magnesium, iron, chromium, aluminium, zinc and manganese (Figure 6-12). 1% steel slag solution washed in de-ionised water (Figure 6-13), analysed via atomic absorption spectrophotometry resulted in more leached calcium ions than other

metal ions, inducing high pH between 9 to over 11.5 at low concentrations of between 0.005% and 1% steel slag (w/v) in de-ionised water (Figure 6-14).

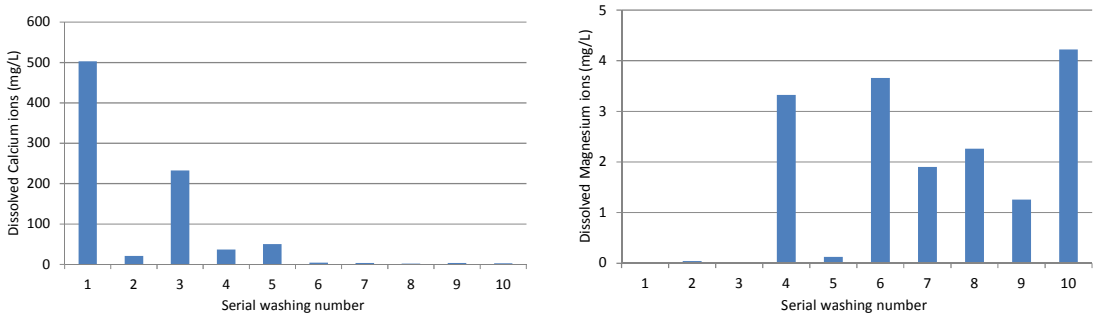


Figure 6-13 Serial washings of 1% (w/v) Steel slag in de-ionised water analysed with atomic absorption spectroscopy (AAS), Mg & Ca (shown in Figure 6-8) negative detection results for Fe, Cr, Al, Zn and Mn

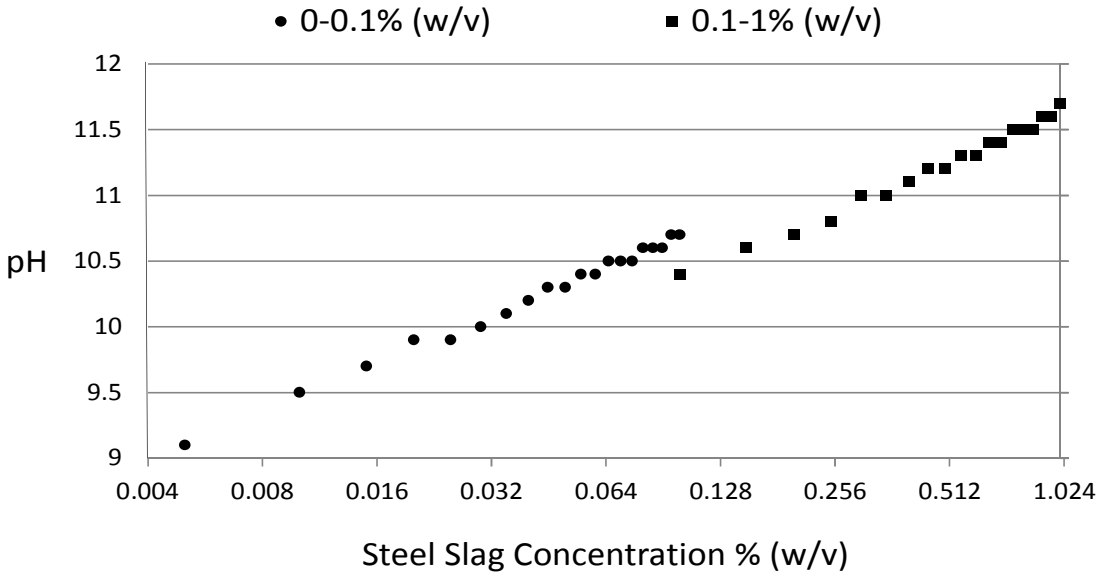


Figure 6-14 Steel slag solution pH at ranges between 0.005% and 1% (w/v). Two dilution ranges between 0-0.1% and 0.1%-1% were prepared.

20 l of de-ionised water exposed to 5h 30 min simulated flue gases recorded a pH of 3.4 (+/-0.5). This acidified water was retained in order to calculate pH neutralisation volumetric analysis with 1% steel slag wash.

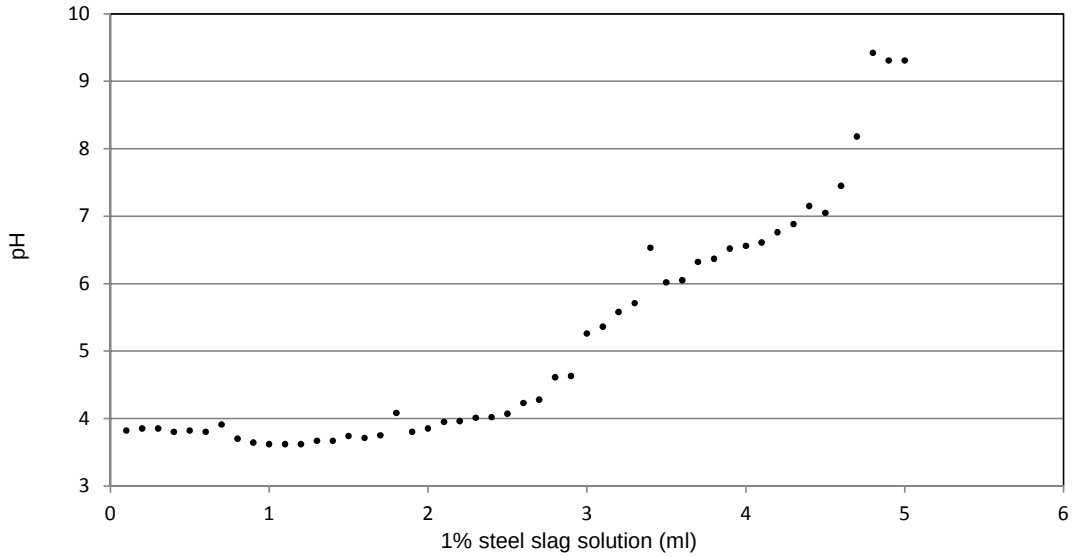


Figure 6-15 – Experimental determination of volumes required for neutralisation of 25 ml de-ionised water exposed to simulated coal combustion flue gas for 5 h 30 min (pH 3.4) with 1% (w/v) washed steel slag (pH 11.5)

Figure 6-15 shows the pH of successive dilution ranges of 25 ml volumes of de-ionised water exposed to 5 h 30 min simulated coal flue combustion gases with incremental volumetric inclusions of 1% steel slag wash. It was discovered that approximately 5 ml of 1% washed steel slag were required to neutralise 25 ml of flue gas water from pH 3.4 to pH 9 (i.e. (20 % volumetric dilution factor). Figure 6-16 shows the time oriented volumetric increase within 2 l conical flasks in response to the loss of 50 ml of solution for sampling and addition of 250-300 ml 1% washed steel slag solution to neutralise pH.

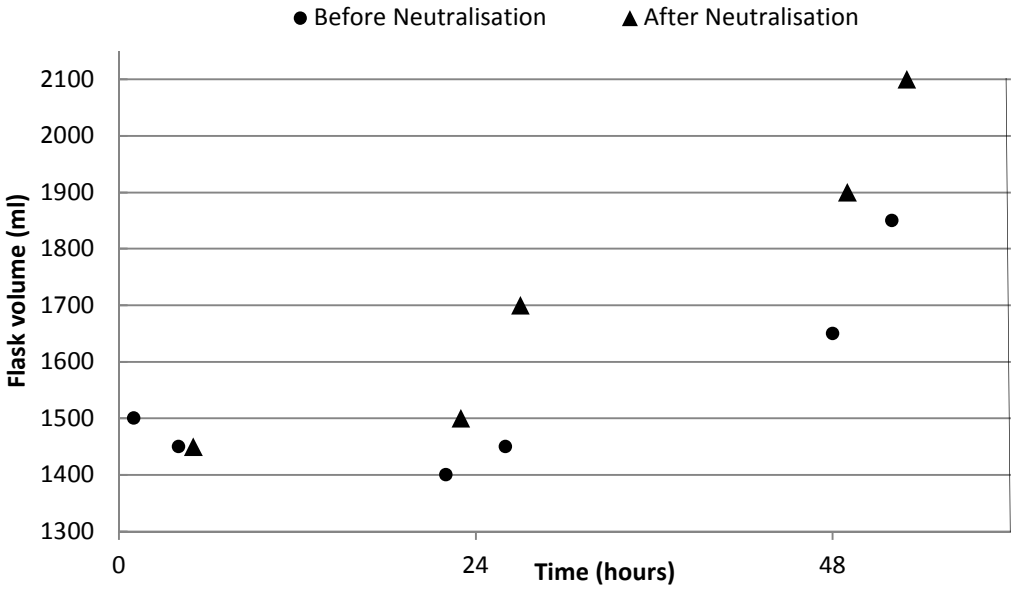


Figure 6-16 Incremental conical flask volume of de-ionised water exposed to simulated flue gases for 3 h per day with pH neutralisation dosing twice per day.

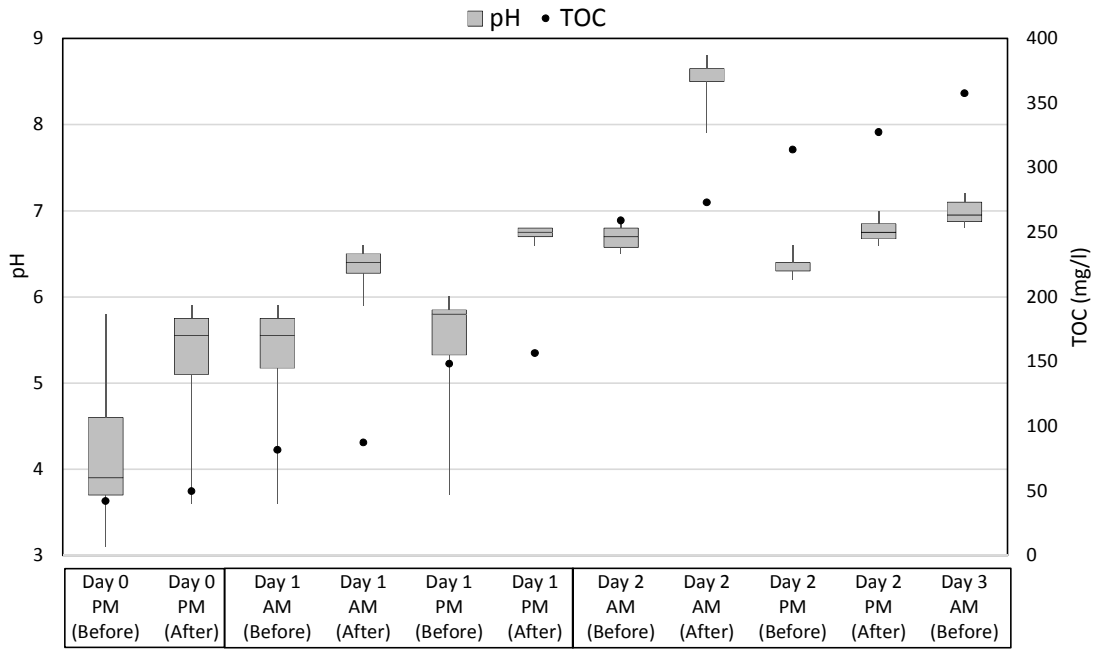


Figure 6-17 *Chlorella vulgaris* growth in a 20 l carbouy. pH changes with daily neutralisation reactions between washed steel slag solution and microalgae culture exposed to 3 h per day of simulated flue gas.
Daily samples measured total organic carbon.

Figure 6-16 and 6-17 show that a chronological neutralising dilution could be offset by the growth rate of the microalgae within the media.

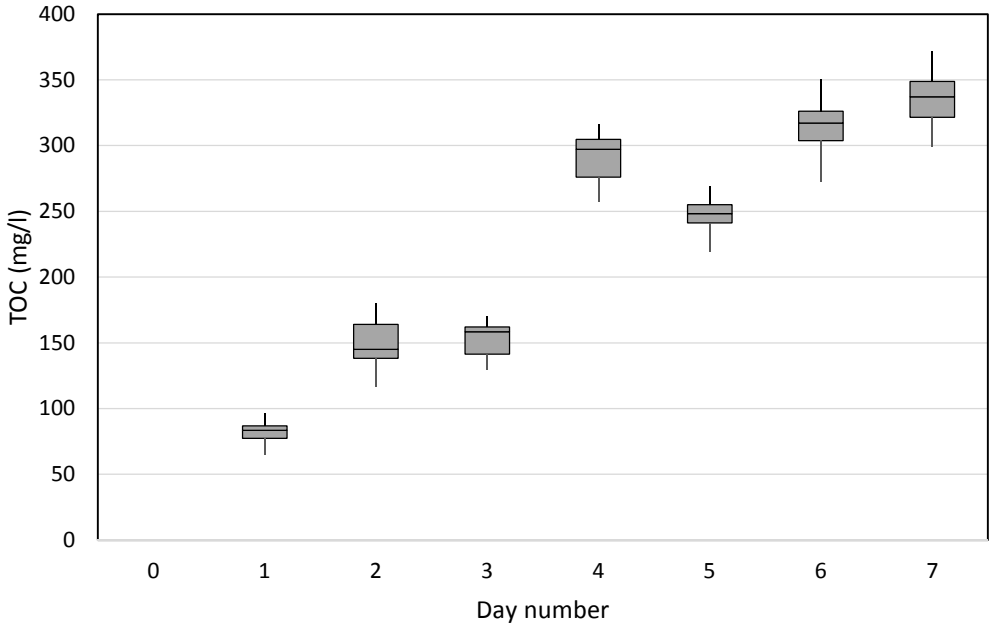


Figure 6-18 Total carbon (mg/l) of each 2 l flask at daily intervals

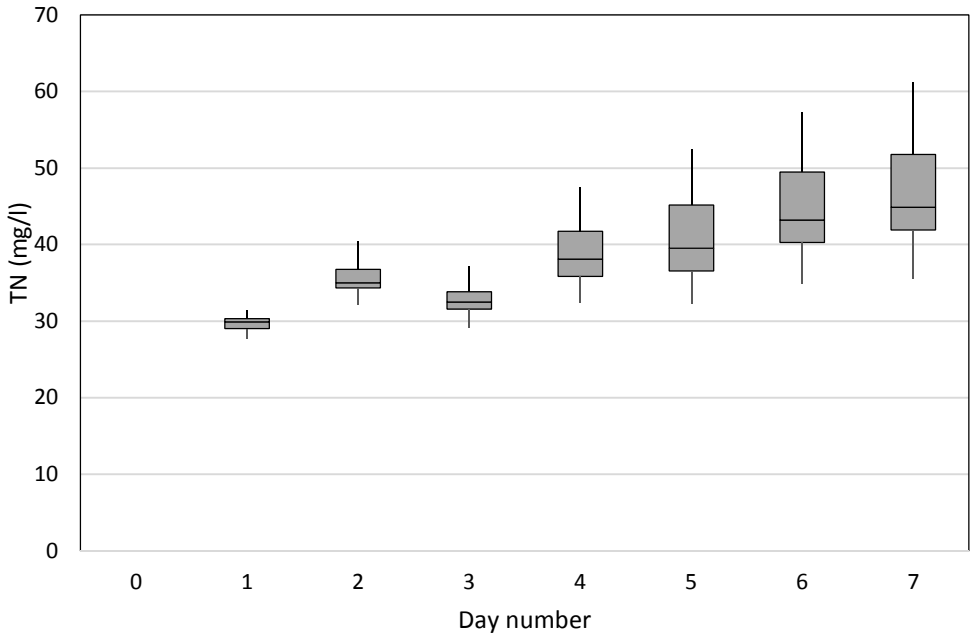


Figure 6-19 Total nitrogen (mg/l) of each 2 l flask at daily intervals

Total nitrogen increase in Figure 6-19 equates to NO_x supply, indicating that NO_x supply alone may be a sufficient form of dissolved nitrogen for microalgae growth.

6.5 Discussion

6.5.1 **Nutrient Trials**

Major sources of utilisable nitrogen and phosphorus in waste industrial processes have already been identified as nutrient sources for microalgae. 0.5% (v/v) inclusion of crude anaerobic digestate was selected to allow for maximum nutrient delivery without compromising light penetration into darker colouration of higher concentration nutrient media. Both filtration and centrifugation had no effect in reducing the darker colouration of crude anaerobic digestate. Anaerobically digested distillery waste from sugar cane molasses contains melanoidin, a polymer formed by the amino-carbonyl reaction in food processing and preservation. The polymer is not easily degraded by microorganisms though widely distributed in nature (Kumar and Chandra, 2006). The anaerobic digestate in this trial was derived from UK Commercial supermarket food products, many of which contain baked products with sugar and amino acid complexes such as bread and refined sugar products which have undergone the Maillard reaction browning process, which concentrate in anaerobic digestate. Commercial anaerobic digestate used in this study was derived from UK supermarket food waste and also contains melanoidin, the dark pigmented complex mixture of uncharacterised polymer molecules responsible for the dark colouration in both the anaerobic digestate and cooked malts from brewery waste in this trial. Growth of microalgae in anaerobic digestate necessitates the dilution of anaerobic digestate both to promote light penetration for phototrophic microalgae (Marcilhac *et al.* 2014), but also to dilute the high nutrient content which would otherwise inhibit growth (Azov & Goldman, 1982). Light penetration depends not only on the colour of the culture media influent, but can also be reduced by too high a concentration of microalgae (Fallowfield and Garrett, 1985). Despite the importance of colour and turbidity of anaerobic digestate nutrient concentration and nutrient recycling efficacy, scientific literature on the characteristics of the

digestate and its impact on the growth of microalgae are rare (Marcilhac *et al.* 2014).

Sterilisation of non-axenic organic media was necessary to reduce risks of pathogenic loading, particularly in the environmentally exposed protozoan containing sample of organic dairy manure. Autoclaved sterilisation of anaerobic digestate media for 20 minutes at 121°C resulted in losses of ammoniacal and nitrate based nitrogen by volatilisation in the region of 20%. However, there exists a trade-off between a reduced growth rate of microalgae as a result of pathogenic grazing and increased energy inputs into the culturing cycle to prevent the manifestation of contamination. In this trial, it should be noted that not all media were equal in their constituent compositional delivery of macronutrients as depicted in Figure 6-5. Consequently, although this trial did not compare nutrient media of equal macronutrient composition concentrations, it did analyse similar dilution values from crude nutrient media into their comparative equal dilution ratios. In this trial, anaerobic digestate sterilised media was the optimal nutrient source, however the study also observed that sterilisation was not obligatory as endemic bacterial communities involved in anerobic digestion process in the region of 60°C and their subsequent culture in microalgae media at 23°C did not reduce growing cell counts of microalgae or induce observed intracellular flocculation. The increase in growth rate of microalgae cultured on sterile rather than non-sterile media in this study suggests that there could be a macronutrient (C, N & P) non-pathogenic competition between microalgae and endemic AD bacteria. Indeed, though the true mechanisms of action still need to be demonstrated, the apparent antagonism between microalgae and nitrifying bacteria appears to favour microalgae (Marcilhac *et al.* 2014). Most of the recent scientific literature reports anaerobic digestion of microalgae as a mixed biomass feedstock for digestion, rather than using anaerobic digestate as a nutrient source to grow microalgae biomass. Methanogenic bacteria form part of the plethora consortia of anaerobic digestion living microbes, and their co-existence with microalgae in the natural environment has been reported as floating mats on the surface effluent of hot alkaline springs where fast growing young surface biomass pushes older, deeper buried biomass below the surface causing self-shading,

biomass decomposition and methanogenesis via the synergistic effects of methanogens with phototrophic microalgae biomass (Ward, 1978).

The stationary growth phase in this trial was observed by day 4 onwards, plausibly induced by light limitation as a result of the non-optimal design construct of the 20 l carbouy photobioreactor. Organic carbon in the liquid phase constituted microalgae, yeast cells or co-habiting filamentous bacteria in association with microalgae culture and visible by light microscopy at high magnification (x400). The surfactant nature of adhesion of low-molecular weight alcohol molecules to surfaces of aeration bubbles could be justified as a proposed method for rapid reduction in liquid organic carbon loading at the gas-liquid interface. This phenomenon of liquid to atmospheric molecular transfer has been reported in the natural environment during ocean bubble bursting, whereby submicron atmospheric aerosol particles were constituted of organic carbon hydroxyls, alcohol groups, characteristic of saccharides and similar to biogenic carbohydrates (Russell *et al.* 2010). In such a potential scenario in this study, bubble ruptures breaking the surface tension at the gas-liquid surface of the vessel could release low-carbon mass alcohol into aerosol airborne droplets for later atmospheric dispersal, corresponding to a lowered TOC time-orientated response in aqueous solution, hence explaining the reduction in TOC on days 2 and 3 in Figure 6-6. This could be tested by introducing a negative control of the brewery waste without inoculated microalgae to test if this happens without the extra variable of carbon consuming microalgae.

Yeast cells walls contain beta-glucan and mannan sugar polysaccharides as well as embedded cell wall proteins (Hohmann, 2002). In this study the [phosphate] in the brewery waste was 380 mg/l which is comparable with [phosphate] found in other studies, 57-326 mg/l (Raposo *et al.* 2010). Nitrogen and phosphorous levels mainly depend on the handling of raw material and the amount of spent yeast present in the effluent, as well as cleaning chemicals between brewing batches (Driessen and Vereijken, 2003).

The mechanism for the decrease in total nitrogen from over 50 mg/l to less than 30 mg/l over the first three days of experimentation is not fully understood,

however structural and enzyme degradation processes are considered to act on yeast cell walls to release embedded cellular proteins. Another possible explanation for rapid carbon loss from solution could be due to yeast cell flocculation, intercellular cell surface coagulation and potential cell senescence mediated by hypo-osmotic shock during rapid transfer from pure yeast and alcohol culture to a more dilute aqueous microalgae culture media via. Yeast adaptations to nutrient availability and stress responses, including the response to osmotic shock, are related phenomena (Hohmann, 2002). Yeast nutrient limitation (such as very low glucose levels) or availability of only poor nutrient sources (such as ethanol instead of glucose as a carbon and energy source or proline instead of ammonium as a nitrogen source) stimulates responses that lead to the adaptation or reprogramming of cellular metabolism, which involves several signalling systems with partially overlapping function such as the adjustment of cellular metabolism at the transcriptional and post-transcriptional levels in response to the availability or quality of carbon sources (Deschenes, 1999). Though the physiological role of the yeast aquaporins is not well understood, Aqy2p is thought to play a role in water efflux during turgor control during rapid growth and under low-osmolarity conditions (Calamita, 2000). There is increasing evidence that weakening of the cell wall results in activation of a salvage pathway leading to compensatory changes, or the cell wall integrity pathway which modulates increased synthesis of chitin, glucan and cross-linking of glucan and proteins in the cell wall (Smits *et al.* 1999). Further work could investigate the yeast cell wall response to a range of osmotic conditions mediated by changes in the concentration of brewery waste, or specifically yeast cell mass percentage per liquid volume. This could be attained by dilution of brewery waste to attain variable concentration of mass per unit volume of whole cell yeast followed by transfer to variable osmotic conditions of microalgae nutrient media. In-situ environmental scanning electron microscopy of formaldehyde preserved yeast cell walls is a potential analysis technique for identification of structural morphology of cell walls.

Despite yeast cells being a rich source of nitrogen (2.1%), much of this cellular nitrogen is unavailable for uptake by microalgae as the nitrogen is organically

bound into the cell wall proteins (13%) of yeast (Northcote & Horne, 1952). Organic carbon deposition within the liquid media and organic carbon attached to solid surfaces of the cultivation vessel was found to not be uniformly distributed, showing preference for solid surface attachment. It is hypothesised that surface ionic charge attraction induces yeast biofilm formation and colonisation. The transition between suspended organic carbon vertically in the water column (living cells in exponential growth phase) and flocculation of cells near maximum cell density (10^6 - 10^7 cells/ml) by sedimentation and deposition of organic carbon at the base of the reacting vessel was observed. Flocculation occurred irrespective of liquid media pH variation and was mediated across all organic nutrient formulations in late exponential phase.

In this research, liquid growth media was consistently sampled 10 cm from the surface of the reacting vessel, form the main culture volume, rather than directly adjacent to the vessel walls. Aeration sparged gas/liquid mixing of the vessel acted to mix vessel-adhered flocculated cells within the liquid media though cells still adhered to solid vessel surfaces out of direct range of immediate gaseous-liquid vortices. A market application for the removal of internal microalgae biofilm which can occur with some species in PBR's, is the utilisation of microsphere cleaning beads within the liquid phase of the PBR to prevent the internal biofilm formation.

Nitrate enters microalgae cells as a positively charged complex which requires greater energy expenditure than uptake of ammonium, in addition once inside the cell nitrate is required to be reduced to ammonium. The fate of carbon and nitrogen in algal metabolism with relation to photosynthesis and amino acid production is complex (Flynn, 1991). Total ammonia in aqueous solution consists of two principle forms, ammonium ion (NH_4^+) and un-ionised ammonia (NH_3). The relative concentrations of these two forms are pH dependent, as described by equation 6-3; the ratio of un-ionised ammonia to ammonium ions increases 10-fold for each unit rise in pH and by about 2-fold for each 10°C rise in temperature between 10°C and 30°C (Erickson, 1985).

Equation 6-3 : Ammonium Dissociation equilibrium

$$K'_a = [NH_3][H^+] / [NH_4]$$

The relative concentrations of the two forms are also temperature dependent (Emerson *et al.* 1975).

Equation 6-4: Temperature dependent ammonia dissociation constant

$$pK_a = 0.09108+2729.92/(273.2+T)$$

Combining pH and temperature of a freshwater solution, the un-ionised ammonia fractions can be calculated (Clement & Merlin, 1995).

Equation 6-5: Unionised NH_3 (%)

$$= 100 / (1 + 10^{(pKa - pH)})$$

During this study, pH was recorded within the diurnal range of 7-10 for the duration of the experiment (Figure 6-9). At a constant experimental temperature of 25°C (Equation 6-4), pK_a was 9.25. As such, theoretically complete conversion of ammoniacal nitrogen will be dissociated into free ammonia at a pH of 9.25, as in Equation 6-5. Using Equation 6-5 and corresponding ammonia concentration values from Figure 6-5, of AD, BW, DM and F2 respectively of 8.6, 3.4, 3.2 and 57.1 mg/l, free ammonia NH_3 and ammonium NH_4^+ is represented in the following table:

Table 6-3 pH variable NH3 and NH4+ available nitrogen in Anaerobic digestate (AD), f/2, Dairy waste (DW) and Brewery Waste (BW)

		pH 7		pH 8		pH 9		pH 9.24	
	$NH_3 + NH_4^+$ (mg/l)	NH_3 (mg/l)	NH_4^+ (mg/l)	NH_3 (mg/l)	NH_4^+ (mg/l)	NH_3 (mg/l)	NH_4^+ (mg/l)	NH_3 (mg/l)	NH_4^+ (mg/l)
AD	8.6	0.4	8.2	0.6	8	2.5	6.1	8.1	0.5
DW	3.4	0.1	3.3	0.3	3.1	1.0	2.4	3.2	0.2
BW	3.2	0.1	3.1	0.2	3.0	0.9	2.3	3.0	0.2
f/2	57.1	2.4	54.7	4.2	52.9	16.5	40.6	54	3.1

Contrary to the findings of Mosier, 1978 who reported that photosynthesis was limited by 50% due to free ammonia toxicity (IC_{50}) at 0.6 mg/l ammonia in *Chlorella ellopsoide;* at NH_3 concentrations of between 20 and 250 mg/l there were no significant differences in specific growth rates and maximal cell densities of *Chlorella vulgaris* were obtained (Tam, 1996). It was also found that adaptation to growth in media containing 750 mg/l of ammonia and tolerance of a strain whose growth was inhibited by 50% at 1500 mg/l ammonia (Przytocka-Jusiak *et al.* 1976).

In this study, free ammonia was not thought to be within toxic ranges for this species of *Chlorella vulgaris* (Table 6-3). NH_3 concentrations applied via higher volumetric application of media at a concentration greater than 0.5% could have been tolerated by the algae. Light attenuation could have been a limiting factor impeding algal growth if a more concentrated culture density were used. In this study 20 l carboys constituted the experimental PBR system. This is not an optimal PBR in terms of light attenuation, due light restricted light transmission at higher culture densities. In addition, the light source was provided on one vertical side of the carboy alone. The cross sectional diameter of the carboys was over 30 cm. In early exponential growth at low cell density, this is not considered to correlate to reduced growth, however as the cell culture density increases later in the growth cycle, light deficiency is hypothesised to become more significant over availability of nitrogen as the predominating factor limiting growth. The light path or the distance that a light source can permeate into the culture before its light energy becomes diminished for *Spirulina* using an artificial light source of 80 $\mu mol/m^{-2}/s^{-1}$ was 1.2 cm (Eriksen, N., 2008). Even sunlight in a 2.3 g/l culture of *Spirulina* only has a light path of 10 cm (Eriksen, N., 2008). Consequently in this study using artificial light at 200 $\mu mol/m^{-2}/s^{-1}$, light rather than provision of nitrogen or toxicity of ammonia is considered to be negatively affecting growth rates, particularly from day 3 of the experiment onwards when cell density diminished the light penetration. A potential remediation of this could be LED lighting which has developed a strong market presence over the last few years, providing strong light intensity at higher efficiency than fluorescent tube lighting which has additional energy losses as heat. Combination red, blue, infrared and white LED's

have been used to simulate the light spectrum wavelength in sunlight (Kohraku and Kurokawa, 2006).

6.5.2 **Simulated flue gas trials**

Coal is a considerable polluting form of energy generation (Table 6-4); however dislocation from appropriation of this form of energy in the near future would arguably instigate a significant social, political and economic challenge to maintain current levels of energy provision. As shown in Table 6-4, SOx and NOx pollutants are more widely emitted in coal than either natural gas or oil.

Table 6-4 Pollutants of natural gas, oil and coal. Fossil fuel emission levels (pounds/billion BTU of energy input). (EIA, 1998)

Pollutant	Natural Gas	Oil	Coal
Carbon dioxide	117,000	164,000	208,000
Carbon monoxide	40	33	208
Nitrogen oxides	92	448	457
Sulphur oxides	1	1122	2591
Particulates	7	84	2744
Mercury	0.00	0.007	0.016
Total	117,140	165,687.007	214,000.016

Steel slag is a widely available and low cost industrial by-product resource. Washed steel slag was found to contain calcium ions and magnesium ions but not iron, chromium, aluminium and zinc. Steel slag is left to weather in a rainy climate such as the UK before use in construction to reduce incidences of swelling and cracking in final construction products (LaFarge Tarmac 2013, personal communication). The natural weathering processes were simulated in the laboratory with serial washings. Water volumes of washed slag contain a constant calcium ion concentration and pH value which can be produced in batch volumes. This approach contrasts the utilisation of solid steel slag mixed within a growing microalgae culture, whereby the constant gradual chronological release

of ions would induce a more variable and potentially more concentrated end product of calcium hydroxide in batch culture.

Pilot experiments of 25 ml samples found that the volumetric dilution ratio of 1% steel slag solution required to neutralise 25 ml of simulated coal flue gases was 20% (v/v) of a 1% steel slag solution in de-ionised water exposed to 5 h 30 min simulated coal flue combustion gases (Figure 6-15). At this dilution, microalgae growth could benefit from elevated CO_2. Simultaneously, acidic pH from dissolved SO_x and NO_x contaminants could be mitigated by neutralisation, (equations 6-1 and 6-2). Sequential volumetric increase of the culture medium (Figure 6-17) diluted growing microalgae, reducing effects of light attenuation from the exponential growth phase. 1% steel slag is a transparent solution, so there is a trade-off between the dilution of the microalgal culture and pH neutralisation. Moreover, precipitates of $CaCO_3$ were observed forming within solution, intermittently and depending on both dilution ranges and solution pH. Figure 6-17 shows the increase in TOC (derived from microalgae cellular biomass), and simultaneous control of pH to neutral ranges via dilution. TOC pertained to microalgal biomass generation and distinguished from IC from $CaCO_3$ formation. The increase in liquid volume within the flasks due to the neutralisation of aqueous acids of SOx and NOx with washed steel slag over three days, filled the entire volume of 2 litre flasks from an intital 1.5 l culture. In this way, a 1 l microalgae culture doubled to 2 l within 5 days as a result of the dilution effects the steel slag solution.

Open pond microalgae growth systems in hot climates have high evaporation rates with significant water loss, requiring replenishment with supplementary water and nutrients. In the South West USA evaporation comprises an estimated 0.3 cm per day from an open pond system (Davis, 2011). If some replacement water from evaporative losses could be replaced with alkaline slag wash, integral systemic pH control of acidic simulated coal flue combustion gases could potentially be mitigated.

Anaerobic digestate supplied nitrogen to the neutralised flue gases, potentially surplus to requirements, due to the assimilation of highly water soluble calcium

nitrate (Equation 6-2). An investigation of the nutrient requirements of the blue-green alga *Anabaena cylindrical* disclosed that calcium was essential for growth whether nitrate or molecular nitrogen was used (Allen, 1955). High CO_2 concentrations lower the pH in the culture medium which subsequently decreases the activity of extracellular carbonic anhydrase, responsible for the concentration of carbon in microalgae (Singh, 2014).

Time-dose response of neutralisation and biomass dilution has been confirmed from this experiment. However, TOC increased in both the 20 l carbouy and the 2 l flasks, indicating microalgae cell growth. Naked eye visual examination of the microalgae cultures indicated that growth occurred during the simulated coal flue combustion challenge. Hypothetical industrial implementation of this technology would ideally supply full strength coal flue combustion gases directly into a growth culture of microalgae as a carbon sequestration strategy. Although simulated flue gas delivery with this experiment was only run for three hours of per day, acidification control was demonstrated. There is consequently a triple trade-off between:

1. Volume of microalgae culture media and volumetric increase during microalgae batch growth phase.
2. Synchronisation of microalgae growth rates to the incremental dilution of growth media so that despite increase in culture volume microalgae growth rate increases to allow for optimal light penetration into culture.
3. Avoidance of sudden pH shock shifts within culture media or deviation in pH outside the range of 6-8.5.

