AN INVESTIGATION ON THE CO-PRODUCTION OF BIODIESEL AND METHANE FROM MICROALGAE

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ABSTRACT

The use of microalgae biomass for the production of biodiesel has been increasingly investigated mainly because arable land is not required for cultivating this biofuel feedstock. However the large scale production of microalgae biodiesel under present day scenarios is currently considered to be infeasible. This is mainly due to the higher energy and economic costs for microalgae biodiesel production compared with the use of common terrestrial biomass oils (i.e. palm, rapeseeds and soybean oil).

This thesis investigates the production of biodiesel from microalgae biomass using the in-situ transesterification process and the recovery of additional energy from the post transesterified microalgae residues, as a tool for improving the energetics of biodiesel production from this feedstock. The thesis aims to provide preliminary experimental information on biodiesel and subsequently methane production from microalgae which was not previously available in the literature. In addition, a simple renewability assessment of the various in-situ and conventional transesterification methods examined in this study was carried out.

The laboratory experiments examined the influence of important reaction parameters (reaction time, process temperature, reactant concentrations, agitation and the biomass moisture content) on biodiesel production from microalgae using the in-situ transesterification method. The results showed a 5% increase in the biodiesel yields with the use of the in-situ method compared with the conventional transesterification process under similar reaction conditions. Furthermore, results obtained from the in-situ transesterification experiments indicated that continuous reactor stirring, increasing the reacting alcohol to oil molar ratios (≥ 315:1) and increasing the process temperature (≥60°C) led to improved fatty acid methyl ester (FAME) conversions. Total inhibition of in-situ microalgae biodiesel conversion was observed with biomass water content greater than 115% w/w (on the basis of oil weight). However, the optimal excess reacting alcohol requirement of the in-situ process was identified as a factor which could potentially limit any energy gains obtainable with this method, due to the energy demands to facilitate the recovery of the excess alcohol quantities i.e. via distillation methods.

To reduce the process alcohol requirement, modifications to the original in-situ transesterification scheme (via the use of low frequency ultrasound and the integration of co-solvents) were applied. Using similar reaction conditions, the use of ultrasound agitation for the in-situ process was shown to improve the biodiesel yields with reduced molar alcohol to oil ratios (105:1) and reaction times.

Regarding energy recovery from the microalgae residues, the use of the anaerobic digestion process for methane production from the residues post transesterification was examined. The results obtained from the batch anaerobic digestion tests demonstrate that the type of lipid extraction solvent utilised in the conventional transesterification process could inhibit subsequent methane production. A recoverable energy of 8.7-10.5 MJ/kg of dry microalgae biomass residue was obtained after the application of both the conventional and in-situ transesterification processes. Co-digesting the microalgae residues with glycerol led to a 4-7% increase in methane production.

With the use of semi-continuously fed anaerobic reactors, the influence of reaction conditions on the specific methane yield of the microalgae residues was also investigated. These included varying the substrate loading concentrations, co-digestion of the microalgae residues with glycerol, hydraulic retention times and temperatures. It was found that the hydraulic retention period was the most important variable affecting methane production from the residues, with longer periods (> 5 days) corresponding to higher energy recovery. The methane yield was also improved by a reduction in the substrate loading rates and an increase in the proportion of the glycerol fraction co-digested with the microalgae residues.

The raw material and process energy requirements of the up-scaled process were obtained for the different transesterification processes using a commercial chemical engineering software (ASPEN plus®), and a renewability assessment of the various schemes was carried out. The biomass cultivation and biodiesel production process renewability was assessed by comparing the minimum work required to restore the non-renewable resources degraded in the considered process with the useful work available from the main process products. The maximum work obtained from the process products is larger than the restoration work the process is considered renewable. In a present day scenario (with the use of fossil fuel sources for the production of the process raw materials, such as for methanol and sulphuric acid production, and electricity), all the transesterification processes were shown to be non-renewable. The influence of the choice of the electricity generation scheme,

raw material source and the type of heating fuels (including heating and drying technology) on the process renewability was also examined The process renewability of the in-situ transesterification of microalgae lipids to biodiesel was found to improve with the use of renewable electricity, reacting alcohols from biomass fermentation and heat pump technology to facilitate the biomass drying and process heating.

Modifying the in-situ transesterification process i.e. with the use of ultrasound agitation and co-solvents was seen to improve the renewability of biodiesel production compared to the mechanical stirred process with the reacting alcohol alone. The use of the ultrasound agitated in-situ process using diethyl ether was seen to be the most renewable process of all the considered transesterification processes. The positive renewability indicators obtained in this study show promise since further process modifications could be used to optimise biodiesel production using microalgae.

The biomass moisture content was shown to negatively influence the alkyl ester production process with the in-situ method. The use of more effective drying methods i.e. heat pump technologies, was shown to have the potential of helping to achieve better process renewabilities. With the in-situ transesterification reacting alcohol requirement being a major process parameter, research into reactor designs which could facilitate the use of lesser alcohol quantities could potentially increase the renewability of biodiesel production via this method. Investigations into optimising the lipid content and productivities of the microalgae biomass could also improve the use of the in-situ method for microalgae biodiesel production. The improved process renewability would be obtained due to the higher biodiesel outputs (and product exergies) achievable with similar work inputs into the process compared with using microalgae with lesser lipid contents.

This work presented in this thesis could provide a basis for further research in the areas of biodiesel production from microalgae using the in-situ process and methane production from the microalgae residues, post transesterification.

Parts of this thesis have been published in academic peer reviewed journals and conference proceedings as listed in the "list of publications".

LIST OF PUBLICATIONS

- Ehimen E.A., Sun Z., Carrington C.G. Birch E.J., Eaton-Rye J. 2011. Anaerobic Digestion of Microalgae Residues Resulting from the Biodiesel Production Process. Applied Energy DOI: 10.1016/j.apenergy.2010.10.020.

- Ehimen E.A., Sun Z., Carrington C.G. 2010. Use of Ultrasound and Co-solvents to Optimize Microalgae Biodiesel Production via the In-situ Transesterification Process. Accepted Lecture. 8th Euro Fed Lipid Congress Munich, Germany. 21-24 November, 2010.

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NOMENCLATURE LIST

1-B 1-Butanol extracted *Chlorella* residue samples

ACIST In-situ transesterified *Chlorella* residue samples

AOCS American Oil Chemists' Society

APHA American Public Health Association

ASTM American Society for Testing and Materials

B Exergy (MJ/kg)

BMP Biochemical methane potential test

BV/A Ratio of butyric and valeric acid to acetic acid

C/N Carbon to nitrogen ratio

CCD Central composite design

CExC Cumulative exergy consumption

CHP Combined heat and power

C-M Chloroform-methanol extracted *Chlorella* residue samples

CNEx Cumulative net exergy consumption

cSt Centistokes (1 cSt= 10^{-6} m^2/s)

CSTR Continuously stirred tank reactor

d Days

d.w. Dry weight

FAAE Fatty acid alkyl esters

FAME Fatty acid methyl esters

FFA	Free fatty acid content
FID	Flame ionisation detector
g	Relative centrifugal force, expressed as units of gravity
GC	Gas chromatography
h	Hour
ha	Hectare (10000 m^2)
HHV	Higher heating values of the fuel, MJ/kg
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time (d)
ID	Internal diameter
Ir	Renewability indicator
LLE	Liquid-liquid equilibrium
MBTE	Methyl tertiary butyl ether
min	Minutes
MM_{oil}	Average molecular mass of the microalgae oil
MM_{FA}	Mean molecular mass of the constituent lipid fatty acids of the microalgae oil
MR %	Microalgae residues in substrate (as % of the total VS of the digested substrate)
N	Number of theoretical distillation stages (quantity of liquid reflux/quantity of distillate product from the distillation column)
NRRs	Non-renewable resources
P/A	Propionic to acetic acid ratios
PBR	Photobioreactor
R^2	Root squared value

rbcL Ribulose- 1, 5-biphosphate carboxylase

RMSE Root mean square error

rpm Revolutions per minute

RR Reflux ratio

RSM Response surface methodology

SCD Substrate loading concentration in digester (kg VS substrate/m^3)

SG Specific gravity

SHP Separate heat and power

SMER Specific moisture extraction ratio (kg moisture extracted/kWh consumed)

sp Species

TAG Triacylglycerol

TG Triglyceride

TS Total solids (g/g sample)

TSP Triple super-phosphate

UASB Up-flow anaerobic sludge bed

VFA Volatile fatty acids

VLE Vapour-liquid equilibrium

VS Volatile solids (g/g sample)

Wp Useful work available from a process product

Wr Restoration work required for a process

1. THESIS INTRODUCTION: STUDY GOALS AND AIMS

1.1. Microalgae as Feedstocks for Biofuel Production

Motivated by increasing global interest in renewable fuels, research into the use of non-traditional "second generation" biomass feedstocks for biofuel production has been on the increase due to the fact that these resources are not expected to impinge on human food production and supply (Hall, 1986). Of the second generation biomass sources, the exploitation of microalgae for the production of fossil fuels substitutes is being increasingly investigated (Chisti, 2007). Reasons supporting the use of this feedstock for biofuel production include:

- Arable land is not required for microalgae biomass production. Microalgae cultivation could potentially be carried out on marginal land (i.e. arid or semi-arid land areas) not suitable for food production (Chisti, 2007).

- Fresh water sources are not required for its cultivation i.e. can utilise brackish or saline water bodies for which there are fewer competing demands (Pedroni et al., 2001; Grobbelaar, 2000).

- Compared to conventional terrestrial energy crops the biomass productivity and photosynthetic efficiency are higher (Pirt, 1986; Goldman, 1980).

- The ability to control the microalgae cultivation process for the production of specific macromolecular components (Illman et al., 2000).

Furthermore, the photosynthetic requirement of carbon dioxide (CO_2) by the microalgae biomass provides an additional advantage with the potential of the microalgae production facilities also serving as a CO_2 capture tool (Pedroni et al., 2001; Benemann, 1997). Prospective large scale microalgae biomass production units could be integrated to utilise CO_2 from flue gases of industrial and power plants as have been demonstrated in laboratory experiments in the literature (i.e. Maeda et al., 1995; Brown, 1996).

The nutrient requirement for microalgae cultivation could also be supplied by the use of nutrient-rich waste, for example treated municipal wastewater streams. Large scale microalgae

production units could thereby provide secondary waste treatment benefits (Benemann et al., 1987), and assist in reducing the biomass cultivation costs.

The use of microalgae as a feedstock for biofuel production is not novel, with work done on the production of methane (CH_4) using this biomass reported over 50 years ago (Meier, 1955; Golueke et al., 1957). However, most of the work on microalgae derived fuels has been relatively recent and carried out since 1978, with the Aquatic Species Programme (ASP) initiated by the U.S. Department of Energy (DOE) and Solar Energy Research Institute (SERI) largely contributing to the present knowledge base microalgae fuels (Sheehan et al., 1998).

A range of conversion technologies are applicable for the production of energy-carriers from microalgae biomass, depending on the intended energy application. These processes can be classified into three main groups: biological (i.e. fermentation), chemical (i.e. extraction, transesterification), and thermal (i.e. drying, pyrolysis). This thesis will be primarily concerned with the chemical and biological fuel production schemes, in particular, examining the use of microalgae biomass for the production of biodiesel via the transesterification process and also the conversion of the resulting biomass residues to methane (CH_4) using the microbial anaerobic digestion process.

Depending on the microalgae specie and cultivation conditions, the microalgae biomass has varying macromolecular composition. The uniform average chemical composition, 50% protein, 30% carbohydrate and 20% lipid, was reported by Goldman (1980) for both freshwater and marine microalgae when light is limiting and all required nutrients are available in excess. Similar to terrestrial oil bearing biomass, the microalgae lipid fraction contains neutral lipids i.e. triacylglycerols and fatty acids which primarily serve storage functions in the microalgae cell (Hu et al., 2008).

This thesis focuses primarily on the use of the microalgae triacyglycerol (and fatty acid) content for the production of a petro-diesel substitute: biodiesel.

Biodiesel (fatty acid alkyl esters) produced via the transesterification (and/or esterification) of triacylglycerols (and fatty acids) with lower alcohols have been successfully used directly, or blended with petro-diesel, in conventional diesel engines without affecting the performance significantly or requiring modifications to the engine before its use (Demibras, 2007).

The potential for the use of microalgae biomass for biodiesel production has been examined (i.e. Nagle & Lemke, 1990; Miao & Wu, 2006; Chisti, 2007, 2008a), and the advantages of this feedstock relative to common terrestrial oil sources have been highlighted. The economics of the

microalgae biomass cultivation and biodiesel production process is important in determining the competitiveness of this feedstock. With the cost of the biomass feedstock oil contributing to 60-75% of the final biodiesel cost (Mata et al., 2010), the relatively higher cost of microalgae oil (when compared to traditional oil sources) is considered to be one of the factors potentially limiting its use (Chisti, 2007). The production economics of microalgae derived biofuels is however still being disputed, with some previous investigations indicating that microalgae oil is cost competitive with current conventional oil sources and petro-diesel (Huntley & Redalje, 2007).

Likewise, assessments of the overall energetics of biodiesel production using microalgae biomass have been shown to vary depending mainly on the biomass production and harvesting methods used. Ehimen (2010)[1], using the fossil energy inputs to the energy value of the biofuel (i.e. MJ fossil fuel input/MJ microalgae biodiesel), demonstrated that more fossil derived energy was consumed for the production of biodiesel from microalgae than the biofuel produces under specific process assumptions. That study used the assumption of microalgae cultivation and biodiesel production in a present day scenario. This involved the use of centrifugation methods for the biomass harvesting and process raw materials derived from fossil fuels. The findings of that study (Ehimen, 2010) were similar to those of Reijnders (2008) which assessed empirical data for the energy requirement for biofuel production from microalgae. The use of waste or organic derived nutrients in the biomass cultivation stages, and renewable generated electricity for the process electrical energy requirement are schemes put forward by Ehimen (2010), which could potentially lead to an improved energy ratio of produced-energy to fossil-energy input for microalgae biodiesel. On the other hand, the results of Chisti (2008b) showed that a net energy gain for microalgae biodiesel energy content over the fossil fuel inputs into the process is achievable if appropriate biomass production and harvesting methods are used. For example the use of sedimentation followed by vacuum belt filters for the biomass harvesting instead of centrifuges as analysed in Reijnders (2008) and Ehimen (2010), was shown to improve the energy output to fossil energy inputs ratio.