Application of Steel slag for water remediation has been used for pH control in environmental wetland systems whom investigated buffering of alkaline steel slag leachate across a natural wetland (Mayes, Younger and Aumo, 2006). Concentrated CO_2 streams produced by cement or power plants which produce a pure form of CO_2, void of NOx and SOx emissions could be used to cultivate algae (Hasanbeigi, Price and Lin, 2012). However, this study is the first known report of using washed steel slag as a pH remediating agent for aqueous NOx and SOx acidity within coal flue combustion gases for the purposes of microalgae

biomass production. This provides a platform for further scope and research within this area. Maintenance of a high concentration of microalgae is an ideal scenario for purposes of biomass harvesting. However, the addition of steel slag wash as a pH neutralisation agent requires 20% (v/v) dilution to neutralise pH and mitigate against coal flue gas contaminants. Future work could consider a low pH / high CO_2 tolerant species such as *Chlorococcum littorale* (Iwasaki, Kurano and Miyachi, 1996). Despite the low pH of between 4 and 6 in this experiment, *Chlorella* are known to be a pH-hardy microalgae species. A copper mine in Wales sampled in the summer of 2006 with a pH of between 2.4 and 2.7 identified six species of freshwater microalgae, the second most prolific of which was a *Chlorella* (López-Rodas *et al.* 2008). This research advocates a different approach to solving a problem which has been tested in laboratory experimental conditions with efficacy to achieve a carbon content of 350 mg/l despite a low pH and time-orientated pH dosage shifts. Future work would consider optimisation of pH via periodic feedback dosage and sensory mechanism which maintain culture pH at a steady state. Fulfilment of this into an experimental context, it would be envisaged that microalgae growth rates could further benefit from increased CO_2 delivery. The caveat is that pilot scale experimentation would need to be scaled to simulate an industrial production scenario.

6.6 Conclusions

This chapter has described the conduct of experiments to investigate potential mechanisms for integration of waste technologies for microalgal biomass production, namely steel slag and coal flue combustion gases for integration into microalgae cultivation. Capacity for macronutrient sources other than utilisation of synthetic nutrients has been presented, and three industrial by-product examples of manure, anaerobic digestate and brewery waste have been investigated experimentally. The provision of CO_2 to enhance growth rate of microalgae has used industrial waste CO_2 simulated as coal flue combustion gases.

Ion chromatography identified analytes of 0.5% AD having 9 mg/L ammonium, 20 mg/l nitrate and 10 mg/l potassium. f/2 contained 57 mg/l ammonium and 11

mg/l nitrate. 0.5% Dairy manure contained 3.5 mg/l ammonium and 4.5 mg/l potassium. 0.5% brewery waste contained 3 mg/l ammonium and 19 mg/l phosphate. Anaerobic digestate contained the most complete macronutrient profile, which could be included to contain brewery waste to supplement levels of phosphate.

AD was the optimal microalgae nutrient. Low nitrogen in dairy manure is attributed to the open collecting pond of the organic dairy farm, causing volatile nitrogen loss and parasitic and bacterial grazing. Contamination of un-sterilised nutrient media induced parasitic feeding on microalgae, causing bioflocculation to prevent accurate cell counts. Sterilisation of AD media resulted in volatisation of nitrogen content but eliminated protozoan culture infection, though heterotrophic nutrition with bacterial contamination in non-sterilsed AD was considered to be attributed to a reduced TOC accumulation. Mixing of cultures was paramount for distribution of cells either in surface contact with internal vessel walls or distribution within the media. Uneven cell mixing within the culture media could underestimate affect cell counts of samples where cell adhesion to the culture vessel occurred.

Coal flue combustion gas dissolved in de-ionised water acidified the water to between pH 2 and 4. 1% Steel slag (w/v) in de-ionised water was pH 11.5 attributed to the calcium oxide content in steel slag of 40%. Serial washings of 1% (w/v) steel slag in de-ionised water were tested for Fe^{2+}, Cr^{3+}, Al^{3+}, Zn^{2+}, Mg^{2+} & Ca^{2+} metal ions with AAS, showing Ca^{2+} ion dissolution from steel slag. Microalgae grew to 300-350 mg/l in 7 days with a daily 3 h of simulated flue gas and pH neutralistion with washed steel slag. pH sudden shifts with neutralisation and acidification to below pH 6 was thought to compromise growth. If a feedback mechanism for culture dilution and neutralistion could occur, then microalgae could grow with greater vigour.

7 Hydrothermal liquefaction of microalgae biomass harvested via two de-watering and drying methods

7.1 Introduction

Hydrothermal liquefaction (HTL) is a direct thermal conversion of carbonaceous solid biomass mixed with water or other liquids into liquid bio-oil and water solubles using heat and pressure. Due to the high pressure water remains in a liquid state so that the dielectric constant is increased and the density decreased relative to water at normal temperature and pressure, resulting in hydrocarbons becoming more water-soluble (Peterson, 2008). Liquid bio-oil or bio-crude is a complex mixture of oxygenated hydrocarbons. Biomass thermo-treated via HTL directly converts biomass into liquid bio-oil, avoiding pre-steps of drying and oil extraction. Bio-oil produced using microalgae biomass also contains nitrogen. The processing pathway of HTL is particularly applicable to wet biomass feedstocks, such as microalgae, which reduces the necessity for additional drying energy of the feedstock prior to processing, as required in alternative thermochemical conversion processes (Elliot *et al.* 2013).

Harvesting is arguably the most critical and challenging state of microalgae biomass production. Current microalgae de-watering techniques and biomass recovery for downstream processing involve a multi-stage process of 1) primary harvesting or bulk harvesting, 2) secondary de-watering or thickening, 3) drying. Of these 3 processes, drying is the most energy intensive (Sharma *et al.* 2013). The low-cost harvesting technology described in Chapter 4 is a one-step process. This harvesting process is able to concentrate microalgae biomass in a culture media from 1-2 g/l (0.1-0.2%) to 100 g/l (10%) and thereafter subsequent drying to zero humidity biomass either with additional heat, freeze drying or without additional energy input via ambient dehydration. Microalgae biomass feedstock at initial concentrations of 10% solids (w/v) provides optimal water content for direct application to HTL downstream processing. Operating conditions for HTL usually range from 250 to 375°C, 10-20 MPa and microalgae mass fractions of 5-20% in the slurry feed, which can be achieved while consuming only 12% of the energy needed for their complete dewatering by conventional de-watering

processes (Xu *et al.* 2011). The ideal concentration of 10% mass per unit volume of water in microalgae is the same range as the concentration of microalgae harvested immediately following the low-cost GIF harvesting process described in chapter 4. It is sometimes considered that because HTL is a “wet” processing technology, this negates the requirement for bulk harvesting and thickening initial processing procedures, however this is not true. The requirement for harvesting microalgae from their culture density of 1-2 g/l to ideal HTL processing concentration of 10% (w/v) uses harvesting processes which could presently be either centrifugation, tangential filtration, flocculation or derivatives thereof. A reduction in the energetic or economic cost of microalgae harvesting would improve the efficiency of the microalgae to biofuels process engineering pathway. GIF harvesting offers a potential solution to this problem.

Extraction of alginate or pectin from seaweed or terrestrial crops uses sodium carbonate (McHugh, 1987). It is likely that unreacted chemical residues of sodium carbonate will be present in the dissolved filtrate of extracted alginate or pectin gels, especially if a crude wet extraction rather than a complete commercial style dry alginate or pectin extraction is used. Where used, sodium carbonate is typically added at 1% in HTL processing as a buffer reagent or catalyst (Zhu *et al.* 2013), notably a similar concentration is used for alginate and pectin extraction from raw feedstock. Sodium carbonate has multiple benefits as a catalyst for use in HTL. Besides being a low-cost and widely available, (Yu *et al.* 2014) states the following advantages of inclusion of Na_2CO_3 into the HTL reaction chamber: it might increase the complexity of oil product by recombining relatively small organic compounds together to form large organic compounds; a 52% carbon recovery with Na_2CO_3 catalyst compared to 32% carbon recovery at 240˚C; less than 2% carbon recovery in solid residue with Na_2CO_3 compared to 18% solid residue carbon recovery without a catalyst. The use of Na_2CO_3 as a catalyst therefore provides justification for advanced nutrient recycling of post HTL reaction water solubles into new batches of microalgae culture media advocated by Biller, 2012. In comparison to metal catalysts which showed carbon deposition and mineral mixing on the surface of metal catalysts (Yu *et al.* 2014), sodium carbonate is a low-cost replaceable alkaline catalyst.

The fact that traces of unreacted Na_2CO_3 residues are likely to be present in GIF harvested microalgae, means that application via the route of HTL for downstream processing of microalgae into bio-oil offers potential bio-oil conversion benefits as outlined above. A further benefit of HTL is that it destroys any pathogenic or any other potential contaminating organisms which may have been present during the non-axenic batch culture of microalgae on waste nutrients. In this chapter a comparison is made between 2 harvesting methods for microalgae biomass for bio-oil production using hydrothermal liquefaction.

7.2 Methods

7.2.1 **Microalgae & lipid extraction**

Microalgae samples analysed included *Chlorella vulgaris, Chlorella salina, Nannochloropsis salina* and *Tetraselmis chui.* These species were chosen for being oleaginous, representative of both seawater and freshwater and with a previous research focus directed towards microalgae biofuels. Microalgae stock culture volumetric availability and the logistical timing of experimentation also influenced the decision of which species to investigate for the effect of harvesting processes on lipid profile. Microalgae were cultivated during the spring and summer months, cultured in 25 cm diameter polyethylene tubular bags in the glasshouse at Cranfield University. Microalgae were harvested either using the GIF harvesting process described in chapter 4 or by disk stacked centrifugation (GEA Westfalia Separator), bench-top centriguation and freeze drying. A direct method of lipid extraction from 1 g of biomass was used for all samples (O'Fallon *et al.* 2007). Though this technique was not originally applied to microalgae lipids, the application has been used in recent years by other authors for extraction of lipids from microalgae (Chakraborty *et al.* 2012). To compare biomass analysis of HTL bio-oil and algal oil harvested by the two methods, GC lipid peak profiles were analysed according to GIF harvesting (GIF) and conventional centrifuged and freeze dried (CFD) harvesting methods. Table 7-1 shows the microalgae processing groups used both for algal oils (G) and algal-HTL bio-oil analysis (B).

Table 7-1 Classification of microalgae species, culture nutrients, harvesting method groups for algae lipid and algae bio-oil lipid analysis. Extraction method - Centrifuged & freeze dried (CFD), Gel induced filtration (GIF) harvesting. Extraction point – algal lipids extracted directly following microalgae harvesting (G), lipids extracted after HTL bio-oil formation (B).

Sample number	Classification	Extraction Point (B / G)	Microalgae species	Harvest method	GIF extraction agent	Culture Nutrients
1	CFD *Chl.v*	B & G	*Chlorella vulgaris*	Centrifuge/Freeze dried	n/a	f/2 + micro
2	CFD *Chl.v*	B & G	*Chlorella vulgaris*	Centrifuge/Freeze dried	n/a	f/2 + micro
3	GIF *Chl.v*	B & G	*Chlorella vulgaris*	GIF	Purified Alginate	f/2 + micro
4	GIF *Chl.v*	B & G	*Chlorella vulgaris*	GIF	Purified Alginate	Whole cell brewery waste
5	GIF *Chl.v*	B & G	*Chlorella vulgaris*	GIF	Purified Alginate	Clarified brewery waste
6	GIF *Chl.v*	B & G	*Chlorella vulgaris*	GIF	Purified Alginate	Organic dairy manure
7	Crude GIF *Chl.v*	B & G	*Chlorella vulgaris*	GIF	Crude S.Beet Pectin	f/2 + micro
8	GIF *Chl.s*	B & G	*Chlorella salina*	GIF	Purified Alginate	f/2 + micro
9	CFD *Nanno.c*	B & G	*Nannochloropsis salina*	Centrifuge/Freeze dried	n/a	f/2 + micro
10	CFD *Tet.c*	B & G	*Tetraselmis chui*	Centrifuge/Freeze dried	n/a	f/2 + micro
11	CFD *Tet.c*	B & G	*Tetraselmis chui*	Centrifuge/Freeze dried	n/a	Anaerobic Digestate
12	Crude GIF *Tet.c*	B & G	*Tetraselmis chui*	GIF	Crude Citrus Pectin	f/2 + micro
13	Crude GIF *Tet.c*	B & G	*Tetraselmis chui*	GIF	Crude S.beet Pectin	f/2 + micro
14	Crude GIF *Tet.c*	B & G	*Tetraselmis chui*	GIF	Crude *Macrocystis pyrifera* Alginate	f/2 + micro
15	GIF *Tet.c*	B & G	*Tetraselmis chui*	GIF	Purified Alginate	f/2 + micro

7.2.2 **HTL process**

The laboratory HTL experimental equipment is shown in Figure 7-1, comprising of an outer pot filled with sand as a heat conductor for heat transfer to the inner pot of the assembly. The batch reactor was inserted into the inner pot after reaching a temperature of 280˚C. The set-up was placed on a ceramic heating plate. The top of the equipment was covered with a steel insulating plate to minimise heat loss. The temperature profile of the sand was monitored with a probe thermocouple.

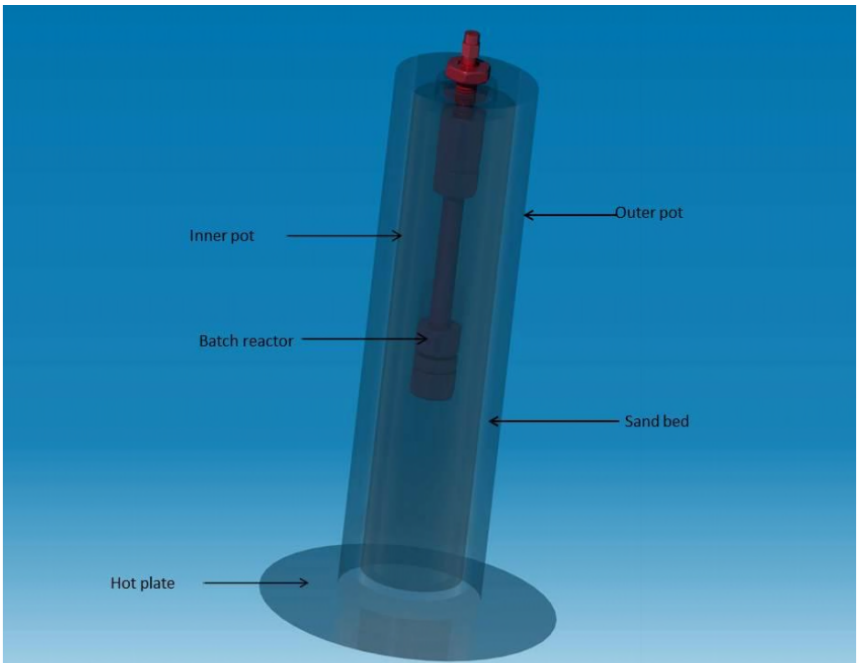


Figure 7-1 Experimental HTL set-up (Guharoy, 2013)

A 10 ml stainless steel Swagelok reactor vessel was used as a “bomb” pressure vessel immersed in a sand bath. This comprised a conventional $^{1}/_{2}$ inch Hoke Gyrolock (SS 316 grade). The 2D view of the Swagelok reactor is shown in Figure 7-2. The length of reactor was 170 mm with an inner diameter of the pipe 10.28 mm. According to the manufacturer, the design temperature range of the material is between (-235˚C to +426˚C). The ceramic hot plate with a basal heat source attained a maximum temperature of 450˚C.

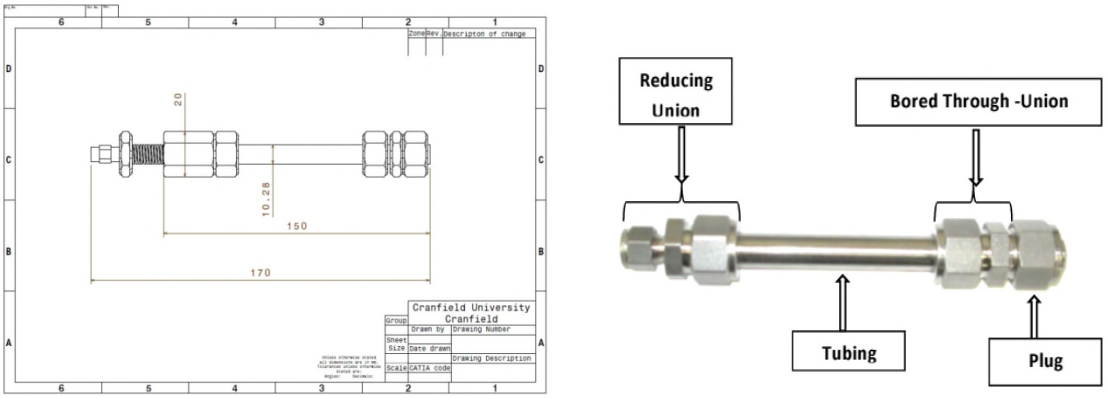


Figure 7-2 Batch reactor "Bomb"

The outer pot was wrapped with mineral wool for insulation, the inner pot was placed inside the outer pot and the inner space filled with sand and tightly packed.

The batch reactor was loaded at 20% (w/v) with 1.8 g of dried microalgae biomass and 9 ml of de-ionised water and sealed. 20% (w/v) proportion has been used by other author's in microalgae HTL experiments (Jena & Das, 2011). On attainment of 280˚C the batch reactor was quickly introduced into the inner pot and left for 20 min for HTL to occur. Post-HTL, the reactor vessel was quenched in a water bucket. On return to room temperature, gases were vented and liquids retained for GC and TOC analysis. Firstly, water soluble fractions were poured from the reactor, centrifuged to remove particulate matter and retained for TOC analysis. Secondly, 3 ml of laboratory grade methanol was placed into the reactor, it was re-sealed and vortexed for 5 min. Dissolved lipids were retained for FAME extraction (O'Fallon *et al.* 2007), using 2.3 ml instead of 5.3 ml of methanol as 3 ml had already been used from the bio-oil extraction procedure. The HTL process flow is described further in Figure 7-3.

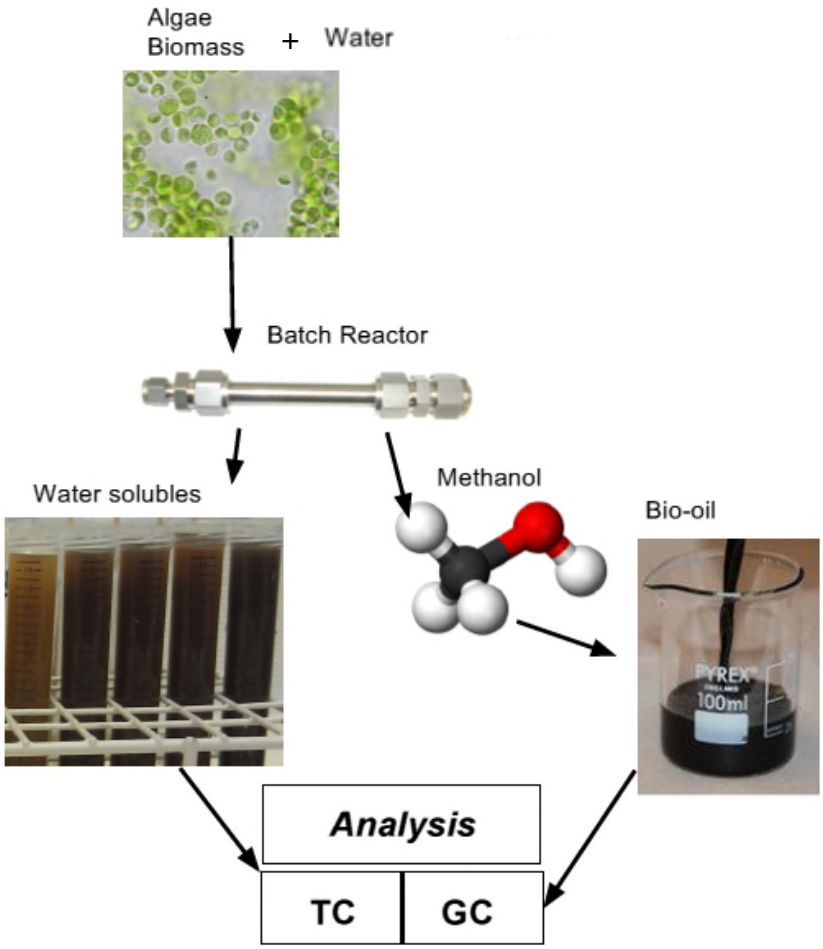


Figure 7-3 HTL flow diagram

FAME analysis of extracted lipid fractions analysed by GC was used to verify lipids, and water solubles were analysed for TOC. Lipid extraction from biomass samples and FAME analysis on GC was performed as described in 4.3.6.

7.2.3 **Data Analysis**

Chromatogram peak values from GC analysis of FAME 37 Supelco® standard lipids between C6 and C24 were quantified using area normalisation by Shimadzu® lab solutions software. Known peaks correlating to the FAME mix standard were quantified as total lipid percentage FAME, unclassified peaks were grouped as other unknown lipids. Statistical analysis of significance was performed using the general linear analysis model, Statistica® version 12, with significance at $p<0.05$. All assumptions of the general linear model were satisfied.

Results

Tables 7-2 & 7-3 reveals the distribution of FAME within microalgae samples sampled by centrifugation & freeze drying, GIF harvesting and the same microalgae samples which were converted into bio-oil via HTL prior to FAME and lipid analysis. Peaks in the GC chromatogram were characterised with the FAME 37 standard shown in Appendix Figure A-2. A prominence of C14-C15, C16-C17 and C20 lipids were detected across all microalgae groups.

The general linear model statistical analysis showed that most of the saturated and unsaturated lipids between C4 and C24 were not statistically significant between both harvesting methods (GIF and CFD) and extraction point (algal oil and HTL bio-oil) as shown in the statistical results in Appendix Figure A-7. There were some detected statistical differences between treatments, however. The harvesting methods of GIF and CFD were different for C18:3n3 ($p=0.035$). This lipid was only detected in FAME's extracted from algal oil treatments, at concentrations less than 1% in all but one treatment. Statistical analysis of differences between the extraction point of algal oils and HTL bio-oils showed significance between C15 ($p=0.019$), C18:3n3 ($p=0.012$), C21 ($p=0.005$), C22 ($p=0.004$), C22-1n9 ($p=0.001$), C20-4n6 ($p=0.0003$) and 'other lipids' ($p=0.0001$). Of these, C15, C20-4n6 and 'other lipids' had the highest percentage compositional differences, with the bio-oil treatments showing higher detection of C15 (5% average total composition) and C20-4n6 (10% average total composition) than the algal oil treatments. Conversely, the algal oil treatments had more unclassified 'other lipids' than the bio-oil treatments.

The GC process for determination of FAME classification used percentage area normalisation. Percentage area normalisation calculates the percentage of lipids relative to the total lipid fractions detected over the course of the whole chromatogram. As a consequence of this detection and analysis procedure, some of the lipids from the bio-oil groups had higher percentage detection of the core FAME, as a result of not having so many smaller peaks in the chromatogram. This was not representative of the distinction between the FAME in the bio-oil

and microalgae lipid extractions due to the variance in the percentage area normalisation method using the manufacturers' integral software.

Bio-oil via HTL has a range of chemical reactions re-polymerising hydrocarbon complexes. So, lipid fractions and other chemical compounds distinct from those in FAME 37 are formed. Such compounds cannot be quantified because their associated peaks were at different retention times to the FAME 37 standard. Consequently, these peaks were therefore grouped together as unclassified lipids. Area normalisation was chosen for quantification purposes rather than the inclusion of an internal standard, because of the high composition of unknown lipids in the sample profiles.

Table 7-2 Total percentage FAME per sample. *Chlorella vulgaris*, *Chlorella salina*, *Nannochloropsis salina*, *Tetraselmis chui*.
CFD-Centrifuged & freeze dried, GIF-Gel induced filtration harvesting, P.A-Purified Alginate, C.S.P-Crude Sugar beet Pectin, C.C.P-Crude Citrus Pectin, C.M.P.E-Crude *Macrocystis pyrifera* Extract, f/2 media, W.C.B.W-Whole Cell Brewery Waste, C.B.W-Clarified Brewery Waste, O.D.W-Organic Dairy Manure, A.D-Anaerobic Digestate.

Microalgae species	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.s*	*Nanno.s*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*
Harvest method	CFD	CFD	GIF	GIF	GIF	GIF	GIF	GIF	CFD	CFD	CFD	GIF	GIF	GIF	GIF
GIF extraction	n/a	n/a	P.A	P.A	P.A	P.A	C.S.P	P.A	n/a	n/a	n/a	C.C.P	C.S.P	C.M.P.E	P.A
Nutrient	f/2	f/2	f/2	W.C.B.W	C.B.W	O.D.M	f/2	f/2	f/2	f/2	A.D	f/2	f/2	f/2	f/2
C6	0.04	0.00	0.01	0.06	-	-	0.03	0.01	0.09	0.00	0.00	-	0.30	-	0.15
C8	0.01	0.02	0.01	0.10	0.21	0.28	0.02	0.02	0.01	0.00	0.00	0.07	0.33	0.07	0.45
C10	-	0.01	0.04	0.07	-	0.30	0.04	0.01		0.00	0.01	0.01	0.83	0.09	0.07
C11	0.00	0.03	0.01	-	-	-	0.01	0.02	0.00	0.00	0.02	0.00	0.60	-	-
C12	0.00	-	0.01	0.09	0.23	0.28	0.01	0.03	0.01	0.00	0.01	0.00	-	-	-
C13	0.00	1.23	0.17	0.42	0.12	0.52	0.36	1.93	4.09	0.27	0.07	0.33	1.11	0.59	3.19
C14	0.05	0.04	0.01	0.08	0.15	0.68	0.04	0.12	0.01	0.04	0.01	0.03	0.35	0.59	0.50
C14:1	0.32	1.20	0.17	0.51	0.91	12.58	1.06	0.50	0.28	0.42	16.38	18.41	14.92	22.67	28.17
C15	1.83	1.48	2.88	6.96	0.64	4.02	1.72	1.67	0.25	0.31	1.06	2.99	0.38	0.95	0.79
C15:1	3.07	0.13	0.04	9.53	3.04	5.29	0.14	0.11	0.33	0.10	3.51	2.31	1.61	2.39	0.12
C16	2.12	0.05	0.14	0.35	0.73	0.57	0.50	0.03	1.28	2.57	0.17	1.99	1.56	0.78	2.40
C16:1	32.08	6.93	4.16	5.41	6.49	15.55	0.97	7.46	7.66	41.97	2.52	35.92	9.74	2.79	0.13
C17	10.62	16.77	22.77	0.38	1.13	17.78	19.86	19.04	0.10	7.72	11.07	7.87	9.88	8.66	0.25
C17:1	1.37	2.20	1.77	0.36	1.04	17.32	2.40	3.09	0.14	1.15	3.13	1.77	1.79	2.10	1.88
C18:1n9t	0.13	0.53	0.19	0.09	0.71	0.47	0.31	0.37	0.14	0.03	0.12	0.08	1.24	0.93	0.94
C18	0.11	0.27	0.14	0.09	0.34	-	0.27	0.15	0.09	0.06	0.09	0.06	0.56	0.74	0.54
C18:1n9c	0.16	0.12	0.09	0.07	0.40	0.20	0.35	0.26	0.14	0.11	0.12	0.15	0.44	0.10	1.01
C18:2n6t	0.15	0.14	0.05	0.19	0.26	-	0.15	0.06	0.14	0.11	0.04	0.10	0.22	0.20	0.87
C18:2n6c	0.47	0.20	0.02	-	1.75	1.54	0.24	0.07	13.06	0.05	0.05	0.15	0.14	0.31	0.30
C20	0.56	0.16	3.06	1.45	0.89	-	0.92	0.65	0.18	0.34	0.47	0.03	1.46	1.68	1.60
C18:3n6	0.26	0.27	0.32	0.26	-	-	1.01	0.16	0.11	0.02	0.17	0.01	0.29	0.20	0.07
C20:1	0.14	0.12	0.03	0.27	0.27	-	0.56	0.08	0.14	0.01	0.02	0.01	-	-	-
C18:3n3	0.04	0.03	0.65	1.18	0.53	0.57	0.30	0.06	0.04	0.03	0.00	0.01	-	-	-
C21	0.03	0.17	0.10	-	0.23	-	0.15	0.05	0.02	0.12	0.00	0.02	-	-	-
C20:2	0.28	0.04	0.01	-	0.12	-	0.17	0.01	0.11	0.28	0.01	0.02	-	0.11	-
C20:3n6	0.16	0.22	0.05	0.08	-	-	0.20	0.05	-	-	0.01	0.15	-	-	0.15
C22	0.17	0.31	0.90	0.49	0.18	0.43	0.05	0.07	0.04	0.05	0.01	0.48	-	-	-
C22:1n9	0.18	0.57	0.01	0.12	0.37	0.58	0.01	0.02	0.02	0.09	0.01	0.06	0.23	0.33	0.24
C20:3n3	0.63	0.09	2.65	0.75	0.86	1.33	0.44	0.04	0.12	0.76	0.09	0.76	0.47	0.27	0.51
C20:4n6	0.33	0.09	0.16	0.77	1.49	2.31	0.08	0.09	0.02	0.01	0.03	0.01	1.04	1.52	0.30
C23	0.09	0.02	0.01	-	4.00	1.46	0.07	0.02	0.01	0.05	0.24	0.05	-	-	-
C22:2	0.12	0.57	9.61	2.48	0.47	-	1.50	0.53	-	0.01	-	0.13	0.34	0.44	
C20:5n3	0.01	0.06	-	-	5.50	-	-	0.01	-	0.02	-	-	-	-	-
C24	0.01	0.00	0.05	-	-	-	0.06	-	0.00	0.04	-	0.01	-	-	-
Other unclassified lipids	44.43	65.95	49.73	67.40	66.94	15.91	65.99	63.19	71.37	43.23	60.52	26.00	50.18	51.48	55.35

Table 7-3 HTL Bio-oil - Total percentage FAME per sample. *Chlorella vulgaris*, *Chlorella salina*, *Nannochloropsis salina*, *Tetraselmis chui*.

CFD-Centrifuged & freeze dried, GIF-Gel induced filtration harvesting, P.A-Purified Alginate, C.S.P-Crude Sugar beet Pectin, C.C.P-Crude Citrus Pectin, C.M.P.E-Crude *Macrocystis pyrifera* Extract, f/2 media, W.C.B.W-Whole Cell Brewery Waste, C.B.W-Clarified Brewery Waste, O.D.W-Organic Dairy Manure, A.D-Anaerobic Digestate.

Microalgae species	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.v*	*Chlor.s*	*Nanno.s*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*	*Tetra.c*
Harvest method	CFD	CFD	GIF	GIF	GIF	GIF	GIF	GIF	CFD	CFD	CFD	GIF	GIF	GIF	GIF
GIF extraction	n/a	n/a	P.A	P.A	P.A	P.A	C.S.P	P.A	n/a	n/a	n/a	C.C.P	C.S.P	C.M.P.E	P.A
Nutrient	f/2	f/2	f/2	W.C.B.W	C.B.W	O.D.M	f/2	f/2	f/2	f/2	A.D	f/2	f/2	f/2	f/2
C6	-	3.98	-	-	-	-	-	-	-	-	-	-	-	-	-
C8	-	-	1.08	-	-	-	-	0.57	-	-	-	-	-	-	-
C10	-	-	-	-	-	-	-	0.33	-	-	-	-	-	-	-
C11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C13	11.79	1.42	-	1.42	2.76	2.88	-	-	1.76	2.94	0.72	-	-	2.50	-
C14	1.06	23.15	-	-	-	-	-	0.42	-	-	-	-	-	-	4.17
C14:1	-	-	7.07	18.90	-	-	3.32	2.54	14.68	24.53	24.68	20.03	23.85	21.88	
C15	1.40	4.45	1.46	5.21	13.96	5.33	2.49	0.27	-	14.59	2.88	3.09	13.04	1.57	30.37
C15:1		2.61	2.42	5.17	-	-	4.80	-	-	2.03	2.03	2.77	1.20	-	15.54
C16	1.61		5.52	2.07	2.46	1.93	15.05	1.37	0.82	1.73	2.29	24.18	-	3.08	
C16:1	8.18	9.51	6.27	16.88	11.03	11.07	9.07	-	4.44	10.63	34.37	1.79	27.16	15.08	-
C17	2.22	3.47	16.16	16.78	10.43	9.66	7.70	0.97	1.07	5.74	7.38	8.21	8.34	4.41	21.54
C17:1	2.17	0.89	-	-	2.83	0.79	19.64	0.72	-	1.58	1.47	1.90	1.74	-	7.99
C18:1n9t	-	-	-	-	2.36	-	-	-	-		0.76	-	1.24	-	-
C18	1.15	-	-	-	-	0.72	-	0.61	-	1.82	-	-	-	-	-
C18:1n9c	-	-	-	-	-		0.01	0.07	1.81	0.01	-	-	-	-	-
C18:2n6t	10.19	-	-	-	0.86	3.29	-	-	-	-	-	-	-	-	-
C18:2n6c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C20	-	4.15	3.33	0.89	3.958109	-	-	0.14	1.29	1.95	1.37	-	-	-	-
C18:3n6	-	-	-	2.67	-	-	-	3.49	-	-	-	-	-	-	-
C20:1	2.95	9.58	1.03	-	-	17.68	0.87	83.39	-	-	-	-	-	-	-
C18:3n3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C20:2	-	4.99	-	-	-	-	-	-	-	-	-	-	6.736312	-	-
C20:3n6	1.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.65
C22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C22:1n9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C20:3n3	-	-	5.31	-	-	-	-	-	-	-	-	-	-	-	-
C20:4n6	8.26	30.50	21.88	0.349914	20.69	10.40	17.24	0.01	10.33	8.10	4.37	12.10	-	15.68442	
C23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C22:2	-	-	-	-	2.46	-	-	-	-	-	-	-	-	-	-
C20:5n3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C24	-	-	4.01	-	-	-	-	-	-	-	-	-	-	-	-
Other unclassified lipids	47.99	1.31	24.47	29.66	26.21	36.25	19.81	5.11	63.80	24.34	17.68	25.94	16.70	35.80	19.74

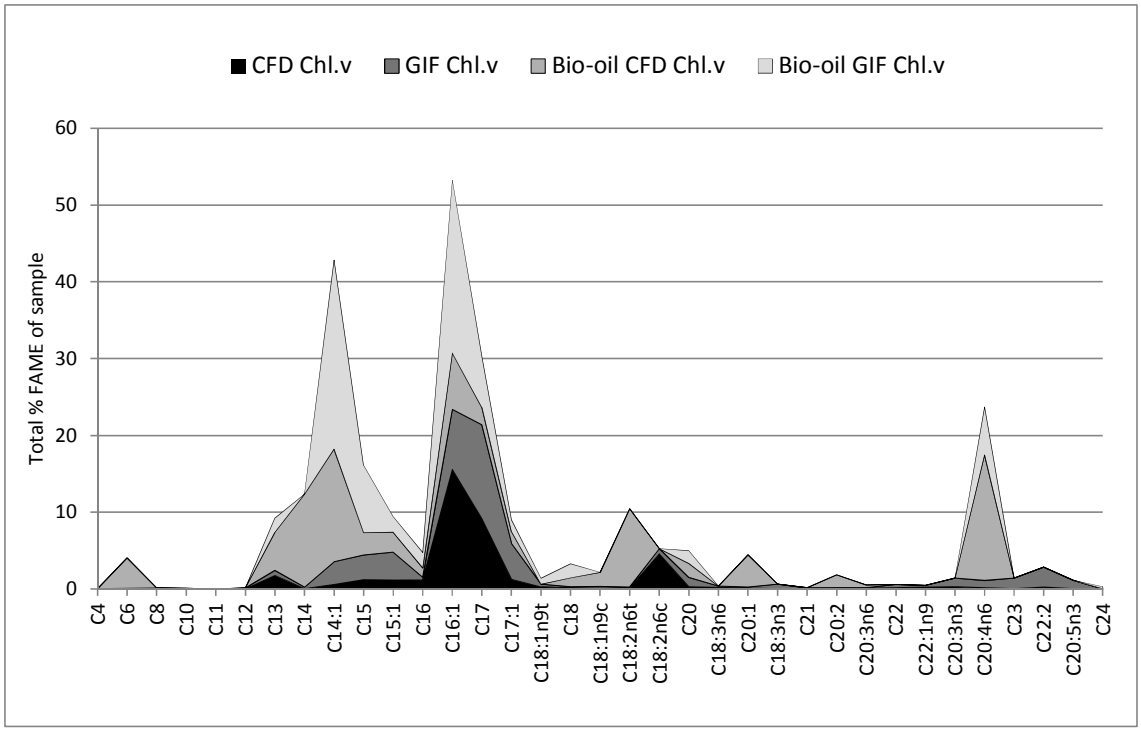


Figure 7-4 Total % FAME of *Chlorella* centrifuged & freeze dried, GIF harvested oils and HTL Bio-oils

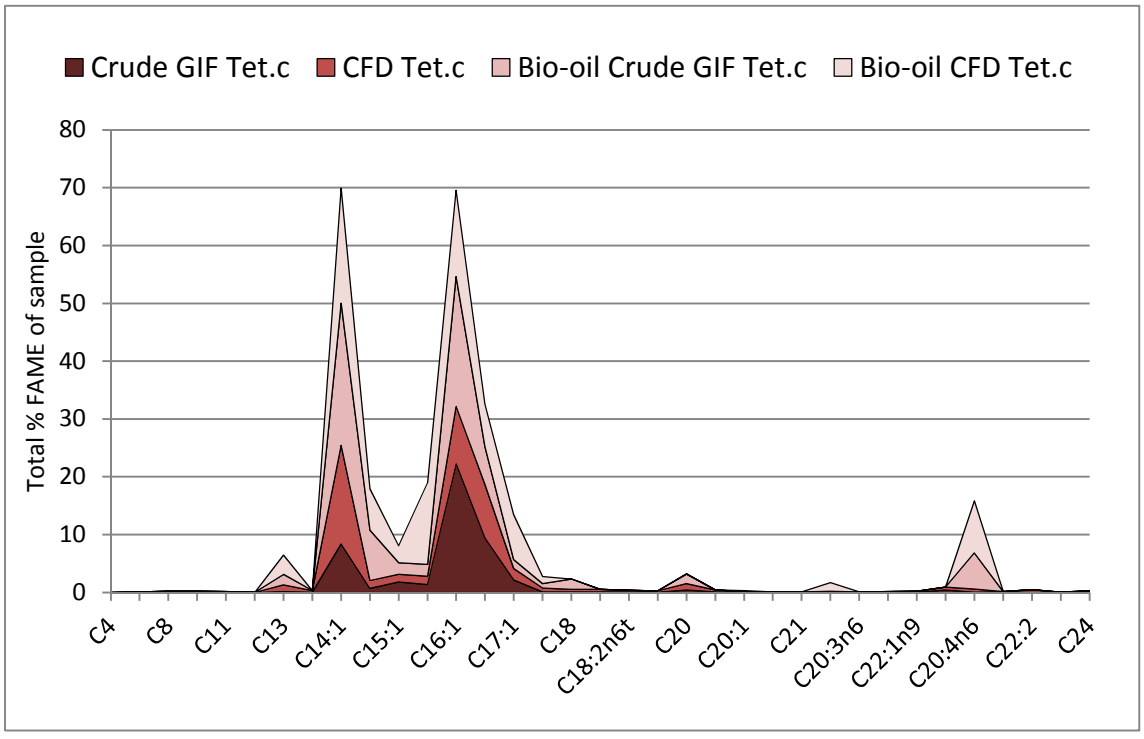


Figure 7-5 Total % FAME of *Tetraselmis* centrifuged & freeze dried, GIF harvested oils and HTL Bio-oils

Average total % FAME's are plotted in figures 7-4 and 7-5. The grouped titles correspond to the predominant microalgae within the group (freshwater or marine), though they are composed of multi-species groups this is not considered to be as indicative of FAME content as factors including culturing methods, harvesting techniques and physico-chemical parameters of HTL operation. The decision for group formation was affected by physical availability of the stock biomass, but also the extraction agent used for GIF harvesting. Both purified gel extracts and crude extracts of alginate and pectin were efficacious at harvesting and preserving the integrity of lipid profile and there was no obvious distinction between lipid profiles in any of the treatments. This result for difference between treatments was a beneficial result as it demonstrated that the GIF harvesting process and ambient drying methods used in this trial did not detrimentally affect the lipid quantity or quality of the analysed FAME.

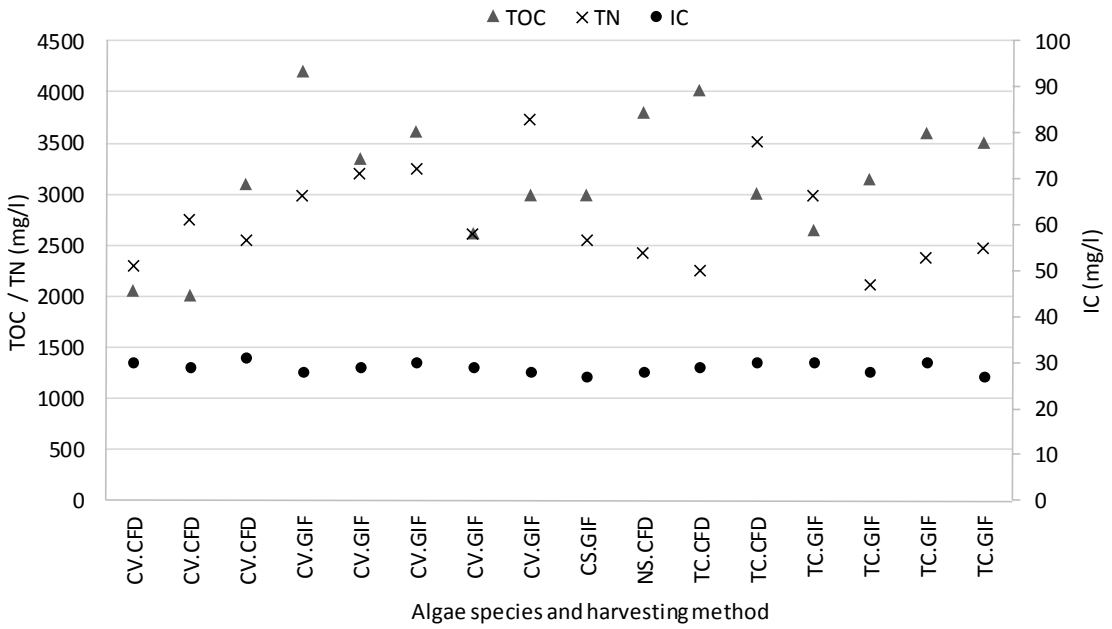


Figure 7-6 post-HTL water soluble analysis of *Chlorella vulgaris* (CV), *Chlorella salina* (CS), *Nannochloropsis salina* (NS), *Tetraselmis chui* (TC) harvested by centrifugation and freeze drying (CFD) and gel induced filtration (GIF)
Total organic carbon (TOC), Inorganic carbon (IC) and total nitrogen (TN) analysis.

Figure 7-6 displays the results of TOC, TC, IC & TN from centrifuged water solubles extracted from the HTL batch reactor vessel. It can be seen that inorganic carbon is negligible compared to total organic carbon. Such levels of TOC at between 2-4 g/l surpass TOC levels of established stationery phase cultured microalgae at cell densities ready for harvest.

7.3 Discussion

Reddy *et al.* 2013 stated that the yield of bio-oil was greater than the amount of lipid FAME present in the microalgae biomass, suggesting that bio-oil yield was incremented from protein hydrolysis. HTL of microalgae biomass is a relatively new area of research with great potential as a primary biofuel production system for later secondary processing and upgrading. Previous research has compared HTL of both high protein and highly oleaginous microalgae species. *Nannochloropsis salina* is an oleaginous microalgae and *Spirulina platensis* is a protein rich species. Both of these have a protein content of around 50%, yet post-HTL, bio-oil yields are near 40% for both species, indicating that species-nascent oil content is not the prevailing factor for HTL conversion to bio-oil. Rather, it was concluded that HTL bio-oil yield was more determined by the growth rate of the species than by the protein, lipid and oil composition of the algae (Barreiro, 2013).

According to (Du *et al.* 2012) and many other authors', lipid accumulation mainly occurs at stationary phase rather than exponential phase of microalgae culture. Consequently, the growth phase of microalgae is a significant factor for FAME accumulation in direct lipid extraction in table 7-2 and figures 7-4 and 7-5.

An aqueous co-product carbon composition of 3.92 g/l was reported by Jena *et al.* 2011, which falls within the same range of TOC reported in this study (Figure 7-6). Total nitrogen composition of post-HTL water solubles is also in the range of many synthetic and non-synthetic microalgae culturing media. This TOC/TN content contributes to the brown colouration of the water, but more importantly encourages nutrient recycling and justifies the potential energy-nutrient-cost trilemma of life cycle assessment in downstream processing of microalgae biomass for application to biofuel using an integrated production system.

Volumetrically at 80-90%, water is the major component of HTL reactants. With substantial dissolved carbon in the form of organic compounds, much of this carbon is available for recycling into successive batches of microalgae culture. In a similar way for the need to dilute AD, post-HTL water solubles also require dilution to support growth of microalgae. This has been investigated by other authors'.

An aqueous co-product nutrient additive containing substantial levels of nitrogen, phosphorus, potassium and other minerals and essential micronutrients required for algae growth was diluted with water at 0.2% (v/v) or 500 x dilution concentration providing growth of *Chlorella minutissima* at 0.52 g/l or half as much as standard BG11 medium (Jena *et al.* 2011). In a similar experiment, Chlorophyll a absorbance of *Spirulina* grown in post-HTL (300˚C operational temperature) water at 400x dilution showed good growth but was not able to grow at 50 x and 100 x dilutions possibly due to inhibitory effects of organic phenol compounds. A fourfold increase in biomass concentration was observed in the first 7 days with *Chlorella vulgaris* using the same HTL water and processing parameters diluted 50, 100 and 400 x (Biller *et al.* 2012).

Future work would amplify the mass of microalgae harvests so that pooling of samples would not be necessary, with a greater diversity of species to be tested. It would also be of interest to evaluate the effect of the HTL process on a pure alginate sample, to understand if any of the carbohydrate fractions are converted into FAME, and if so what fractional composition they account for. Work has been done on HTL of seaweed species including sugar kelp, *Laminaria saccharina*, (Bach, Sillero, Tran and Skjermo, 2014) and *Laminaria digitata, Laminaria saccharina, Laminaria hyperborean*, *Alaria Esculenta* (Anastasakis and Ross, 2015). Potassium and sodium naturally present in macroalgae might be catalyzing the reactions as their hydroxides and carbonates are catalysts commonly used during hydrothermal liquefaction of terrestrial biomass (Anastasakis and Ross, 2015). Carbohydrates will be converted to various phenolic compounds during the hydrothermal treatment (Luijkx, Van Rantwijk and Van Bekkum, 1993). The additional component of alginate which binds to the

microalgae during GIF harvesting in this study, adds extracellular polysaccharide to the final harvested product constitution. Sequential hydrothermal liquefaction of *Chlorella sorokiniana* has been developed to isolate polysaccharides with minimal degradation (Chakraborty, McDonald, Nindo and Chen, 2013). These authors' found that the maximum yield of ethanol precipitated insoluble polysaccharides was obtained at 160˚C. Alginate does not contain oil, though the degradation and re-polymerisation reactions occurring under heat and pressure could form bio-oil, and this may account for some of the difference in FAME profile between the conventionally harvested and GIF harvested microalgae samples in this study. *Botryococcus braunii* liquefaction by HTL at 300˚C and 10MPa in the presence of sodium carbonate yielded 57-64% oil with a calorific value of 45.9 MJ/kg, close to that of petrodiesel (Dote, Sawayama, Inoue, Monowa and Yokoyama 1994). Similarly, *Dunaliella tertiolecta* with a 78.4% water content using HTL at 340˚C and 10MPa in H_2 for 60 min yielded 35-37% oil with an energy density of 35-36 MJ/kg (Minowa, Yokoyama, Kishimoto and Okakura, 1995).

HTL experimental work has been conducted on both macroalgae and microalgae. It is logical to investigate the reason for the additional processing steps involved for the HTL of GIF harvested microalgae in comparison to HTL of macroalgae alone. GIF harvested microalgae can use an alginate extract of macroalgae (or terrestrial pectin). If alginate is used, HTL of GIF harvested microalgae is similar to the HTL of microalgae with an added fractional component of macroalgae derived polysaccharides. One benefit already discussed for HTL of GIF harvested microalgae is the use of Na_2CO_3 as a processing by-product of GIF harvesting and potential indirect catalyst during HTL. Na_2CO_3 is initially present as the vector for the extraction of alginate from macroalgae, and subsequently during the HTL process as a trace inclusion from sodium alginate solution used during GIF harvesting. Another advantage of using GIF harvested microalgae instead of macroalgae is that post harvested biomass is between 10-20% solids (w/v). This is the ideal viscosity and solids loading for integration with HTL and positively correlates with the energy conversion efficiency (Barreiro, 2013). Furthermore, although GIF microalgae at post-harvest has a 20% solids content, it remains as a liquid and is able to be pumped, which eases potential scalability and industrial

commercial issues with potential increased volumetric capacity. By contrast, macroalgae is a coarse product. Chopping of macroalgae in wet form is inconsistent and cannot guarantee a uniform particle size because biomass pieces are lubricated and move in a non-Newtonian fluid characteristic. Chopping macroalgae in dry form provides better particle uniformity, but there may still be some particles too large to pass through the narrow feed pipes of the HTL unit and potentially reactor vessel. In a scaled up commercial production scenario, attention must be given to ensuring that there are no blockages within HTL transportation and reactor vessel pipework which could otherwise reduce production efficiency.

Conclusions

GC-FID analytics utilise reference standards for peak identification. With a complex mixture of organic lipids in both microalgae oil and bio-oil, any reference standard cannot capture and interpret the complete range of biochemicals present within a sample, as evident by results in this study. By comparison, Gas Chromatography-Mass Spectrometry (GC-MS) provides finer substance identification cross-referenced against a database of defined chemical substances. Further work would ideally interpret results using GC-MS or in combination with GC-FID. Otherwise a reference standard with lipid profiles of typical bio-oil products containing the complex mixture of parafins, oleofins, hydrocarbons, alcohols, aromatic hydrocarbons, esters, furans and ketones in addition to the FAME 37 reference standard would complement a fuller understanding of the biochemical and thermo-chemical conversion profile.

However the main beneficial conclusion of this chapter is that GIF harvesting and drying does not adversely compromise or modify the lipid profile of microalgae lipids as detected to a level of statistical significance with the major identified lipids.

7.4 Acknowledgements

The author is grateful for the practical contribution from Freda Onakpoya and Utsab Guharoy with work undertaken in this chapter. Specifically, the author would like to acknowledge their individual contribution. Utsab Guharoy designed and constructed the experimental HTL vessel and heating equipment outlined in 7.2.2. Freda Onakpoya processed microalgae biomass samples using the HTL equipment, and subsequently methylated these HTL bio-oil samples prior to analysis using gas chromatography, total carbon and nitrogen.

8 Biomass Processing Economics and Solar Integrated Hydrothermal Liquefaction of microalgae

This chapter has contributed together with other academics' work for publication in the Journal of Applied Energy, Techno-economic analysis of solar integrated hydrothermal liquefaction of microalgae, Vol. 166, p.19-26 (2016).

8.1 Introduction

Preceding chapters have analysed methods and mechanisms for the growth, harvesting and processing of microalgae into bio-oil. This chapter considers the economic implications of how microalgae biomass is grown and converted into bio-oil or other potentially viable bio-products.

Analysis firstly looks at the proposed batch scale growth of microalgae using the hypothetical scaled alginate-pectin immobilised growth system described in chapter 3. The experimental alginate disk method was investigated to reduce consumable cost expenditure of experimental testing in the laboratory, and simultaneously provide answers to this proposed growth system if it were to be scaled in production. Aside from whether or not an immobilised gel matrix can achieve flotation; large-scale extensive growth application within lakes or the sea is only feasible depending on the material cost inclusion and economics of scaled production systems which is addressed in this chapter.

Integrated waste technologies have been described in previous chapters. United concepts and experimental findings of previous chapters present an overview of a process system technology using HTL as a conversion pathway and solar thermal concentrating power (CSP) as a heat source. Unification of the processes presented in this thesis with associated energetic and monetary values quantify the transition and conversion divide from experimental interest to the core of a commercial investment proposition.

Energy balance inputs and outputs determining biofuel processing efficiency of microalgae have multiple interpretations from different authors'. Energy efficiency can be identified in terms of fossil fuel inputs or energy used for cultivation, harvesting or drying relative to the final biomass energy content. However,

complete lifecycle analysis (LCA) should also include downstream processing of microalgae biomass into biofuel, practically resulting in more energy consumed than produced due to high production energy inputs for oil extraction, or thermal downstream processing. The energy required for microalgae and biogas production from *Nannochloropsis* has been calculated to be as much as 8-11 times more than the bio-gas energy yield (Razon & Tan, 2011). Key issues for future R&D of microalgae biofuels include both the utilisation of co-products and development of energy efficient thermo-conversion processes (Khoo *et al.* 2013). The original concept of solar-HTL integration for bio-oil rather than conventional solar based electrical energy generation provides comparison between energetic liquid bioenergy yields and electricity generating conventional counterparts. This study investigates thermodynamic and economic factors influencing the unification of HTL and CSP parabolic troughs. CSP parabolic troughs yield a temperature of up to 400°C (Sansom, 2014), whilst beneficial operational temperature requirements for HTL occur within the range of 250-350°C (Elliott *et al.* 2015; Toor *et al.* 2011). Combination of a thermodynamic assessment of parabolic troughs (Manzolini *et al.* 2011; Pitz-Paal *et al.* 2007; Montes *et al.* 2011) with an economic appraisal using experimental field trials of microalgae productivity (Acíen *et al.* 2012; Chisti, 2007; Norsker *et al.* 2011) presents a forecast of the potential viability of this form of technology integration.

Parameters affecting product yield of HTL derived bio-oil include microalgae species, feed ratio of solids to liquid, reaction temperature, holding time, heating rate, cooling rate, presence of catalysts and effective product separation (Barreiro *et al*. 2013). HTL process development from batch to continuous feed reactors has occurred (Elliott *et al.* 2015). Continuous feed systems have advantages of higher feedstock flows and lower process and retention times, lacking uncertainties in heating and cooling rates common in batch run experiments (Jazrawi *et al.* 2015). Development of a continuous feedstock process requires thermal quenching to reduce temperature differentials, ensure preservation of reactant products and optimise the viable and scalable commercial integration into a CSP/HTL production system. Engineering of design parameters encountered in product development will lead to further challenges to facilitate an

understanding of thermal retention, multi-phase flow, feedback control for product optimisation and fluid mechanics of reactant products within the core of the reaction pressure vessel. Microalgae biomass of concentration 10-20% (w/v) is optimal for HTL boundaries of solids loading (Jena *et al.* 2011). Triple energy cost-savings at strategic intervals of product process optimisation include:

• microalgae growth in wastewater & recycled HTL water solubles

• low-cost microalgae harvesting for direct application to downstream processing

• low-energy processing to bio-oil via CSP/HTL

Operational pressures, temperatures and experimental processing variables initiate targeted bio-oil product formation. Thermodynamic and mass balance modelling of conversion trends provides research understanding for inception of biofuel formation. Companies which originally targeted microalgae biofuel 5-10 years ago have now diverted their main product portfolios away from biofuels to align with nutritional and nutraceutical dietary components for either human or animal consumption. This market product transition has been predominantly economically and market driven and has effectively elongated the target timeframe for viable biofuel production from microalgae. HTL beneficially converts protein, carbohydrate as well as lipid component fractions into bio-oil, resulting in bio-oil yields 10-15% above the lipid content of original microalgae (Biller & Ross, 2011). HTL utilises whole cell biomass and any other waste organic matter in the culture media to be chemically degraded into inert hydrocarbon fractions as end products. Integration of solar heat as the imperative vector of biofuel transformation does not jeopardise holistic energetic transformation pathways resulting in a favourable net energy return in the LCA. Inclusion of diluted post-HTL liquid soluble fractions into microalgae growth culture as a complementary mixotrophic nutrient to bio-available nitrogen and phosphate from spent wastewater treatment additionally promotes a favourable LCA net energy return.

8.2 Aims and Objectives

Aims were to identify the optimal economic biomass production and conversion profile with primary consideration of biofuel as an end product. The commercial use of alginate vectored desalination is considered with a potential commercial application. GIF harvesting in chapter 4 is evaluated economically at scale and for use in conjunction with solar concentrated power as a heat integrated hydrothermal liquefaction processing technology using current market costs. The economics of integrating microalgae and hydrothermal liquefaction using solar concentrated power parabolic troughs is investigated. Objectives identify Heat Transfer Fluid (HTF) volume of a conventional electricity generating CSP plant, and replace this core HTF volume with an equivalent volume of an HTL pressure reactor; comparing CAPEX and OPEX costs of an electricity generating CSP plant and a theoretical CSP-HTL bio-oil production facility. Integrated microalgae production costs evaluate the cost-benefit structure and potential investment prospect of such technology integration.

8.3 Methodology

A decision matrix flowchart is presented in Figure 8-1 to outline and establish some of the criterion used to disseminate the most cost effective processing pathway of microalgae growth and subsequent conversion into bio-oil. Gel immobilised mass cultivation at the compositional inclusion required to form a structurally stable matrix is calculated as being too costly for commercial implementation for end usage in bio-oil, though could viably be a production scenario for higher value products for consideration in further research. GIF harvesting is presented as an economic alternative or lower cost processing pathway in comparison to conventional harvesting. Finally, the downstream processing pathway of hydrothermal liquefaction and heat integration from solar energy is evaluated as a proposed cost reduction strategy. The following economic methodology processing step subsections of immobilised microalgae growth, GIF harvesting and solar integrated processing are considered in sequential order.

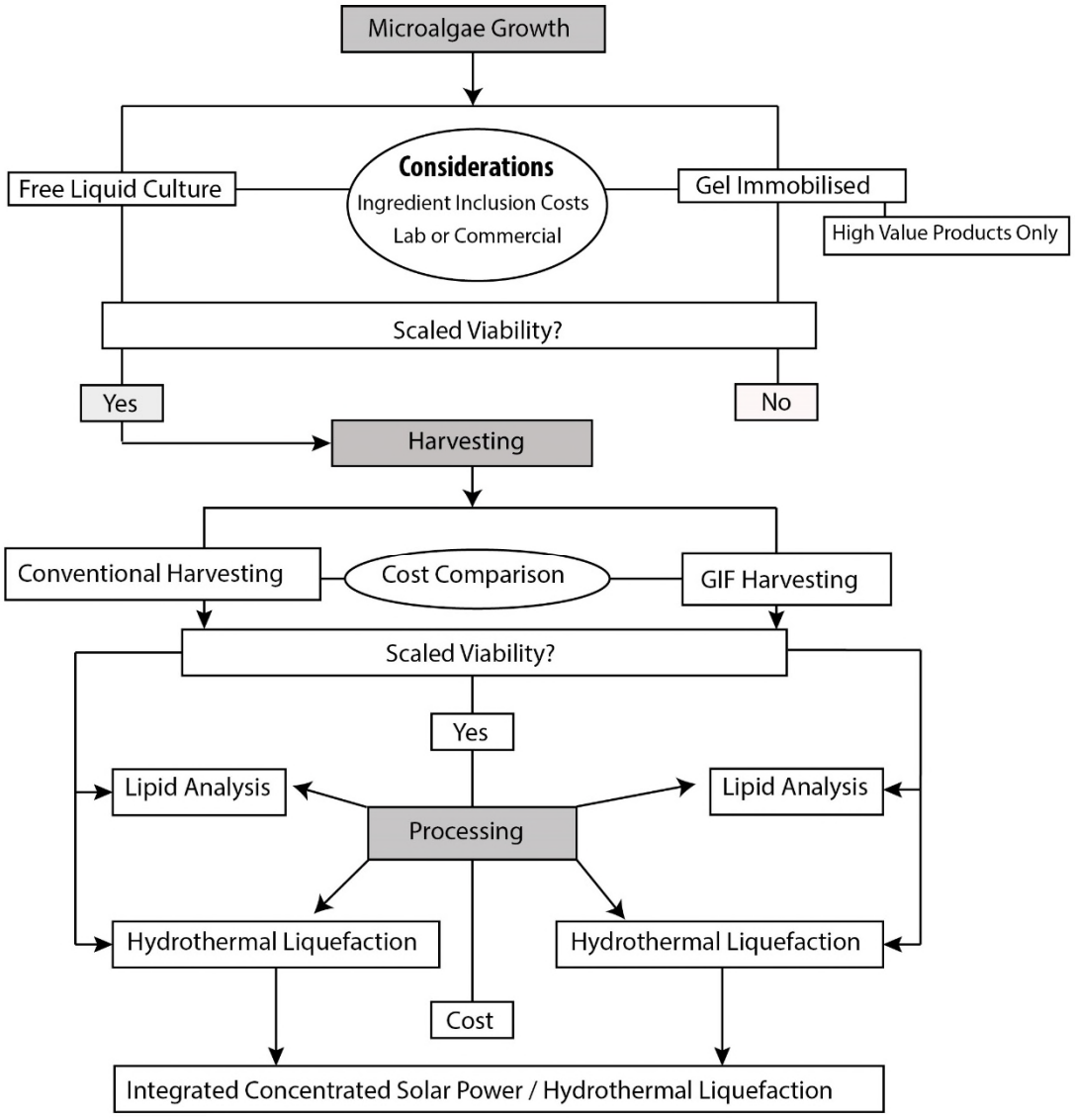


Figure 8-1 Decision matrix flowchart to determine optimal cost effectiveness and processing pathways for microalgae growth, harvesting and processing.

8.3.1 **Immobilised growth economics**

Global market alginate and seaweed prices from January 2013 (Piantini, 2012, Chile), and European traded pectin samples from June 2013 (Herbstreith & Fox GmbH, Germany) were used for current costs. The commercial laboratory price of alginate at £100-150 /kg includes the additional processing of the seaweed raw material to process to a high grade of purity, incorporating additional mark-up

costs by the distributors, traders and refiners of dried seaweeds. For example, a commercial producer of around 40,000 t of dried macroalgae with a retail price of £800 /t equates to approximately £3,000 /t for refined sodium alginate (Piantini, 2012). Wet seaweed contains approximately 40% alginate and there is a 33% recovery of sodium alginate from dried seaweed (Vauchel *et al,* 2008). With strong wind or tropical sunshine, seaweed can be dried with low net-energy inputs although the harvesting of natural non-farmed seaweeds requires high numbers of workers and manual labour intensity. Calcium chloride is a “waste” by-product of the Solvay process for production of Sodium Carbonate (soda ash), widely used in the paper, glass, detergents industries (Trypuć and Bialowicz, 2011), also for sulphur dioxide removal in power stations. Sodium carbonate is widely used in UK sewage treatment works. Commercial UK use of 10% sodium carbonate solution has been quoted at £177 per 1000 l or sodium bicarbonate powder (40 x 25 kg bags) at £320 (Operations coordinator, Alpheus Environmental Ltd, 19.8.15).

Economic evaluation of floating hydrogel cultivation scalability used current raw commodity ingredient prices (a fluctuating cost). Fabrication material inclusion levels were decided by experimental structural integrity longevity trials with variable alginate (0.5% - 2%) and glycerol (1% - 10%) inclusion levels in disks. The economic forecast model excludes additional manufacturing processes, equipment and labour. These additional costs were only to be added for secondary analysis in the eventuality that major production ingredient costs proved to be favourable. Gel disk methodology is described in 3.2.4. After the 10-day longevity incubation period, disks were removed from artificial seawater and analysed visually be eye for durability and structural integrity.

8.3.2 **GIF harvesting economics**

GIF harvesting data from was firstly extrapolated with commercial market values of gelling materials to estimate the potential cost of scaled production from the laboratory to a small business production scale. 200 ml samples of *Tetraselmis chui* were harvested with crude SBP extract and analysed with varying incremental volumes of SBP between 4 ml and 30 ml. A secondary GIF

harvesting forecast utilised an alginate and SBP concentration of 1.5% and 5%, with corresponding prices of £5000 /t and £37.20 /t respectively.

The higher market estimate of £300 /t for de-molassed sugar beet was used for economic forecasting. Agricultural feed prices fluctuate at frequent time intervals and are dependent on global commodity markets, crop yields, and tonnage purchased. However, the market estimate of £300 /t for de-molassed sugar beet was a conservative and realistic reflection of current market prices. A soluble fibre content of pectin at 12.4% was used. The forecast model assumed that non-digestible fibre could be re-integrated into the feed chain. In so doing, the market value of sugar beet feedstock was reduced proportionately by 12.4% and likewise the same proportionate costs were allocated to sugar beet pectin (SBP), i.e. £37.20 /t. This assumption was based on account of SB already being sold into established animal feed markets and also that the inclusive component of sodium carbonate is a recognised animal feed additive. A further assumption is that the newly formed pectin extracted feed product can fetch equivalent market prices as unmodified sugar beet. Extrapolated commercial values are scaled estimates based on laboratory experimentation using crude extracts of laboratory extracted SBP for GIF harvesting on 10 l batches of microalgae culture.

Finally, amplification of productivity forecasts alginate and pectin to hypothetically harvest 1 t microalgae. 2% sodium carbonate to extract alginate and pectin from seaweed and terrestrial biomass respectively, and 1% calcium chloride solutions were modelled with microalgae cultures prior to filtration. Assumptions included 4 rates of microalgae production of 0.5, 2, 5 g/l (phototrophic) and 50 g /l (heterotrophic). Calcium chloride cost was £220 /t, sodium carbonate was £320 /t. Alginate and pectin costs varied between £700 and £3000 /t depending on source, microalgae productivity and culture volume. Microalgae cost forecasts were based on a sales price of £500, £2500 and £7000 /t. Gross revenue was calculated as the negative total harvesting costs plus sales price per metric tonne of microalgae biomass. Additional capital expenditure costs and operational microalgae productivity and labour costs were not included in the harvesting forecast.

8.3.3 **Solar integrated HTL processing economics**

HTL requires attainment of temperatures in the range of 300˚C and pressures of 15-20 MPa (Elliott *et al.* 2015; Toor *et al.* 2011). This pressure is achieved by heating unpressurised water in a pressure proof vessel to 300˚C. No additional pre-pumping or pre-pressurisation is required. Figure 8-2 shows the experimental comparison between pressure and temperature in a 250 ml lab-scale HTL pressure vessel (Parr Instrument Company) with 100 ml of water at atmospheric pressure before heat was applied. The pressure vessel supported pressures up to 465˚C corresponding to 4650 psig (32 MPa), therefore the range of pressures and temperatures required for HTL of microalgae biomass is well within the specifications of this manufactured pressure vessel.

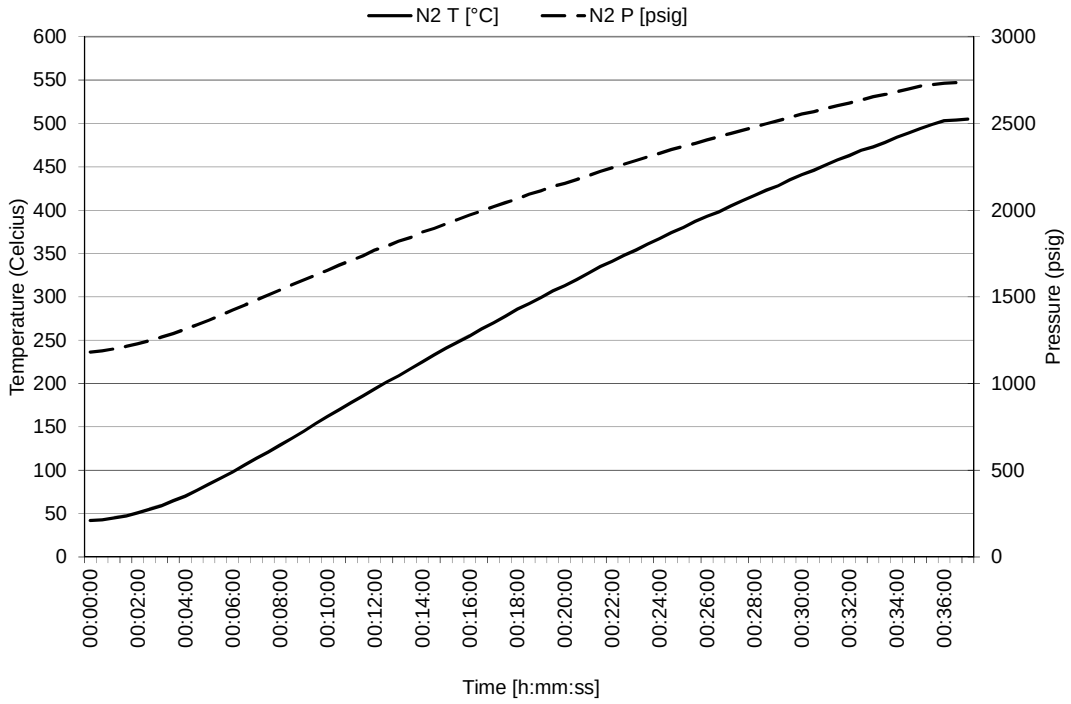


Figure 8-2 Atmospheric pressure increase with temperature rise in a pressure vessel

If heat and a suitable containment pressure vessel alone are the requirements for HTL reaction kinetics, then integrated energy processing technologies using Concentrating Solar Thermal power (CSP) offers a viable solution.