A comparison of the use of microalgae as a potential bioenergy feedstock (energetic and economic) relative to other biomass sources is not the aim of this thesis. Rather biofuels from microalgae is assumed to be just one of the potential fossil fuel replacement strategies which can be

[1] This published study, which was carried out at the start of the project period when the research questions were still being formed, is presented in Appendix A (CD-ROM).

applied. It is proposed to be used in conjunction with other products as a biofuel source and not to completely replace other renewable energy sources such as bioethanol, biodiesel from other oil sources, synthetic diesel, hydrogen and renewable electricity. This thesis will deal primarily with biofuel production from microalgae since much information is still required to enable this biomass feedstock to be used more effectively.

To further advance the proposed use and competiveness of microalgae biomass for biodiesel production, Grobbelaar (2000) and Chisti (2007) put forward technological research aspects which could be investigated to support improvements in the energetics, economics and practicality of biofuel production from microalgae. These include:

- Enhancement of the algal biology, with the aim of optimising the microalgae biomass productivity. This could be achieved by strain selection or/and manipulation of the microalgae cells at a molecular and/or genetic level.

- Improved photo-bioreactor engineering.

- Improvements in the biofuel production processes, and the use of a biorefinery based approach for the conversion of the algae biomass, in which every part of the biomass input would be utilised for the production of different fuels or high end products.

This thesis does not deal with the optimisation of the microalgae biomass or oil productivity or the photo-bioreactor technologies. It focuses primarily on the potential improvement of the fuel production process, i.e. 25-40% of the final biodiesel production costs, on the assumption that cheap microalgae biomass is available, as in Huntley & Redalje, 2007. The investigations in this thesis are based on the third approach above, and explore how the application of process modifications to the conventional oil extraction and transesterification process could potentially improve the overall energetics of biodiesel production using microalgae biomass. It also examines the co-production of CH_4 from the post transesterified microalgae residues.

1.2. The Problem

The application of the alternative biodiesel production route 'the in-situ transesterification method' is used in this study of biodiesel production from microalgae biomass. This one-step in-situ transesterification scheme, in which the biomass oil is not extracted prior to biodiesel

production, was chosen for this study, since it had been previously considered and proposed as a biodiesel production route which would aid process simplification, enhance biodiesel yield, while potentially providing cost and waste reductions to biodiesel production (Haas et al., 2004; Haas et al., 2007). A review of the literature revealed a scarcity of published empirical data for the in-situ transesterification of microalgae biomass, which would provide essential information on the biodiesel yields from the use of this method, establishing the influence of various reaction parameters on the biomass-to-fuel conversion process. Before any economic, technological or energetic analysis on the potential for using this biodiesel production route can be conducted, and compared with the more conventional process, experimental information on the influence of various process variables on fatty acid esters production is required.

A major interest in this thesis is to optimise the 'biomass-to-energy' process by using the microalgae residues and the glycerol by-product, obtained from the transesterification process, for CH_4 production by means of an anaerobic microbial gasification process. Although the generation of CH_4 using microalgae biomass residues, and its potential benefits, have been reported in the literature (Chisti, 2008a; Sialve et al., 2009; Neenan et al., 1986), no empirical data on CH_4 production using this residual feedstock have been reported previously. The assessment of the CH_4 yields from microalgae residues in the literature have been solely based on theoretical CH_4 estimations. Although these evaluations provide some information on the potential CH_4 energy yields, the major problem with the use of such methods is the fact that, in realistic situations, the estimated CH_4 yields are usually not obtained (Hansen et al., 2004). The lower practical CH_4 yields are obtained because some process factors which could affect the substrate digestibility, hence limiting energy recovery, are not usually accounted for in the theoretical estimation. These factors include the microalgae composition, nature of the cell wall, and the presence of sodium in marine microalgae species (Sialve et al., 2009). The effect of the preceding biodiesel production process on the fermentation of the microalgae residues cannot also be evaluated without experimental data.

Since microalgae bioenergy (especially biodiesel) research is not yet as established as that for traditional energy crops, and because of the lack of published data, fundamental experimental investigations of CH_4 yields from the microalgae residues are required. These tests must be undertaken before any comparisons can be made of the potential benefits obtainable from the proposed energy recovery scheme.

1.3. Aim of Thesis

Motivated by the lack of experimental data, this thesis sets out to address several fundamental unanswered questions regarding the co-production of biodiesel and CH_4 from microalgae biomass via the use of the in-situ transesterification process. The anaerobic digestion of the downstream residues was also studied. In addition, a simplified thermodynamic assessment of the microalgae-biodiesel production routes, and that of the conventional scheme, will be used to facilitate a comparison of the different conversion approaches.

The research project was designed to meet the following aims:

- Determine the influence of important in-situ transesterification parameters (such as reaction time and temperature, reactant concentrations, agitation, feedstock moisture content) on the microalgae-biodiesel conversion process.

- Based on the results of the initial in-situ trials, routes in the in-situ process which could be improved were identified. Further experimental investigations into the optimisation of the identified routes were carried out by applying modifications to the in-situ process.

- Compare the 'biomass to biodiesel' conversion efficiencies obtained using the in-situ process (and its further modifications) against the use of the conventional extraction and transesterification method.

- Establish the practical optimal CH_4 recovery from the microalgae residues after biodiesel production using the in-situ transesterification method. Investigations on CH_4 production from residues after the conventional route were also carried out. This was carried out to examine the effects, if any, that the preceding extraction or transesterification route would have on the anaerobic digestion of the microalgae residues obtained. It also facilitated a comparison of energy recovery from the residues using both transesterification methods.

- Obtain experimental data for the influence of various digestion factors (i.e. substrate loading concentrations, retention periods, temperature) on the CH_4 yield using laboratory scale continuously stirred reactors.

– Assess the potential process energetic benefits for microalgae biodiesel production using the in-situ transesterification method over conventional method. This was largely carried out using a simplified renewability appraisal, and based on the experimental data obtained in this study.

1.4. Description of the Following Chapters in this Thesis

A literature review is presented in Chapter 2, providing a general introduction to the transesterification and anaerobic digestion processes and previous work in the area of biodiesel and CH_4 production using microalgae. The concept of the in-situ transesterification process is introduced and the potential benefits obtainable from its use for biodiesel production are highlighted. Possible improvements in overall energy recovery via the integration of the anaerobic digestion process with the biodiesel production using microalgae biomass are presented. Some of the major questions which this study addresses are also highlighted in Chapter 2.

An introduction to the representative microalgae specie utilised for this study (*Chlorella sp.*), including the reasons for its selection is presented in Chapter 3. This chapter also describes the laboratory cultivation of the microalgae biomass, detailing the culturing conditions and the techniques utilised in this study. The methods used to characterise the microalgae biomass, its oil content, and the results of this assessment, are presented in Chapter 3.

Chapter 4 covers the experimental evaluation (including the methods and results) of the study of the in-situ transesterification process for microalgae biomass. The conventional biodiesel production from microalgae biomass is also explored in Chapter 4. This was carried out to facilitate a comparison of the in-situ transesterification process with the conventional method.

Based on the findings in Chapter 4, possible improvements in the in-situ transesterification route are considered in Chapter 5. A comparison of the results obtained by use of the modified in-situ process with the results in Chapter 4 was also presented.

An investigation of the potential CH_4 yields obtainable from the post-transesterified *Chlorella* residues, using laboratory batch reactors, is presented in Chapter 6. The influence of the preceding oil extraction or transesterification method on the substrate digestibility and CH_4 production was also examined in this chapter.

The influence of various operational parameters on the anaerobic digestion process and CH_4 yields under semi-continuous reactor loading conditions are presented in Chapter 7.

The minimum process energy requirement of the biodiesel processes (in-situ, modified in-situ, and conventional methods) were estimated in Chapter 8. This was carried out using commercial chemical engineering software using the empirical data obtained in this study. Heat integration methods (pinch analysis) are utilised to assess the possibilities of using the process heat streams reduce the overall energy demand of the biodiesel production processes. An assessment of the energy demand for the anaerobic digestion of the microalgae residues was also presented.

Using an exergy approach, and the process renewability, the potential energetic benefits of the different conversion methods in this study was analysed in Chapter 9. The limitations and difficulties encountered during the investigations in this thesis are presented in Chapter 10 and recommendations for future research are presented.

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2. LITERATURE REVIEW: MICROALGAE BIOMASS PRODUCTION, THE IN-SITU TRANSESTERIFICATION AND ANAEROBIC DIGESTION PROCESSES

2.1. Introduction

With uncertainties on the long term availability of fossil fuel sources (i.e. petroleum), fluctuations in petroleum prices, there has been an increasing trend globally to find alternative energy sources to replace fossil fuels. Biofuels production and use has been proposed as one of the schemes which could meet this goal. Owing to the widespread use of petro-diesel as a transport and industrial fuel, the replacement of this petroleum derived fuel with economically competitive, technically compatible and easily available biofuels has been promoted (Srivastava & Prasad, 2000).

Naturally occurring biomass oils have been proposed as an alternative to petro-diesel (Srivastava & Prasad, 2000). However, the use of biomass oils in diesel engines (direct and indirect injection types) is regarded as an unsuitable and inadequate replacement, mainly due to their unfavourable physical and chemical characteristics. The high viscosities of biomass oils, their fatty acid and free fatty acid composition, plus their low volatility are undesirable. In addition, their contamination and thickening of the lubricating oil, and gum formation during storage and fuel combustion, inhibit their use as engine fuels (Demirbaş, 2007, Meher et al., 2006). Furthermore, at reduced environmental temperatures (i.e. during winter), an increase in the viscosity of the untreated biomass oils can be observed even to the point of solidification (Demirbaş, 2007). This implies that during cold periods, before and during use, the oil tanks must be kept at suitable temperatures that guarantee the right oil viscosity for the fuel injection system (GSES, 2005). This can be achieved via a modification of the oil storage tank to use heat from the engine or auxiliary heating systems to maintain the oil temperature. If not, poor atomisation of the fuel occurs, leading to incomplete combustion and deposition of carbon, risking engine damage (GSES, 2005).

These limiting factors technically hinder the use of unmodified biomass oils in conventional diesel engines since modifications to the engines must be made before the direct use of these oils could be considered. The uptake of this alternative fuel is therefore inhibited by the capital investments

involved in the engine and car manufacturing industry. The auto-industry would most likely not undertake such system modifications to accommodate biomass oil use, which is not yet widespread and not accepted globally. As a result, if the biomass oils are to be introduced into the traditional diesel fuel market without the need for engine modifications, the chemical and physical properties of these oils must be upgraded to match those of petro-diesel.

To improve the characteristics of the biomass oils, three main processes have been proposed and explored to facilitate its conversion to a fuel product comparable with petro-diesel. These are: pyrolysis, microemulsification and transesterification (Fukuda et al., 2001; Srivastava & Prasad, 2000; Ma & Hanna, 1999).Of these methods, biodiesel production via the transesterification of biomass oils is currently the most common method used in the production of suitable petro-diesel replacements (Srivastava & Prasad, 2000). An improvement in the fuel qualities of the biomass oil is facilitated via its conversion to biodiesel (fatty acid alkyl esters, FAAE). Examples of fuel properties improvements attained include an increase in the higher heating value (HHV), a reduction in its viscosity and improved low temperature properties of the biodiesel when compared with the original biomass oil (Fukuda et al., 2001).

The advantages of biodiesel as a petro-diesel substitute also include the renewability and biodegradability of the fuel; lower sulphur and aromatic emissions on combustion and higher combustion efficiency, flash point, and lubricity of the biodiesel when compared to petro-diesel (Ma & Hanna, 1999; Knothe et al., 2006; Demirbaş, 2007). Table 2.1 shows a comparison of the chemical properties of biodiesel and petro-diesel.

Table 2.1. Comparison of the chemical properties of biodiesel and petro-diesel (Demirbaş, 2007)

Chemical property	Biodiesel (FAME)	Petro-diesel
Ash (wt %)	0.002-0.036	0.006-0.010
Sulphur (wt %)	0.006-0.020	0.020-0.050
Nitrogen (wt %)	0.002-0.007	0.0001-0.003
Aromatics (vol %)	0	28-38
HHV(MJ/kg)	39.2-40.6	45.1-45.6

Commercial production and use of biodiesel is increasing globally, due to the fact that it can be produced using existing technologies and distribution can be facilitated using established petro-diesel distribution channels. In the European Union (EU), biodiesel currently accounts for 82% of the total biofuels production in the region, with its global production estimated to be > 35×10^9 l (Bozbas, 2008).A closer look at the transesterification process is carried out in the next section (2.2).

2.2. The Transesterification Process and Biodiesel Production

The transesterification process (or alcoholysis) involves the conversion of the biomass oils (mainly composed of triacylglycerol, TAG) to monoesters of lower alcohols. This is facilitated by a displacement reaction using alcohol, with glycerol formed as a by-product. Monohydric aliphatic alcohols with 1-8 carbon atoms are usually employed in the transesterification process. Methanol and ethanol most commonly used as the reaction alcohol, mainly due to the costs and availability of these alcohols (Fukuda et al., 2001).

For the reversible step-wise transesterification process, a 3 to 1 stoichiometric molar ratio of alcohol to triglyceride is required for the reaction to proceed. Excess alcohol reactants, than stoichiometrically required, are usually employed to shift the reaction equilibrium to the right, favouring the formation of the alkyl esters (see Figure 2.1 below). When methanol is used as the reacting alcohol (i.e. methanolysis), fatty acid methyl esters (FAME) are formed. Figure 2.1 illustrates the generalised chemistry involved in the transesterification reaction, using methanol as the reacting alcohol. Where R represents the functional hydrocarbon group of the fatty acid in the triglyceride.

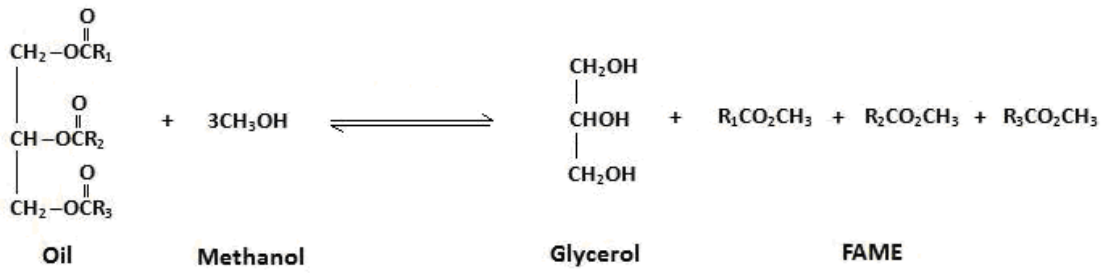

Figure 2.1. A generalised transesterification equation illustrating the use of methanol as the reaction alcohol for the production of fatty acid methyl esters (FAME) and glycerol from biomass oils (triglycerides).

The transesterification reaction is usually carried out in the presence of catalysts. Alkaline, acidic, biological (lipases) or heterogeneous catalysts are usually utilised to improve the transesterification reaction rates for a variety of biomass oils (Meher et al., 2006; Al-Zuhair, 2007; Ma & Hanna, 1999). The alkaline catalysts, sodium hydroxide (NaOH), sodium methoxide (CH_3ONa), potassium hydroxide (KOH) and potassium methoxide (CH_3OK), are more commonly used for the transesterification of high quality biomass oils. For alkaline alcoholysis, a catalyst concentration (NaOH and KOH) in the range of 0.4-2% w/w (on the basis of the oil weight) was reported to be usually applied for alkyl ester production in research and industry (Meher et al., 2006).

For biomass oils with a relatively high free fatty acid (FFA) content (> 0.5%, on the basis of the total oil mass), the acid catalysed transesterification is more suitable (Sprules & Price, 1950). This will be explained further in section 2.3. Commonly used as acidic transesterification catalysts are the inorganic acids: sulphuric (H_2SO_4), hydrochloric (HCl) and phosphoric (H_3PO_4) acids. Al-Widyan & Al-Shyoukh (2002) studied the acidic catalysed transesterification at different acid concentrations using H_2SO_4 and HCl with waste vegetable oils and ethanol as the reaction feedstocks. That study showed that with regards to alkyl ester formation, the use of H_2SO_4 (1.5-2.25M concentration range) was the better than HCl as acid catalyst under the same reaction conditions.

The use of biological catalysts (i.e. enzymes) has been studied, with the aim of overcoming some drawbacks facing the more conventional chemical catalysed biodiesel production routes. These include its energy requirement, catalyst and glycerol recovery, treatment of the process waste water, as well as the use of low quality oil feedstocks (particularly for the alkaline catalysed process). Both extracellular and intracellular enzymes (lipases) can be applied to catalyse the transesterification process, with various types of reacting alcohols employed (Fukuda et al., 2001).

Lipases from microbial and fungi origins are commonly used for the biological biodiesel production process (Al-Zuhair, 2007). For example, *Candida rugosa*, *Pseudomonas flouorescens*, *Rhizopus oryzae*, *Burkholderia cepacia*, *Aspergillus niger*, *Thermomyces lanuginose* and *Rhizomucor miehei* are easy to obtain via simple fermentation processes and a few purification steps. A comparative study of lipases from various sources was carried out by Iso et al. (2001). This study showed that *Pseudomonas flouorescens* lipase exhibited the highest enzymatic activity for the production of biodiesel when compared with the use of *Pseudomonas cepacia*, *Mucor javanicus*, *C. rugosa* and *Rhizopus niveus* after a reaction time of 5 h. The experiments involved the use of 1-propanol and 1-butanol as the reacting

alcohol with a reaction temperature of 50°C (Iso et al., 2001). Iso et al. (2001) also showed that the use of immobilised lipases exhibited a higher activity than free lipases because the enzyme active sites became more effective when the enzymes were dispersed on the surface of the carrier particle in the immobilisation scheme (Iso et al, 2001).

Another option for converting biomass oils to biodiesel is the supercritical transesterification process (based on the properties of the reacting alcohol), which is facilitated at high reaction temperatures and pressure (> 240°C and >8 MPa respectively) without catalysts (Saka & Kusdiana, 2001). This technology has been demonstrated to have the shortest reaction times to reach a near complete equilibrium conversion of the oils to alkyl esters (120-240 s), and higher fuel product yields compared to the conventional process (Saka & Kusdiana, 2001). This process exploits the findings that transesterification reaction rates, and fatty ester yields have been shown to be significantly improved with increases in the reaction temperatures to the supercritical temperatures of the reacting alcohol (Demirbaş, 2002). Furthermore, unlike the alkaline catalysed process, the supercritical process is insensitive to the presence of high levels of FFAs and moisture in the oil feedstock. This is due to the simultaneous transesterification of the triglycerides and esterification of the FFAs to alkyl esters (Al-Zuhair, 2007). The supercritical transesterification method also has an added advantage that its products require lower levels of purification treatments, compared to those needed for the catalysed methods (Al-Zuhair, 2007).

A comparison of some of the major reaction conditions and products using catalytic (inorganic and biological) and non catalytic transesterification processes are shown in Table 2.2.

Table 2.2. Comparison of various transesterification methods[2] (Al-Zuhair, 2007, Fukuda et al., 2001, Saka & Kusdiana, 2001)

	Alkaline Catalysed	Acid catalysed	Enzyme catalysed	Supercritical method
Reaction Temperature (K)	303-338	338	303-313	> 513
Reaction Pressure (MPa)	0.1	0.1	0.1	> 8.09
Reaction Time (min)[3]	60-360	4140	Varied	2-4
Effect of free fatty acid content	Saponified products	Methyl esters, water	Methyl esters	Methyl esters, water

The exact values of the reaction variables presented in Table 2.2 are for the demonstrations performed by the cited references. For the acid catalysed and supercritical transesterification processes, water is formed as a process by-product due to the esterification of the free fatty acids resulting in methyl esters and water production.

2.3. Transesterification Mechanism and Kinetics

As shown in Figure 2.2, the reaction mechanism for the alkali transesterification process can be highlighted in three steps (Ma & Hanna, 1999). The first step involves an attack on the triglyceride molecule by the alkoxide ion resulting in the formation of a tetrahedral intermediate. The tetrahedral intermediate then reacts with the alcohol in the second step to facilitate the regeneration of the alkoxide ion. The third step involves the formation of a fatty acid ester (biodiesel) and a diglyceride brought about by a rearrangement of the tetrahedral intermediate.

[2] Transesterification carried out with methanol as the reaction alcohol.

[3] Time required for achieving maximum equilibrium FAME conversion yields

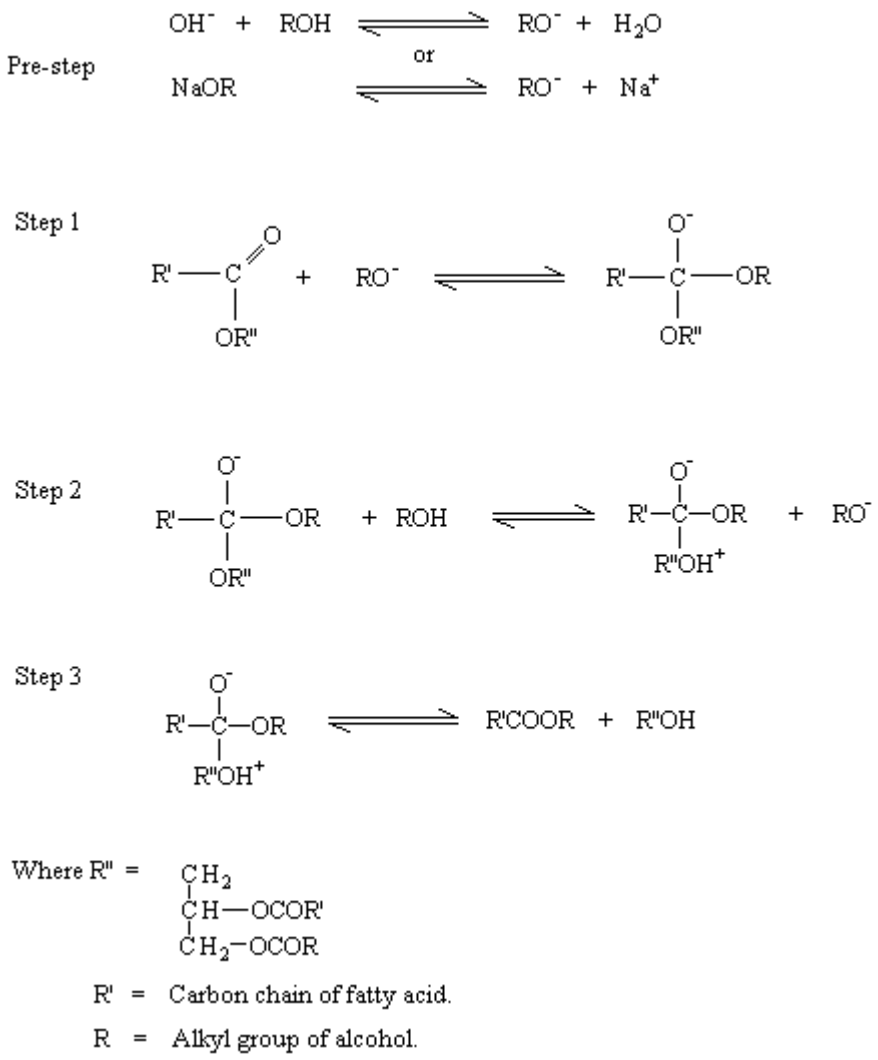

Figure 2.2. Mechanism of the alkaline catalysed transesterification process (Ma & Hanna, 1999).

Prior to the first step, the mixture of inorganic alkaline catalysts, i.e. sodium hydroxide NaOH, with alcohol results in the formation of the alkoxide ion (Sridharan & Mathai, 1974) as shown as the "pre-step" in Figure 2.2. The more commonly employed alkaline catalysed transesterification process is however limited by its sensitivity to the water and FFAs in the feedstock. (Al Zuhair, 2007).

Under alkaline conditions, water presence may result in a saponification reaction, causing a consumption of the catalyst, by the formation of the fatty acid salts and glycerol. This leads to emulsion formation which could increase the difficulty of the downstream biodiesel processing. The FFAs content of the biomass oils can also react with the alkali catalysts to form soaps.

The use of acidic catalysis in contrast, has been demonstrated to be insensitive to FFAs, but has not received as much attention compared to the alkaline catalysed reactions due to its relatively slower conversion rates (Meher et al., 2006).

The mechanism for the inorganic acid catalysed transesterification is shown in Figure 2.3 (a-c). The acid catalyst facilitates (a) the protonation of the carbonyl group of the glycerol esters leading to carbocation formation, (b) after which a neucleophilic attack of the alcohol produces a tetrahedral intermediate, and (c) the diglyceride is then eliminated from this intermediate resulting in the formation of a fatty acid ester, with the catalyst regenerated.

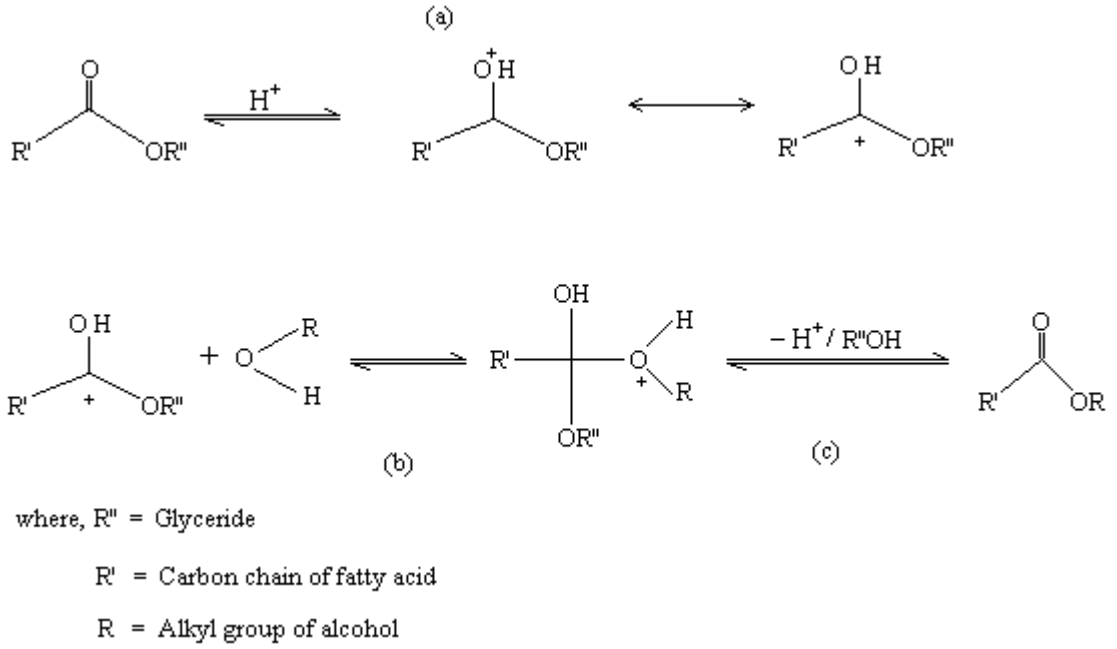

Figure 2.3. Mechanism of the acid catalysed transesterification reaction.

In the presence of excess alcohol, with acid or alkaline catalysts, the forward reaction follows pseudo-first order reaction kinetics with the reverse demonstrated to be second order (Meher et al., 2006). The reaction rate constants are much higher for the alkaline catalysed reactions than for those using acid catalysts, with the rate constants for both systems increasing with an increase in the amount of catalyst used (Ma & Hanna, 1999).