CSP is able to concentrate solar energy using highly reflective mirrors to generate high temperature thermal energy in excess of 400°C. In conventional CSP power plants, heat energy is diverted into steam or gas turbines to produce commercial electricity. There are four main types of CSP systems - parabolic troughs, parabolic dishes, flat mirror heliostats and linear Fresnel reflectors. Of these, parabolic troughs are most widely used for electricity power generation. CSP parabolic troughs contain a heat transfer fluid (HTF) which can either be molten salts (mixtures of $NaNO_3$ and KNO_3) or synthetic oil. The most important characteristics of HTF are specific heat capacity, density, corrosivity, viscosity, freezing temperature and thermal stability (Manzolini *et al.* 2011).

Capital expenditure (CAPEX) investment costs of a large parabolic trough plant range from USD $4.3-8.5 /W (Guerrero-Lemus & Martínez-Duart, 2012). A 5 MW CSP plant based on a trough width of 2.26 m, 15 rows of troughs of 90 m spaced 4 m apart, provides a total trough length of 1350 m and 5694 kW output. Such a CSP plant provides a heat exposed HTF volume of 4343 l. With an operational expenditure (OPEX) investment value of USD $8 /W, this facility would have a capital investment cost of USD $45.5M. World electricity output from CSP flat-lined at less than 400 MW from 1990 to 2006, then surged to almost 1800 MW by 2011, (Guerrero-Lemus & Martínez-Duart, 2012), consequently the technology is in a state of recent expansion and as such there still remains some ambiguity concerning the economics of production systems.

According to (Putt *et al.* 2011), and a figure quoted by other authors', 20 g dry mass/m^2/day^{-1} for a high rate algal pond is a typical range of productivity. Therefore, a 1 hectare site could produce in the region of 180-200 kg biomass per day. With a ratio of 20% solids in HTL, this could provide 1000 l feedstock per day. The HTF volume space is ideally suited for the reaction core of HTL of biomass as it reaches the required temperature and it would avoid pumping contents far from the solar field therefore removing the requirement for a heat exchanger. HTF could be replaced by microalgae biomass and water as the reactant components of HTL. Inputs and discharge on either end of each linear row of parabolic troughs would suffice for batch or semi-continuous processing.

With a diurnal thermal HTL capacity for 3 h either side of midday, a batch process time of 1 h to fill and run the reaction core, another 1 h to empty and prepare the core for the next batch sample, then it would be possible to achieve 3 batch runs per day, 9-11am, 11-1pm and 1pm-3pm. Assuming a production time and change around time of HTL equipment being 2 h, then CSP plant size for processing of HTL feedstock would be less than the requirements of using CSP for electrical energy generation.

8.4 Results

8.4.1 **Immobilised gel growth**

Table 8-1 shows a macro-economic evaluation of alginate hydrogel bio-polymer cultivation. Results show that the costs of production are almost threefold greater than the production incomes per cycle, using an alginate concentration of 2%. This is not a viable business model nor worth additional investigation for an end product targeting microalgae biofuels, though feasibly justified for higher value end-products. A reduction in the solid alginate gel concentration below 2% increases the fragility of the structured gels and would not be practically viable. Figure 8-3 shows disks tested at 4 concentrations of alginate and 4 concentrations of glycerol for 10-day longevity durability on exposure to artificial seawater.

Minimum inclusions were found to be 1.5% alginate and 2% glycerol to avoid ruptured disks. Replacement of higher value alginate with lower value pectin is also not a complete substitution because pectin gels are more fragile than alginate gels (Table 3-1). Table 8-1 shows scaled costs using alginate at wholesale crude extract price of £ 5 /kg.

Table 8-1 Macro-economic evaluation of alginate hydrogel bio-polymer cultivation

a	Average thickness (mm)		10
b	Surface Area of disks (cm^2)	$\pi \times r^2$	346.4
c	Number of disks per (m^2)	10000/*b*	28.87
d	Weight of Coco per disk (g)		6
e	Volume of gel per disk (ml)		300
f	Dry weight of gel per disk (g)		6
g	1 hectare (m^2)		10000
h	Mass of Coco for 1 hectare (kg)	*(c x d x g)/1000*	1732.3
i	Mass of dry gel for 1 hectare (kg)	*(c x f x g)/1000*	1732.3
j	Volume of wet gel for 1 hectare (m^3)	*((e/1000)/1000) x c x g*	86.6
k	Volume of glycerol for 1 hectare (m^3)	*j x 0.05*	4.33
l	Volume of $CaCl_2$ for 1 hectare (m^3)	*k x 0.6*	52
m	Density of Glycerol (g/L)		1260
n	Density of $CaCl_2$ (g/L)		1020
o	Coco cost per tonne (£)		250
p	Cost of 1 hectare Coco production (£)	*o x (h/1000)*	433
q	Cost of Alginate per tonne (£)		5000
r	Cost of 1 hectare Alginate production (£)	*q x (i/1000)*	8662
s	Cost of glycerol (£)		600
t	Cost of 1 hectare glycerol production (£)	*(m/1000) x k x s*	3274
u	Cost of $CaCl_2$ (£)		300
v	Cost of 1 hectare $CaCl_2$ production (£)	*(n/1000) x l x (u x 0.02)*	318
w	**Total cost of 1 hectare production (£)**	***p + r + t + v***	**12,687**
x	Volume of production in 1 hectare (m^3)	*J*	
y	Microalgae production @ 2g/L/cycle (kg)	*(j x 1000) * 0.002*	173
z	Biomass total per cycle (kg)	*y + h + i + (k*m)*	9095
aa	Bio-crude production per cycle (L)	*(z x 0.3) x 1.125*	3069
bb	Bio-crude production per m^2 per cycle (L)	*aa/10000*	0.307
cc	Cycle length (days)		10
dd	Bio-crude production per m^2 per day (L)	*bb/10*	0.0307
ee	Density of bio-crude (g/L)		875
ff	Cost of bio-crude per litre (£)		1.4
gg	**Production income per cycle (£)**	**aa x ff**	**4,297**

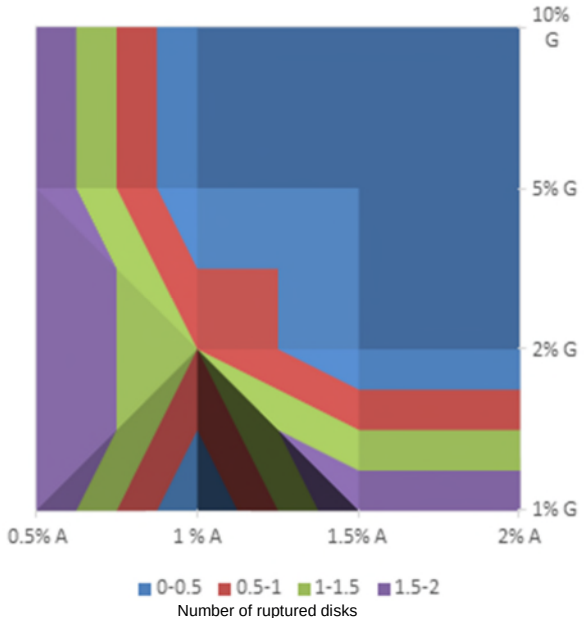


Figure 8-3 - Surface contour plot of 32 alginate-glycerol hydrogel composite disks ranging between 0.5-2% Alginate (A) and 1-10% Glycerol (G)
showing the number of ruptured disks (0-2 per treatment) following a 10-day longevity tolerance trial to artificial seawater exposure.

8.4.2 GIF harvesting and desalination

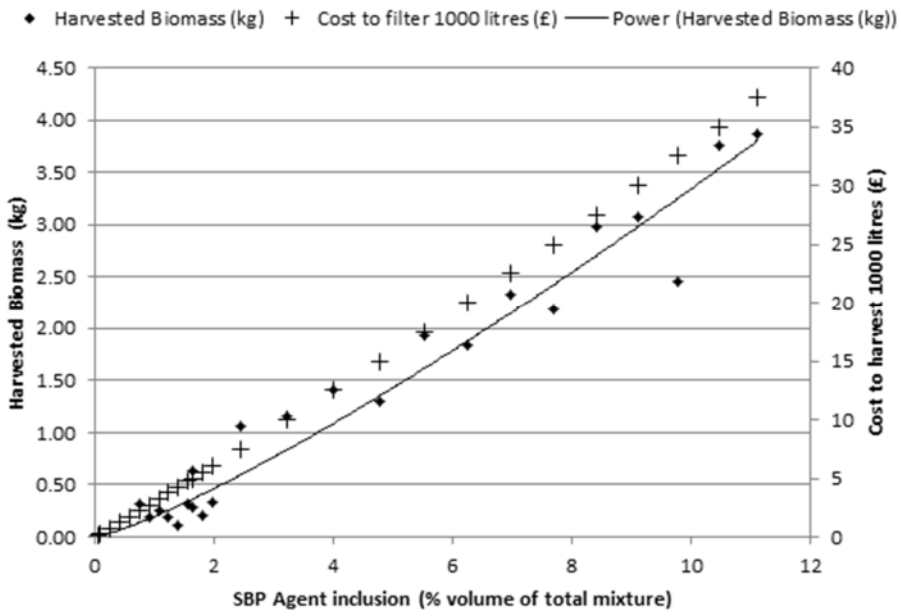


Figure 8-4 Experimental cost forecast based upon 200 ml samples, <1% - 10% range of crude SBP inclusion as percentage of total mixture to harvest *Tetraselmis chui* and extrapolated costs of scaled harvesting projected yields to 1000 l culture.

Compositions of hydrogel formulations are worth investigating further due to the relative cost fluctuations of the respective ingredients, some of which are low grade by-products of industrial food and feed processing. Though this mechanism of production has proven to be uneconomically viable in the present form for biofuels, development of foam technologies would simultaneously reduce inclusion of ingredients and improve flotation characteristics. Patents for foam flotation dissolved protein separation systems for the separation of metallic ions from aquarium water (Guido, 1972) and seawater purification (Gesellschaft, 1973) have been developed. An alternative strategy to achieve a commercially viable production system would be to increase the cost return per unit volume or mass on the microalgae product composition with high value biochemical. For a high value antioxidant or unsaturated lipid end product from microalgae, this approach may be worthy of further research and development.

Of the three crude-extracted gelling agents, SBP was the most effective for GIF harvesting. Citrus Pecting (CP) and *Macrocystic pyrifera* crude extracted alginate also functioned for GIF harvesting. In a theoretical commercial scale-up of this technology, Table 8-2 and 8-3 show how economic forecasting translates the costs of the incipient ingredients. Table 8-2 shows that alginate is not cost effective. Table 8-3 shows pectin has potential profitability given the low cost of this resource compared with use as a microalgae harvesting mechanism. Cost comparisons are made with other microalgae flocculants for waste water treatment as there is no similar comparison for this specific technique elsewhere. Henderson, 2007 states polyDADMAC coagulant for waste water treatment costs £0.40 per 1000 l of treatment, and aluminium sulphate costs £0.80 per kg or £0.83 per 1000 l. Ometto, 2014 states that microspheres cost £10,000 per t and enzymes cost £1,000 per t. By comparison sugar beet pectin is significantly cheaper.

Extrapolated productivity of this technology to a commercial scenario forecasted a loss using alginate, but a profit using pectin.

Table 8-2 GIF harvesting with alginate – OPEX production economies of scale

			Test tube scale	**Small business scale**
a	Volume of Alginate gel (ml)		8	5,000,000,000
b	Alginate dry gel (g)	a x 1.5%	0.12	75,000,000
c	Volume of $CaCl_2$ (ml)	a x 1.5	12	7,500,000,000
d	Volume of algae culture (ml)	a x 5	40	25,000,000,000
e	Final volume (l)	(a+c+d)/1000	0.06	37,500,000
f	Alginate cost £0.03/kg (5g/l) (£)	5 x (b/1000)	0.0006	375,000
g	CaCl2 cost (£)	0.3 x 0.02 x (c/1000)	0.000072	45,000
h	Harvested microalgae (kg)	(d x 0.0025)/1000	0.0001	62,500
i	Bio-crude production (l)	((b/1000)+h) x 0.3 x 1.125	0.000074	46,406
J	Bio-crude production (ml)	I x 1000	0.07425	46,406,250
k	Bio-crude income @ (£1.4/l)	I x 1.4	0.000104	64,969
l	**Profit / (Loss)**	**k – (f+g)**	**(0.00057)**	**(355,031)**

Table 8-3 GIF harvesting with SBP – OPEX production economies of scale

			Test tube scale	**Small business scale**
a	Volume of SBP gel (ml)		8	5,000,000,000
b	SBP dry gel (g)	a x 5%	0.4	250,000,000
c	Volume of $CaCl_2$ (ml)	a x 1.5	12	7,500,000,000
d	Volume of algae culture (ml)	a x 5	40	25,000,000,000
e	Final volume (l)	(a+c+d)/1000	0.06	37,500,000
f	SBP cost £0.0372/kg (5g/l) (£)	0.0372 x (b/1000)	0.000015	9300
g	CaCl2 cost (£)	0.3 x 0.003 x (c/1000)	0.000018	11,250
h	Harvested microalgae (kg)	(d x 0.0025)/1000	0.0001	62,500
i	Bio-crude production (l)	((b/1000) +h) x 0.3 x 1.125	0.00017	105,469
J	Bio-crude production (ml)	i x 1000	0.16875	105,468,750
k	Bio-crude income @ (£1.4/l)	i x 1.4	0.000236	147,656
l	**Profit / (Loss)**	**k – (f+g)**	**0.000203**	**127,106**

Alginate vectored desalination efficiency would require repeated consecutive treatments in order to achieve levels of overall desalination from seawater salinity to potable water. This is in comparison to reverse osmosis water treatment plants which can achieve this process in a one step, albeit, energy intensive process. If alginate desalination is found to be economically viable at a larger scale of production, particularly with respect to low-cost brine remediation, then these findings have implications for treatment of saline water either alone or in combination with incumbent desalination technologies. Process improvements in operational efficiency with modified methodology or equipment could offer a potential future solution for commercial desalination processes. This technology could also be a low-cost brine remediation technology from the waste outputs of

conventional desalination processes prior to disposal in the natural environment in order to protect marine flora, fauna and ecology.

Table 8-4 Cost forecast for microalgae growth, alginate extraction and GIF harvesting costs for scaled production of 1 t microalgae biomass

	GROWTH		EXTRACTION		
COST FORECAST	Microalgae Productivity (g/l)	Volume of water needed to grow 1 t algae (m^3)	[0.5%] Alginate/Pectin required to harvest water for 1 t algae (t)	[2%] Na_2CO_3 to extract alginate/pectin required to harvest volume of water needed to grow 1 t algae (t)	Cost of Na_2CO_3 (£)
	0.5	2000	2	0.67	213
	2	500	0.5	0.17	53
	5	200	0.2	0.07	21
	50	20	0.02	0.007	2

	HARVEST				
COST FORECAST	Cost of Alginate/Pectin per t crude biomass (£)	Cost of Alginate/Pectin to harvest volume of water to grow 1 t algae (£)	[1%] $CaCl_2$ required to harvest volume of water needed to grow 1 t algae (t)	Cost of $CaCl_2$ (£)	Total alginate/pectin extraction and algae harvesting costs /t algae (£)
	1213	8087	4	880	8967
	1053	1755	1	220	1975
	1021	681	0.4	88	769
	1002	67	0.04	8.8	76

Table 8-5 Gross operating revenue for GIF harvesting process of 1 t microalgae biomass excluding capital infrastructure expenditure and labour

	Scenario 1 - Gross revenue /t (Alginate fixed price £3 K /t)				
			Low sales price	Medium sales price	High sales price
SALES FORECAST	Microalgae Productivity (g/l)	Total alginate/pectin and algae harvesting costs /t (£)	£500 /t	£2500 /t	£7000 /t
	0.5	6000	-5500	-3500	1000
	2	1500	-1000	1000	5500
	5	600	-100	1900	6400
	50	60	440	2440	6940
	Scenario 2 - Gross revenue /t (Na_2CO_3 extracted alginate £1 K /t)				
			Low sales price	Medium sales price	High sales price
SALES FORECAST	Microalgae Productivity (g/l)	Total alginate/pectin and algae harvesting costs /t (£)	£500 /t	£2500 /t	£7000 /t
	0.5	8967	-8467	-6467	-1967
	2	1975	-1475	525	5025
	5	769	-269	1731	6231
	50	76	424	2424	6924
	Scenario 3 - Gross revenue /t (Na_2CO_3 extracted pectin £ 0.7 K /t)				
			Low sales price	Medium sales price	High sales price
SALES FORECAST	Microalgae Productivity (g/l)	Total alginate/pectin and algae harvesting costs /t (£)	£500 /t	£2500 /t	£7000 /t
	0.5	6087	-5587	-3587	913
	2	1255	-755	1245	5745
	5	481	19	2019	6519
	50	47	453	2453	6953

Tables 8-4 and 8-5 show a productivity forecast for alginate or pectin extracted biomass used to hypothetically harvest 1 t microalgae. 2% sodium carbonate was used as the sole chemical to extract alginate from seaweed (or pectin from terrestrial biomass) respectively, as described in detail in 4.3.1. Though potentially a low-cost harvesting process, benefit is incurred from a reduced volume of microalgae culture as a smaller volume of alginate and GIF harvesting chemicals can be used. However, further work would need to identify the validity of using the GIF harvesting method at higher productivities of microalgae biomass in heterotrophic production systems. The forecast model therefore considers a range of microalgae productivities between 0.5 g/l (phototrophic) and 50 g/l (heterotrophic, from a species such as *Chlorella protothecoides*). Costs were

calculated as present day market values. Calcium chloride cost was £220 /t, sodium carbonate was £320 /t. Alginate and pectin costs varied between £700 and £3000 /t depending on source, extraction methodology, microalgae productivity and associated culture volume. Microalgae cost forecasts were based on a sales price of £500, £2500 and £7000 /t. Sales prices align with present day market prices and cover the range span of prices, from cheaper than microalgae can be produced, to a high baseline commodity price of microalgae biomass for feed and fuel applications. The highest price range of £7000 /t is still below some higher value products which can fetch considerably more than this, for example astaxanthin and long chain unsaturated omega-3 oils, albeit refined extracts from whole cell biomass. As depicted visually in figure 1-2, low production of high value speciality products are thought to be more orientated to microalgae biomass. These are also the currently commercially viable products from microalgae rather than lower value food and feed products. Tables 8-4 and 8-5 focus only on the main GIF harvesting processing components of a potential scaled production scenario. This permits a standalone appraisal and further understanding of the validity of the ranges of costs and crude commodity ingredients. The valuation of alginate at £1000 /t for seaweed, pectin at £700 /t and $CaCl_2$ at £220 /t are conservative higher-end cost estimates. If carefully sourced as waste materials from select food industries for terrestrial pectins, marine carbohydrates or mining waste for $CaCl_2$ and Na_2CO_3 and incorporated into an alternative waste recycling business model, it is plausible that these costs could be reduced further, making potential revenue more than that shown in Tables 8-4 and 8-5. Additionally, bulk buying could further lower wholesale costs.

8.4.3 **Solar integrated Hydrothermal Liquefaction**

A 1 hectare CSP site is in-excess of requirements for the requirement to process 200 kg microalgae biomass per day. 1000 l daily production of HTL feedstock comprising 200 kg dry weight microalgae and 800 l of water is significantly less than the 4343 l of HTF volume on a 1 hectare CSP electricity plant. 350 l HTF volume would be attained from 108 m of solar parabolic troughs, which if used for electricity generation would produce 456 kW of heat output. A CSP plant of

this size being used for HTL application would be able to process the 200 kg of daily biomass estimated production. With 3 cycles per day of 333 l per batch, then such a CSP plant would be suffice to requirements, and take up less than 0.2 hectares of land space for the solar processing component of operations. Using the capital expenditure investment costs of USD $4.3 – 8.5/W (Guerrero-Lemus & Martínez-Duart, 2012), then this plant would cost between USD $1.9-3.9M. However such a plant would actually cost less than these figures as the stipulated capital investment costs are for a conventional CSP plant including associated infrastructure in addition to the solar array such as heat exchangers and power turbines. Consequently, estimated capital investment costs using CSP direct steam generation (DSG) in the HTF of the solar field constitute between 52-64% of the total investment, the rest being land, indirect costs and the power infrastructure (Pitz-Paal *et al.* 2007). CSP DSG rather than synthetic oil or molten salts in the HTF identifies proportionally allocated costs of the solar field and the integrated solar combined cycle with a gas turbine (Montes *et al.* 2011).

Energy output (kW) from a CSP parabolic collector and HTF volume with prior consideration of all physical operating parameters and heat losses are presented in Figure 8-5 (Data from Manzolini *et al.* 2011. Database by Sansom, 2014).

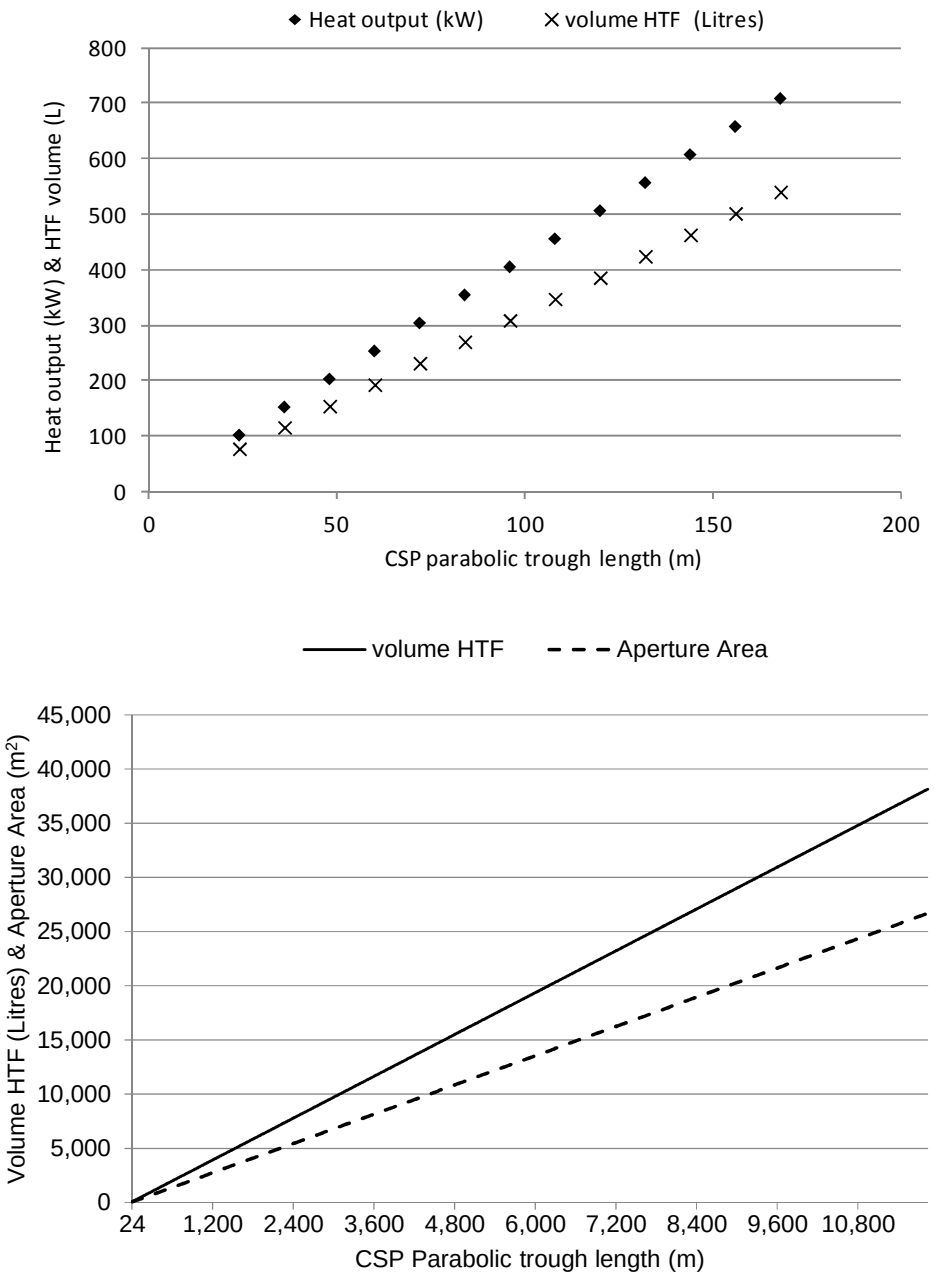


Figure 8-5 Heat output (kW) and volume of Heat Transfer Fluid (HTF) (l) from CSP parabolic trough (up to 200 m trough length scale above, large scale below)

The solar component alone of Montes' study, Table 8-6 highlights differences in estimated investment costs, operational costs, space, plant size, sales turnover and investment payback period of these two scenarios in comparison to the use of HTL for CSP based on 1 hectare of microalgae production. There are wide variations in the cost of microalgae dependent on many factors. Table 8-7 summarises some of these variations.

Table 8-6 Production and economic forecast of 3 CSP scenarios

	Pitz-Paal (2007) CSP Parabolic trough	**Montes (2011) CSP Parabolic trough**	**This study CSP Parabolic + HTL**
Power plant size (MW)	47MW	75MW	64,000 litres bio-oil/year
HTF substance	Direct steam generation	Direct steam generation	Water + (Free) Algae biomass
Solar aperture (m^2)	337,076	537,887	250
Site size (hectares)	144	230	<0.2
Solar Field (€/m^2)	190	200	200
Power generation block (€/kW)	700	984	0
Land (€/m^2)	2	2	2
O&M (% investment cost/year)	1	1	100
Total spend (€)	**101 M**	**188 M**	**108 K**
Turnover @ €0.25/kW or €2/L	**11.8 M**	**18.8 M**	**128 K**
Investment payback (years)	**8.6**	**10**	**0.9**

Table 8-7 Algae production costs and cost estimates (Christenson and Sims, 2011).

Type	Production method	Cost ($ kg^{-1})	Notes/Assumptions	Reference
Cost	Open pond	5	Spirulina production	Benemann (2008)
Cost	Open pond	3.60[a]	Dunaliella production	Brennan and Owende (2010)
Estimate	Attached culture	0.70-0.97	Using dairy wastewater	Pizarro et al. (2006)
Estimate	Open pond	6.93[a]	Netherlands location	Norsker et al. (2011)
Estimate	Closed PBR	5.81[a]	Netherlands location	Norsker et al. (2011)
Estimate	Open pond	3.8	Free CO_2	Chisti (2007)
Estimate	Closed PBR	2.95	Free CO_2	Chisti (2007)
Forward-looking estimate	Open pond	1.79[a]	Free CO_2 and growth media, 60% improved photosynthetic efficiency, Dutch Antilles location	Norsker et al. (2011)
Forward-looking estimate	Closed PBR	0.98[a]	Free CO_2 and growth media, 60% improved photosynthetic efficiency, Dutch Antilles location	Norsker et al. (2011)
Forward-looking estimate	Open pond	0.6	100 x increased production for better economy of scale	Chisti (2007)
Forward-looking estimate	Closed PBR	0.47	100 x increased production for better economy of scale	Chisti (2007)

[a] Calculated using a conversion factor of 1.4 dollars per Euro.

Using the €Euro adjusted range of costs of microalgae production at USD \$0.6 /kg^{-1}, \$0.98 /kg^{-1}, \$5.81 /kg^{-1} and \$6.93 /kg^{-1} from Table 8-7 (Norsker *et al.* 2011 and Chisti, 2007), the CSP HTL component of Table 8-6 is re-defined with inclusive representative costs of microalgae production (Table 8-8).

Table 8-8 Production and economic forecast scenarios of CSP parabolic trough + HTL

	CSP Trough + HTL	**CSP Trough + HTL**	**CSP Trough + HTL**	**CSP Trough + HTL**
Author - microalgae production cost	(Norsker, 2011)	(Norsker, 2011)	(Norsker, 2011)	(Chisti, 2007)
Power plant size production (litres bio-oil/year)	64,000	64,000	64,000	64,000
HTF substance	Water + Algae biomass	Water + Algae biomass	Water + Algae biomass	Water + Algae biomass
Microalgae dry weight production from 1 hectare (kg/year)	64,000	64,000	64,000	64,000
Microalgae growth system	Open pond	Open pond	PBR	Open pond
Latitude	51.4°N	12.2°N	12.2°N	Not stated
Microalgae production cost (€/kg)	4.95	1.28	0.7	0.43
Microalgae production cost/year	316,800	81,920	44,800	27,520
Solar aperture (m^2)	250	250	250	250
Site size (hectares)	<0.2	<0.2	<0.2	<0.2
Solar Field cost (€/m^2)	200	200	200	200
Land, O&M costs (€)	58,000	58,000	58,000	58,000
Total (€) CAPEX	**50 K**	**50 K**	**50 K**	**50 K**
Total (€) OPEX	**375 K**	**140 K**	**103K**	**86 K**
Total spend (€)	**425 K**	**190 K**	**153 K**	**136 K**
Turnover @ €2/L	**128 K**	**128 K**	**128 K**	**128 K**
Investment payback (years)	**Invalid**	**Invalid**	**3**	**1.5**

Table 8-8 compares costs of microalgae biomass at 4 different values of cost of production, with inclusive infrastructure investment costs of CSP and HTL. At a microalgae production cost of €1.28 /kg and €4.95 /kg the investment is never repaid and the business is unviable. At a microalgae production cost of €0.43 /kg and €0.7 /kg the investment is repaid in 1.5 and 3 years respectively with an IRR of 556% and 110%. This compares favourably to an investment payback of 8-10 years for CSP for electrical energy generation with an IRR of 8 and 10%. CSP

HTL is therefore an application of CSP research which is worthy of further investigation.

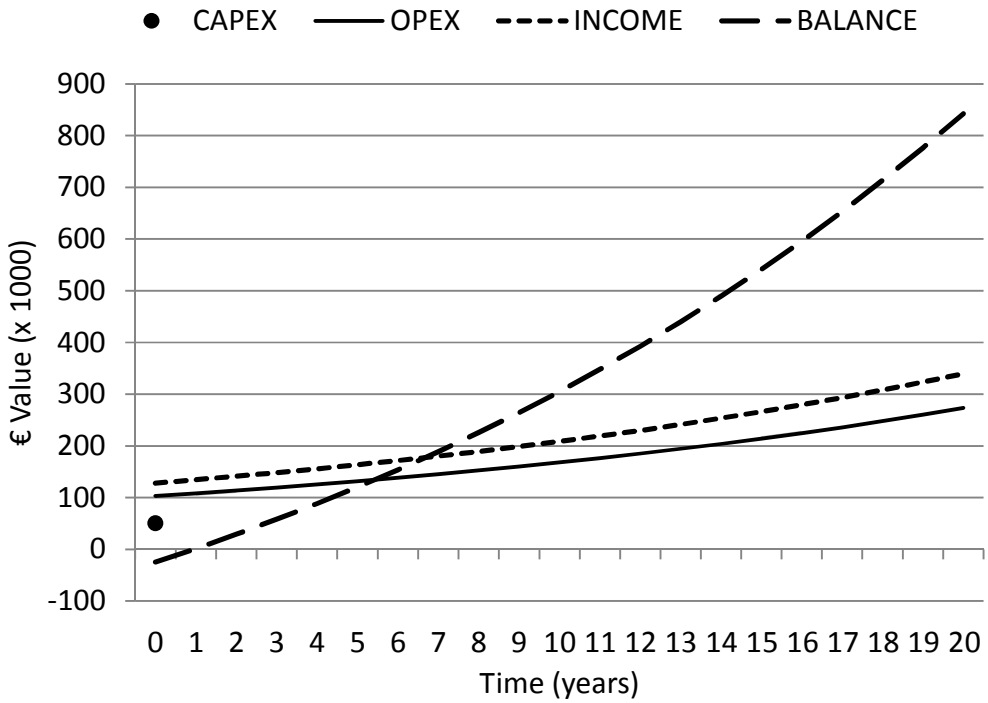


Figure 8-6 Cash-flow forecast of bio-oil produced via CSP & HTL. Microalgae growth conditions: PBR at 12.2˚N with cost of production €0.70/kg (Table 8-8).

The Dutch economic productivity investigation used as the basis for the cash forecast in Figure 8-6 an inflation rate of 5%, and microalgae production cost based on growth in the Dutch Antilles in the tropics (Norsker *et al.* 2011).

8.5 Discussion

Crude laboratory extraction procedure of SBP from SB could further be improved, therefore assumptions for subsequent harvest yield gains are realistic. However, improved refinement extraction processes would achieve only modest yield gains which for laboratory operations would not warrant the significant extra cost in pumps, filters and fluid processing capacity for the low volumes of batch processed feedstock required for experimentation. Commercial alginate extraction businesses have already made this extra investment to optimise their extraction yield and have safeguarded intellectual property knowledge behind their extraction technology.

Economic considerations must also include sub-rendered products as well as input feedstock nutrients, as profitability of microalgae for lower valued products is finely balanced. Indeed, combining microalgae cultivation with wastewater treatment can improve water quality and simultaneously provide biomass for biofuels or other products, but it remains to be demonstrated that the economics and energy returned on energy invested of the combined systems support its development (Beal *et al.* 2012; Lundquist *et al.* 2010; Wiley, 2011). All experimental biomass processing material used were non-toxic and food grade.

A challenge of unification of microalgae production, HTL and CSP is scale. Though conventional CSP for electricity generation is more efficient for larger-sized CSP plants, there is no commercial benefit to have excess thermal processing capacity above required biomass processing capacity of available feedstock. Electrical generation CSP is already established, whereas CSP for HTL of microalgae biomass is a novel technology. Prudence in capital expenditure costs requires matching microalgae biomass production with plant size accordingly.

Comparison between CSP DSG and CSP HTL is a valid one, because the main difference between the solar field components of the HTF is the replacement of water with microalgae and water at 20% inclusion (w/v). CSP parabolic trough plants continue to strive for cost improvements in their infrastructure investment and operations. Replacement of HTF using DSG is one such research and development, though not yet applied at commercial wide scale production. The major bottleneck constraint to the use of DSG instead of molten salt or synthetic oil is energy storage (Feldhoff *et al.* 2012). The application of CSP HTL does not suffer from this constraint, as the HTL product is the end energy product in itself. However, investment-cost strategies should include the complete integration of microalgae production costs integrated into the production forecasts.