2.4. Variables Affecting the Transesterification Process

Apart from the catalyst type (or supercritical method) employed, the progress of the transesterification reaction, can be affected by various reaction conditions. Previous demonstrations and discussions in the literature on some of the important parameters, and their influence on the alcoholysis process are described below.

2.4.1. Molar ratio of alcohol to oil

The molar ratio of reacting alcohol to triglycerides is one of the most important variables affecting the fatty acid ester yield of the transesterification reaction. As mentioned in section 2.2, a stoichiometric ratio of 3 moles of alcohol to 1 mole of triglyceride is required for the production of 3 moles of fatty acid alkyl esters and 1 mole of glycerol. Since the transesterification reaction is a reversible reaction, increasing the reacting alcohol concentration is used to drive the reaction to the right, favouring product formation. Greater ester formation and shorter reaction times have been obtained with increases in the ratio of alcohol to oil feedstocks (Klass, 1998), but the type of catalyst used is also considered to be closely associated to the alcohol requirement (Meher et al., 2006).

Freedman et al. (1986) demonstrated that a 30:1 molar ratio of butanol to soybean oil was required for the acid catalysed reaction, while a molar ratio of only 6:1 was required for the alkaline catalysed process to achieve similar oil to ester conversions of 93-96% (w/w) with similar reaction times. Likewise, using a reacting ratio of 6 moles of alcohol to 1 mole of triglyceride was shown by Klass (1998) to result in an 'oil to FAAE' conversion of 98% using alkaline catalysts (NaOH) and methanol after a reaction period of 1 h. Al-Widyan & Al-Shyoukh (2002) showed that an increase in the reacting molar ratio of alcohol (25-100% excess ethanol) to triglyceride, using acid catalysts (2.25M HCl), led to increased fatty acid ester formation with a shorter time (< 4 h).

The molar ratio of the alcohol to oil employed in the transesterification process was reported to have no effect on the acid (an indication of the FFA content), peroxide (an indication of the oxidative rancidity), saponification (an indication of the chain length) and iodine (an indication of the degree of unsaturation) values of the alkyl ester produced (Tomasevic & Marinkovic, 2003). However, increasing the reacting molar ratio of alcohol to oil could interfere with the downstream

biodiesel-glycerol separation process. This could hinder the use of cheap gravitational separation techniques due to the increased solubility of the fatty esters in the alcohol, thus increase the costs of the biodiesel recovery (Ma & Hanna, 1999).

The effect of the molar ratio of the reacting alcohol to oil for the enzymatic catalysed process has been described in the literature. Shimada et al. (1999) demonstrated that the use of molar alcohol (methanol) to oil ratios > 3:1 negatively affected the ester conversion process. A similar finding was described by Noureddini et al., (2005) although the optimal molar alcohol (methanol) to oil ratio was much higher at 7.5:1. Al-Zuhair (2007) concluded that in general, for enzyme catalysed transesterification processes, the reacting alcohol should be added in quantities that do not result in deactivation of the lipases.

Demirbaş (2002) studied the application of the supercritical method for the production of methyl esters from cottonseed oil using different molar ratios of oil to alcohol (1:1, 1:3, 1:9, 1:20 and1:40). The study showed that the use of the reacting oil to alcohol ratios of 1:40 exhibited the highest yields, with a near complete methyl ester conversion (<99%, w/w) observed after a reaction time of 200s, the results being similar to those of Saka & Kusidiana (2001).

2.4.2. Effect of free fatty acid and moisture content

For the alkaline catalysed transesterification, it was first demonstrated by Wright et al. (1944) that the oil feedstock should have an acid value < 1 and all the reacting materials (oil and alcohol) should be anhydrous. If the acid value were > 1, more alkaline catalysts would be required to neutralise the FFAs. Moisture in the reacting species results in soap formation, consuming the alkaline catalyst thereby reducing the catalyst efficiency (Ma & Hanna, 1999). The resulting soaps also lead to increases of the viscosity and to gel formation in the product, as well as increasing the difficulty of the glycerol separation process (Freedman et al., 1984). Other research (Bradshaw & Meuly, 1944; Feuge & Grosse, 1949; Freedman et al., 1984) stressed that dry oil with FFA contents < 0.5% was required to facilitate the alkaline transesterification process. Ma et al. (1998) agreed with this FFA content limit for alkaline transesterification. Using beef tallow as the oil feedstock, Ma et al. (1998) investigated the influence of FFA (0.0-0.6%, w/w) and moisture (0.0-0.9% H_2O, w/w) on the alkaline catalysed transesterification process. Based on the study results, the authors recommended that the FFA of beef tallow aimed for transesterification be kept below 0.5% (w/w), with a moisture content of < 0.06 % (w/w).

By contrast, for oil feedstocks with a high FFA content, the acid catalysed process has been found to be insensitive to high FFA contents (Canakci & Gerpen, 2001). However an increase in the moisture content of the feedstock oil or reacting alcohol might negatively affect the biodiesel yields of the transesterification process. This is due to the water in the transesterification solution competing with the reacting alcohol for the acidic catalyst's hydrogen ions, thereby limiting their availability for catalysis (Loreto et al., 2005). As noted by Loreto et al. (2005), since the inorganic acid catalyst (H_2SO_4 used in that study) has a strong affinity for water, it is likely that it would interact preferentially with the water in the reaction vessel, than with the alcohol, leading to a reduction in the formation of the ester product.

When enzyme catalysts are used, the moisture content of the reacting species has been found to significantly affect the conversion process, with reduced lipase activity (for a variety of lipases) observed in the absence of water (Kaieda et al., 1999, 2001; Shimada et al., 1999; Noureddini et al., 2005). A minimum amount of water was found to be required for the activation of the enzyme and consequently for biodiesel formation. However, at higher water content levels, and depending on the particular lipase employed, a gradual decrease in the enzyme activity was shown by Al-Zuhair (2007). With regard to the FFA content of the oil feedstocks, the use of lipases has been demonstrated to be capable of converting > 99% of the FFAs in the biomass oil to fatty acid esters (Sanchez & Vasudevan, 2006).

Kusidana and Saka (2004) reported on supercritical transesterification of rapeseed oil and methanol at specific conditions of temperature, pressure and time: 350°C, 43 MPa and 240 s respectively. Using a molar ratio of oil to alcohol of 1:42, the presence of water (0-6%, w/w oil weight) was shown to have no effect on the methyl ester yields, with similar near complete conversions (>99.9%) consistently achieved, regardless of the oil moisture content. Feedstock oils with a high FFA contents were also shown to be converted (>99.9%, w/w) to methyl esters under these conditions (Kusidana & Saka, 2001).

2.4.3. Reaction temperature and time

Biodiesel conversion and yields have been found to be strongly influenced by the reaction temperature and time, depending on the type of biomass oil used (Ma & Hanna, 1999). Freedman et al. (1984) examined the use of three different reaction temperatures (32, 45 and 60°C) for the alkaline catalysed transesterification (using 1% NaOH) of soybean oil with methanol. These authors

showed that after 0.1 h the ester yields were 64, 87 and 94% (w/w) respectively at these temperatures. After 1 h, the FAME yields were observed to be identical for the 60 and 45°C runs (≈96% methyl ester conversion, w/w) and only slightly lower for the reaction carried out at 32°C (≈90% methyl ester conversion, w/w). After 4 h, the percentage methyl ester conversion from the 32°C run was seen to slightly exceed that of the other temperature levels. Using CH_3ONa as the alkaline catalyst, a methanol to oil ratio of 6:1 and reaction temperature and time of 60°C and 1 h, the percentage methyl ester conversions were demonstrated to be similar (93-98%) for the four biomass oils (soybean, sunflower, cottonseed and peanut oil) studied (Freedman et al., 1984). Srivastava and Prasad (2000) noted that given enough time the transesterification process will proceed to near completion (>99%, w/w) even at ambient temperatures. However, for most transesterification processes, the reaction temperature is commonly set close to the boiling point of the reacting alcohol at atmospheric pressure.

For the acidic transesterification (using 1% H_2SO_4 as catalyst) of soybean oil and butanol, it was shown by Freeman et al. (1986) that the conversion begins at a slow rate, proceeds at a faster rate and then slows again as the reaction nears completion for all temperatures investigated (77-117°C). A similar trend was also observed for alkaline catalysed reactions (Freedman et al., 1986). This trend was also shown by Ma et al. (1998), who studied the transesterification of beef tallow with methanol under alkaline catalysis. That study showed that the reaction was slow in the first minute due to the mixing and dispersion of the methanol into the beef tallow, proceeding to faster rates from 1-5 mins, the reaction becoming slower thereafter, reaching maximum conversion values at 15 mins (Ma et al., 1998).

Depending on the particular lipase employed, the enzyme catalysed process is normally conducted at the optimum enzyme temperatures, usually in the range of 30-40°C (Fukuda et al., 2001). Increasing the temperatures past this level could lead to protein denaturation and a loss in the catalytic activity of these biological catalysts. The enzymatic methyl ester production process was shown to require as little as 7 h for a continuous operated reactor using Immobilized *Candida Antarctica* to achieve a 92-94% ester conversion (Shimada et al., 1999), but up to 80-90 h for a batch fed reactor using a variety of lipases to attain an 80-100% methyl ester conversion (Kaieda et al., 2001).

Saka & Kusdiana (2001) demonstrated that using the supercritical method (with methanol and rapeseed oil) at a reaction temperature of 350°C, over 40% of the oil had been transesterified after a reaction time of 30 s, while at 240 s over 95% was converted to methyl esters. Examining the

influence of the reaction temperature on the supercritical transesterification of different biomass oils, Demirbaş (2002) showed that increasing the reaction temperatures and pressures past the thermodynamic critical point of the alcohol had a considerable influence on the methyl ester conversions.

2.4.4. Process mixing

Process mixing is another factor with the potential to influence the progress and efficiency of the transesterification of oil into fatty acid esters. At the start of the transesterification reaction, the oil and alcohol-catalyst mixture are immiscible, resulting in two liquid phases (alcohol and oil). Since the transesterification reaction occurs in the alcohol phase, stirring is required to facilitate phase mixing and initiate the conversion process (Meher et al., 2006). Mixing is considered to be particularly important during the early stages of the reaction where poor diffusion between the two phases limits the reaction rates (Srivastava & Prasad, 2000).

On the other hand, the need for stirring is expected to reduce with increasing alkyl ester formation, since the ester product acts as a mutual solvent for both reactants, forming a single phase system (Srivastava & Prasad, 2000). Ma et al. (1998) showed that for the alkaline transesterification of melted beef tallow no reaction was observed without process mixing. Furthermore, when the NaOH-methanol solution was added to the transesterification reactor while stirring was in progress and the reactor mixture was completely mixed, the stirring speed used was seen to be insignificant (Ma et al., 1998).

2.5. Alternative Biodiesel Feedstocks: The use of microalgae biomass

The production of biodiesel globally is predominantly dependent on the use of edible vegetable oils or animal fats, such as rapeseed oil, soybean oil, palm oil and tallow (Srivastava & Prasad, 2000). These oils are already used for human consumption, and also as industrial feedstocks for the cosmetic, food and chemical industries.

The cultivation and use of these edible oils (also referred to as first generation oil sources) for biodiesel production has generated controversies, mainly associated with the possibility of edible oil price increases, their availability for food uses and supply security. Other issues such as land use

and practices involved in the biomass cultivation, and the ethics of the use of these edible oil sources for biodiesel production (food-fuel debate), especially with regards to poorer economies, have also being raised (Moore, 2008).

With the current global petro-diesel demands, the potential biodiesel market far surpasses the availability of commonly used biomass oils not designated to meet competing processes i.e. the food industry (Mata et al., 2010). To meet this demand, and to facilitate the economic competitive production of biodiesel, alternative schemes, such as the use of non-edible biomass oils, have been proposed (Demirbaş, 2007).

Oils (lipids) from the photosynthetic aquatic biomass, microalgae, have received interest as a potential candidate for the production of biodiesel, due to the fact that non-edible oils used solely as biodiesel feedstocks could be obtained from the large scale cultivation of the microalgae biomass without requiring arable land or competing with human food requirements. The proposed large scale production of microalgae biomass for bioenergy purposes is not without its problems (i.e. economic, operational and energetic cost requirements) as highlighted in the literature (van Beilen, 2010; Reijnders, 2009; Chisti, 2007). This should, however, not be discouraging, but viewed as indicative of the research work still required for successful large-scale biofuel production from this feedstock.

As mentioned in Chapter 1, this investigation of the use of microalgae biomass for biodiesel production is not aimed at completely replacing biodiesel from all other potential oil bearing feedstocks. Rather it is proposed to be a useful alternative which must be explored further for the production of non-edible oil which can be used for biodiesel synthesis.

2.6. Microalgae: Biology, Oil synthesis and Biomass Cultivation

2.6.1. Biology of microalgae

Algae are a broad term used to refer to a relatively large group of aquatic organisms which mainly use the process of photosynthesis for the production of organic materials. These organisms are abundant in nature and can be found in all geographical locations and climates, including the Polar Regions (Kirst & Wiencke, 1995). The term microalgae is used to describe a group of photosynthetic organisms which contain chlorophyll *a*, a thallus not differentiated into roots, stems

and leaves (Lee, 1989) and also the oxygenic photosynthetic bacteria i.e. the cyanobacteria (blue–green algae). Over 40,000 microalgae species have been identified and classified on the basis of their structure and biology. There are two major groups: (i) the Prokaryotes (lacking membrane bound organelles such as mitochondria, plastids, and Golgi bodies), which include the classes Cyanophyceae and Prochlorophyceae (cyanobacteria) and (ii) the Eukaryotes (with membrane bound organelles) with the classes Chlorophyta (green algae), Bacillariophyceae (diatoms), Xanthophyceae (yellow-green algae), Chrysophyceae (golden-brown algae), Rhodophyceae (red algae), Phaeophyceae (brown algae), Dinophyceae (dinoflagellates), Rhaphidophyceae (chloromodas), Prymnesiophyceae and Eustigmatophyceae (Tomaselli, 2006).

Microalgae biomass cultures have mineral nutrition requirements similar to those of higher plants with two major forms of nutrition, autotrophy and heterotrophy (Becker, 1994). Autotrophic nutrition is characterised by the organisms obtaining all their growth requirements from inorganic compounds. This may be further differentiated into photoautotrophs which utilise light for their energy production process and chemoautotrophs, which obtain energy via the oxidation of inorganic compounds. Most algae are photoautotrophic since they utilise the photosynthetic process for the fixation of inorganic carbon dioxide (CO_2) for their energy and biomass macromolecule synthesis. Heterotrophs on the other hand, are incapable of producing energy from inorganic compounds so rely on organic compounds which have been produced by other organisms. Another form of heterotrophy called auxotrophy, is also exhibited by some microalgae, in which the organisms require very small quantities of organic compounds such as, amino acids and vitamins (Becker, 1994).

Mixotrophic nutrition has also been shown by microalgae cultures, where the energy derived is obtained either by photosynthesis (autotrophy) and/or through oxidation of organic compounds (heterotrophy) (Becker, 1994). As expected for those cultures, both CO_2 and organic compounds are required to sustain growth. No distinct switch between these modes of nutrition was however seen in cultures exhibiting mixotrophic nutrition, with the autotrophic and heterotrophic routes both seen to be present at all times, with the exception of periods of total darkness (Grobbelaar, 2006). Lee et al. (1996) reported mixotrophic growth by *Chlorella sorokiniana* cultures during the day, where both glucose and CO_2 were consumed, and at night the algae utilised the glucose present in the growth medium as its energy source.

2.6.2. Mass cultivation of microalgae

The mass cultivation of microalgae to produce biomass for energy conversion purposes requires the operation of large scale production systems where yield is a principal concern. The need to optimise the algae photosynthetic or biomass production processes is necessary, since a large increase in the achievable productivities of the microalgae production systems would in turn lead to a better biomass output and hence a possible improvement of the energy potential of the cultures.

The photosynthetic activities and biomass productivities of algal cultures can be greatly influenced by a number of factors. Environmental issues such as the availability of light, the pH of the medium and the temperature play important roles. The supply of nutrients required for algal growth and the dissolved oxygen levels in the aqueous environment are important conditions which must be controlled for efficient algal production (Becker, 1994). Downstream processes such as biomass harvesting also need to be considered in the design of microalgae cultivation reactors.

Although this thesis does not consider optimisation of the mass culturing of microalgae, an understanding of the factors influencing its large scale cultivation provides relevant background information on biofuel production from this feedstock and would assist in subsequent discussions in this thesis. Accordingly, a brief discussion of these factors (especially the factors affecting large scale operations) are included in this section.

To improve the practicality and acceptance of the microalgae production systems for energy production, the biomass cultivation unit design should be cost effective and competitive with currently used terrestrial biomass production systems. The construction of microalgae cultivation reactors is presently faced with high productivity and lower costs trade-off issues. For example, the choice of using more expensive high performance reactor designs (optimised to improve photosynthesis and biomass productivities) compared with the use of systems which are cheaper to operate but suffer from lower biomass productivities, due to factors such as culture contamination.

There are two basic designs which are predominantly used for the large scale cultivation of microalgae: open and closed systems. The open systems are differentiated from the closed reactors by having a significant portion of the algal culture exposed to the atmosphere, whereas the latter is contained within a growth medium with very little or no direct contact with the atmosphere (Grobbelaar, 2000).

2.6.2.1. Open systems

Open microalgae reactors are mainly large outdoor systems, usually characterised by the use of natural water bodies or constructed uncovered outdoor units. In natural water bodies i.e. lakes and ponds, when suitable environmental conditions and nutrient levels are present (especially in the case of high nutrient loading), microalgae growth can occur freely, leading to algal blooms. These blooms could be harvested and exploited for various applications, with the water bodies serving as highly productive systems. This is particularly in cases where the water bodies are shallow and mixing can effectively be achieved by winds. Such natural bodies can also be manipulated, such as the construction of unstirred cultivation ponds with specified cultivation parameters (Lee, 1997). In cases where the lakes or ponds exhibit specific selective chemical characteristics such as high (or low) pH or salinity, the presence of monospecific microalgae is possible (Becker, 1994). These unstirred ponds (natural or manmade) are the most economical and least technical production systems in terms of operation. Min Thein (1993) reports the growth of *Arthrospira* in monospecific cultures throughout the year in four (old) volcanic craters in Myanmar. The current global production of microalgae using such natural systems is reported to be 30 metric-t/annum (Lee, 1997). Due to the low productivity of unstirred ponds, it is however not considered a suitable scheme for large scale microalgae biomass production (Becker, 1994).

The commercial cultivation of microalgae is predominantly carried out in open systems due to the low costs of construction and operation, and also owing to the durability of these microalgae reactors (Richmond, 1999). Based on local conditions and the construction materials available, different types of open microalgae reactors can be designed, varying in shape, size, inclination and mode of agitation (Becker, 1994). The three main types of open stirred cultivation unit designs, presently utilised for microalgae production (Becker, 1994), are (see Figure 2.4):

a) Circular ponds, with the use of a rotating arm for stirring of the medium.
b) Raceway or oblong ponds, which can either be installed as a single unit or merged to form a larger unit with the mixing of the medium achieved by the use of a paddle wheel, propellers or air lift pumps.
c) Inclined or sloped ponds, which are often constructed to have meandering or winding paths. Agitation of the algal cultures in this type of system is achieved by gravity flow and also by the use of pumps depending on the set up. The culture suspension in this system

would flow downwards where it might need to be pumped to the top of the slope (Richmond, 1999).

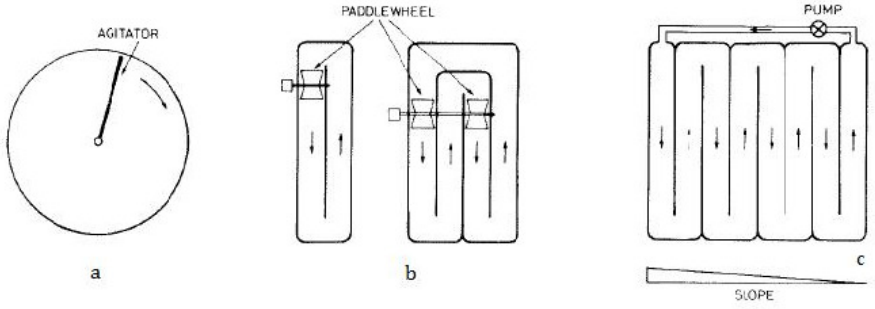

Figure 2.4. Schematic representation of major algal microalgae open cultivation system designs (Becker, 1994). (a) The circular pond with the rotating mixer; (b) Single and joined oblong ponds with paddle wheels; (c) Sloped meandering ponds with circulating pumps.

Although the use of open microalgae cultivation systems, especially in locations with suitable climatic conditions, seem favourable in terms of cost considerations, these systems are typically affected by the problems of low year-round productivity and contamination by other competing microorganisms i.e. other algal species, rotifers and grazers (Becker, 1994).

2.6.2.2. Closed systems or photobioreactors

To overcome limitations such as evaporative water loss, a lack of process temperature control, and biological contamination encountered in open systems, the use of covered cultivation systems i.e. photobioreactors (PBRs), have been considered for the large-scale production of microalgae. PBRs, as reported earlier, do not have the cultures directly exposed to the atmosphere. Instead the growth medium is enclosed in a transparent vessel (i.e. tubes). This characteristic protection from the external environment provides an added attribute of reducing or preventing water losses by evaporation, aiding temperature control of the biomass cultivation (Richmond & Becker, 1986).

Microalgae can be grown photoautotrophically, heterotropically or mixotrophically in PBRs, depending on the availability of light and nutrients. These systems can be located outdoors to utilise sunlight or indoors where light supply is facilitated by artificial light sources i.e. fluorescent tubes or using fibre optics (Pulz & Scheibenbogen, 1998). The use of artificial lightning for the consideration of large scale microalgae cultivation for biofuel production might however prove disadvantageous with regards to the process economics and energetics. PBRs can be largely classified in terms of their design, and also on the basis of their operation (Tredici, 2006), such as:

- Flat or tubular

- Horizontal, inclined, vertical or spiral

- Manifold or serpentine. The manifold PBR consists of a series of parallel tubes connected at the ends by two manifolds (one for collection of the culture suspension and the other for distribution). On the other hand, the serpentine PBR has several straight tubes connected in a series by U-bends to form a flat loop which can be arranged vertically or horizontally

Further classification of PBRs on account of their construction materials can be made, such as the use of glass vessels or plastic bags for the biomass cultivation.

Closed cultivation systems are, however, faced with the major disadvantage of high capital requirements for construction, energy and maintenance, especially when compared to open ponds (Borowitzka, 1999). They are also considered to be more difficult to scale up (Borowitzka, 1999).

The scaling up issue may prove to be a problem for the use of closed systems for the large scale cultivation of microalgae aimed at energy production, since the biomass has to be produced at a cost where it can compete favourably with fossil fuel sources and currently available terrestrial biofuel feedstocks. Furthermore, based on the type and transparency of the PBR construction material, the intensity of the light reaching the microalgae cultures in the media suspension may be reduced (Richmond & Becker, 1986) resulting in an overall lowered light utilisation efficiency by the microalgae. Mechanisms for reducing dissolved oxygen build-up in PBRs must also be considered, to prevent decreased cell yield or even culture death owing to photo-inhibition and photo-oxidation (Richmond, 2006).

Even though a major reason supporting the use of PBRs for microalgae production is the potential to limit biological contamination (except in cases where the PBR design integrates a sterilisation function) these closed systems have been reported as not much better equipped than

open microalgae cultivation systems for overcoming contamination by unwanted organisms (Tredici, 2006; Richmond, 1999).

2.6.3. Harvesting of microalgae biomass

A brief introduction will be included in this chapter on the harvesting of microalgae biomass, since it is also a challenge faced in the large scale production of microalgae biomass.

Microalgae cell recovery was reported by Gudin & Therpenier (1986) to account for at least 20-30% of the total cost of microalgae biomass production. This is mainly as a result of the small size of the microalgae cells (3-30 μm) and their low concentration in the culture suspension (< 50 mg/l) (Molina Grima et al., 2006).

Major methods utilised for the removal and concentration of these dilute microscopic organisms from the growth medium include: centrifugation, electroflotation and chemical flocculation (followed by sedimentation or air flotation), continuous belt filtration, vibrating and stationary screens, sand bed filtration, and autoflocculation (Richmond & Becker, 1986).

Of the systems mentioned above, only a few have the potential to be developed into low cost, efficient harvesting methods. Microalgae production systems require a harvesting process or a combination of methods adapted to fit the organism being cultivated and is also based on the intended use of the microalgae biomass (Gudin & Chaumont, 1991). Chisti (2008a) put forward the use of a combination of microalgae biomass sedimentation techniques followed by a belt filtration process to achieve low cost and energetically efficient harvesting of the microalgae biomass for bioenergy applications.

Depending on the energy conversion process in which the microalgae biomass is to be used, it might require a dehydration step after harvesting to improve its use. The use of a sun drying process in locations with suitable climatic conditions, as well as other methods such as spray-and drum-drying have been reported by Molina Grima et al. (2006).

2.7. Microalgae Biomass Productivities and Lipid Content

Maximum microalgae yields of > 50 g (dry weight, d.w) $m^{-2} d^{-1}$ (180 t dry microalgae biomass $ha^{-1} annum^{-1}$) was reported for various open and closed cultivation systems (Grobbelaar, 2000; Lee et al., 1995). It is however unclear how sustainable these biomass yields (in the long term) are since the growth periods were not reported. For closed PBRs, biomass production rates as high as 79 g (d.w) $m^{-2} d^{-1}$ was reported by and Tredici & Zitelli (1998) with the PBR maintained for a period of 6 d. Although much higher biomass productivities were reported for open cultivation systems, practical yields of between 15-25 g (d.w) $m^{-2} d^{-1}$ was reported by Goldman (1980) to be very common for outdoor microalgae cultures cultivated for long periods (> 30 d). The microalgae biomass yields are however still an issue of dispute in the literature (i.e. van Bielen, 2010). Since this thesis is not concerned with optimising biomass production, average demonstrated yields will be used in the analyses carried out in this study. Biological and technological considerations which can be manipulated to optimise the microalgae productivity, in turn improving the energy potential of the feedstock were put forward by Grobbelaar (2000).

Under optimal growth conditions, and depending on the species, microalgae synthesise fatty acids, primarily aimed for the formation of glycerol based membrane lipids which constitute 5-20% of the microalgae dry cell weight (Hu et al., 2008). The major membrane lipids, the glycosylglycerides, are found in the chloroplast, with the phosphoglycerides significantly located in the plasma membrane and endoplasmic membrane systems (Harwood, 1998; Wada & Murata, 1998). However, under stress or specific controlled culture conditions, many microalgae species have their lipid biosynthetic pathway altered to favour the production and accumulation of neutral lipids, mainly in the form of TAGs up to 20-50% of their dry cell weight (Hu et al., 2008). For example, *Chlorella vulgaris* cells grown under nitrogen starved conditions has been shown to have its lipid fraction more than double, with a corresponding reduction in the carbohydrate content (Illman et al., 2000). The TAGs and fatty acids found in microalgae are similar to those of commonly cultivated terrestrial oil crops, and are mainly composed of unsaturated fatty acids i.e. palmitoleic (C16:1) oleic (C18:1), linoleic (C18:2) and linolenic acids, as described by Meng et al. (2009). Saturated fatty acids such as palmitic (C16:0) and stearic acid (C18:0) are also present to a lesser extent (Meng et al., 2009). These fatty acids may vary in the number and position of double (or triple) bonds on their carbon backbone. A list of major fatty acids found in different algae

groups are highlighted in Hu et al. (2008). The oil contents (% of microalgae dry cell) for different microalgae species is shown in Table 2.3.