It is plausible that the economics shown in Table 8-8 can be further improved by including low cost nutrients, a waste source of CO_2, and low-cost harvesting technology. Synthetic nutrients at €333 /m^3 of media, a Westfalia centrifuge investment of €183,000 /hectare capacity, and a CO_2 cost of €330 /t of algae

biomass produced were factored into the economic calculation (Norsker, 2011). These components comprised 31% of total major equipment costs, yet still with this chosen production cost strategy, a final microalgae production cost of €0.7 /kg was achieved. An even lower-cost production strategy could theoretically reduce microalgae production cost below €0.7 /kg, and improve performance profitability via the solar HTL processing pathway further than that described by Norsker.

Another economic evaluation of cost of microalgae production in Spain indicated a production cost of €69 /kg. This is as a result of their higher operational and processing costs such as added carbon dioxide, synthetic nutrients, centrifugation, freeze-drying and labour costs. It was concluded by the Spanish trial that process automation, low-labour costs, waste flue-gases and waste nutrients could reduce production costs further (Acíen *et al.* 2012).

Preliminary experiments at Cranfield described in chapter 7 have demonstrated that bio-oil from HTL of microalgae collects on the inner surface of a tubular pressure vessel whereas the higher volumetric component of carbonaceous water soluble fractions become concentrated within the inner core. The aqueous solubles are easily removed, whereas the bio-oil is more viscous in texture. There is scope for re-dilution of water soluble carbon fractions and usage into microalgae culture. Following pumping or pouring of water soluble carbon fractions, bio-oil deposition in contact with solid internal vessels can be removed by compression.

8.6 Conclusions

Unity of an experimental approach for technology integral processing using hydrothermal liquefaction of microalgae biomass and solar concentrating power has not been reported in literature. This chapter has proposed such an approach with an analysis of the reported energetic and economic costs of producing HTL bio-oil from microalgae and solar CSP. It has been found that theoretical profitability could be achieved by an integrated application of microalgae growth in wastewater & recycled HTL water solubles, low-cost microalgae harvesting for direct application to downstream processing and low-energy processing to bio-oil via CSP/HTL. Further experimental work would validate this assumption.

9 **Conclusions**

This thesis has integrated multiple components of the growth, harvesting and processing of microalgae with consideration, but not exclusive use, for biomass application in advanced biofuels. During the conduct of this research there have been “blind alleys” and “U-turns” in order to develop the construct of reverting to a reduction in the use of energy and cost of processing; not least because of failures to achieve the desired outcome of floating, low-density gels for massive scale offshore cultivation of microalgae biomass at low-cost, in a sacrificial embedded nutrient batch fed harvestable matrix. Furthermore, in an integrated study, some of the initiating research ideas which formed, developed and outlined experimental transition have not even been included in this text, because their research links are not closely enough aligned to the continuity theme of this thesis. From the outset, research aims were scoped at the huge, modern and yet to be solved challenge of an alternative biomass production and processing scenario for advanced liquid biofuels from microalgae. It is hoped that this communication provides a contribution for continued research into this worthy ambition. This thesis provides linking ideas and unique experimental methodology which can help others continue to build upon the resolution of microalgae biofuel research and development.

This work has investigated at laboratory experimental scale how integrated waste technologies are able to reduce the energetic and economic cost of biofuel production from microalgae. These findings have been escalated to theoretical mass scaled production systems using similar technology applications. Whilst an economic business model requires further scaled and applied research to be comparable with other forms of renewable liquid combustibles, components of 6 currently established industries (Anaerobic Digestion, Brewing, Coal, Dairy farming, Steel and the Seaweed industry) have been demonstrably united with microalgae production and processing as a proposed symbiosis for value-added product generation.

Present philosophy for the application of reducing the production costs of integrated technologies of microalgae farming and biofuel production consider:

- Compartmentalisation of “algae” into either microalgae or macroalgae for their respective harvesting and downstream production processes.
- Regarding microalgae specifically, advocating the integrated bio-refinery concept for fractionation of cell biomass into its’ variable value components.

It is recognised that innovation in this sector will be central to development of novel technology. Original contribution to knowledge within this thesis has highlighted the way in which microalgae and macroalgae can be united in processing technologies for the same ending purpose rather than compartmentalised into their two respective class fractions. This thesis also advocates how downstream processing for microalgae biomass into bio-oil is able to process whole cell biomass rather than preliminary extraction of higher value compounds from microalgae and thereafter division of sub-products. Although a pre-step of higher value component extraction is not disregarded as a possible additional revenue stream.

The purpose of this research has been to contribute to industrial process unification with respect to microalgae culturing, harvesting and conversion into biofuel.

- Anaerobic digestion has demonstrably shown to be a complete replacement to synthetic nutrient media and was identified as the most superior organic growth nutrient in this research. Growth profiles of batch culture of microalgae grown in anaerobic digestate nutrients were maximal between days 2-4. Integration of anaerobic digestate nutrients with waste CO_2 from simulated coal flue combustion gases has been demonstrated.
- Brewery waste nutrient was found to be high in phosphate, a nutrient with predictions for future scarcity as a global commodity. However, microalgae did not grow well in media from this waste resource, considered to be due

to the integrity of the yeast cell wall for retention of both nitrogen and phosphorus in cell wall and nuclear proteins. 0.5% (w/v) of brewery waste was compared to concentrations of other organic waste products.

- Steel slag from the steel production industry is a massive volumetric resource with huge global stockpiles. This research has identified a potential strategy to use washed ions from this resource to neutralise pH in combination with another severe problematic global gaseous waste, carbon dioxide from combustion of coal. NO_x from simulated coal flue combustion gases dissolved in solution formed nitric acid and reacted with calcium hydroxide to form the water soluble fertiliser calcium nitrate. This provided a renewable source of nutrient for growing microalgae. In contrast, sulphuric acid was neutralised by calcium oxide to provide an optimal pH for microalgae. The caveat to this component of industrial waste integration research was that rapid pH adjustments to growth media with periodic dilutions were probably not beneficial to growing microalgae which justifies why only 0.3 g/l biomass was yielded after 7 days of batch growth.
- Endemic microorganism populations within organic dairy manure nutrients were the main constraint to nutrient delivery to microalgae which required pre-treatment. Dairy manure was also found to be the most nutrient deplete of all organic nutrients investigated. Where cattle and dairy cows are herded on pastureland in the UK; collection, retention and waste nutrient concentration of animal excreta can be more challenging due to space and wide dispersal. Also, environmental precipitation can dilute nutrients. Conversely, in the US where intensive livestock rearing occurs on high proteinaceous animal feeds in high numbers of livestock per unit area of land space, collection, processing and nutrient sterilisation and concentration of animal waste is more commercially feasible and plausible as a renewable nutrient.
- Alginates and Pectins are known to be able to support the structural growth of microalgae. The retention of batch cultured nutrients and use of these bioresources in a novel harvesting process has been demonstrated. Whilst

not cost-effective for attached or ionic gelation floating cultivation system, pectins have been shown to be cost-effective as a harvesting agent for microalgae.

- Hydrogels have been found to have ionic inducing properties affecting nutrient retention within their gel matrix as well as influencing the external aqueous ionic environment in their vicinity. Ionotropic gelation of alginate has demonstrated interesting properties for partial desalination of variable concentrations of seawater. Hydrogel composites in association with coir fibre have incremental structural resistance against deformation tested in accordance with British standards for geotextile membranes. If further research could reduce the volumetric inclusion of alginate with foaming technologies, this method could present a potentially controllable cultivation strategy for microalgae similar to the natural algal blooms and foams shown in the introductory chapter. This could become a new carbon neutral anthropogenic industry of ocean algaculture as opposed to ocean aquaculture which is already established.
- A novel low-cost harvesting method using gels proved that harvesting and atmospheric drying does not adversely compromise or modify the profile of microalgae lipids.
- The newly developed low-cost harvesting method process can be integrated with HTL of microalgae to form bio-oil. The post-harvest 10-15% solids (w/v) is the required solids ratio for HTL loading. The liquid characteristics of post harvested product promotes fluid pumping, and scale-up viability.
- Integrated technologies of CSP and HTL have potential applications for an energy saving thermo-chemical conversion process for production of bio-oil, though this is unlikely to be a profitable enterprise until the cost of producing microalgae falls in the range of €0.7-1 /kg.
- Methodology employed during the practical experimentation during this thesis has developed a range of novel procedures. These include the structural integrity testing of bio-composite gel disks, a new low-cost method to harvest microalgae, an investigation of an ionic and density

phase separation process in seawater, remediation of simulated coal flue combustion gases with industrial by-products and gas chromatography lipid, carbon & nitrogen analysis of microalgae harvested in a unique manner and processed via hydrothermal liquefaction.

9.1 Further Research

Anaerobic digestion is presently proposed as a means for digesting microalgae biomass as a mechanism for phytoremediation and waste water cleaning. AD also provides macronutrients for growing microalgae in mass culture. AD produces CO_2 and CH_4 in the approximate proportions of 40% and 60%. The effect of remediating CO_2 with microalgae culture to combine AD gas, AD nutrients and microalgae cultivation is of interest for further investigation.

Sterilisation of organic nitrogen media was necessary to eliminate pathogenic loading. However the extent of atmospheric volatisation of nitrogen losses and ways to mitigate such losses are of concern to preserve integrity of nitrate and ammonia for delivery to microalgae culture.

Integrity of nitrogen and phosphorus in the cell wall and bound organelles of yeast cells is considered to be a factor affecting nutrient bio-availabilty in microalgae culturing. Prior digestion of yeast cells to release these valuable nutrients into solution could act to remediate brewery waste further than whole-cell utilisation.

A reformation of experimental procedure which reflects a continual mechanistic pH chemostat style feedback to control sudden pH shifts would better investigate control of pH changes from CO_2 in coal flue gas than by having more than one varying parameter (CO_2 concentration and pH). A priority for continuation of the research of integrating coal flue combustion gases and washed steel slag is to be able to have a pH control feedback mechanism which can simultaneously monitor pH solution in microalgae culture and feedback volumetric injection of washed steel slag solution to evenly control pH flux. There are a concoction of variable reactions which occur in the reaction of washed steel slag with simulated coal flue combustion gases based on the diversity of SO_x and NO_x aquatic

chemistry. A deeper understanding of the stoichiometry quantification of coal flue gas aqueous chemical reactions would help to design a process engineered system to integrate these technologies and application of industrial waste resources.

The low-cost harvesting of microalgae biomass warrants further investigation for the anticipated cost-reduction in microalgae production that it could viably achieve. This process has only been tested in a few species using two filter types. More filter types and other species for testing are certainly required to validate the process further for wider application. Appropriation of such further research should ideally be accompanied by a related commercial partner such as pectin producer, food producer, an interested processor of waste nutrients or downstream product developer.

Combination of CSP and HTL is a fascinating concept which presently has no previously known experimental research applications. As such, further research would help to understand the combination of these technologies better. There remain many questions to this application of concentrated solar power for which bio-oil could be harnessed cheaper, especially when integrated with other components of growth and harvesting of microalgae included within this thesis.

There are context and limitations of this research. One of these relates to the multi-disciplinary coverage of research activities and the many further research questions that findings from this thesis uncover. Research aims have investigated an integrated process systems engineering approach to the problem of delivery of biofuels from microalgae. Full completion would involve scaled demonstration with costings and could deliver an exciting commercial investment proposition, though this has yet to be demonstrated. The wide application of research has covered methods of culturing, harvesting and conversion into biofuel with insight into novel applications of applied research and integrated processing. Experimentation by virtue has combined various multidisciplinary research themes.

Another main limitation relates predominantly to the scale of production and extrapolation of research findings from lab scale to field scale. The intention for

research findings from this thesis has been for them to be applied and scaled-up from small to commercial, or alternatively to take the technology from the laboratory to industry. Scaling of technologies requires persuasion, money and risk. It is recognized that such attributes fall outside the remit of pure technology innovation and science application into the diverse world of other working life disciplines.

The present energy conundrum is humanities' greatest quest. Microalgae are merely one proponent of biofuel generation, amongst a plethora of life forms. This thesis thoroughly endorses their continued propagation.

REFERENCES

Acíen, F. G., Fernández, J. M., Magán, J. J., and Molina, E. (2012), Production cost of a real microalgae production plant and strategies to reduce it, *Biotechnology advances,* Vol. 30. p. 1344-1353.

Ahlgren, S., Baky, A., Bernesson, S., Nordberg, Å., Norén, O., and Hansson, P. A. (2008), Ammonium nitrate fertiliser production based on biomass – environmental effects from a life cycle perspective, *Bioresource Technology*, Vol. 99, No. 17, p. 8034-8041.

Aldridge, J., van de Molen, J. and Forster, R. (2012), Wider ecological implications of Macroalgae cultivation, *The Crown Estate*, UK.

Allen, M. B., & Arnon, D. I. (1955), Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica Lemm*, *Plant Physiology*, Vol. 30, No.4, p. 366.

Alonso-Rodríguez, R. and Páez-Osuna, F. (2003), Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California, *Aquaculture,* Vol. 219, Issues 1-4, p. 317-336.

Amaro, H. M., Guedes, A. C., Malcata, F. X., (2011), Advances and perspectives in using microalgae to produce biodiesel, *Applied Energy*, Vol. 88, No. 10, p. 3402-3410.

Amin, S. (2009), Review on biofuel oil and gas production processes from microalgae, *Energy Conversion and Management*, Vol. 50, No. 7, p. 1834-1840.

Anastasakis, K., & Ross, A. B. (2011), Hydrothermal liquefaction of the brown macro-alga *Laminaria saccharina*: effect of reaction conditions on product distribution and composition, *Bioresource technology*, Vol. 102, No. 7, p.4876-4883.

Anastasakis, K., & Ross, A. B. (2015), Hydrothermal liquefaction of four brown macro-algae commonly found on the UK coasts: an energetic analysis of the

process and comparison with bio-chemical conversion methods, *Fuel*, Vol. 139, p.546-553.

Andersen, R. A. (2006), Algal Culturing Techniques, Elsevier.

Audsley, E. and Wilkinson, M. (2014), What is the potential for reducing national greenhouse gas emissions from crop and livestock production systems? *Journal of Cleaner Production*, Vol. 73. p. 263-268.

Austin, L., Bower, J. J., Muldoon, C., (1996), The controlled release of leukaemia inhibitory factor (LIF) from alginate gels, Proceedings of International Symposium on Controlled Release in Bioactive Materials, Vol. 23, p. 739–740.

Ayala, F. and Vargas, T. (1987), Experiments on Spirulina culture on waste-effluent media and at the pilot plant. In Twelfth International Seaweed Symposium (pp. 91-93), Springer Netherlands.

Azov, Y., Goldman, J. C. (1982), Free ammonia inhibition algal photosynthesis in intensive cultures, *Applied Environmental Microbiology,* Vol. 43, No. 4, p. 735-739.

Babel, S. and Takizawa, S. (2010), Microfiltration membrane fouling and cake behavior during algal filtration, *Desalination*, Vol. 261, No.1, p. 46-51.

Bach, Q. V., Sillero, M. V., Tran, K. Q. and Skjermo, J. (2014), Fast hydrothermal liquefaction of a Norwegian macro-alga: Screening tests, *Algal Research*, Vol. 6, p. 271-276.

Bajpai, S. K. and Sharma, S. (2004), Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions, *Reactive and Functional Polymers*, Vol. 59 , No. 2, p. 129-140.

Baranov, V. S., Locev, V. N., Guernet, N. A., Kuchumova, R. P., Kuchumov, A. M. (1967), Method to obtain sodium alginate, USSR Patent 707,561.

Barnes, H. A. (1989), Shear thickening (“Dilatancy”) in suspensions of nonaggregating solid particles dispersed in Newtonian liquids, *Journal of Rheology*, Vol. 32, p. 329-366.

Barreiro, L. D., Prins, W., Ronsse, F. and Brilman, W. (2013), Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects, *Biomass and Bioenergy,* Vol. 53, p. 113-127.

Barranguet, C. H., Veuger, B., Van Beusekom, S., Marvan P., Sinke, J. J, Admiraal, W., (2005), Divergent composition of algal-bacterial biofilms developing under various external factors, *European Journal of Phycology,* Vol. 40, p.1–8.

Barsanti, L., and Gualtieri, P. (2006), *Algae: Anatomy, Biochemistry and Biotechnology*, CRC Press.

Bauer, F. F., Govender, P. and Bester, M. C. (2010), Yeast flocculation and its biotechnological relevance, *Applied microbiology & biotechnology, V*ol. 88, p. 31-39.

Beal, C. M., (2012), Energy Return on Investment for Algal Biofuel Production Coupled with Wastewater Treatment, *Water Environment Research*, Vol. 89, No. 9, p. 692-710. doi:10.2175/106143012X13378023685718.

Becker, E. W. (1994), Microalgae: biotechnology and microbiology, Vol. 10, Cambridge University Press.

Bekatorou, A., Psarianos, C. and Koutinas, A. (2006), Production of food grade yeasts, *Food Technology & Biotechnology*, Vol. 44, No. 3, p. 407-415.

Bender, J. and Wagner, N.J. (1996), Reversible shear thickening in monodisperse and bidisperse colloidal dispersions, *Journal of Rheology,* Vol. 40, No. 5, p. 899-916.

Ben-Amotz, A., (2004), Industrial production of microalgal cell-mass and secondary products – major industrial species: Dunaliella. In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science Ltd., Oxford, pp. 273–280.

Benemann, J., (2003), Biofixation of CO_2 and greenhouse gas abatement with microalgae – technology roadmap, US Dept. Energy Report.

Benemann, J. and Oswald, W. J. (1996), Systems and Economic analysis of microalgae for conversion of CO_2 to biomass, Final report to the Department of Energy, Pittsburgh Energy Technology Center, US.

Bescond, M. P. (1948), Procédé de traitement des tiges de luminaire verte ou séche afin d'obtenir des alginates de soude. Republique Française Patent 940,035.

Biller, P. and Ross, A. B. (2011), Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content, *Bioresource Technology*, Vol. 102, p. 215-225.

Biller, P., Ross, A. B., Skill, S. C., Lea-Langton, A., Balasundaram, B., Hall, C., Riley, R. and Llewellyn, C. A. (2012), Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process, *Algal Research,* Vol. 1, No.1, p. 70-76.

Bixler, H. J. and Porse, H. (2011), A decade of change in the seaweed hydrocolloids industry, *Journal of Applied Phycology*, Vol. 23, No. 3, p. 321–335.

Blum, A. (2009), Efficient use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research,* Vol. 112, No. 2, p. 119-123.

Bligh, E. G. and Dyer, W. J. (1959), A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*, Vol. 37, No. 8, p. 911-917.

Bodemeier, R., Chen, H., Paeratakul, O. (1989), A novel approach to the oral delivery of micro- or nanoparticles, *Pharmaceutical Research*, Vol. 6, p. 413–417.

Borowitzka, M. A. (1999), Commercial production of microalgae: ponds, tanks, tubes and fermenters, *Journal of Biotechnology*, Vol. 70, p. 313-321.

Borowitzka, M. A. (2006), Biotechnological & Environmental Applications of Microalgae, Murdoch University, http://www.bsb.murdoch.edu.au/groups/beam/BEAM-Appl0.html

Borowitzka, M. A (2013), High value products from microalgae – their development and commercialisation, *Journal of Applied Phycology,* Vol. 25, p. 743-756.

Borowitzka, M. A. and Moheimani, N. R. (2013), Sustainable biofuels from algae, *Mitigation and Adaptation Strategies for Global Change*, Vol. 18, No. 1, p. 13-25.

Bott, T, R. (2011), Industrial biofouling, B.V, Elsevier, Amsterdam.

Box, G. E. P. and Hunter, J. S. (1957), Multifactor experimental designs for exploring response surfaces, *Annals of Mathematical statistics,* Vol. 28, p. 195-242.

Brennan, L. and Owende, P., Biofuels from microalgae - a review of technologies for production, processing and extraction of biofuels and co-products, (2010), *Renewable Sustainable Energy Reviews*, Vol. 14, No. 2, p. 557-577.

Brown, L. M. (1996), Uptake of carbon dioxide from flue gas by microalgae, *Energy Conversion and Management*, Vol. 37, No. 6-8, p. 1363-1367.

Cai, S., Hu, C., Du, S. (2007), Comparisons of growth and biochemical composition between mixed culture of alga and yeast and monocultures, *Journal of Bioscience and Bioengineering*, Vol. 104, No.5, p. 391–397.

Calamita., G. (2000), The *Escherichia coli* aquaporin-Z water channel, *Molecular Microbiology*, Vol. 37, p. 254–262.

Cannell, M. G. R. (2003), Carbon sequestration and biomass energy offset: theoretical, potential and achievable capacities globally, in Europe and the UK, *Biomass and Bioenergy,* Vol. 24, No. 2, p. 97-116.

Cardinale, B. J. (2011), Biodiversity improves water quality through niche partitioning. *Nature,* Vol. 472, No. 7341, p. 86–89.

Çelekli, A. and Dönmez, G. (2006), Effect of pH, light intensity, salt and nitrogen concentrations on growth and β-carotene accumulation by a new isolate of Dunaliella sp. *World Journal of Microbiology & Biotechnology*, Vol. 22, No. 2, p. 183-189.

Chakraborty, M., Miao, C., McDonald, A. and Chen, S. (2012). Concomitant extraction of bio-oil and value added polysaccharides from C*hlorella sorokiniana* using a unique sequential hydrothermal extraction technology. *Fuel*, Vol. 95, p. 62-70.

Chang, K. J L., Rye, L., Dunstan, G. A., Grant, T., Koutoulis, A., Nichols, P. D. and Blackburn, S. I. (2015), Life cycle assessment: heterotrophic cultivation of thraustochytrids for biodiesel production, *Journal of Applied Phycology*, Vol. 27, p. 639-647.

Charcosset, C. (2009), A review of membrane processes and renewable energies for desalination, *Desalination,* Vol. 245, No. 1, p. 214-231.

Chater, P. (2013), Presentation at British Phycological Society Annual meeting, A synthetic model gut system to study macronutrient digestion *in vitro*, 7-10 July 2013, Belfast.

Cheirsilp, B., Suwannarat, W., Niyomdecha, R. (2011), Mixed culture of oleaginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock, *Nano Biotechnology,* Vol. 28, No. 4, p.362–368.

Chen, P. H. and Oswald, W. J. (1998), Thermochemical treatment for algal fermentation, *Environment International*, Vol. 24, No. 8 p. 889-897.

Cheng, P. F., Ji, B., Gao, I., Zhang, W., Wang, J., Liu, T., (2013), The growth, lipid and hydrocarbon production of *Botryococcus braunii* with attached cultivation, *Bioresource Technology*, Vol. 138, p. 95-100.

Cheung, H. Y., Ho, M. P., Lau, K. T., Cardona, F. and Hui, D. (2009), Natural fibre-reinforced composites for bioengineering and environmental engineering applications, *Composites Part B: Engineering*, Vol. 40, No. 7, p. 655-663.

Cheung, Y. H. and Wong, M. H. (1981), Properties of animal manures and sewage sludges and their utilisation for algal growth, *Agricultural Wastes,* Vol. 3, No. 2, p. 109-122.

Chisti, Y. (2007), Biodiesel from microalgae, *Biotechnology Advances*, Vol. 25, No. 3, p. 294-306.

Chisti, Y. (2008), Biodiesel from microalgae beats bioethanol, *Trends in biotechnology*, Vol. 26, No. 3, p. 126-131.

Chiu, S. Y., Kao, C. Y., Huang, T. T., Lin, C. J., Ong, S. C., Chen, C. D., Chang, J. S., and Lin. C. S. (2011), Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulphur dioxide from flue gas using *Chlorella* sp. cultures. *Bioresource Technology*, Vol. 102, No. 19, p. 9135-9142.

Choi, B. Y., Park, H. J., Hwanf, S.J. and Park, J. B. (2002), Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents, *International Journal of Pharmaceutics,* Vol. 239, p. 81-91.

Choi, W. C., Yoo, S. A., Oh, I. H., Park, A. H. (1998), Characterisation of extracellular flocculating substance produced by a planktonic cyanobacterium, *Anabaena sp. Biotechnology Letters,* Vol. 20, No. 7, p. 643-646.

Choi, S. K., Lee, J. Y., Kwon, D. Y. & Cho, K. J. (2006). Settling characteristics of problem algae in the water treatment process. *Water Science & Technology*, Vol. 53, No. 7, p. 113–119.

Christenson, L. and Sims, R. (2011), Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, *Biotechnology Advances*, Vol. 29, No. 6, p. 686-702.

Chui, S. Y., Kao, C. Y., Huang, T. T., Lin, C. J., Ong, S. C., Chen, C. D., Chang, J. S. and Lin, C. S. (2011), Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulphur dioxide from flue gas using *Chlorella sp.* cultures, *Bioresource Technology,* Vol. 102, p. 9135-9142.

Clark, D. E., Green, H. C. (1936), Alginic acid and process of making same. US patent 2,036,922.

Clement, B. and Merlin, G. (1995), The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed, *Science of the Total Environment,* Vol. 170, No. 1-2, p. 71-79.

Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P. and Del Borghi, M. (2009), Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production, *Chemical Engineering and Processing: Process Intensification*, Vol. 48, No.6, p. 1146-1151.

Cornelissen, S., Koper, M. and Deng, Y. (2012), The role of bioenergy in a fully sustainable global energy system, *Biomass and Bioenergy,* Vol. 41, p. 21-33.

Coward, T., Lee, J. G. M. and Caldwell, G. S. (2014), The effect of bubble size on the efficiency and economics of harvesting microalgae by foam flotation, *Journal of Applied Phycology*, p. 1-10.

Cunningham, A. and Buonnacorsi, G. A. (1992), Narrow-angle forward light scattering from individual algal cells: implications for size and shape discrimination in flow cytometry, *Journal of Plankton Research,* Vol. 14, No. 2, p. 223-234.

Damadian R. (1971), Tumour detection by Nuclear Magnetic Resonance, *Science,* Vol. 171, No. 3976, p. 1151-1153.

Danquah, M. K., Ang, L., Uduman, N., Moheimani, N., and Forde, G. M. (2009), Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration, *Journal of Chemical Technology and Biotechnology*, Vol. 84, No.7, p. 1078-1083.

Davey, P. T., Hiscox, W. C., Lucker, B. F., O'fallon, J. V., Chen, S. and Helms, G. L. (2012), Rapid triacylglyceride detection and quantification in live micro-algal cultures via liquid state H NMR, *Algal Research*, Vol. 1, No. 2, p. 166-175.

Davis, R., Aden, A., Pienkos, P. T. (2011), Techno-economic analysis of autotrophic microalgae for fuel production, *Applied Energy*, Vol. 88, No. 10, p.3524-3531.

Davis, S. J., Caldeira, K. and Matthews, H. D. (2010), Future CO_2 emissions and climate change from existing energy infrastructure, *Science,* Vol. 329, No. 5997, p. 1330-1333.

Day, J. G., Slocombe, S. P. and Stanley, M. S. (2012), Overcoming the constraints to enable the exploitation of microalgae for biofuels. *Bioresource Technology*, Vol.109, p. 245-251.

Delucchi, M. A. (2003), A Lifecycle Emissions Model (LEM): Lifecycle emissions from transportation fuels, motor vehicles, transportation modes, electricity use, heating and cooking fuels, and materials, *Institute of transportation Studies.*

Deschenes, R. J., Lin, H., Ault, A. D. and Fassler, J. S. (1999), Antifungal properties and target evaluation of three putative bacterial histidine kinase inhibitors*, Antimicrobial Agents Chemotherapy*, Vol 43, p. 1700–1703.

Devrimci, H. A., Yuksel, A. M. and Sanin, F. D. (2012), Algal alginate: A potential coagulant for drinking water treatment, *Desalination*, Vol. 299, p. 16-21.

Dodd, M. C., Zuleeg, S., Von Gunten, U., Pronk, W. (2008), Ozonation of source-separated urine for resource recovery and waste minimization: Process modelling, reaction chemistry, and operational considerations. *Environmental Science and Technol*ogy, Vol. 42, No. 24, p. 9329 - 9337.

Dong, Q-L., Zhao, X-M. (2004), In situ carbon dioxide fixation in the process of natural astaxanthin production by a mixed culture of *Haematococcus pluvialis* and *Phaffia rhodozyma*, *Catalyst Today*, Vol. 98, No. 4, p.537–544.

Dote, Y., Sawayama, S., Inoue, S., Minowa, T., Yokoyama S-Y., Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction, *Fuel,* Vol. 73, p. 1855-1857.

Doucha, J. (2005), Utilization of flue gas for cultivation of microalgae (*Chlorella sp.*) in an outdoor open thin-layer photobioreactor, *Journal of Applied Phycology*, Vol 17, No. 5, p. 403-412.

Draget, K. I., Smidsrød, O. and Skjåk-Bræk, G. (2005), Alginates from algae, *Biopolymers Online*, Wiley.

Draget, K. I., Skjåk-Bræk, G., Smidsrod, O. (1997), *International Journal of Biological Macromolecules* Vol. 21 p.47.

Driessen, W. and Vereijken, T. (2003), Recent developments in biological treatment of brewery effluent, *The Institute and Guild of Brewing Convention,* Livingstone, Zambia.

Du, Z., Hu, B., Shi, A., Ma, X., Cheng, Y., Chen, P., Liu, Y., Lin, X., Ruan, R. (2012), Cultivation of a microalga *Chlorella vulgaris* using recycled aqueous phase nutrients from hydrothermal carbonization process, *Bioresource Technology*, Vol. 126, p. 354-367.

Ducat, D., Way, J. and Silver, P. (2011), Engineering cyanobacteria to generate high-value products, *Trends in Biotechnology*, Vol. 29, No. 2, p. 95-103.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); (2010). Scientific Opinion on the safety of sodium carbonate (soda ash) for all species, *EFSA Journal*, Vol. 8, No. 7, p. 1695-1702.

Ehimen, E. A., Sun, Z. F., Carrington, C. G., Birch, E. J. and Eaton-Rye, J. J., 2011, Anaerobic digestion of microalgae residues resulting from the bioduesel production process, *Applied Energy*, Vol. 88, No. 10, p. 3454 – 3463.

Einstein, A. (1905), A New Determination of molecular dimensions (University of Zurich dissertation), In: *The collected papers of Albert Einstein, Volume 2, The Swiss years: Writings, 1900-1909, English Translation*, Anna Beck, Translator: Peter Havas, Consultant.

Eldridge, R. J., Hill, D. R. A. And Gladman, B. R. (2012), A comparative study of the coagulation behaviour of marine microalgae, *Journal of Applied Phycology* DOI 10.1007/s10811-012-9830-4.

Elqin, Y. M. (1995), Encapsulation of urease enzyme in xanthan–alginate spheres, *Biomaterials*, Vol. 18, p. 1157–1161.

Elliott, D. C., Hart, T. R., Schmidt, A. J., Neuenschwander, G. G., Rotness, L. J., Olarte, M. V., Zacher, A. H., Albrecht, K. O., Hallen, R. T. and Holladay, J. E. (2013), Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor, *Algal Research*, Vol. 2, No. 4, p. 445-454.

Elliott, D. C., Biller, P. Ross, A. B, Schmidt, A. J. and Jones, S. B. (2015), Hydrothermal liquefaction of biomass: Developments from batch to continuous process, *Bioresource Technology*, Vol. 178, p. 147-156.

Emerson, K., Russo, R. C., Lund, R. E. and Thurston, R. V. (1975), Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature, *Journal of the Fisheries Research Board of Canada,* Vol. 32, No. 12, p. 2379-2383.

Ercoli, L., Mariotti, M., Masoni, A. and Bonari, E. (1999), Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of Miscanthus, *Field Crops Research*, Vol. 63, No. 1, p. 3-11.

Erickson, R. J. (1985), An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms, *Water Research*, Vol. 19, No. 8 p. 1047-1058.

Eriksen, N. T. (2008), The technology of microalgal culturing, *Biotechnology Letters*, Vol. 30, p. 1525-1536.

European Biodiesel standard DIN EN 14214

European Biodiesel standard DIN EN 14103

European Parliament. Communication from the commission to the European Parliament, the council, the European Economic and Social Committee of the regions, Consultative Communication on the Sustainable Use of Phosphorus, Brussels, July 2013, COM (2013) 517 final.

European Parliament. Council of the European Union (2010) Directive 2010/75/EU integrated pollution prevention control directive http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:334:0017:0119:en:PDF (accessed 1.8.13).

European Parliament, EU Standard EN1484, (1997). Water analysis – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC). European committee for standardisation, Brussels.

European Parliament, EU Standard EN12260, (2003), Determination of bound nitrogen, following oxidation to nitrogen oxides. European committee for standardisation, Brussels.

European Parliament, Council of the European Union (2000) Water Framework Directive, 2000/60/EC http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:327:0001:0001:EN:PDF (accessed 1.8.13)

European Parliament, Council of the European Union (2009), EU Directive 2009/28/EC Promotion of the use of energy from renewable sources, http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009L0028&from=EN (accessed 4.12.14).

FAO (Food and Agriculture Organization of the United Nations), (1997), Renewable Biological Systems for Alternative Sustainable Energy Production, FAO Agricultural Services Bulletin-128

Fallowfield, H. J. and Garrett, M. K. (1985), The photosynthetic treatment of pig slurry in temperate climatic conditions: a pilot plant study, *Agricultural Wastes*, Vol. 12, No. 2, p. 111-136.

Fargione, J., Hill, J., Tilman, D., Polasky, S. and Hawthorne, P. (2008), Land clearing and the Biofuel Carbon Debt, *Science*, Vol. 319, 29th Feb.

Feldhoff, J. F., Schmitz, K., Eck, M., Schnatbaum-Laumann, L., Laing, D., Ortiz-Vives, F. and Schulte-Fischedick, J. (2012), Comparative system analysis of direct steam generation and synthetic oil parabolic trough power plants with integrated thermal storage, *Solar Energy*, Vol. 86, No. 1, p. 520-530.

Ferreira, M., Coutinho, P., Seixas, P., Fábregas, J. and Otero, A. (2009), Enriching rotifers with “premium” microalgae *Nannochloropsis gaditana*, *Marine Biotechnology*, Vol.11, No. 5, p. 585-595.

Field, C. B., Campbell, J. E. and Lobell, D. B. (2008), Biomass energy: the scale of the potential resource, *Trends in ecology & evolution*, Vol. 23, No.2, p. 65-72.

Flynn, K. J. (1991), Algal carbon-nitrogen metabolism: a biochemical basis for modelling the interactions between nitrate and ammonium uptake. *Journal of Plankton Research,* Vol. 13. No. 2. p. 373-387.

Fouilland, E. (2012), Biodiversity as a tool for waste phycoremediation and biomass production. *Reviews in Environmental Science and Biotechnology,* Vol. 11, No. 1, p. 1-4.

Galindo-Rosales, F. J., Rubio-Hernández, F. J. and Sevilla, A. (2011), An apparent viscosity function for shear thickening fluids, *Journal of Non-Newtonian fluid mechanics,* Vol. 166, No. 5, p. 321-325.