Table 2.3. Oil contents of selected microalgae species (Chisti, 2007)

Microalgae species	Oil Content (% microalgae dry weight)
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis suecica	15-23

The synthesised TAGs usually do not play a cellular structural role like the membrane glycerolipids. They instead primarily serve an energy storage function and are deposited in densely packed lipid bodies which are located in the cytoplasm of the microalgae cell (Hu et al., 2008). Microalgae lipid metabolism, in particular the biosynthesis of fatty acids and TAGs in microalgae, is considered to be poorly studied in comparison with that of higher terrestrial plants (Hu et al., 2008). Since, similar genes and enzymes involved in lipid metabolism have been isolated from both microalgae and higher terrestrial plants, broad generalisations on the microalgae lipid biosynthesis, based on that of higher plants, have been made (Hu et al. 2008). The pathways proposed for the synthesis of fatty acids and TAGs have been detailed in the literature (i.e. Ratledge, 1988; Hu et al., 2008; Ohlrogge & Browse, 1995). However, as highlighted by Hu et al. (2008), molecular level

research on microalgae lipid metabolism is still fragmentary, with investigations continuing. This thesis therefore does not look further at this topic.

2.8. Microalgae Oil vs. Commonly Used Oil Feedstocks

Although the microalgae lipid fraction is dependent on the specific microalgae strain and can be improved by altering the culture or growth conditions (Spoeher & Milner, 1948), the uniform average lipid composition of 20%, as reported by Goldman (1980) for both freshwater and marine microalgae, will be used for the general fuel yield investigations and comparisons in this section. With an average areal biomass productivity of 20 g (d.w) m^{-2} d^{-1} (72 t (d.w) ha^{-1} a^{-1}) (Goldman, 1980), the oil yield of microalgae compares favourably with that of common terrestrial oil feedstocks (t oil/ha/annum), currently used for biodiesel production, as shown in Figure 2.5.

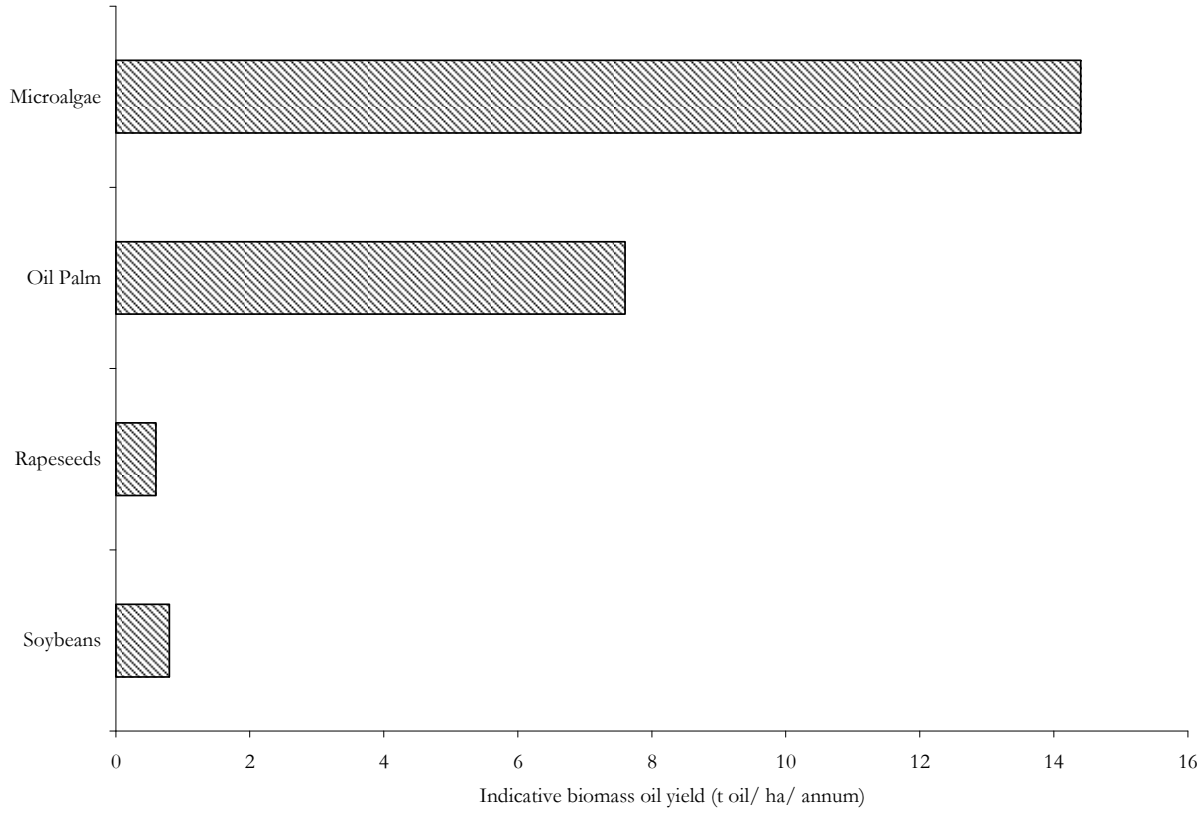

Figure 2.5. Oil yield comparisons of microalgae derived oil with conventional oil sources.[4]

[4] The annual terrestrial biomass oil yields are based on generalised scenarios of best yields (Hall, 1986).

With an average microalgae oil yield of 14.4 t/ha/annum, oil production from microalgae biomass was seen to result in ≈ 21 times more oil per unit area compared with that derived from rapeseeds cultivation (Figure 2.5). The practical transesterifiable oil available from the microalgae biomass is however dependent on the particular microalgae species, biomass cultivation conditions, and the extraction methods and solvents used in obtaining the microalgae oil.

2.9. Biodiesel from Microalgae Biomass

The use of microalgae derived oils for the production of fatty acid esters has been described by Chisti (2007) as one of the most promising feedstocks, with the potential to meet petro-diesel replacement targets without encroaching on arable land suitable for food production. Research and publications from the aquatic species program (ASP) of the U.S. Department of Energy (DOE) and Solar Energy Research Institute (SERI) have largely aided in the promotion of the use of microalgae lipids for the production of biodiesel.

The ASP project investigated the large scale cultivation of microalgae, the lipid profiles of various candidate microalgae species, and their suitability for biodiesel production. The project also assessed the economic and technology requirements for microalgae biofuel production. Future research investigations which could potentially improve biodiesel production from microalgae were also highlighted. Reports from this project i.e. Benemann et al. (1982), Feinberg (1984), Hill & Feinberg (1984) and Neenan et al. (1986), contributed significantly to research into the potential for biodiesel production from microalgae biomass.

A practical laboratory scale production of biodiesel using microalgae lipids (from *Chaettoceros muelleri* and *Monoraphidium minutum*) was first demonstrated in the literature by Nagle & Lemke (1990), using the conventional route. This involves the extraction of the lipids from the microalgae biomass followed by its conversion to alkyl esters and glycerol. The extraction of algae lipids can be carried out using commonly employed methods and solvents to produce a reaction feedstock similar to vegetable oils. Nagle & Lemke (1990) showed that using 1-butanol as the extraction solvent, the highest and most consistent extraction efficiency (≈90%), and a lipid purity of about 94%, was achieved. Alternatively, a hexane/2-propanol mixture (40% hexane, 60% 2-propanol(v/v)), and an ethanol/1-propanol mixture (95% ethanol, 5% 1-propanol (v/v)), showed

average net extraction efficiencies of 78% and 73 % respectively, both exhibiting a lipid fraction purity of about 0.90 (w/w) (Nagle & Lemke, 1990).

The transesterification to fatty acid esters was then carried out on the extracted microalgae lipids using the same methods used currently for biodiesel production from vegetable oils. Results of the methanolysis studies carried out by Nagle & Lemke (1990) indicated that the most important variable in the microalgae lipid conversion process was the type of catalyst used. The use of acid catalysts (HCl) was demonstrated to result in a higher FAME yield per microalgae lipid input compared to alkaline catalyst (NaOH) use under the same reaction conditions. This was attributed to the high FFA content of microalgae lipids (Miao and Wu, 2006), which would contribute to a reduction in the conversion efficiency with alkaline catalysts use, as presented in section 2.3. The use of inorganic acids as the reaction catalysts is largely considered for microalgae lipid transesterification, due to its insensitivity to the FFA content of this oil feedstock. This is advantageous since both the biodiesel producing transesterification and esterification reactions are facilitated by acidic catalysis. The influence of the concentration of the catalyst, the reaction time and the temperature, on the transesterification reaction was also studied by Nagle & Lemke (1990). An optimal equilibrium methyl ester yield from microalgae oil was obtained using 0.6 N HCl in methanol for 0.1 h at a reaction temperature of 70°C (Nagle & Lemke, 1990). The conventional transesterification of microalgae lipids was also demonstrated by Miao and Wu (2006), who showed that the use of a 100% acid catalysts (H_2SO_4, w/w)), a methanol to oil molar ratio of 56:1, and reaction temperature and time of 30°C and 4 h respectively, gave the best methyl ester yields.

The use of enzymatic catalysis for microalgae lipid conversion to biodiesel has also been demonstrated. Li et al. (2007) investigated the enzymatic catalysed transesterification (using immobilised lipases from *Candida sp.* 99-125) of *Chlorella protothecoides* oil (44.3-48.7% lipid content, on the basis of the biomass d.w) recovered from large scale heterotrophic reactors (750-11000 L). The results from this study showed that using 75% lipase (12000 U/g[5], based on the lipid quantity), a reacting molar ratio of methanol to oil of 3:1, reactor agitation at 180 rpm and batch feeding the reactor three times, a 98.15% conversion of the microalgae oils to methyl esters was achieved in 12 h.

[5] U/g is a means of quantifying the lipase. With 1 U= 1μmol FAME produced/minute per g of the reacting oil weight.

The application of the supercritical method for the transesterification of neutral lipids extracted from *Chlorella protothecoides* biomass was demonstrated by Demirbaş (2009). The results of this study however did not provide any indication of the influence of the various reaction parameters on the conversion of the microalgae oil to biodiesel, or the percentage conversion yields achievable from the feedstock using the supercritical method.

In order to assess the suitability of replacing petroleum diesel with biodiesel obtained from microalgae biomass, certain fuel properties such as its heating value, density, kinematic viscosity and flash point need to be determined to ascertain its suitability. Table 2.4 shows a comparison of fuel properties of biodiesel derived from microalgae with petroleum diesel.

The fuel properties of microalgae derived biodiesel can be seen to be largely similar to that of petro-diesel (Miao & Wu, 2006). The heating value and density of the microalgae biodiesel (Miao & Wu, 2006) was however observed to be better than those of methyl esters obtained from common biomass oils, such as soybean and palm oil as described by Srivastava & Prasad (2000).

Table 2.4. Fuel properties of microalgae derived biodiesel compared to petro-diesel (No. 2) (Miao & Wu, 2006)

Properties	Microalgae derived biodiesel[6]	Petro-diesel
Density (kg/l)	0.864	0.838
Kinematic viscosity (cSt) at 40°C	5.2	1.9–4.1
Flash point (°C)	115	75
Solidifying point (°C)	-12	-50–10
Cold filter plugging point (°C)	-11	-30
Higher Heating value (MJ/kg)	41	40–45

[6] *Chlorella protothecoides* oil and methanol used for the transesterification reaction.

Biodiesel fuel properties are largely influenced by the fatty acid composition of the microalgae lipids, with the desired properties i.e. viscosity, directly related to the degree of unsaturation and length of the fatty acid carbon chains (Hill & Feinberg, 1984). One of the characteristics of microalgae lipids is the variety of the fatty acids contained in them, with a high degree of unsaturation in the longer chained fatty acids and higher concentration of shorter chained saturated fatty acids observed in some species (Hill and Feinberg, 1984). The presence of a high concentration of polyunsaturated fatty acids in the lipids would increase susceptibility to oxidation during storage, with the short chained fatty acids resulting in a fuel with lower melting points, with a lower cloud point and viscosity (Hill & Feinberg, 1984). Likewise, vulnerability to oxidative polymerization is minimised with increased lipid saturation, resulting in improved storage characteristics for the microalgae derived biodiesel (Hill & Feinberg, 1984). Demirbaş (2009) suggests that the lower melting points exhibited by the biodiesel from microalgae lipids with high levels of polyunsaturated fatty acids results in a fuel with better cold weather properties (i.e. lower cold filter plugging point) compared with biodiesel produced using common biomass oils rich in monosaturates and saturated fatty acids.

For the large scale production of microalgae biodiesel, it is proposed that cheap and energetically efficient conversion technologies are applied to overcome the disadvantageous costs associated with the microalgae biomass production. The relatively high costs of the lipase catalysis (Fukuda et al., 2001), and the prohibitive operational and energetic costs of the supercritical method (Marchetti & Errazu, 2008), when compared with the inorganic catalysed routes, limits the short-medium term commercialisation of those methods and will therefore not be examined further in this thesis. In addition, safety concerns about the use of supercritical transesterification would further hinder its use for microalgae biodiesel production (Saka, 2006). The use of inorganic catalysis for the transesterification process was solely considered for use in this study.

Investigations into the improvement of biomass productivities and yield (i.e. via genetic manipulation), and photo-bioreactor design, are major research areas which have the potential to provide cheaper microalgae biomass. This thesis will however concentrate on the potential for optimising the fatty acid ester production route using inorganic catalysts.

An alternative to the conventional transesterification process, which was not examined earlier in section 2.2, the 'in-situ' transesterification method, will be the main focus in the investigations on microalgae biodiesel production in this thesis.

2.10. The In-situ Transesterification Process

The in-situ process facilitates the conversion of the biomass oil to FAAE directly from the oil bearing biomass, thereby eliminating the solvent extraction step required to obtain the oil feedstock, as in the conventional method (Figure 2.6). This biodiesel production scheme could aid in the simplification of the fuel conversion process, potentially reducing the overall process cost, hence aiding in the reduction of the final fuel product costs (Haas et al., 2007).

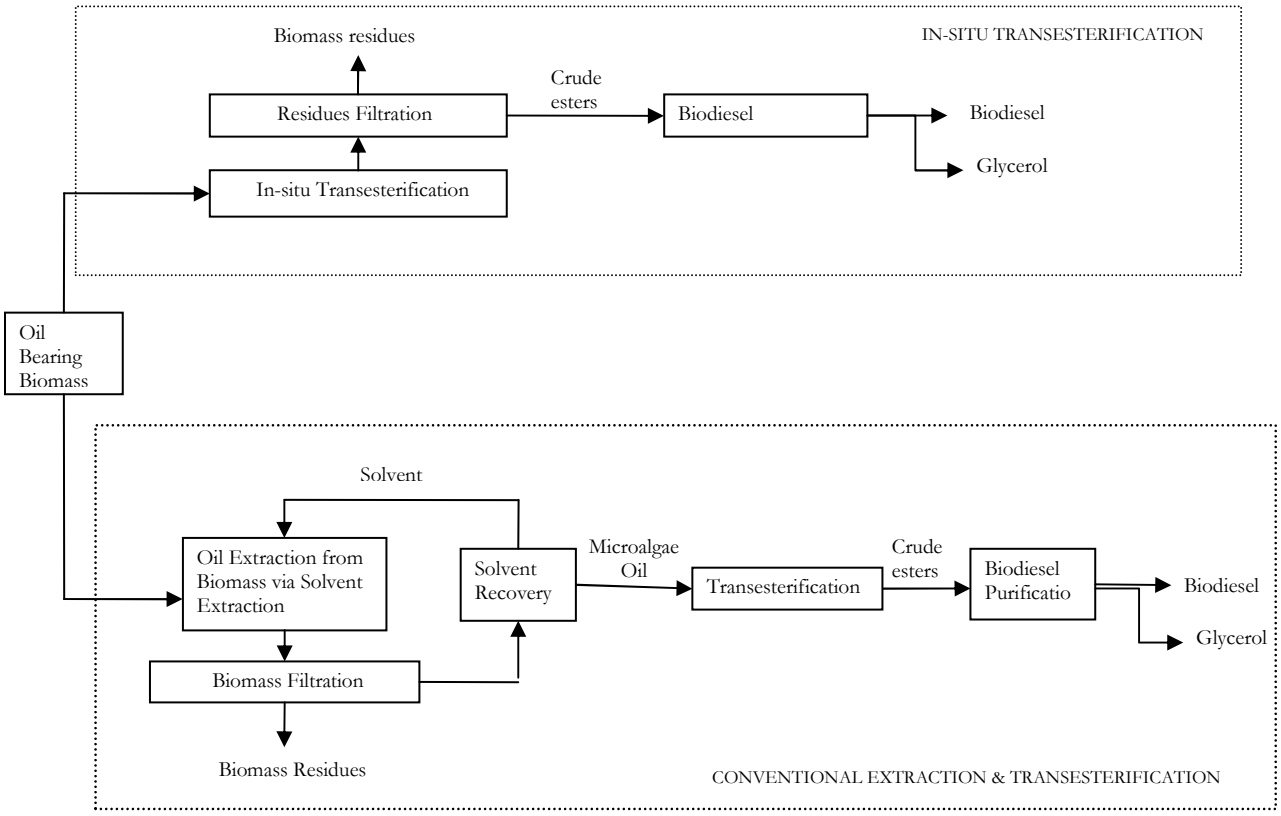

Figure 2.6. An overview of the conventional and in-situ transesterification processes.

This method may be especially advantageous for use with microalgae, since the extraction of microalgae lipids is usually accomplished by solvent extraction, rather than cheaper physical extraction methods (for example, expellers), as utilised in conventional oil crops (Johnson & Wen, 2009).

The transesterification of the oil in the biomass (sunflower seeds) directly has been shown to result in increased biodiesel yields, when compared to those obtained via the conventional route (Harrington & D'Arcy-Evans, 1985a). Using the in-situ method, these authors demonstrated an increase in alkyl ester yields of up to 20% compared to the conventional process under similar reaction conditions. This improvement in the ester yields was considered by the authors to be attributable to the improved accessibility of the oil in the biomass by the acidic medium (Harrington & D'Arcy-Evans, 1985b).

Haas et al. (2007) further suggested that the process wastes and pollution emanating from biodiesel production could also be reduced by the use of this method. However, practical investigations on the of the application of the in-situ method using different microalgae species were lacking in the literature. Part of this study will aim to provide useful preliminary information on the application of the acid catalysed in-situ transesterification process for the production of biodiesel using microalgae. Previous work reported in the literature on the use of this transesterification method for biodiesel production from oil bearing biomass will be briefly highlighted in this section.

The in-situ transesterification of macerated sunflower seeds (in methanol) was also studied by Siler-Marinkovic & Tomasevic (1998) who studied the effect of using two temperatures, 30°C and 64.5°C, and a range of test reaction conditions for the in-situ transesterification process. The alcohol (methanol) to oil molar ratio varied from 100:1 to 300:1, the catalyst (H_2SO_4) concentration ranged from 16% to100% (on the basis of the oil) and the reaction time was 1-4 h. Within the limits of the conditions studied, the best FAME yields (98.2%), based on the oil content of the sunflower seed, was obtained at a molar ratio of methanol to oil of 300:1, an acid catalyst concentration of 100% and a reaction time of 1 h (Siler-Marinkovic and Tomasevic, 1998).

The use of the acid in-situ transesterification scheme has also been demonstrated for FAAE production from soybean and high FFA containing rice bran oil (Kildiran et al., 1996; Özgül-Yücel & Türkay, 2002).

2.10.1. Microalgae biodiesel via the in-situ transesterification process: Study goals

Due to the biomass maceration and varied oil composition, the findings from previous studies on the in-situ transesterification of common oil bearing biomass cannot be directly used to

predict the influence of the different reaction parameters with microalgae use. As a result specific information is required on the influence of important reaction variables on the in-situ microalgae transesterification process.

Since the main driver for the use of the in-situ transesterification process is its possible application as a cost and process energy reducing scheme for microalgae derived biodiesel, this thesis will mainly consider reaction variables that potentially influence the cost of the in-situ process. This study will aim to provide information on the in-situ transesterification operating conditions that could provide the best fatty acid ester yields, while also having the least material and energy requirements, and consequently lowest process costs.

2.11. Utilisation of Post Transesterification By-products

In addition to the biodiesel and the glycerol by-product formed during the transesterification process, large amounts of solid microalgae biomass residues are also produced following the lipid extraction and/or fuel conversion process.

The major question "What to do with the microalgae residues after the transesterification process?" arises.

As considered by Dubinsky et al. (1980), these protein-rich microalgae residues have the potential to be incorporated in livestock feeds where they could replace the more commonly used soy and fish meals. However, since the large scale microalgae production is proposed to be combined with wastewater treatment facilities, the suitability and acceptance of the use of the microalgae residues for animal feed production may be restricted.

Where this option is not available or feasible, the residual biomass would be considered as waste with its disposal cost, adversely affecting the already unfavourable economics for biodiesel production from microalgae (Chisti, 2007).

The primary limitation facing the large scale production of biodiesel from microalgae is mainly associated with the high energy inputs required for the microalgae biomass production, harvesting and biodiesel production stages (Chisti, 2007). The further extraction of energy from the residual biomass could serve as a means of increasing energy production from microalgae, possibly leading to a reduction of the overall process costs and wastes.

In particular, methane produced via the anaerobic microbial gasification of the residual microalgae biomass has potential as a secondary biofuel, which could contribute to the energy requirements of the microalgae biomass production and fuel processing stages (Shifrin & Chisholm, 1980; Chisti, 2007, 2008). However, no prior investigation of the use of the microalgae biomass residues resulting from the biodiesel production process was found in the literature.

On the other hand, a number of demonstrations on the use of the anaerobic digestion process for the production of CH_4 using untreated microalgae biomass as the process feedstock have been described (Golueke et al., 1957; Oswald & Golueke, 1960; Uziel, 1978; Samson & LeDuy, 1982, 1983a, 1983b, 1986; Chen, 1987; Sanchez & Travieso, 1993; Chen & Oswald, 1998; Yen & Brune, 2007).

Before presenting the study goals of this thesis on the use of the microalgae residues post transesterification as a primary feedstock for CH_4 production, a brief elucidation of the anaerobic fermentation process and CH_4 production will first be presented in the next section (2.12).

2.12. Methane Production and Anaerobic Digestion of Biomass

Methane production from microalgae residues can be effected by a multi-stage biological conversion process involving the anaerobic degradation of the complex polymers and compounds of the biomass to lower molecular weight intermediates, which are then converted to CH_4 and carbon dioxide (CO_2).

The catabolism of the biomass substrate is facilitated by a consortium of bacteria which respire anaerobically i.e. utilise inorganic electron acceptors such as nitrate (NO_3^-), sulphate (SO_4^{2-}) and CO_2 instead of oxygen (O_2) in their electron transport chain. Based on the composition of the biomass feedstock, the resulting gaseous mixture, commonly called biogas, is composed primarily of CH_4 and CO_2, together with small concentrations of secondary gases, including hydrogen sulphide (H_2S), hydrogen (H_2), ammonia (NH_3), nitrogen (N_2), other trace gases and water vapour.

The anaerobic digestion of organic matter is a complex process which can be broadly divided into three major stages: hydrolysis and acidogenesis (phase I), acetogenesis (Phase II), and methanogenesis (Phase III,) as shown in Figure 2.7 (Hill, 1982; Hill et al., 1987).

The first step involves the hydrolysis of the long chain polymers of the organic matter, i.e. proteins, lipids and polysaccharides, to their corresponding oligo- and monomer units, i.e. peptides and amino acids, fatty acids and glycerol, and monosacharides, respectively. The hydrolysis step is usually carried out by microbial extracellular enzymes. The production of these enzymes is, however, energy demanding and as a result only occurs when the environment is depleted of easily accessible carbon sources (Klass, 1998)

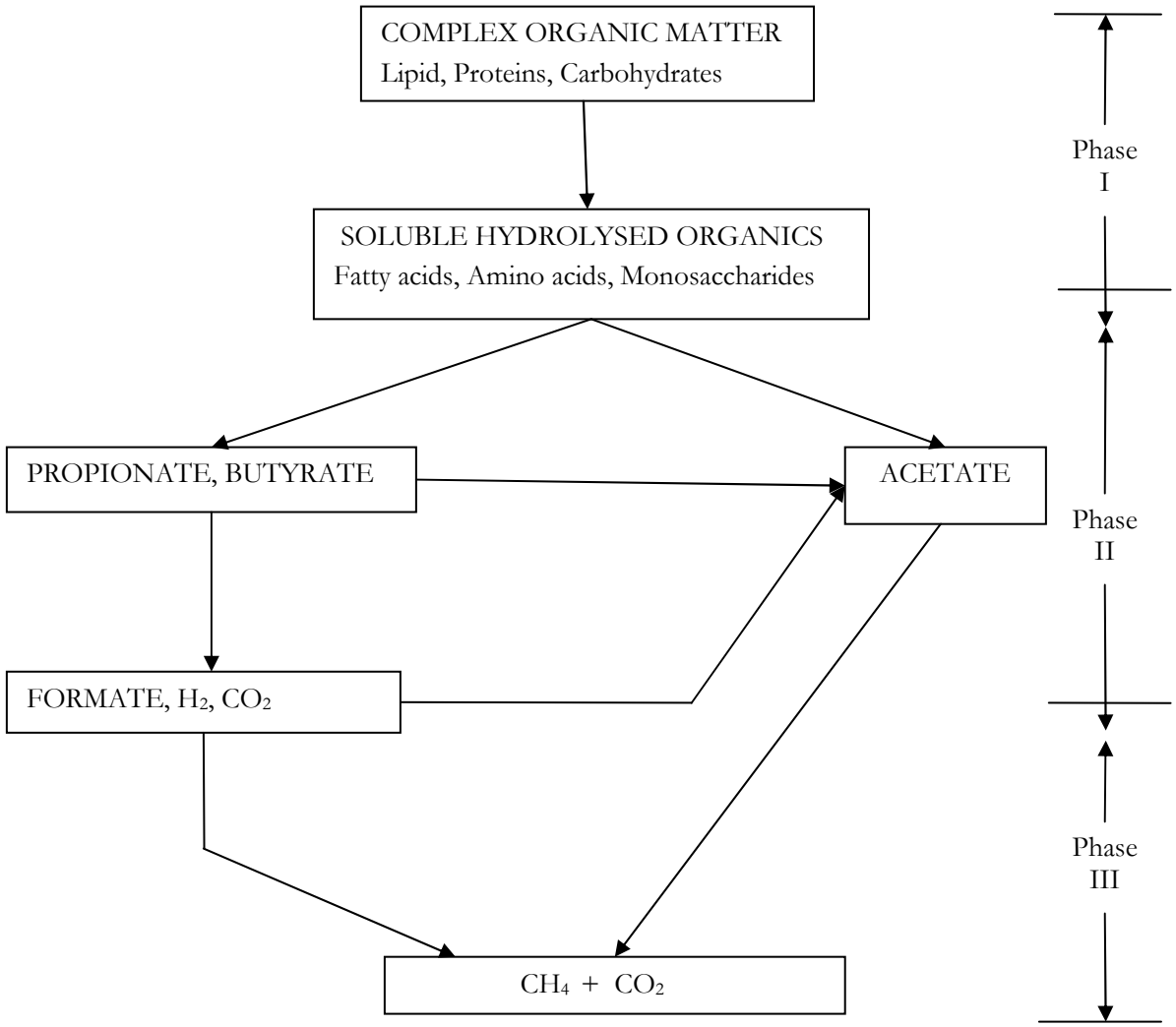

Figure 2.7. Anaerobic digestion of organic matter.

The products resulting from the hydrolysis step are then further degraded to volatile fatty acids (VFAs). The formation of short chained alcohols is also facilitated in this step (Conrad, 1999). Hydrogen (H_2), CO_2 and formate production have been demonstrated to be promoted concomitantly with the VFA production, as reported by Klass (1998).