Gao, Y., Gregor, C., Liang, Y., Tang, D. and Tweed, C. (2012), Algae Biodiesel – a Feasibility Report, *Chemistry Central Journal*, Vol. 6, S1.

Gardea-Torresdey, J., Becker-Hapak, M. K., Hosea, J. M. and Darnall, D. W. (1990), Effect of chemical modification of Algal Carboxyl groups on metal ion binding, *Environmental Science Technology*, Vol. 24, p. 1372-1378.

Garnham, G. W., Codd, G. A. and Gadd, G. M. (1992), Accumulation of cobalt, zinc and manganese by the estuarine green microalga *Chlorella salina* immobilised in alginate microbeads, *Environmental Science and Technology,* Vol. 26, No. 9, p. 1764-1770.

Gesellschaft, M. P. (1973), Method and apparatus for purifying seawater. Patent number US3772192.

Genzer, J. and Edimenko, K. (2006), Recent developments in superhydrophobic surfaces and their relevance to marine fouling: a review. *Biofouling,* 22, 339–60.

Gerardo, M. L., Oatley-Radcliffe, D. L. and Lovitt, R. W. (2014), Minimizing the energy requirement of dewatering *Scenedesmus* sp. By Microfiltration: Performance, costs, and feasibility, *Environmental Science and Technology,* Vol. 48, p. 845-853.

Goh, A. (1984), Production of microalgae using pig waste as a substrate, In: Barclay W. R., McIntosh, R. P., *Algal biomass technologies*, J. Cramer, Colorado

Goldman, J. C. Riley, C. B. and Dennett M. R., (1982), The effect of pH in intensive microalgal cultures. II. Species competition, *Journal of Marine Biology and Ecology,* Vol. 57, p. 15-24.

Gombotz, W. R. and Wee, S. F., (2012), Protein release from alginate matrices, *Advanced drug delivery reviews*, Vol. 64, p. 194-205.

Gomez, C. G., Pérez Lambrecht, M. V., Lozano, J. E., Rinaudo, M. and Villar, M. A. (2009), Influence of the extraction-purification conditions on final properties of alginates obtained from brown algae (Macrocystis pyrifera)*, International Journal of Biological Macromolecules,* Vol. 44, No. 4, p. 365-371.

Gouaux, E., MacKinnon, R. (2005), Principles of selective ion transport in channels and pumps, *Science,* Vol. 310, No. 5753, p. 1461-1465.

Gray, C. J., Dowsett, J. (1998), Retention of insulin in alginate gel beads, *Biotechnology and Bioengineering*, Vol. 31, p. 607–612.

Greenwell, H. C., Laurens, L. M. L., Shields, R. J., Lovitt, R. W. and Flynn, K. J. (2010), Placing microalgae biofuels on the priority list: a review of the technological challenges. *Journal of the Royal Society Interface*, Vol. 7. p. 703-726.

Grima, E. M., Belarbi, F. G., Fernandez, A., Medina, A. R. And Chisti, Y., (2003), Recovery of microalgal biomass and metabolites: process options and economics, *Biotechnology Advances*, Vol. 20, p. 491-515.

Guerrero-Lemus, R. & Martínez-Duart, J. M. (2012), Renewable Energies and CO_2 Cost Analysis, *Environmental Impacts and Technological Trends*, 2012 Edition, Vol. 3, Springer.

Guharoy, U. (2013), Design of a lab-scale hydrothermal liquefaction set-up and oil extraction from microalgae biomass, MSc Thesis, Cranfield University, Cranfield.

Guido, H. (1972), Foam flotation separation system particularly suitable for separating dissolved protein compounds and toxic metallic ions from aquarium water. Patent number US3669883.

Guiry, M. D. (2012), How many species of algae are there? *Journal of Phycology,* Vol. 48, p.1057-1063.

Hameed, A. M. S., Ebrahim, O. H. (2007), Biotechnological potential uses of immobilized algae. *International Journal of Agriculture & Biology*, Vol. 9, No. 1, p. 183-192.

Han, R., Li, H., Li, Y., Zhang, J., Xiao, H. and Shi, J. (2006), Biosorption of copper and lead ions by waste beer yeast, *Journal of Hazardous materials*, Vol. 137, No. 3, p. 1569-1576.

Hanrahan, G. and Lu, K. (2006), Application of factorial and response surface methodology in modern experimental design and optimisation, *Critical reviews in analytical Chemistry,* Vol. 36, p. 141-151.

Hasanbeigi, A., Price, L. and Lin, E. (2012), Emerging energy-efficiency and CO_2 emission-reduction technologies for cement and concrete production: A technical review. *Renewable and Sustainable Energy Reviews*, Vol. 16, No. 8, p. 6220-6238.

Haug, A., (1964), Composition and properties of alginates, *Republic of Norway Institution of Seaweed Research,* Vol. 30, p. 123.

Harris, E. H. (1989), The *Chlamydomonas* sourcebook, Academic Press.

Henderson, R. K., Parsons, S. A. and Jefferson, B. (2008). Surfactants as bubble surface modifiers in the flotation of algae: Dissolved air flotation that utilises a chemically modified bubble surface, *Environmental science and technology*, Vol. 42, No. 13, p. 4883-4888.

Hernández-Carmona, G., McHugh, D. J., Arvizu-Higuera, D. L., Rodríguez-Montesinos, E. (1999), Pilot plant scale extraction of alginate from *Macrocystis*

pyrifera. 1. Effect of pre-extraction treatments on yield and quality of alginate, *Journal of Applied Phycology*, Vol. 10, p. 507-513.

Heywood, J. B. (2006), What are the options for decreasing demand for oil and lowering greenhouse gas emissions in cars and light trucks? *Scientific American,* Vol. 295, No. 3.

Ho, K-C, Kang, S-H, Lam, I. H. Y., Hodgkiss, I. J. (2003), Distribution of *Alexandrium tamarense* in Drake Passage and the threat of Harmful Algal Blooms in the Antarctic Ocean, *Ocean and Polar Research, Vol. 25, 4, p. 625-631.*

Hoek, C. (1995), *Algae: An Introduction to Phycology*. Cambridge University Press.

Hohmann, S. (2002), Osmotic stress signalling and Osmo-adaptation in yeasts, Microbiology and Molecular Biology Reviews, p. 300-372.

Hubbert, M.K., (1956), Nuclear energy and fossil fuels: API Conference, San Antonio, TX, (March 7–9, 1956). Later published as Publ. no. 95, Shell Development Co. (June 1956).

Hughes, A. D., Kelly, M. S., Black, K. D and Stanley, M. S. (2012), Biogas from macroalgae: is it time to revisit the idea? *Biotechnology for Biofuels*, Vol. 5, p. 86.

Hutson, N. D., Krzyzynska, R. and Srivastava, R. K. (2008), Simultaneous Removal of SO_2, NO_x, and Hg from Coal flue gas using a $NaClO_2$-Enhanced Wet Scrubber, *Industrial Engineering Chemical Research,* Vol. 47, p. 5825-5831.

Iannuccelli, V., Coppi, G., Bernabei, M. T. and Cameroni, R. (1998), Air compartment multiple-unit system for prolonged gastric residence. Part 1: Formulation study, *International Journal of Pharmaceutics,* Vol. 174, p. 47-54.

International Energy Agency (2014), *Renewable Energy medium term market report 2014*, Market analysis and forecasts to 2020.

4th IPCC Report http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf (accessed 1.8.13).

Iwasaki, I., Kurano, N., and Miyachi, S. (1996), Effects of high-CO_2 stress on photosystem II in a green alga, *Chlorococcum littorale*, which has a tolerance to high CO_2, *Journal of Photochemistry and Photobiology B: Biology*, Vol. 36, No.3, p. 327-332.

Jazrawi, C., Biller, P., He, Y., Montoya, A., Ross, A. B., Maschmeyer, T. and Haynes, B. S. (2015), Two-stage hydrothermal liquefaction of a high-protein microalga, *Algal Research*, Vol. 8, p.15-22.

Jena, U., Das, K. C., & Kastner, J. R. (2011), Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*, *Bioresource Technology*, Vol. 102, No.10, p. 6221-6229.

Jena, U. and Das, K. C. (2011), Comparative Evaluation of Thermochemical Liquefaction and Pyrolysis for Bio-oil production from microalgae. *Energy & fuels*, Vol. 25, No. 11, p. 5472-5482.

Jena, U., Vaidyanathan, N., Chinnasamy, S., and Das, K. C. (2011), Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. *Bioresource Technology,* Vol. 102, No. 3, p.3380-3387.

Johnson, M. B. and Wen, Z. (2010), Development of an attached microalgal growth system for biofuel production, *Applied microbiology & biotechnology,* Vol. 85, No. 3, p. 525 – 534.

Jung, K. A., Lim, S. R., Kim, Y. and Park, J. M. (2013), Potentials of macroalgae as feedstocks for biorefinery, *Bioresource Technology,* Vol. 135, p. 182-190.

Kaštánek, F. (2010), In-field experimental verification of cultivation of microalgae Chlorella sp. using the flue gas from a cogeneration unit as a source of carbon dioxide, *Waste Management and Research*, Vol 28, No. 11, p. 961-966.

Kazamia, E., Czesnick, H., Nguyen, T. T. V., Croft, M. T., Sherwood, E. and Sasso, S. (2012), Mutualistic interactions between vitamin B_{12}-dependent algae and heterotrophic bacteria exhibit regulation, *Environmental Microbiology,* Vol. 14, No. 6, p. 1466-1476.

Kebede-Westhead, E., Pizarro, C. and Mulbry, W. W. (2006), Treatment of swine manure effluent using freshwater algae: production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates, *Journal of Applied Phycology*, Vol. 18, No.1, p.41-46.

Khan, S. A., Hussain, M. Z., Prasad, S. and Banerjee, U. C. (2009), Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews*, Vol. 13, No. 9, p. 2361–2372.

Khawaji, A. D., Kutubkhanah, I. K. and Wie, J. M. (2008), Advances in seawater desalination technologies, *Desalination,* Vol. 221, No. 1, p. 47-69.

Khoo, H. H., Koh, C. Y., Shaik, M. S., & Sharratt, P. N. (2013). Bioenergy co-products derived from microalgae biomass via thermochemical conversion–Life cycle energy balances and CO_2 emissions, *Bioresource Technology*, Vol. 143, p. 298-307.

Kiørboe, T. and Hansen, J. (1993), Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exoplymeric material, *Journal of Plankton Research*, Vol. 15, No. 9, p. 993-1018.

Knuckey, R. M., Brown, M. R., Robert, R., Frampton, D. M. F, (2006), Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds, *Aquacultural Engineering*, Vol. 35, p. 300-313.

Kohraku, S. and Kurokawa, K. (2006), A fundamental experiment for discrete-wavelength LED solar simulator, *Solar energy materials and solar cells*, Vol. 90, No.18, p.3364-3370.

Körner, S., Das, S. K., Veenstra, S. and Vermaat J. E. (2001), The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba, Aquatic Botany*, Vol. 71, p. 71-78.

Kropat, J., Hong-Hermesdorf, A., Casero, D., Ent, P., Castruita, M., Pellegrini, M., Merchant, S. S. and Malasarn, D. (2011), A revised mineral nutrient supplement increases biomass and growth rate in Chlamydomonas reinhardtii, *The Plant Journal*, Vol. 66, No. 5, p. 770 - 780.

Kumar, P. and Chandra, R. (2006), Decolourisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures of *Bacillus* spp, *Bioresource Technology*, Vol. 97, No. 16, p. 2906-2102.

Kumar, V. Wati, L., FitzGibbon, F, Nigam, P. Banat, I. M., Singh, D. and Marchant, R. (1997), Bioremediation and decolorisation of anaerobically digested distillery spent wash, *Biotechnology Letters,* Vol. 19, No. 4, p. 311-313.

Lam, M. K. and Lee. K. T. (2012), Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production, *Applied Energy*, Vol. 94, p. 303–308.

Lam, M. K., Lee, K. T. and Mohamed, A. R. (2012), Current status and challenges on microalgae-based carbon capture. *International Journal of Greenhouse Gas Control*, Vol. 10, p. 456-469.

Larsen, B., Salem, D. M. S. A., Sallam, A. E., Mishrikey, M. M, Beltagy, A. I. (2003), Characterization of the alginates from algae harvested at the Egyptian Red Sea coast (2003), *Carbohydrate Research* Vol. 338, p.2325-2336.

Latour, R. A. (2004), Biomaterials: protein-surface interactions. *Encyclopedia of biomaterials and biomedical engineering. New York: Marcel Dekker;*

Lau, P. S., Tam, N. F. Y. and Wong, Y. S. (1998), Effect of Carrageenan immobilisation on the physiological activities of *Chlorella vulgaris, Bioresource Technology*, Vol. 63, p.115-121.

Lavoie, A. and J. de la Noue. (1983), Harvesting microalgae wtih chitosan, *Journal of World Mariculture Society*, Vol. 14, p. 685-694.

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

Lee, K., and Lee, C. G. (2001), Effect of Light/dark cycles on wastewater treatments by microalgae, *Biotechnology & Bioprocess Engineering,* Vol. 6, p. 194-199.

Lee, S., Ang, W. S. and Elimelech, M. (2006), Fouling of reverse osmosis membranes by hydrophilic organic matter: implications for water reuse. *Desalination*, Vol. 187, No. 1, p. 313-321.

Lee, S., Boo, C., Elimelech, M. and Hong, S. 2010. *Comparison of fouling behaviour in forward osmosis (FO) and reverse osmosis (RO). Journal of membrance science*. Vol. 365, Issue 1-2 p. 34-39.

Lee, Y. S. and Wagner, N. J. (2003), *Dynamic properties of shear thickening colloidal suspensions, Rheologica Acta*, Vol. 42, No. 3, p. 199-208.

Lewis, J., Salam, F., Slack, N., Winton, M., Hobson, L. (2011), Product options for the processing of marine macro-algae – Summary Report: The Crown Estate, 44 pages, ISBN:978-1-906410-31-5.

Liang, Y., Sarkany, N., Cui, Y. (2009). Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions, *Biotechnology letters,* Vol. 31, p. 1043-1049.

Li, L., Fang, Y., Vreeker, R., Appelqvist, I. and Mendes, E. (2007), *Re-examining the Egg-box model in Calcium-Alginate gels with X-ray diffraction, Biomacromolecules* Vol. 8, No. 2, p. 464-468.

Li, S., Xu, T., Sun, P., Zhou, Q., Tan, H. and Hui, S. (2008), NO_x and SO_x emissions of a high sulphur self-retention coal during air-staged combustion, *Fuel,* Vol. 87 No. 6, p. 723-731.

Liu, J., Huang, J., Sun, Z., Zhong, Y., Jiang, Y. and Chen, F. (2011), Differential lipid fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofiniensis:* Assessment of algal oils for biodiesel production, *Bioresource Technology,* Vol. 102, No. 1, p. 102-110.

Liu, T., Wang, J., Hu, Q., Cheng, P., Ji, B., Liu, J., Chen, Y., Zhang, W., Chen, X., Chen, I., Gao, I. Ji, C., Wang, H. (2013), Attached cultivation technology of microalgae for efficient biomass feedstock production, *Bioresource Technology*, Vol. 127, p. 216-222.

Ling, G. N. (1963), A Physical Theory of the Living State: the Association-Induction Hypothesis*, Academic Medicine,* Vol. 38, No. 6, p. 530.

Lobban, C. S., Harrison, P. J., Duncan, M. J., (1985), The Physiological Ecology of Seaweed, Cambridge University Press.

Loeblich L. A. (1982), Photosynthesis and pigments influenced by light intensity and salinity in the halophilic *Dunaliella salina* (Chlorophyta). *Journal of Marine Biology Association* U.K. Vol. 62 p.493-508.

López-Rodas, V., Marvá, F., Costas, E. and Flores-Moya, A. (2008), Microalgal adaptation to a stressful environment (acidic, metal-rich mine waters) could be due to selection of pre-selective mutants originating in non-extreme environments, *Environmental and Experimental Botany*, Vol. 64, No. 1, p. 43-48.

Luijkx, G. C., Van Rantwijk, F. and Van Bekkum, H. (1993), Hydrothermal formation of 1, 2, 4-benzenetriol from 5-hydroxymethyl-2-furaldehyde and D-fructose, *Carbohydrate research*, Vol. 242, p. 131-139.

Lukachyov, O. P., Pochkalov, V. K. (1965), Method to obtain alginate from brown algae, USSR Patent 200, 416.

Lundquist, T. (2010), A Realistic Technology and Engineering Assessment of Algae Biofuel Production, Energy Biosciences Institute, Univeristy of California, Berkeley, p. 178.

Maeda, K. (1995), CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae, *Energy Conversion and Management*, Vol 36, No. 6-9, p. 717-720.

Mallick, N. (2002), Biotechnological potential of immobilised algae for wastewater N, P and metal removal: A Review, *Biometals*, Vol. 15, p.377-390.

Manzolini, G., Giostri, A., Saccilotto, C., Silva, P. and Macchi, E. (2011), Development of an innovative code for the design of thermodynamic solar power plants part A: Code description and test case, *Renewable Energy*, Vol. 36, No. 7, p. 1993-2003.

Marcilhac, C., Sialve, B., Pourcher, A-M, Ziebal, C., Bernet, N. and Béline, F, (2014), Digestate colour and light intensity affect nutrient removal and competition phenomena in a microalgal-bacterial ecosystem, *Water Research*, Vol. 64, p. 278-287.

Marshall K, C. (1984), Microbial adhesion and aggregation. Springer-Verlag, Berlin.

Martinsen, A., Skjak-Braek, G., Smidsrod, O. (1989), Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads, *Biotechnology and Bioengineering*, Vol. 33, p. 79–89.

Masuda, K., Sah, R. L., Hejna, M. J. and Thonar, E. J. (2003), A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: The alginate-recovered-chondrocyte (ARC) method, *Journal of Orthopaedic Research*, Vol. 21, No. 1, p. 139-148.

Mata, T. M., Martins, A. A., Caetano, N. S., (2010), Microalgae for biodiesel production and other applications: a review, *Renewable Sustainable Energy Reviews*, Vol. 14, No. 1, p. 217-232.

Mata, T. M., Melo, A. C., Simões, M. and Caetano, N. S. (2012), Parametric study of brewery effluent treatment by microalgae *Scenedesmus obliquus, Bioresource Technology*, Vol. 107, p. 151-158.

Mayes, W. M., Younger, P. L. and Aumo, J. (2006), Buffering of alkaline steel slag leachate across a natural wetland, *Environmental Science and Technology,* Vol. 40, No. 4, p.1237-1243.

Mayfield, S. P. and Franklin, S. E. (2005), Expression of human antibodies in eukaryotic micro-algae, *Vaccine*, Vol. 23, No. 15, p. 1828-1832.

McGovern, R. K. Lienhard, V. J. H. (2014), On the potential of forward osmosis to energetically outperform reverse osmosis desalination, *Journal of Membrane Science*, Vol. 469, p. 245–250.

McHugh, D. J. (1987), Production, properties and uses of alginates. Production and Utilization of Products from Commercial Seaweeds. *FAO. Fish. Tech. Paper*, Vol. 288, p. 58-115.

McHugh, D. J. (2003). A guide to the seaweed industry FAO Fisheries Technical Paper 441. *Food and Agriculture Organization of the United Nations, Rome*.

McKay, C. P., Friedmann, E. I., Gómez-Silva, B., Cáceres-Villanueva, L., Andersen, D. T. and Landheim, R. (2003), Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: Four Years of observations including the El Niño of 1997-1998, *Astrobiology*, Vol. 3, No. 2, p. 393-406.

McLaughlin, N. B, Hiba, A., Wall, G. J. and King, D. J. (2000), Comparison of energy inputs for inorganic fertilizer and manure based corn production, *Canadian Agricultural Engineering,* Vol. 42, No. 1, p. 9-18.

Mendoza, H., de la Jara, A., Freijanes, K., Carmona, L., Ramos, A. A., de Sousa Duarte, V., Varela, J. and Carlos, J. (2008), Characterization of *Dunaliella salina* strains by flow cytometry: a new approach to select carotenoid hyperproducing strains, *Electronic Journal of Biotechnology*, Vol. 11, No. 4, p. 5-6.

Mennini, N., Furlanetto, S., Maestrelli, F., Pinzauti, S., & Mura, P. (2008), Response surface methodology in the optimization of chitosan–Ca pectinate bead formulations, *European journal of pharmaceutical sciences,* Vol. 35, No.4, p. 318-325.

Mercer, P. and Armenta, R. (2011), Developments in oil extraction from microalgae, *European Journal of Lipid Science and Technology*, Vol. 113, p.539-547.

Minowa, T., Yokoyama, S-Y., Kishimoto, M. and Okakura, T. (1995), Oil production from algal cells of *Dunaliella tertiolecta, Fuel,* Vol. 74, p. 1735-1738.

Mitchell, J. R. and Blanshard, J. M. V., (1976), *Journal of Texture Studies*, Vol. 7, p.341-351.

Moheimani, N. R. (2013), Inorganic carbon and pH effect on growth and lipid productivity of *Tetraselmis suecica* and *Chlorella sp.* (Chlorophyta) grown outdoors in bag photobioreactors, *Journal of Applied Phycology,* Vol. 25, p. 387-398.

Moheimani, N. R. and Borowitzka, M. A. (2006), The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds, *Journal of Applied Phycology*, Vol. 18, No. 6, p. 703-712.

Möhler, O., Georgakopoulos, D. G., Morris, C. E., Benz S., Ebert, V., Hunsmann, S., Saathoff H., Schnaiter, M. and Wagner, R. (2008), Heterogeneous ice nucleation activity of bacteria: new laboratory experiments at simulated cloud conditions. *Biogeosciences*, Vol. 5, No. 5, p.1425–1435.

Molina Grima, E., Acién Fernández, F. G., García Camacho, F., Camacho Rubio, F., Chisti, Y. (2000), *Journal of Applied Phycology*, Vol. 12, p. 355–368.

Molina Grima, E., Belarbi, E. H., Acién, F. G., Robles Medina, A., Chisti, Y., (2003), Recovery of microalgal biomass and metabolites: process options and economics, *Biotechnology Advances,* Vol. 20, p. 491-515.

Montes, M. J., Rovira, A., Muñoz, M. and Martínez-Val, J. M. (2011), Performance analysis of an integrated solar combined cycle using direct steam generation in parabolic trough collectors, *Applied Energy*. Vol. 88, No. 9, p. 3228-3238.

Montgomery, D., 2006. Design and Analysis of Experiments, 5th Edition. John Wiley & Sons.

Morais, M.G. (2011), Biofixation of CO_2 from synthetic combustion gas using cultivated microalgae in three-stage serial tubular photobioreactors, *Zeitschrift fur Naturforschung* - Section C, *Journal of Biosciences*, Vol. 66 C, No.5-6, p. 313-318.

Morris, E. R., Rees, D. A., Thom, D. and Boyd, J. (1978), Chiroptical and stoichiometric evidence of a specific, primary dimerisation process in alginate gelation, *Carbohydrate research,* Vol. 66, No. 1, p. 145-154.

Mosier, A. R. (1978), Inhibition of photosynthesis and nitrogen fixation in algae by volatile nitrogen bases, *Journal of Environmental Quality*, Vol. 7, No. 2, p. 237-240.

Mulbry, W., Westhead, E. K., Pizarro, C. and Sikora, L. (2005), Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer, *Bioresource Technology,* Vol. 96, No. 4, p. 451-458.

Mulbry, W., Kondrad, S., Pizarro, C. and Kebede-Westhead, E. (2008), Treatment of dairy effluent using freshwater algae: Algae productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresource Technology*, Vol. 99, No. 17, p. 8137-8142.

Nagase, H. (2001), Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae, *Biochemical Engineering Journal*, Vol. 7, No.3, p. 241-246.

Naik, S. N., Goud, V.V., Rout, P. K. and Dalai, A. K. (2010), Production of first and second generation biofuels: A comprehensive review, *Renewable and Sustainable Energy Reviews,* Vol. 14, No. 2, p. 578-597.

Nakamura, Y., Taniguchi, A., Okada, S. And Tokuda, M. (1998), Positive growth of phytoplankton under conditions enriched with steel-making slag solution, *ISIJ International*, Vol. 38, No. 4, p. 390-398.

Nielsen, E. S. and Jensen, E. A. (1957), Primary oceanic production: the autotrophic production of organic matter in the oceans, Vol. 1, p. 49-135.

Noike, T., Goo, I. S., Matsumoto, H., Miyahara, T, (2004), Development of a new type of anaerobic digestion equipped with the function of nitrogen removal, *Water Science Technology*, Vol. 49, p. 143-179.

Norsker, N. H, Barbosa, M. J., Vermuë, M. H. and Wijffels, R. H. (2011), Microalgal production – A close look at the economics, *Biotechnology Advances*, Vol. 29, No. 1, p. 24-27.

Northcote, D. H. and Horne, R. W. (1952), The chemical composition of the yeast cell wall, *Biochemical Journal*, Vol. 51, No. 2, p. 232-236.

A. Nussinovitch, Polymer Macro- and Micro-Gel Beads: Fundamentals and applications, (2010), Chapter 2, Bead formation, strengthening, and modification, ISBN: 978-1-4419-6617-9

O'Fallon J. V., Busboom, J.R., Nelson, M. L. and Gaskins, C.T. (2007), A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science,* Vol. 85, No. 6, p.1511–1521.

O'Grady, J., Morgan, J. A. (2011), Heterotrophic growth and lipid production of *Chlorella protothecoides* on glycerol, *Bioprocess and biosystems engineering,* Vol 34, No. 1, p. 121-125.

Olaizola M, (2003), Commercial development of microalgal biotechnology: from the test tube to the marketplace, *Biomolecular Engineering,* Vol. 20, p. 459-466.

Olaizola M, Huntley, M. E. (2003) In: Fingerman M., Nagabhushman R., editors, Biomaterials and bioprocessing, Enfield: Science Publishers, p.143-164.

Olajire, A. A. (2010), CO_2 capture and separation technologies for end-of-pipe applications – A Review, *Energy*, Vol. 35, No. 6, p. 2610-2628.

Ometto, F. (2014), Microalgae to energy: Biomass recovery and pre-treatments optimisation for biogas production integrated with wastewater nutrients removal, PhD Thesis, Cranfield University, Cranfield.

Onakpoya, F. O. (2014), Study of bio oil from hydrothermal liquefaction of microalgae harvested by two different paths, MSc Thesis, Cranfield University, Cranfield.

Papone, T., Kookkhunthod, S., Leesing, R. (2012), Microbial oil production by monoculture and mixed cultures of microalgae and oleaginous yeasts using sugarcane juice as substrate, *World Academy of Science Engineering and Technology*, Vol. 64, p.1127–1131.

Passow, U., Alldredge, A. L. (1995), Aggregation of a diatom bloom in a mesocosm: the role of transparent exopolymer particles (TEP). Deep Sea Res Part II: *Topical Studies in Oceanography,* Vol. 42 p. 99–109.

Passow, U. (2002), Transparent exopolymer particles (TEP) in aquatic environments, *Progress in Oceonography,* Vol. 55, No. 3, p. 287-333.

Patil, V., Tran, K-Q., Giselrød, H. R. (2008), Towards sustainable production of biofuels from microalgae, *International Journal of Molecular Science*, Vol. 9, No. 7, p.1188-1195.

Pearce, M. (2014), New patent application: *Processing of microalgae*, UK Patent Application No.1402298.2, filed 11th February 2014.

Pearce, M., Brennan, F. (2015), Novel findings in desalination, *Desalination,* Vol. 360, p. 13-18.

Pearce, M., Shemfe, M. and Sansom, C., (2016), Techno-economic analysis of solar integrated hydrothermal liquefaction of microalgae, *Applied Energy,* Vol. 166, p. 19-26.

Peterson, A. A., Vogel, F., Lachance, R. P., Froling, M., Antal, M. J., Tester, J. W. (2008), Thermochemical biofuel production in hydrothermal media: a review of sub- and supercritical water technologies, *Energy & Environmental Science,* Vol. 1, p. 32-65.

Piantini R. (2012), *Personal Communication*, Antofagasta, Chile.

Pienkos, P. T. (2007), *The potential for biofuels from algae,* Algae Biomass Summit, San Francisco, CA. 15th November 2007, National Renewable Energy Laboratory.

Pires, J. C. M., Goncalves, A. L., Martins, F. G., Alvim-Ferraz, C. M. and Simoes, M. (2013), Effect of light supply on CO_2 capture from the atmosphere by *Chlorella vulgaris* and *Pseudokirchneriella subcapitata, Mitigation and adaption strategies for Global change*, Vol. 19, p. 1109-1117.

Pittman, J. K. Dean, A. P., Osundeko, O, (2011), The potential of sustainable algal biofuel production using wastewater resources, *Bioresource Technology,* Vol. 102, No. 1, p. 17-25.

Pitz-Paal, R., Dersch, J., Milow, B., Tasilez, F., Ferriere, A., Langnickel, U. and Popel, O. (2007), Development steps for parabolic trough solar power technologies with maximum impact on cost reduction. *Journal of Solar Energy Engineering*, Vol. 129, No. 4, p. 371-377.

Pizarro, C., Mulbry, W., Blersch, D. and Kangas, P. (2006), An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent, *Ecological engineering*, Vol. 26 No.4, p. 321-327.

Powell, E. E., Mapiour, M. L., Evitts, R. W., & Hill, G. A. (2009), Growth kinetics of *Chlorella vulgaris* and its use as a cathodic half cell, *Bioresource Technology*, Vol. 100, No.1, p. 269-274.

Prieto, A., Cañavate, J. and Garcia-Gonzalez, M. (2011), Assessment of carotenoid production by *Dunaliella salina* in different culture systems and operation regimes, *Journal of Biotechnology*, Vol. 151, No. 2, p. 180-185.

Prince, P. A., Ricketts, C. and Thomas, G. (1981), Weight loss in incubating albatrosses and its implication for their energy and food requirements, *Condor*, Vol. 83, p. 238 – 242.

Przytocka-Jusiak, M. (1976), Growth and survival of *Chlorella vulgaris* in high concentrations of nitrogen. *Acta Microbiologica Polonica,* Vol. 25, p. 287-289.

Pulz, O. (2001), Photobioreactors: production systems for phototrophic microorganisms, *Applied Microbiology and Biotechnology,* Vol. 57, p.287-293.

Putt, R., Singh, M., Chinnasamy, S. and Das, K. C. (2011), An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. *Bioresource Technology*, Vol. 102, No. 3, p. 3240-3245.

Quinby-Hunt, M. S. and Wilde, P. (1987), Modelling of dissolved elements in seawater, *Ocean Science and Engineering,* Vol. 11, No. 3&4, p. 153-251.

Raposo, M. F. D. J., Oliveira, S. E., Castro, P. M., Bandarra, N. M. and Morais, R. M. (2010), On the utilization of microalgae for brewery effluent treatment and possible applications of the produced biomass, *Journal of the Institute of Brewing*, Vol. 116, No.3, p. 285-292.

Ras, M., Laardon, L., Bruno, S., Bernet, N. and Steyer, J-P. (2011), Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris, Bioresource Technology,* Vol. 102, No. 1, p. 200-206.

Rawat, I., Ranjith, K. R., Mutanda, T., Bux, F, (2011), Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, *Applied Energy*, Vol. 88, No. 10, p. 3411-3424.

Razon, L. F. and Tan, R. R. (2011), Net energy analysis of the production of biodiesel and bio-gas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*, *Applied Energy*, Vol. 88, No. 10, p. 3507-3514.

Rebhun, M. and Lurie, M. (1993), control of organic matter by coagulation and floc separation, *Water Science Technology*, Vol. 27, No. 11, p. 1-20.

Reitan, K. I., Rainuzzo, J. R., Øie, G., and Olsen, Y. (1997), A review of the nutritional effects of algae in marine fish larvae, *Aquaculture*, Vol. 155, No.1, p. 207-221.

Reddy, H. K., Muppaneni, T., Rastegary, J., Shirazi, S. A., Ghassemi, A. and Deng, S. (2013), Hydrothermal extraction and characterization of bio-crude oils from wet *Chlorella sorokiniana* and *Dunaliella tertiolecta, Environmental Progress and sustainable energy*, Vol. 32. No. 4, p. 910-915.

Richmond, A. (1999), Physiological principles and modes of cultivation in mass production of photoautotrophic microalgae, *Chemicals from Microalgae*, p. 353-86.

Richmond, A. (2000), Microalgal biotechnology at the turn of the millennium: A personal view, *Journal of Applied Phycology*, Vol. 12, p. 441-451.

Roesijadi, G., Jones, S.B., Snowden-Swan, L.J., Zhu, Y., (2010), Macroalgae as a Biomass Feedstock: A Preliminary Analysis (PNNL-19944).

Ross, A. B., Jones, J. M., Kubacki, M. L. and Bridgeman, T. (2008), Classification of macroalgae as fuel and its thermochemical behaviour, *Bioresource Technology,* Vol. 99, Issue 14, p. 6494-6504.

Ruiz-Marin, A., Mendoza-Espinosa, L. G. and Stephenson, T. (2010), Growth and nutrient removal in free and immobilised green algae in batch and semi-continuous cultures treating real wastewater, *Bioresource Technology,* Vol. 101, No. 1, p. 58-64.

Rupérez, P. and Toledano, G., (2003), Indigestible fraction of edible marine seaweeds, *Journal of the Science of Food and Agriculture*, Vol. 83, p. 1267-1272.

Russell, L. M., Hawkins, L. N., Frossard, A. A., Quinn, P. K. and Bates, T. S. (2010), Carbohydrate-like composition of submicron atmospheric particles and their production from ocean bubble bursting, *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 107, No. 15, p. 6652-6657.

Ryu, B. G., Kim, K., Kim, J., Han, J. I. and Yang, J. W. (2013), Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, *Aurantiochytrium sp.* KRS101. *Bioresource Technology,* Vol. 129, p. 351-359.

Salim, S., Bosma, R. And Vermue, M. H. (2011), Harvesting microalgae by bio-flocculation, *Journal of Applied Phycology*, Vol. 23, p. 849-855.

Salim. S., Vermuë, M. H and Wijffels, R.H. (2012), Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation, *Bioresource Technology*, Vol.118, p. 49-55.

Sansom, C. (2014), *Personal communication*, Cranfield University.

Santos, C. A. And Reis, A. (2014), Microalgal symbiosis in biotechnology, *Applied Microbiology and Biotechnology*, Vol. 98, p.5839–5846.

Sarmiento, J. L., Gruber, N. (2006), Ocean biogeochemical dynamics, Princeton University Press.

Schenk, P. M., Thomas-Hall, S. R., Stephens, E, (2008), Second generation biofuels: high-efficiency microalgae for biodiesel production, *Bioenergy Research*, Vol. 1, No. 1, p. 20-43.

Schlarb-Ridley, B. and Parker, B. (2013), A UK Roadmap for Algal Technologies, NERC-TSB, UK.

Secconi, M. G. (1967), Procédé de fabrication d'alginates pariant des cystosires ou algues similaires, et produit industriel obtenu, Republique Française, Patent 1,464, 840.

Shah, H. N., Mody, I. C., Rao, A. V. (1967), Seasonal variation of viscosity of sodium alginate from *Sargassum* spp. and the preparation of high viscosity alginates, *Indian Journal of Science and Technology*, Vol. 5, p. 269–270.

Sharma, K. K., Garg, S., Li, Y., Malekizadeh, A. and Schenk, P. M. (2013), Critical analysis of current microalgae dewatering techniques. *Biofuels* Vol. 4, No. 4, p. 397-407.

Sheehan, J., Dunahaym T., Benemann, J. and Roessler, P. (1998), A look back at the U.S. Department of Energy's Aquatic Species Program – Biodiesel from Algae, prepared for U.S. Department of Energy's Office of Fuels Development, NREL, USA.

Sholkovitz, E. R. (1976), Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater, *Geochimica et Cosmochimica Acta*, Vol. 40, No. 7, p. 831-845.

Sialve, B., Bernet, N. and Bernard, O. (2009), Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable, *Biotechnology Advances*, Vol 27, No. 4, p. 409-416.

Silva, M. A. D., Bierhalz, A. C. K. and Kieckbusch, T. G. (2009), Alginate & Pectin composite films crosslinked with Ca^{2+} ions: Effect of the plasticizer concentration, *Carbohydrate Polymers,* Vol. 77, No. 4, p. 736-742.

Sim, T., Goh, A. and Becker, E. W. (1988), Comparison of centrifugation, dissolved air flotation and drum filtration techniques for harvesting sewage-grown algae, *Biomass*, Vol. 16, No. 1, p. 51-62.

Simon, N., Le Bot, N., Marie, D., Partensky, F. and Vaulot, D. (1995), Flourescent In Situ Hybridisation with rRNA-Targeted Oligonucleotide Probes to identify small Phytoplankton by Flow Cytometry, *Applied and Environmental Microbiology,* Vol. 61, No. 7, p.2506-2513.

Simon N., Cras A. L., Foulon, E., Lemée, R. (2009), Diversity and evolution of marine phytoplankton, *Comptes rendus biologies*, Vol. 332, No. 2, p. 159–170.

Simpson, A. P. and Lutz, A. E. (2007), Exergy analysis of hydrogen production via steam methane reforming, *International Journal of Hydrogen Energy*, Vol. 32, No. 18, p. 4811-4820.

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

Six, C. Finkel, Z. V., Irwin, A. J. and Cambpell, D. A. (2007), Light variability illuminates niche partitioning among Marine Picocyanobacteria, DOI: 10.1371/journal.pone.0001341.

Skorupskaite, V., Makareviciene, V. and Levisauskas, D. (2015), Optimisation of mixotrophic cultivation of microalgae *Chlorella sp.* For Biofuel production using response surface methodology, *Algal Research,* Vol. 7, p. 45-50.

Smil, V. (1999), Long-range perspectives on inorganic fertilizers in global agriculture, *Travis P. Hignett Memorial Lecture, IFDC, Muscle Shoals, Alabama.*

Smits, G. J., Kapteyn, J. C., Van den Ende, H. and Klis, F. M., (1999), Cell wall dynamics in yeast, Current. *Opinions in Microbiology*, Vol. 2, p.348–352.

Smith, V. H., Sturm, B. S. M., Denoyelles, F. J. and Billings, S. A. (2010), The ecology of algal biodiesel production*, Trends in Ecology & Evolution*, Vol. 25, No. 5, p. 301-309.

Sommer, C. (2014), Global Dairy Developments – An overview on milk prices and milk production costs worldwide, *International Farm Comparison Network Dairy Research Center, Kiel, Germany, Tri-State Dairy Nutrition Conference*, April 14-16, 2014.

Sorrell, S., Miller, R., Bentley, R. and Speirs, J. (2010), Oil futures: A comparison of global supply forecasts, *Energy Policy*, Vol. 38, No. 9, p. 4990-5003.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambet, A. (2006), Optimisation of *Nannochloropsis oculata* growth using the response surface method, *Journal of Chemical Technology and Biotechnology*, Vol. 81, p.1049-1056.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambet, A. (2006), Commercial Applications of Microalgae, *Journal of Bioscience and Bioengineering*, Vol. 101, No.2, p.87–96.

Spiegel, M., Noordam, M.Y. and Fels-Klerx, H. J. (2013), Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production, *Comprehensive Reviews in Food Science and Food Safety,* Vol.12, No. 6, p. 662-678.

Spilling, K., Seppala, J. and Tamminen, T. (2011), Inducing autoflocculation in the diatom *Phaeodactylum tricornutum* through CO_2 regulation, *Journal of Applied Phycology*, Vol. 23, p. 959-966.

Spinosa, L. (Ed.), (2011), Wastewater sludge: a global overview of the current status and future prospects, IWA Publishing.

Stanley, M. (2013), Seaweed to Biofuel: Towards a sustainable supply chain, 21st May 2013, SAMS, Oban.

Stein, J. R. (Ed.). (1979), *Handbook of Phycological Methods. Culture methods and growth measurements*, Vol. 1, CUP Archive.

Stephenson, P. G., Moore, C. M., Terry, M. J., Zubkov, M. V., Bibby, T. S., (2011), Improving photosynthesis for algal biofuels: toward a green revolution. *Trends in Biotechnology,* Vol. 29, No. 12, p. 615–623.

Syres, K., Johnston, E., Curtin, D. and Gilkes, R. J. (2010), A new perspective on the efficiency of phosphorus fertilizer use. In *Proceedings 19th World Congress of Soil Science, Soil solutions for a changing world,* p. 172-175.

Sukenik, A. And Shelef, G. (1983), Algal Autoflocculation – verification and proposed mechanism, *Biotechnology and Bioengineering*, Vol. 26, p. 142-147.

Sze, J., London, J., Shilling, F., Gambirazzio, G., Filan, T. and Cadenasso, M. (2009), Defining and Contesting Environmental Justice: Socio-natures and the Politics of Scale in the Delta, *Antipode*, Vol. 41, No.4, p.807-843.

Sze, P., 1993. A Biology of the algae, second Ed. Wm. C. Brown Publishers.

Takeda, H. (1991), Sugar composition of the cell wall and the taxonomy of *Chlorella* (Chlorophyceae), *Journal of Phycology,* Vol. 27, p. 224-232.

Tam, N. F. Y. & Wong, Y. S. (1996), Effect of ammonia concentration on growth of *Chlorella vulgaris* and nitrogen removal from media, *Bioresource Technology,* Vol 57, p. 45-50.

Tammam, A. A., Fakhry, E. M. & El-Sheekh, M. (2013), Effect of salt stress on antioxidant system and the metabolism of the reactive oxygen species in *Dunaliella salina* and *Dunaliella tertiolecta, African Journal of Biotechnology, V*ol. 10, No. 19, p. 3795-3808.

Tang, D., Han, W., Li, P., Miao, X., and Zhong, J. (2011), CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels, *Bioresource technology*, Vol. 102, No. 3, p. 3071-3076.

Tarleton, E. S. and Wakeman, R. J., (1993) Understanding Flux decline in cross flow microfiltration, Part 1 - Effects of particle and pore size, *Chemical Engineering Research and Design*, Vol. 71, No.4, p. 399-410.

Taylor, R. L., Rand, J. D. And Caldwell, G. S. (2012), Treatment with Algae extracts promotes flocculation and enhances growth and neutral lipid content in Nannochloropsis oculata – a candidate for biofuel production, *Marine Biotechnology*, DOI 10.1007/s10126-012-9441-8.

Thomas, S. F., Rooks, P., Rudin, F., Atkinson, S., Goddard, P., Bransgrove, R., Mason, P. T. and Allen, M. J. (2014), The Bactericidal Effect of Dendritic Copper Microparticles, Contained in an Alginate Matrix, on *Escherichia coli.* PLoS ONE 9(5): e96225. doi:10.1371/journal.pone.0096225.

Tipping, E. (2002), *Cation binding by humic substances*, (Vol. 12), Cambridge University Press.

Toledo, E. J. L., Ramalho, T. C. and Magriotis, Z. M. (2008), Influence of magnetic field on physical-chemical properties of the liquid water: Insights from experimental and theoretical models, *Journal of Molecular Structure*, Vol. 888, No. 1-3, p. 409-415.

Tomlinson, P. R. and Branston, J. R. (2014), The demand for UK beer: estimates of the long-run on- and off-trade beer price elasticities, *Applied Economics Letters*, Vol. 21, No. 3, p. 209-214.

Toor, S. S., Rosendahl, L. and Rudolf, A. (2011), Hydrothermal liquefaction of biomass: A review of subcritical water technologies, *Energy,* Vol. 36, p. 2328-2342.

Tredici, M. (1999), in: M.C. Flickinger, S.W. Drew (Eds.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation, Wiley, p. 395–419.

Trypuć, M., Bialowicz, K. (2011), $CaCO_3$ production using liquid waste from Solvay method, *Journal of Cleaner Production,* Vol. 19, No. 6, p. 751-756.

Turner, J. A. (2004), Sustainable Hydrogen production, *Science,* Vol. 305, No. 5686, p.972-974.

DECC UK Energy in Brief (2014), https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/350941/UK_Energy_in_Brief_2014_revised.pdf (accessed 24.9.14).

DECC UK Energy Trends, 6 (2016): Renewables www.gov.uk accessed 9.1.16

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M. and Hoadley, A, (2010), Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of renewable and sustainable energy*, Vol. *2,* No.1, 012701.

UK Government, (2011), UK parliament briefing paper – biofuels from algae. http://www.parliament.uk/briefing-papers/POST-PN-384/biofuels-from-algae-july-2011 (accessed 1.8.13).

Umeyama, H. and Morokuma, K. (1977), The origin of hydrogen bonding. An energy decomposition study, *Journal of the American Chemical Society,* Vol. 99, No. 5, p. 1316-1332.

US Energy Information Administration EIA (1998), Natural gas issues and trends, http://www.eia.gov/pub/oil_gas/natural_gas/analysis_publications/natural_gas_1998_issues_trends/pdf/it98.pdf (accessed 4.12.14).

Valton, M. (2014), Simulated coal flue combustion flue gas and steel slag for *Chlorella vulagaris* cultivation, MSc Thesis, Cranfield University, Cranfield.

Van den Hende, S., Vervaeren, H., Desmet, S. And Boon, N. (2011), Bioflocculation of microalgae and bacteria combined with flue gas to improve sewage treatment, *New Biotechnology*, Vol. 29, No. 1. p. 23-31.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B. and Muylaert, K. (2012), Flocculation of C*hlorella vulgaris* induced by high pH: Role of magnesium and calcium and procatical implications, *Bioresource Technology,* Vol. 105, p. 114-119.

Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K. (2012), Influence of organic matter generated by Chlorella vulgaris on five different modes of flocculation, *Bioresource Technology*., Vol. 124, p. 508–511.

Vandamme, D., Foubert, I., Muylaert, K. (2013), Flocculation as a low-cost method for harvesting microalgae for bulk biomass production, *Trends in Biotechnology*. Vol. 31, No. 4, p. 233–239.

Vandamme, D., Eyley, S., Van den Mooter, G., Muylaert, K., Thielemans, W. (2015), Highly charged cellulose-based nanocrystals as flocculants for harvesting *Chlorella vulgaris, Bioresource Technology,* Vol. 194, p. 270-275.

Varadarajan, A. S., Philip, P. K., & Ramamoorthy, B. (2002), Investigations on hard turning with minimal cutting fluid application (HTMF) and its comparison with dry and wet turning, *International Journal of Machine Tools and Manufacture*, Vol. 42, No.2, p.193-200.

Vauchel, P., Kaas, R., Arhaliass, A., Baron, R. and Legrand, J. (2008), A new process for extracting alginates from *Laminaria digitata*: reactive extrusion, *Food and Bioprocess Technology*, Vol. 1, No. 3, p. 297-300.

Vauchel, P., Leroux, K., Kaas, R., Arhaliass, A., Baron, R. and Legrand, J. (2009), Kinetics modelling of alginate alkaline extraction from *Laminaria digitata*, *Bioresource Technology,* Vol. 100, No. 3, p. 1291-1296.

Walker, F. T. (1954), Distribution of Laminariaceae around Scotland, *Journal du Conseil,* Vol. 20, No. 2, p. 160-166.

Wang, B., Li, Y., Wu, N., Lan, C. Q., (2008), CO_2 bio-mitigation using microalgae, *Applied Microbiology and Biotechnology*, Vol. 79, No. 5, p. 707-718.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J. and Ruan, R. (2010), Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp*., *Bioresource Technology, V*ol. 101, No. 8, p. 2623–2628.

Ward, (1978), Thermophilic Methanogenesis in a Hot-Spring Algal-Bacterial Mat (71 to 30°C), *Applied and Environmental Microbiology*, Vol. 35, No. 6, p. 1019-1026.

Ward, A. (1945), *Colloids their properties and applications*, Blackie & Son Ltd.

Wee, S.F., Gombotz, W. R. (1994), Controlled release of recombinant human tumor necrosis factor receptor from alginate beads *Proceedings of International Symposium on Controlled Release in Bioactive Materials*, Vol. 21, p. 730–731.

Widjaja, A., Chien, C. C., and Ju, Y. H. (2009), Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*, *Journal of the Taiwan Institute of Chemical Engineers*, Vol. 40, No.1, p. 13-20.

Wiley, P. E., Campbell, J. E. and Mc Kuin, B. (2011), Production of Biodiesel and Biogas from Algae: A Review of Process Train Options, *Water Environment Research*, Vol. 83, No. 4, p. 326-338. doi:10.2175/106143010X12780288628615

Wilkie, A. C. and Mulbry, W. W. (2002), Recovery of dairy manure by benthic freshwater algae, *Bioresource Technology,* Vol. 84, No. 1, p. 81-91.

Wong, M. H. (1981), Chicken manure and blood waste for growing *Chlorella pyrenoidosa, Conservation & Recycling*, Vol. 4, No. 1, p. 9-14.

Woods, J. (2013), "Bioenergy and food security: friend or foe? Evaluating the role of bioenergy in sustainable development through the LACAf and GSB projects", FAPESP week London Brazil-Europe Scientific Cooperation, 27th Sept 2013, The Royal Society, London.

Worden, A. Z., Nolan, J. K. and Palenik, B. (2004), Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component, *Liminology and Oceanography,* Vol. 49, No. 1, p. 168-179.

Wu, J., Contreras, A. E. and Li, Q. (2014), Studying the impact of RO membrane surface functional groups on alginate fouling in seawater desalination*, Journal of Membrane Science,* Vol. 458, p. 120-127.

Wu, Z., Zhu, Y, Huang, W., Zhang, C., Li, T., Zhang, Y., Li, A. (2012), Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium, *Bioresource Technology*, Vol. 110, p. 496-502.

Xie, K., Jia, X., Xu, P., Huang, X., Gu, W., Zhang, F., Yang, S. and Tang, S. (2012), Improved composting of poultry feces via supplementation with ammonia oxidizing *archaea*, *Bioresource Technology,* Vol. 120, p. 70–77.

Xiong, W., Li, X., Xiang, J., & Wu, Q. (2008), High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production, *Applied Microbiology and Biotechnology*, Vol. 78 No.1, p. 29-36.

Xu L, Brilman D. W., Withag J. A., Brem G. and Kersten, S. (2011), Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis, *Bioresource Technology*, Vol. 102, No. 8, p. 5113-5122.

Yao, J., Hu, Y. and Lu, W. (2012), Performance research on coir fibre and wood debris hybrid boards, *Bioresources*, Vol. 7, No.3, p. 4262-4272.

Yeh, K. L. and Chang, J. S. (2012), Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31, *Bioresource technology*, Vol. 105, p. 120-127.

Yildirim, I. and Prezzi, M. (2015), Geotechnical Properties of Fresh and Aged Basic Oxygen Furnace Steel Slag, *Journal of Materials in Civil Engineering,* Vol. 27, No. 12, 04015046.

Yu, G., Zhang, Y., Guo, B., Funk, T. and Schideman, L. (2014), Nutrient flows and quality of Bio-crude oil produced via catalytic hydrothermal liquefaction of low-lipid microalgae, *BioEnergy Research,* p. 1-12.

Zhang, T. S., Yu, Q. J., Wei, J., Li, J. and Zhang, P. (2011), Preparation of high performance blended cements and reclamation of iron concentrate from basic oxygen furnace steel slag, *Resources, Conservation and Recycling* Vol. 56, No. 1, p. 48–55.

Zhu, Y., Albrecht, K. O., Elliott, D. C., Hallen, R. T. and Jones, S. B. (2013), Development of hydrothermal liquefaction and upgrading technologies for lipid-extracted algae conversion to liquid fuels. *Algal Research,* Vol. 2, No.4, p. 455-464.

Zimmerman, W. B., Tesar, V., Bandulasena, H. C. (2011), Towards energy efficient nanobubble generation with fluidic oscillation, *Current opinion in Colloid & Interface Science, Vol.* 16, No. 4, p. 350-356.

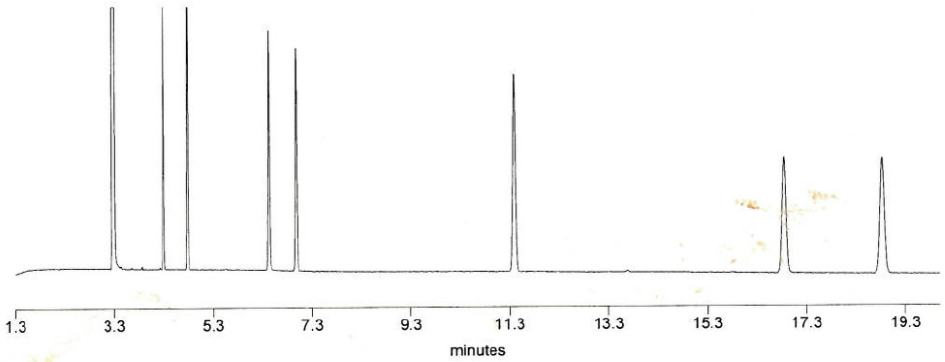
Appendix A

SHIMADZU

GC CAPILLARY COLUMN

PERFORMANCE REPORT

CODE: 60M X 0.25MM ID SHIM-WAX 0.25UM
PART NO: 054428SH
SERIAL NO: 1098625



PERFORMANCE DATA

PEAK	COMPOUND	RETENTION TIME (MIN)	CAPACITY RATIO (K)	KOVATS INDEX
1	SOLVENT	3.28		
2	2-OCTANONE	4.30	0.33	1316.23
3	TETRADECANE	4.78	0.48	1400.00
4	1-OCTANOL	6.42	0.99	1563.88
5	HEXADECANE	6.98	1.16	1600.00
6	NAPHTHALENE	11.39	2.53	1776.23
7	2,4-(DMA)	16.85	4.22	1892.18
8	2,6-(DMP)	18.84	4.84	1923.12

GAS VELOCITY (cm/sec)	31.0
EFFECTIVE PLATES (Peak 8)	144511
THEORETICAL PLATES (Peak 8)	217655
EFFECTIVE PLATES/METRE (Peak 8)	2409
SEPARATION NUMBER(Peaks 7-8)	9.97
SKEW (Peak 8)	1.04

COLUMN SPECIFICATIONS

LENGTH	60 METRE	FILM THICKNESS	0.25 micron
TYPE	BONDED PHASE	MAX.TEMPERATURE	260 deg.C
MATERIAL	FUSED SILICA	I.D.	0.25 mm
PHASE	SHIM-WAX (polar)		

TESTING CONDITIONS

COLUMN TEMPERATURE	145	SAMPLE	MIX B1
DETECTOR TEMPERATURE	280	SAMPLE SIZE	0.1ul
INJECTOR TEMPERATURE	240	SPLIT RATIO	60:1
CARRIER GAS	HYDROGEN	SENSITIVITY	32*10E-12 AFS
INLET PRESSURE	1.41Kg/cm^2	DETECTOR	FID

The enclosed capillary column has been individually tested to guarantee it meets required performance standards. The above chromatogram and data are from the enclosed column.

Figure A-1 – GC column specifications

TraceCERT®
Traceable Certified Reference Materials

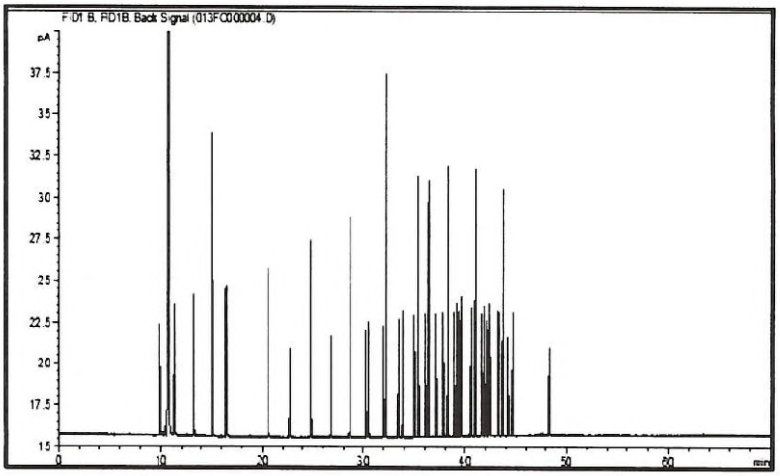
SUPELCO
Solutions within.™
595 North Harrison Road · Bellefonte, PA
16823-0048 USA · Phone(814)359-3441

Certificate of Analysis

Description: Supelco 37 Component FAME Mix, varied conc. in dichloromethane

Part Number: CRM47885
Lot Number: LC06601V
Expiration Date: March 2017
Storage: Freeze

Analytical Method Parameters:
Column: SP2560 100m x 0.25mm x 0.2µm df
100°C (5min) to 240°C (20min) at 4°C/min
Detector: FID, 260°C
Injection Volume:1.0uL



Elution	Analyte	Lot Number	CAS Number	Chromatographic Purity %	Certified Gravimetric Conc µg/ml	Expanded Uncertainty µg/ml	Analytical Conc. µg/ml
1.	Methyl Butyrate	LB99087	623-42-7	99.9	399.7	±10.2	414.0
2.	Methyl Hexanoate	LB78270	106-70-7	99.9	399.5	±7.8	414.3
3.	Internal Standard	N/A	N/A	N/A	N/A	N/A	N/A
4.	Methyl Octanoate	LB97319	111-11-5	99.9	399.5	±9.6	416.4
5.	Methyl Decanoate	LB86422	110-42-9	99.9	399.6	±8.9	410.6
6.	Methyl Uncdecanoate	LB79889	1731-86-8	99.9	199.9	±5.3	208.9
7.	Methyl Laurate	LB97659	111-82-0	99.9	399.7	±9.9	416.1
8.	Methyl Tridecanoate	LC02846	1731-88-0	98.7	197.4	±5.5	207.6
9.	Methyl Myristate	LB97558	124-10-7	99.9	399.6	±9.2	415.7
10.	Myristoleic Acid Methyl Ester	LB96087	56219-06-8	99.9	199.9	±5.3	207.4
11.	Methyl Pentadecanoate	LB84181	7132-64-1	99.9	199.8	±5.0	206.5
12.	Cis-10-Pentadecanoic Acid Methyl	LB92709	90176-52-6	99.0	198.0	±5.1	205.4

A2LA ACCREDITED TESTING Cert# AT-1607
A2LA ACCREDITED REFERENCE MATERIAL PRODUCER Cert# AR-1606

Produced in double accredited laboratory fulfulling
ISO/IEC 17025 and
ISO Guide 34

Page 1 of 3

SIGMA-ALDRICH®

Figure A-2 – GC certified reference standard FAME 37 mix

Elution	Analyte	Lot Number	CAS Number	Chromatographic Purity %	Certified Gravimetric Conc µg/ml	Expanded Uncertainty µg/ml	Analytical Conc. µg/ml
13.	Methyl Palmitate	LC02625	112-39-0	99.9	599.4	±15.0	622.7
14.	Methyl Palmitoleate	LC05477	1120-25-8	99.9	199.9	±5.2	211.8
15.	Methyl Heptadecanoate (1)	LB87552	1731-92-6	99.6	199.2	±5.7	198.9
16.	Cis-10-Heptadecanoic Acid Methyl	LC03559	77745-60-9	98.4	196.9	±5.1	212.7
17.	Methyl Stearate	LB97274	112-61-8	99.9	399.6	±9.9	417.6
18.	Trans-9-Elaidic Methyl Ester	LB93221	2462-84-2	99.7	199.4	±4.9	199.5
19.	Cis-9-Oleic Acid Methyl Ester	LC02936	112-62-9	99.9	399.7	±10.0	417.7
20.	Linolelaidic Acid Methyl Ester	LB98119	2566-97-4	99.9	199.7	±5.1	202.8
21.	Methyl Linoleate	LB92660	112-63-0	99.9	199.9	±5.4	210.1
22.	Methyl Arachidate	LB88395	1120-28-1	99.9	399.7	±10.9	422.2
23.	Gamma-Linolenic Acid Methyl Ester	LB91798	16326-32-2	99.9	199.8	±7.0	208.5
24.	Methyl cis-11-Eicosanoate	LC01495	2390-09-2	99.9	199.9	±5.1	208.1
25.	Methyl Linolenate	LC02491	301-00-8	99.9	199.8	±6.4	209.0
26.	Methyl Heneicosanoate	LB85704	6064-90-0	99.9	198.8	±5.0	207.9
27.	Cis-11,14-Eicosadienoic Acid Methy	LC04789	2463-02-7	99.9	199.8	±6.2	208.2
28.	Methyl Behenate	LC03090	929-77-1	99.7	398.9	±10.4	414.7
29.	Cis-8,11,14-Eicosatrienoic Acid Met	LC05069	21061-10-9	99.8	199.6	±6.7	214.3
30.	Methyl Erucate	LB99614	1120-34-9	99.9	199.8	±4.7	209.1
31.	Cis-11,14,17-Eicosatrienoic Acid Me	LB94324	55682-88-7	99.9	199.8	±6.5	198.9
32.	Methyl Tricosanoate	LB97489	2433-97-8	99.9	199.8	±5.6	206.3
33.	Methyl Cis-5,8,11,14-Eicosatetraeno	LC03262	2566-89-4	98.7	197.3	±6.5	197.2
34.	Cis-13,16-Docosadienoic Acid Meth	LC06471	61012-47-3	99.9	199.8	±6.1	208.5
35.	Methyl Lignocerate	LB90233	2442-49-1	99.9	399.6	±10.5	418.3
36.	Methyl Cis-5,8,11,14,17-Eicosapent	LB95206	2734-47-6	99.8	199.5	±6.4	190.7
37.	Methyl Nervonate	LC03085	2733-88-2	99.9	199.8	±5.7	208.5
38.	Cis-4,7,10,13,16,19-Docosahexaen	LB95142	301-01-9	99.9	199.9	±8.7	209.1

PAS110 Approved Testing Laboratory

PAS110 Certificate of Analysis (Continued)

Client: FERNBROOK BIO
(M455) 158 WASHBROOK ROAD
RUSHDEN
NN10 6AA

Originator: FERNBROOK BIO
WHOLE DIGESTATE

Lab ID: 67047 - 16260
Sample ID: STORAGE TANK 110712
Sample Type: Whole Digestate

Date Received: 13/07/2012
Date Reported: 31/07/2012
Date Sampled: 11/07/12

Characteristics of WD / SL / SF for declaration, without limit values, that influence application rates
(Results on an 'as received' basis)

Parameter	Units	Result	M *	Amount per fresh tonne or m3	Amount applied at an equivalent total Nitrogen application of 250 kg N/ha	Units
pH		8.7	1			
Dry Matter	% m/m	4.22	2	42.20	1788	Kg DM
Loss On Ignition	% m/m	2.65	3	26.50	1123	Kg OM
Total Nitrogen (N)	% m/m	0.59	4	5.90	250	Kg N
Ammoniacal Nitrogen (NH4-N)	mg/kg	5226	5	5.23	221.43	Kg NH4-N
Total Phosphorus (P)	mg/kg	462	6	1.06	44.83	Kg P2O5
Total Potassium (K)	mg/kg	2167	6	2.60	110.18	Kg K2O
Total Magnesium (Mg)	mg/kg	58.2	6	0.10	4.09	Kg MgO
Total Sulphur (S)	mg/kg	214	6	0.54	22.67	Kg SO3
Water Soluble Chloride (Cl)	mg/kg	2595	7	2.60	11.00	Kg WS Cl
Water Soluble Sodium (Na)	mg/kg	1503	7	1.50	6.37	Kg WS Cl
Equivalent field application rate		—		1.00	42.37	tonnes or m3 / ha

*** Method of Test**

1 BS EN 13037
2 BS EN 14346
3 BS EN 15169
4 BS EN 13654-2 (Dumas)
5 SOP Z/004 (soluble in potassium chloride)
6 BS EN 13650 (soluble in aqua regia)
7 BS EN 13652 (soluble in water)

Natural Resource Management Ltd, Coopers Bridge, Braziers Lane, Bracknell, Berkshire RG42 6NS
Tel +44 (0) 1344 886338 Fax + 44 (0) 1344 890972 E-Mail enquiries@nrm.uk.com Web www.nrm.uk.com
Registered in England No. 2577148. Registered office: Coopers Bridge, Braziers Lane, Bracknell, Berkshire RG42 6NS

Figure A-3 – Anaerobic Digestate PAS110 Certificate of Analysis

culturecollection of algae and protozoa

media recipes

f/2 Medium

Stocks		per litre
(1)	$NaNO_3$	75g
(2)	$NaH_2PO_4.2H_2O$	5.65g
(3)	Trace elements (chelated)	
	NA_2 EDTA	4.16 g
	$FeCl_3.6H_2O$	3.15 g
	$CuSO_4.5H_2O$	0.01 g
	$ZnSO_4.7H_2O$	0.022 g
	$CoCl_2.6H_2O$	0.01 g
	$MnCl_2.4H_2O$	0.18 g
	$Na_2MoO_4.2H_2O$	0.006 g
(4)	Vitamin mix	
	Cyanocobalamin (Vitamin B_{12})	0.0005 g
	Thiamine HCl (Vitamin B_1)	0.1 g
	Biotin	0.0005 g

Medium	per litre
$NaNO_3$	1.0 ml
$NaH_2PO_4.2H_2O$	1.0 ml
Trace elements stock solution (1)	1.0 ml
Vitamin mix stock solution (2)	1.0 ml

* Add while stirring

Make up to 1 litre with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl. For agar add 15g per litre Bacteriological Agar. Sterilise by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.

Reference

Guillard RRL & Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran. Can. J. Microbiol. **8**: 229-239.

Note

If you are making up f/2 using stocks received from CCAP, refer to the labels on the stock tubes for volume per litre as it may differ from the above.

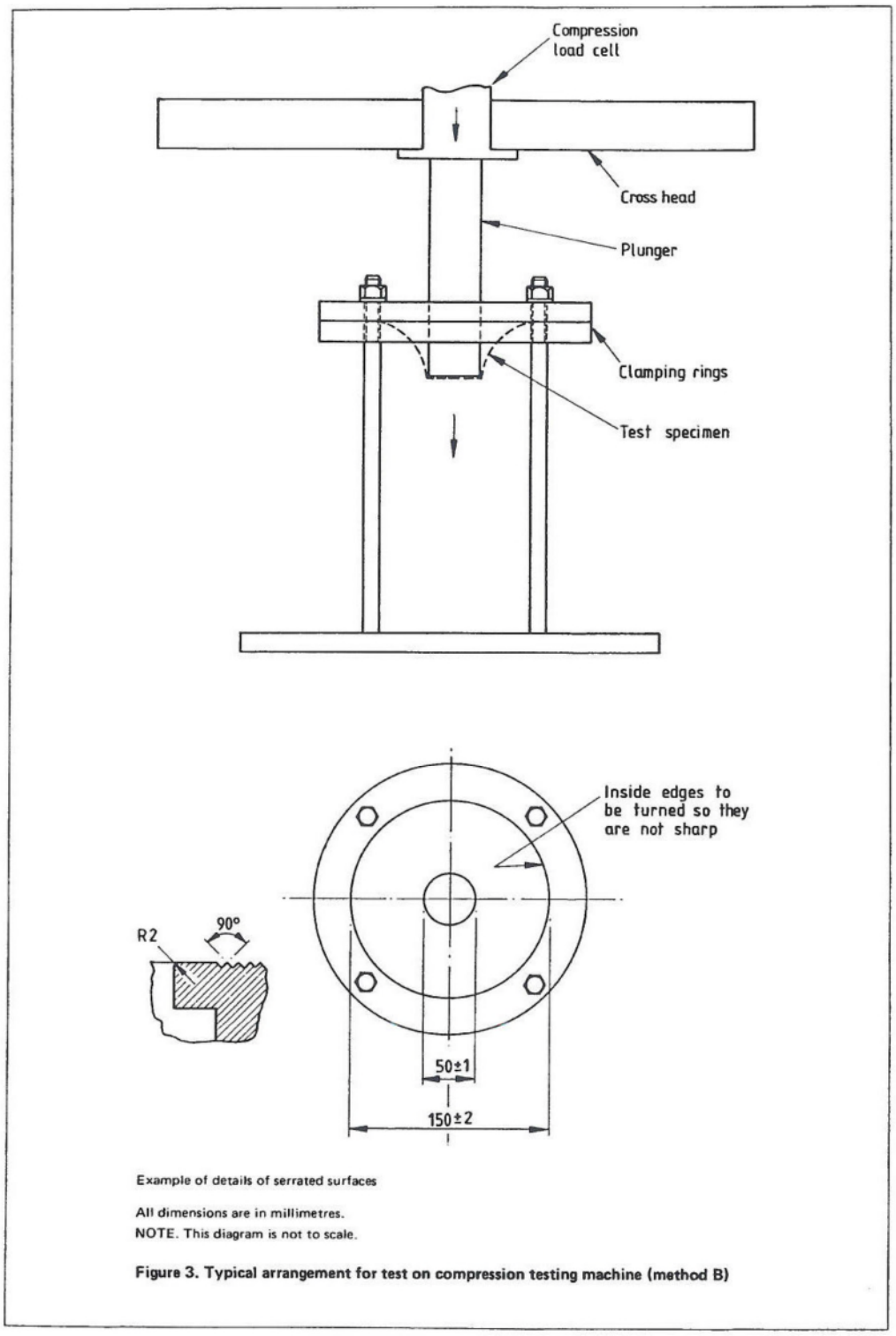
f/40 Medium

For f/40 medium for calcifying *Emiliania*, simply dilute all stock solutions by 1/20th

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

Figure A-4 – f/2 media composition

Licensed Copy: Cranfield University, Cranfield University, 05/02/2013 18:42, Uncontrolled Copy, (c) The British Standards Institution 2012



Example of details of serrated surfaces

All dimensions are in millimetres.
NOTE. This diagram is not to scale.

Figure 3. Typical arrangement for test on compression testing machine (method B)

Figure A-5 – BS6906

Permission to reproduce extracts from British Standards is granted by BSI Standards Limited (BSI). No other use of this material is permitted.

Trident

delivering you growth

Equibeet

Equibeet is a highly palatable, high digestible fibre feed for horses and ponies, ideal for promoting gut health. It is suitable for laminitic animals, 'fussy eaters' and those with poor condition or digestion.

Typical Analysis (on a dry matter basis)

Dry matter (%)	Crude protein (%)	Oil (%)	Starch (%)	Sugar (%)	Fibre (%)	DE (MJ/Kg) (%)
89	9.1	1	0.0	6.0	16.5	11.0

What are you trying to achieve?

Need	Feature	Benefit
Encourage intake	A highly palatable feed.	An excellent feed for 'fussy feeders'.
Gut health	High proportion of the energy as digestible fibre.	Increases energy and vitamin B supply through improved digestion. Suitable for animals with digestive problems or those struggling to maintain or improve condition.
Non-heating		Allows energy intakes to be increased without the problems associated with high grain diets. Promotes calm behaviour - ideal for box rest.
Feed suitable for laminitic or sugar sensitive horses	Low sugar (<5%) content.	Ideal feed for animals requiring a controlled diet, providing the optimum supply of fibre.
Encourage water intake	High water content and a good source of electrolytes.	Excellent feed after hard work. *Equibeet* is useful to encourage horses who often do not want to drink after exercise, or during hot weather, when intake may be inadequate.
Traceability	Produced in the UK from non – GM, UK grown sugar beet.	A short and local supply chain creates peace of mind and stringent quality assurance.

The predicted responses (benefits) assume that the specified nutrient, physical or structural dietary components are limiting livestock performance in the current ration.

t +44 (0)1733 422214
w www.tridentfeeds.co.uk

Suggested feeding rates are produced as a guide only and many other factors may have an overriding effect on animal response; no performance guarantee can be given. Rations should be carefully balanced for energy and protein, contain sufficient forage to maintain rumen function and be fortified with an appropriate vitamin and mineral supplement. Animals must have constant access to clean water.

Figure A-6 – Sugar Beet specifications

Trident

delivering you growth

Recommended daily feed rates (per head basis)

Workload	Pony(350kg)	Hack (500 kg)	Hunter (600kg)
Resting	1.0	1.5	1.7
Light work (occasional hacking)	1.3	1.9	2.3
Moderate work (short schooling/daily hacking)	1.5	2.2	2.6
Training (preparation for competition)	1.8	2.6	3.1
Hard work (competing/full fitness)	2.3	3.3	4.0

Feeding rates are produced as a guide only and should be adjusted to take account of other feeds in your horse or ponies' diet as well as their condition and workload. A minimum of 50%, by weight, of your horse's daily requirement should be forage (grass/hay/silage/chaff) to maintain gut health and function.

Soaking of Equibeet

- *Equibeet* requires **soaking for a minimum of 24 hours**, after which it can be combined with other horse feeds.
- Any horse that has been fed unsoaked sugar beet should be seen by a veterinarian immediately.
- Add 1 litre of water to every 200g of Equibeet (5: 1)and drain off any excess water before feeding.
- Soaked sugar beet should be used up in a couple of days. In hot weather, soaked sugar beet may start to ferment changing its palatability and nutritional value. Only soak enough pellets to last one day in summer months.
- Use each batch of soaked sugar beet completely before preparing the next batch. Do not keep on 'topping up' the sugar beet left to soak.

Availability, handling and storage

Equibeet is available from regional distributors, UK wide, in 20kg bags. It must be stored in a secure shed, bunker, bin or hopper and kept cool, dry and free from vermin. Make sure horses and ponies cannot gain access to unsoaked Equibeet.

Additional information

Method of production

Sugar beet is a root crop, resembling a turnip, which is commonly grown in Northern Europe for sugar production. Once the sugar has been diffused out from the beet, the remaining fibrous residues are dried and then pelleted to produce the final product.

Quality Assurance

Equibeet is FEMAS assured (or a recognised equivalent) and is marketed by Trident who are UFAS-accredited. Equibeet (Dried (sugar) beet feed) is listed under number 4.1.10 in the EU Catalogue of Feed Materials.

t +44 (0)1733 422214
w www.tridentfeeds.co.uk

Suggested feeding rates are produced as a guide only and many other factors may have an overriding effect on animal response; no performance guarantee can be given. Rations should be carefully balanced for energy and protein, contain sufficient forage to maintain rumen function and be fortified with an appropriate vitamin and mineral supplement. Animals must have constant access to clean water.

Trident

delivering you **growth**

Legal disclaimer

Suggested feeding rates are produced as a guide only and many other factors may have an overriding effect on animal response; no performance guarantee can be given. Rations should be carefully balanced for energy and protein, contain sufficient forage to maintain rumen function and be fortified with an appropriate vitamin and mineral supplement. Animals must have constant access to clean water.

Equibeet

Detailed Typical Analysis (fresh basis other than where stated)

Dry matter	%	89.0	Calcium	g/kg	11.00
Oil A	%	0.50	Magnesium	g/kg	1.30
Oil B	%	0.90	Phosphorus	g/kg	0.77
Crude protein	%	8.1	Potassium	g/kg	27.7
Crude protein: DM	%	9.00	Salt	g/kg	1.37
Fibre	%	16.5	Sodium	g/kg	0.43
Ash	%	8.40	Copper	mg/kg	7.00
DE	MJ/kg DM	11.0	Manganese	mg/kg	36.0
NDF	%	38.3	Selenium	mg/kg	0.09
Starch	%	0.00	Zinc	mg/kg	20.5
Sugar	%	5.00	Saturates	% of oil	23.0
			Monounsaturates	% of oil	11.0
			PUFAs	% of oil	66.0
			Long chain PUFAs	% of oil	0.00
			Lysine	% of CP	6.53
			Methionine	% of CP	1.86
			Cysteine	% of CP	1.63
			Histidine	% of CP	3.73
			Threonine	% of CP	6.06

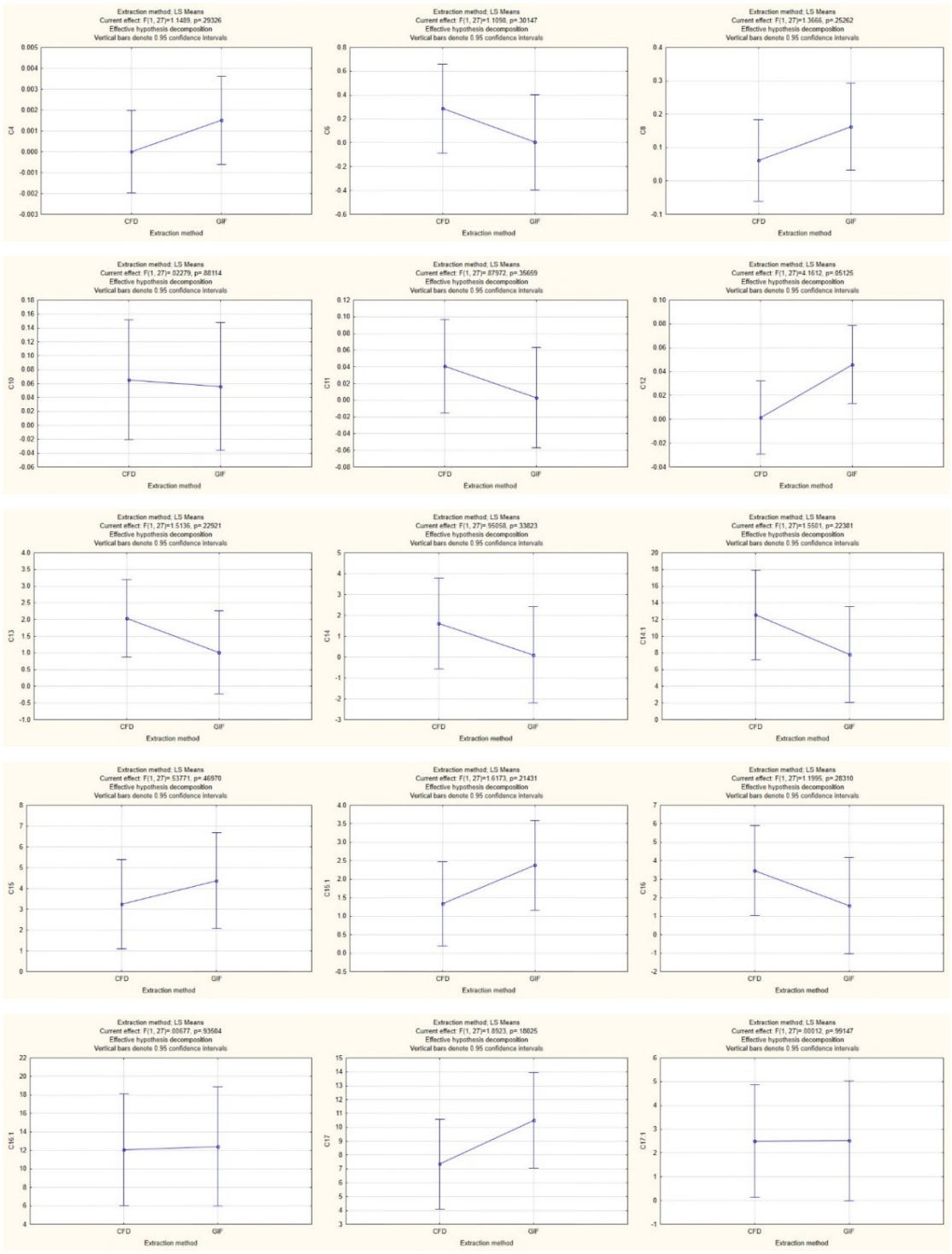
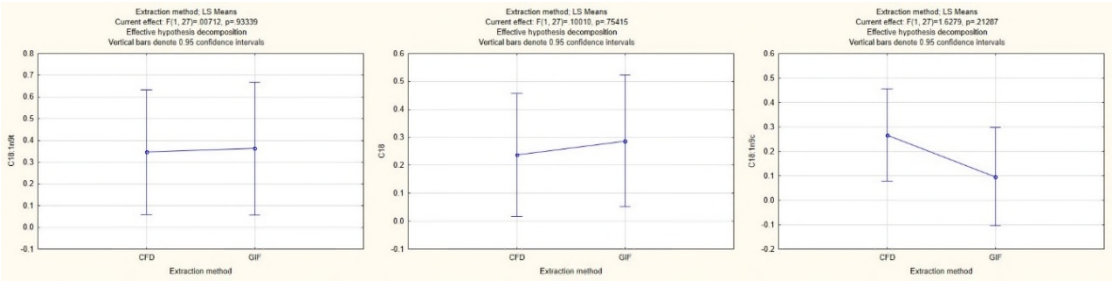
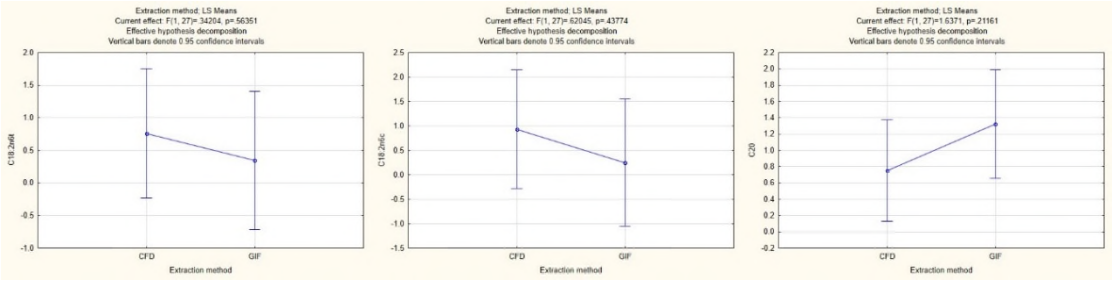
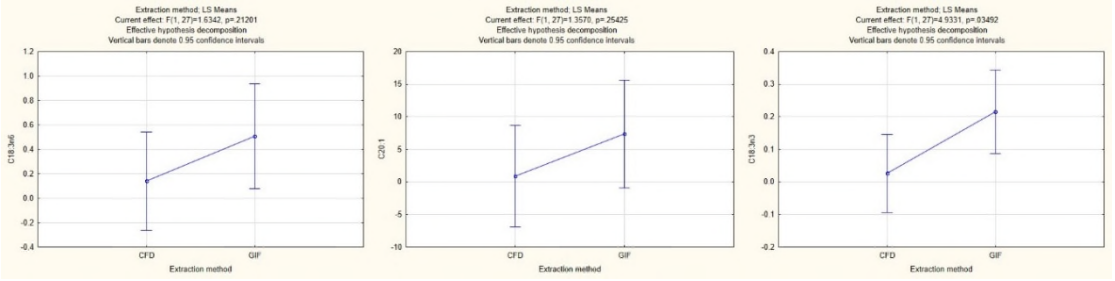
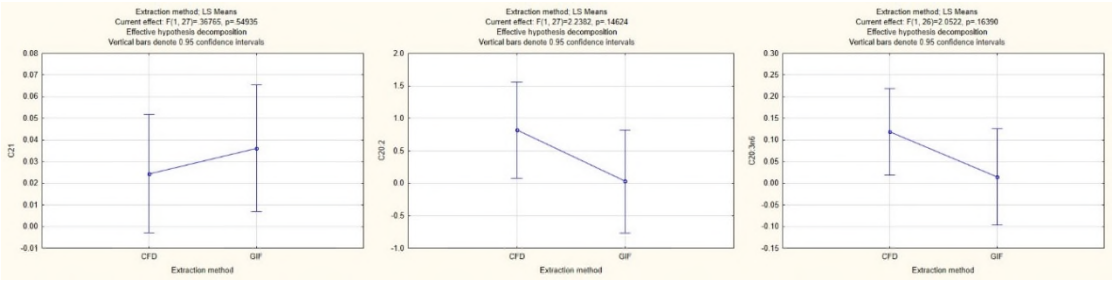


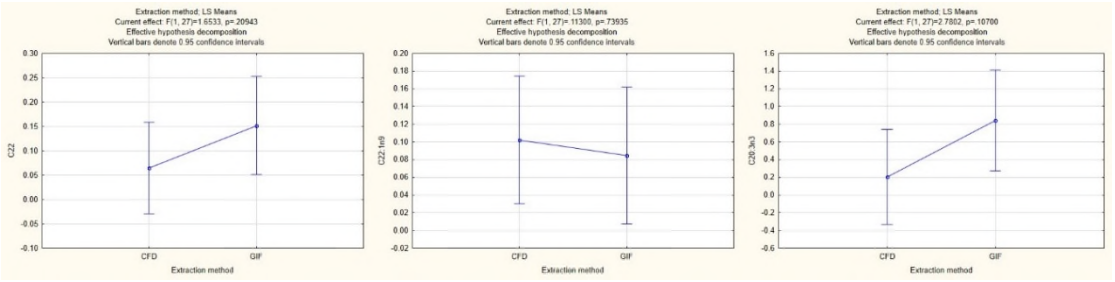
Figure A-7 – HTL & microalgae oil FAME General Linear model statistical analysis

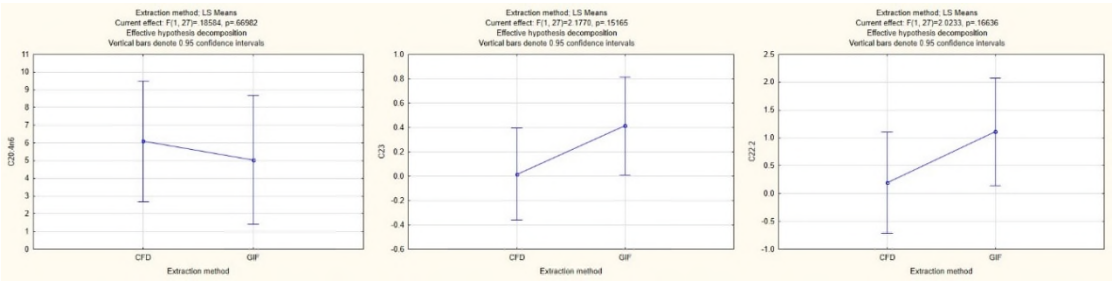

Extraction method; LS Means
Current effect: F(1, 27)=.00712, p=.93339
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:1n9t
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)= 10010, p= 75415
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=1.6279, p= 21287
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:1n9c
CFD
GIF
Extraction method

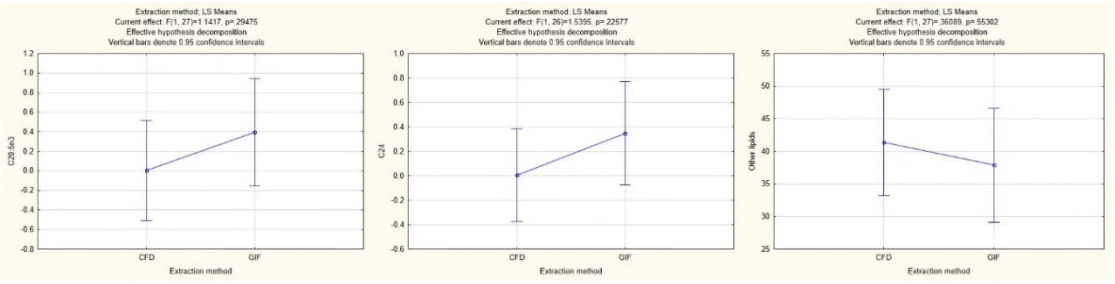

Extraction method; LS Means
Current effect: F(1, 27)= 34204, p=.56351
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:2n6t
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)= 62045, p=.43774
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:2n6c
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=1.6371, p= 21161
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20
CFD
GIF
Extraction method

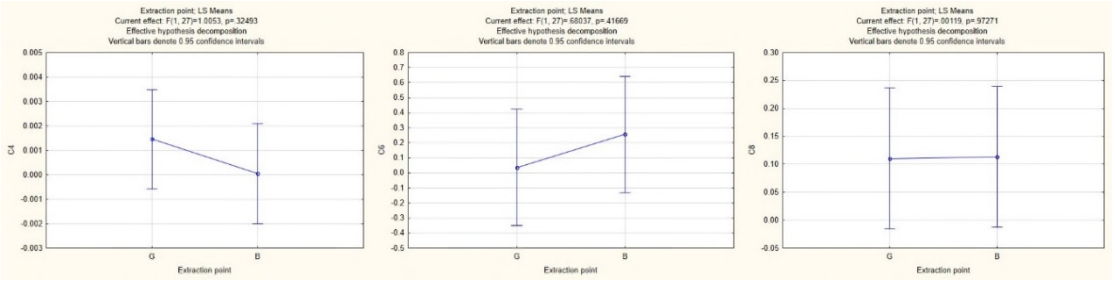

Extraction method; LS Means
Current effect: F(1, 27)=1.6342, p=.21261
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:3n6
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=1.3570, p=.25425
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:1
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=4.9331, p=.03492
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:3n3
CFD
GIF
Extraction method

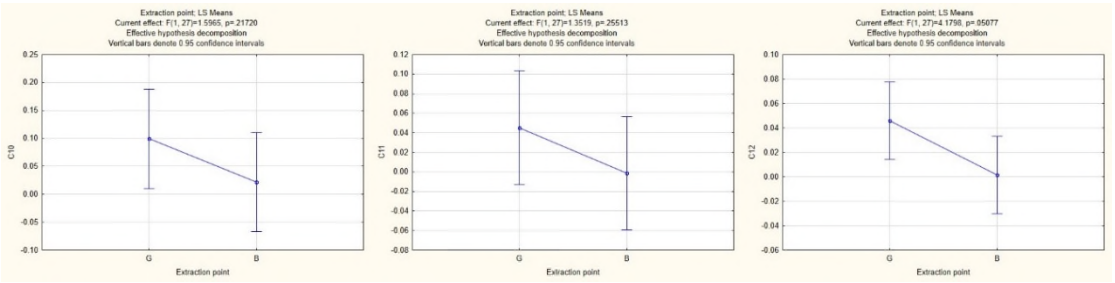

Extraction method; LS Means
Current effect: F(1, 27)=.36765, p=.54935
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C21
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=2.2382, p=.14624
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:2
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 26)=2.0522, p=.16390
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:3n6
CFD
GIF
Extraction method

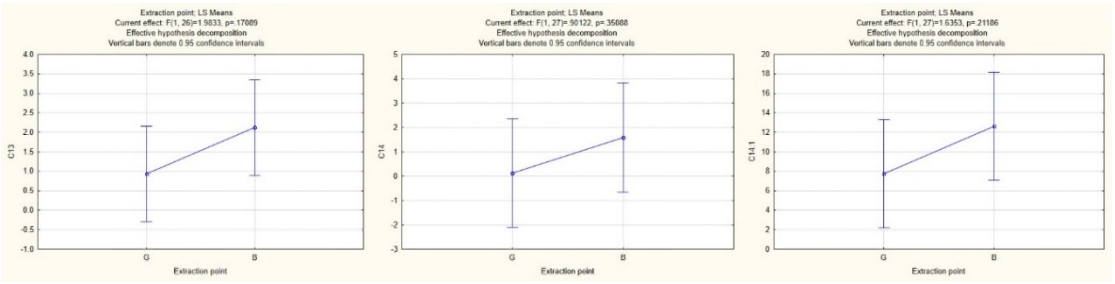

Extraction method; LS Means
Current effect: F(1, 27)=1.6533, p=.20943
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)= 11300, p=.73935
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22:1n9
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=2.7802, p=.10700
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:3n3
CFD
GIF
Extraction method

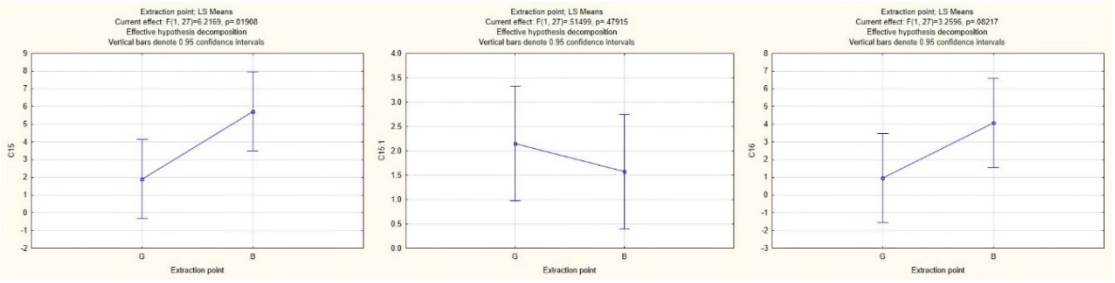

Extraction method; LS Means
Current effect: F(1, 27)= 18584, p=.66982
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:4n6
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=2.1770, p=.15165
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C23
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=2.0233, p=.16636
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22:2
CFD
GIF
Extraction method

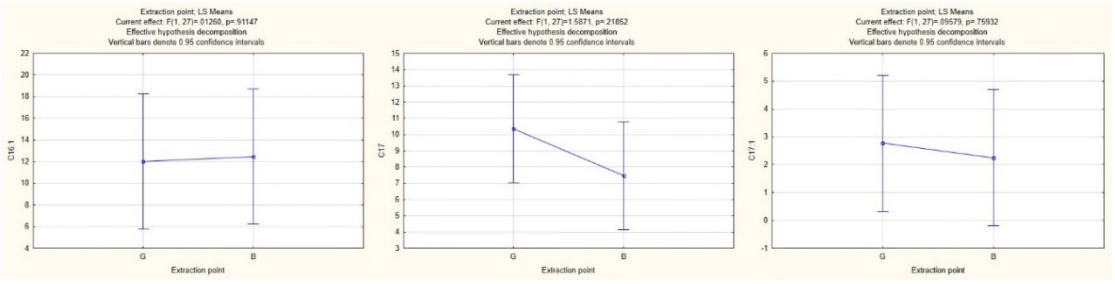

Extraction method; LS Means
Current effect: F(1, 27)=1.1417, p=.29475
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C26:5n3
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 26)=1.5395, p=.22577
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C24
CFD
GIF
Extraction method
Extraction method; LS Means
Current effect: F(1, 27)=.36089, p=.55382
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
Other lipids
CFD
GIF
Extraction method

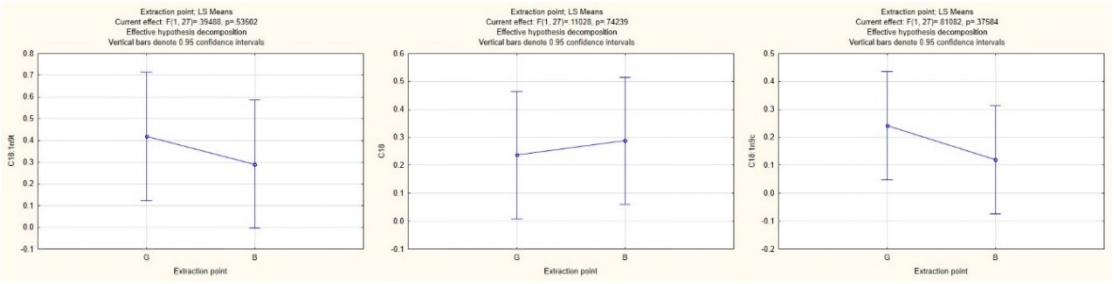

Extraction point; LS Means
Current effect: F(1, 27)=1.0053, p=.32493
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C4
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=.68037, p=.41669
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C6
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=.00119, p=.97271
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C8
G
B
Extraction point

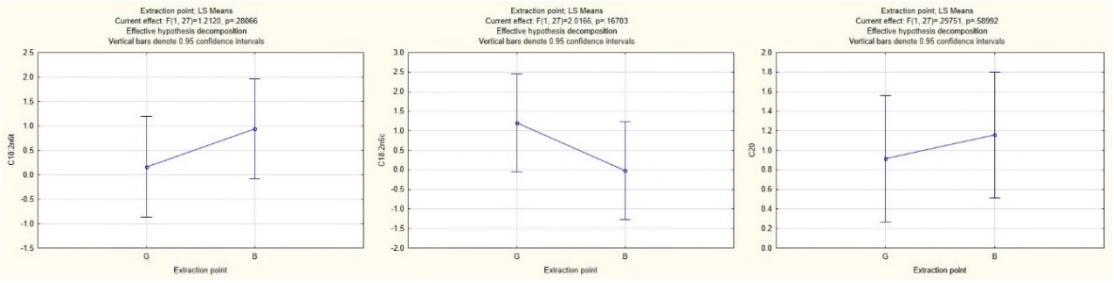

Extraction point; LS Means
Current effect: F(1, 27)=1.5965, p=.21720
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C10
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=1.3519, p=.25513
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C11
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=4.1758, p=.05077
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C12
G
B
Extraction point

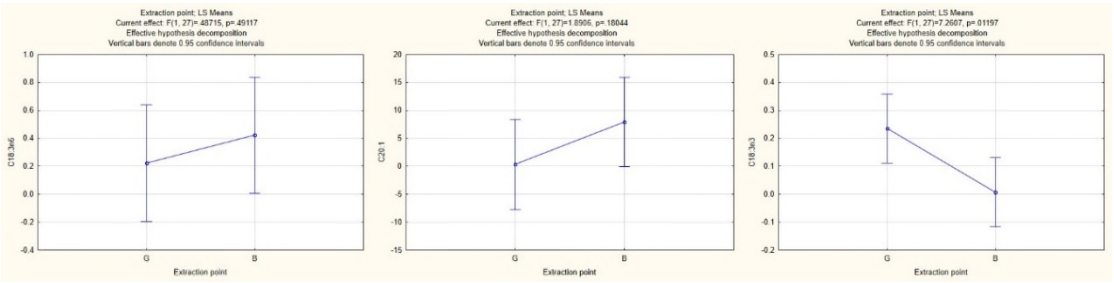

Extraction point; LS Means
Current effect: F(1, 26)=1.9833, p=.17089
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C13
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=.90122, p=.35088
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C14
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=1.6353, p=.21186
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C14:1
G
B
Extraction point

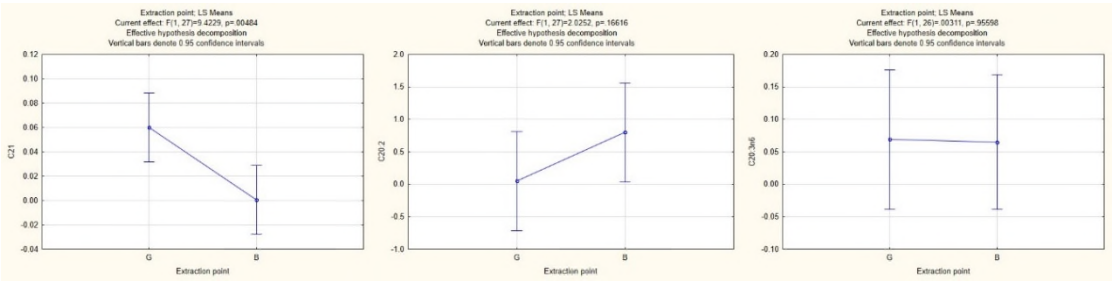

Extraction point; LS Means
Current effect: F(1, 27)=6.2169, p=.01908
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C15
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=.51499, p=.47915
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C15:1
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=3.2596, p=.08217
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C16
G
B
Extraction point

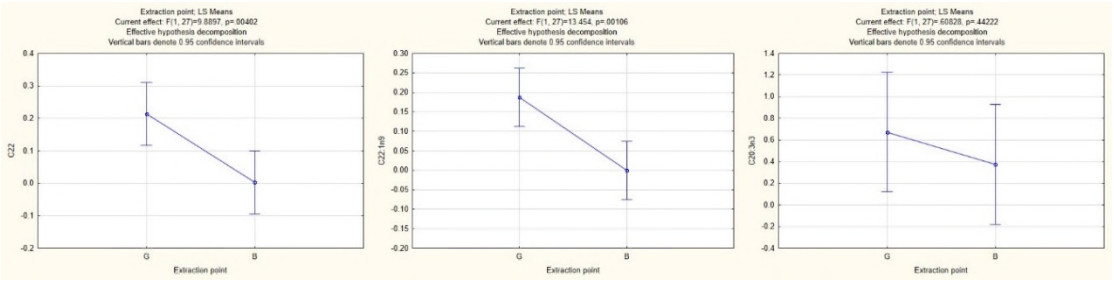

Extraction point; LS Means
Current effect: F(1, 27)=.01260, p=.91147
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C16:1
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=1.5871, p=.21852
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C17
G
B
Extraction point
Extraction point; LS Means
Current effect: F(1, 27)=.09579, p=.75932
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C17:1
G
B
Extraction point

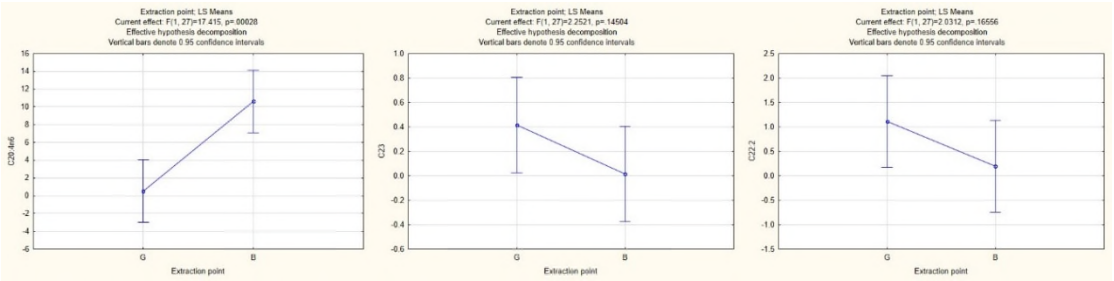

Extraction point; LS Means
Current effect: F(1, 27)= 39488, p=.53582
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:1n9t
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)= 11028, p=.74239
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)= 81082, p=.37584
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:1n9c
Extraction point
G
B

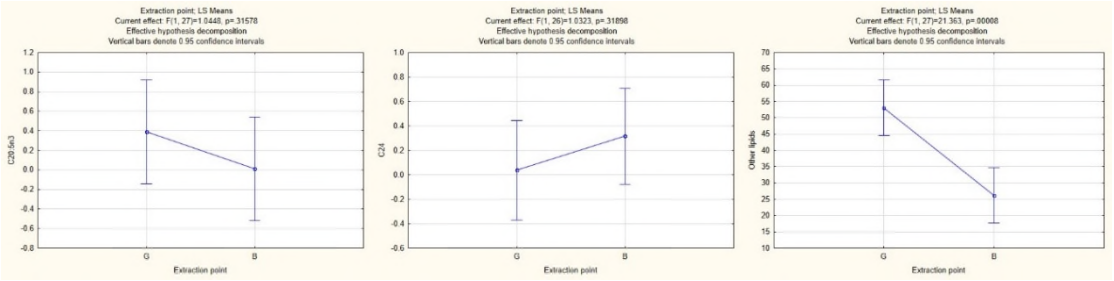

Extraction point; LS Means
Current effect: F(1, 27)=1.2120, p=.28066
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:2n6t
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=2.0166, p=.16703
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:2n6c
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)= 29751, p=.58992
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20
Extraction point
G
B


Extraction point; LS Means
Current effect: F(1, 27)= 48715, p=.49117
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:3n6
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=1.8906, p=.18044
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:1
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=7.2607, p=.01197
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C18:3n3
Extraction point
G
B


Extraction point; LS Means
Current effect: F(1, 27)=9.4229, p=.00484
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C21
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=2.0252, p=.16616
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:2
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 26)=.00311, p=.95598
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:3n6
Extraction point
G
B


Extraction point; LS Means
Current effect: F(1, 27)=9.8897, p=.00402
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=13.454, p=.00106
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22:1n9
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)= 60828, p=.44222
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C20:3n3
Extraction point
G
B


Extraction point; LS Means
Current effect: F(1, 27)=17.415, p=.00028
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C26.fa6
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=2.2521, p=.14504
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C23
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=2.0312, p=.16556
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C22.2
Extraction point
G
B


Extraction point; LS Means
Current effect: F(1, 27)=1.0448, p=.31578
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C25.fa3
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 26)=1.0323, p=.31898
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
C24
Extraction point
G
B
Extraction point; LS Means
Current effect: F(1, 27)=21.363, p=.00008
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
Other lipids
Extraction point
G
B