Acetate formation occurs in the second step, mainly via three primary pathways, as shown in Figure 2.7. These are: the direct fermentation of the degraded monosaccharide products; the reduction of CO_2 with H_2 used as an electron donor; and the fermentation of the intermediate VFA, such as propionic and butyric acids (and lower alcohols), by a group of acetogenic bacteria (hydrogenogens).

The reduction of CO_2 to acetate as the only reaction product is assisted by a group of bacteria called homoacetogens. The direct formation of acetate from VFAs and alcohols is however only energetically favourable when the concentration of H_2 is kept low, which can be achieved by H_2-consuming methanogenic bacteria (Horn, 2000).

The methanogenic bacteria responsible for CH_4 production are strictly anaerobic (i.e. obligate anaerobes), deriving their energy from a restricted number of substrates. The methanogenic bacteria use three main pathways for the production of CH_4: a reduction of CO_2 to CH_4 using H_2 or formate as an electron donor; the direct (acetoclastic) production of CH_4 and CO_2 from acetate by the methanogens (*Methanosarcina* and *Methanothrix*); and the formation of CH_4 and CO_2 from the methyl groups of methanol and methylamines (not shown in Figure 2.7) (Deppenmeier et al., 1996; Archer & Kirsop, 1990).

The energy content of the gas mixture resulting from the anaerobic digestion process is mainly determined by the concentration of CH_4, which has a HHV of 39.3 MJ/m^3 (Klass, 1998). In turn, the CH_4 concentration depends largely on the type and characteristics of the feedstocks employed in the microbial conversion route (Sorensen, 2000).

2.12.1. Variables affecting the anaerobic digestion process

Apart from the important condition of an oxygen free (anaerobic) environment required for the fermentation process to proceed, there are a number of factors that are vital for the favourable breakdown of organic matter to CH_4. These include:

2.12.1.1. Type of substrate or/and co-substrate used

The organic material which is to be digested during the anaerobic process is known as the substrate. Due to variations in their characteristic macromolecular composition, different substrates

have different CH_4 yields. Co-substrates are other degradable organic materials which are digested with the primary substrate with the aim of improving the overall CH_4 production.

The particle size of the substrate is considered to be an important digestion parameter. Reducing the substrate particle sizes improves CH_4 yields, since this increases the surface area available for bacterial activity, and also reduces the fermentation period (Yadvika et al., 2004). Sharma et al. (1988) showed that of five particle sizes (0.088, 0.40, 1.0, 6.0, and 30.0 mm) maximum CH_4 yields were obtained from the digestion of 0.088 and 0.40 mm particle sizes.

2.12.1.2. Temperature

There are three broad temperature ranges in which anaerobic digestion is usually carried out. These are the psychrophilic (Cryophilic), mesophilic and thermophilic, which are facilitated in the temperature ranges of <25°C, 25-40°C and 40-60°C respectively. Most anaerobes have, however, been demonstrated to be most active in the mesophilic and thermophilic temperature ranges (Maurya et al., 1994; Zennaki et al., 1996), with the length of the fermentation period considered to be dependent on the process temperatures. Garba (1996) showed that the methanogenic phase was very sensitive to sudden temperature changes, suggesting that drastic changes of the process temperatures should be avoided to improve CH_4 yields.

2.12.1.3. Substrate loading rates

The CH_4 production in anaerobic digesters has been demonstrated to be dependent on the substrate loading rates. Vartak et al. (1997) showed that CH_4 yields increased with a reduction in the substrate loading rates. Optimum loading rates, beyond which increases in the substrate quantities will not result in proportionately improved CH_4 yields, have been described for different reactor types and sizes, and for various feedstocks (Yadvika et al., 2004).

2.12.1.4. Hydraulic retention time (HRT)

This is the average time spent by the substrate in the anaerobic digester. The use of shorter retention times for the anaerobic digestion process is considered to result in washouts of active

bacterial masses, with corresponding reduced biomass degradation and CH_4 yields, while longer retention times, though assisting improved CH_4 yields, require a large digester volume and hence more capital costs (Sorensen, 2000).

2.12.1.5. pH value

The process pH is an important variable affecting microbial growth during the fermentation process. An operational pH range of 6.8-7.2 for anaerobic digesters is recommended for enhanced CH_4 production (Yadvika et al., 2004). This can be achieved by feeding the reactors at an optimum substrate loading rate (Yadvika et al., 2004). The formation of VFAs and CO_2 during the anaerobic digestion process strongly influences the process pH (Sorensen, 2000). The production of ammonia (NH_4) from the breakdown of proteins could lead to an increase in the process pH (Sorensen, 2000).

2.12.1.6. Carbon/nitrogen (C/N) ratio

The carbon/nitrogen (C/N) ratio is the relationship between the amount of carbon and nitrogen in the biomass material.

C/N ratios between 20 and 40 have been proposed as optimum for CH_4 production (Sorensen, 2000). If the C/N ratio of the organic material to be digested is greater than the proposed ratio, the N content could be insufficient to meet the protein demands for bacterial growth, resulting in limited CH_4 production. Conversely, with a lower C/N ratio, the excess N content could lead to the build up of NH_4. The accumulated NH_4 could lead to an increase in the pH levels of the process, with corresponding toxic consequences for bacterial activity. This is known as bacteria poisoning (Sorensen, 2000).

Stafford et al. (1981) however, described anaerobic digestion systems with C/N ratios as high as 70 without problems. Organic materials with high C/N ratios could be mixed with those with lower to obtain a composite anaerobic digestion input within the desired range.

2.12.1.7. Presence of inhibitory substances

The action of bacteria might be impeded by the presence of substances in the biomass feedstock such as metal salts, antibiotics or disinfectants in manure (Archer & Kirsop, 1990).

2.12.2. Evaluation of the potential for methane production

A number of evaluation techniques are available for the assessment of the CH_4 production potential of biomass feedstocks. These include theoretical estimations, based on the biomass elemental composition (Buswell & Mueller, 1952; Boyle, 1977), and the use of the macromolecular composition, as described by Cowley & Wase (1981).

Although representative maximum CH_4 production values can be obtained using these methods, the estimated CH_4 yields are not usually obtained in realistic situations (Hansen et al. 2004). In most cases lower CH_4 yields are achieved because complete biomass biodegradation is not realised.

Data from practical anaerobic digestion tests are needed for determining achievable CH_4 production rates from biomass feedstocks. For example, the CH_4 production potential can be determined using methods such as the biochemical methane potential (BMP) test (Owen et al., 1979) and the anaerobic degradability potential tests (Shelton & Tiedje, 1984; Hansen et al., 2004; Shanmugan & Horan, 2009).

These batch tests involve the anaerobic digestion of the biomass samples for fixed periods, during which the CH_4 production and content is recorded and analysed. This approach (as well as the use of a semi-continuously fed reactor) was used in this thesis in order to provide initial information on the expected performance of a large scale anaerobic energy recovery system.

2.12.3. Anaerobic digestion of the microalgae biomass post transesterification by-products: Study goals

Chisti (2008) suggested that the recovery of energy from the microalgae residues after biodiesel production could potentially be used to meet most of the energy demands of the microalgae cultivation and the subsequent biodiesel production processes. Based on the average

chemical composition of microalgae biomass, that study estimated theoretically that an average fuel energy of 9360 MJ/metric-t of residual microalgae biomass was potentially recoverable, in the form of CH_4 produced using the anaerobic digestion process (Chisti, 2008).

The potential improvement of the energy efficiency of the microalgae biodiesel process due to CH_4 production from microalgae residues after the lipid extraction and transesterification process was further examined by Sialve et al. (2009). That study identified factors such as the microalgae biochemical composition, nature of the cell wall, the high cellular protein content of the residues and presence of sodium in marine microalgae species, which potentially limit energy recovery from this feedstock using the anaerobic conversion route.

Although other theoretical discussions and analysis on the CH_4 production from microalgae biomass residues post biodiesel production were found in the literature, experimental investigations on the CH_4 yields produced by this feedstock were lacking.

Since, the anaerobic digestion process is a capital intensive scheme, and since microalgae bioenergy research is not as well established as that for conventional energy crops, it is important to make representative practical assessment of the CH_4 yields available from the post transesterified microalgae residues. This will provide useful preliminary information for assessing the design of proposed large scale energy recovery systems using the microalgae residues. Information on the CH_4 yields could then be used in process modelling and cost analysis to determine the feasibility and practicality of such an integrated biodiesel-CH_4 generation scheme for microalgae feedstock.

Using batch and continuous laboratory scaled anaerobic digesters, this thesis aims to provide information on the extent of CH_4 recoverable from the microalgae process residues. The effects of the preceding oil extraction and/or biodiesel production scheme on the CH_4 production from the microalgae residues are also considered in this study. This was undertaken to determine whether or not any improvements or inhibitions in the biomass degradation are achievable by the use of the modified transesterification process (in-situ transesterification), compared with the microalgae residues obtained from the conventional process. In addition, the potential for improving the CH_4 yields via the co-digestion of the microalgae residues with the glycerol produced from the transesterification process is also assessed in this thesis.

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3. CULTIVATION AND CHARACTERISATION OF THE CHLORELLA SP.

3.1. Introduction

3.1.1. The Representative Microalgae Species (Chlorella sp.)

The microalgae *Chlorella* was chosen as the representative species for this work. This was primarily due to the wealth of documented studies detailing the biology, biochemistry, cultivation and industrial applications of this specific genus compared with other microalgae species. *Chlorella* is reported to be the most commercially exploited and marketed microalgae species (Richmond, 1990). Currently 2000 t (dry weight) of *Chlorella* are produced per year globally, principally for use in the health food, aquaculture and cosmetic industries (Spolaore et al., 2006; Apt & Behrens, 1999). Knowledge of the large scale production of *Chlorella* biomass amassed from existing industries is thus expected to play a key role in expanding its mass production for biofuel use. Furthermore, this specie was selected because *Chlorella* has been the principal microalgae species utilised for most bioenergy demonstrations described in the literature, especially for research in biodiesel and methane production, as reported earlier in Chapter 2.

Chlorella is reported to frequently dominate outdoor freshwater cultures as a weed species, even when attempts are made to grow other species (Goldman, 1980). The use of this species as an oil bearing feedstock for this study was also intended to be representative of microalgae biomass that would be cultivated in non-sterile open growth systems, as expected for low-cost biomass production facilities. As described earlier in Chapter 1, it is expected that to improve the practicality and economics of large scale microalgae production systems, it would be integrated with waste water treatment facilities. This is to provide the required nutrients for the biomass growth cheaply. In addition, to optimise the photosynthetic process, the cultivation units could also utilise flue gas carbon dioxide (CO_2) from industries. Such an integrated facility would help to address water remediation and CO_2 emission problems. Surveys carried out on algae populations of waste oxidations ponds by Silva and Papenfuss (1953) and Palmer (1974) showed *Chlorella* to be one of the most abundant and frequently occurring microalgae species in waste oxidation ponds.

Furthermore, the fixation of flue gas CO_2 by *Chlorella sp* has been extensively described in the literature, with this genus considered to be an ideal candidate for the biological capture of CO_2 (Maeda et al., 1995; Doucha et al., 2005; Keffer & Kleinheinz, 2002). The large scale production of microalgae biomass with a large fraction of *Chlorella sp* appears to be feasible using the proposed waste treatment-biomass production facilities and CO_2 emissions. This reinforced the selection of *Chlorella* for this study since it was assumed that the results obtained would be representative of microalgae biomass cultivated in the proposed cheaply operated, unsterilized large scale systems, integrated with waste treatment and carbon fixation infrastructures.

The genus *Chlorella* is favoured in microalgae research in part because it is reported to be cosmopolitan, i.e. distributed globally (Tomaselli, 2006), and also due to its rapid growth rate (cell division every 2 h) (VanDemark & Batzing, 1987). The cells are usually small (i.e. 5-10 μm for *Chlorella vulgaris* (Scragg et al., 2003)), with spherical, unicellular, non-motile cells having a thin cell wall and cup shaped chloroplasts (Tomaselli, 2006). *Chlorella* does not produce zoospores, but reproduces by the formation of autospores (daughter cells, 4-8-16) having a similar shape to that of the parent cell (Tomaselli, 2006). The autospores are released via a collapse of the parent cell wall, after which they can then germinate into vegetative cells (VanDemark & Batzing, 1987).

Regarding culture nutrition, *Chlorella* cultivation in autotrophic, heterotrophic and mixotrophic conditions have been reported in the literature (Grobbelaar, 2006; Lee et al., 1996; Miao &Wu, 2006; Becker, 1994; Li et al., 2007). Under optimum growth conditions the relatively high lipid content reported for this species (as shown in Table 2.3) suits its proposed use, making *Chlorella* a suitable microalgae candidate for biodiesel production. It must, however, be noted that the figures represented in Table 2.3 are only estimates, with the actual chemical composition of the microalgae cells highly dependent on the environmental parameters. For example, significant increases in the lipid content of various *Chlorella* strains have been obtained under cultivation in a low nitrogen medium, with a corresponding reduction in the carbohydrate and/or protein contents (Illman et al., 2000; Scragg et al., 2002; Piorreck et al., 1984; Spoehr & Milner, 1948).

The methods used in the cultivation, harvesting and storage of the *Chlorella* biomass used in this thesis, and its characterisation and the determination of its oil content, are covered in this chapter. The purpose of this chapter is to examine:

- Basic information on the microalgae used in this study.

– The suitability of the selected microalgae species for biodiesel production, and how it compares with reports in the literature.

– The reasons behind the choice of the inorganic catalysts used for the transesterification of the microalgae lipid contents i.e. based on the free fatty acid content of the *Chlorella* oil.

3.2. Materials and Methods

3.2.1. *Chlorella* biomass source

The *Chlorella sp.* cultures were obtained as streaked agar plates from the algal culture collection of the Allan Herbarium, Landcare Research, Nelson, New Zealand as shown in Figure 3.1. The microalgae strain was isolated from lawn soil in Lincoln, NZ[7]. A phylogenetic analysis via sequencing of the *Chlorella* gene rbcL (ribulose-1, 5-biphosphate carboxylase) revealed that the strain used is closely related to *Chlorella vulgaris*[8].

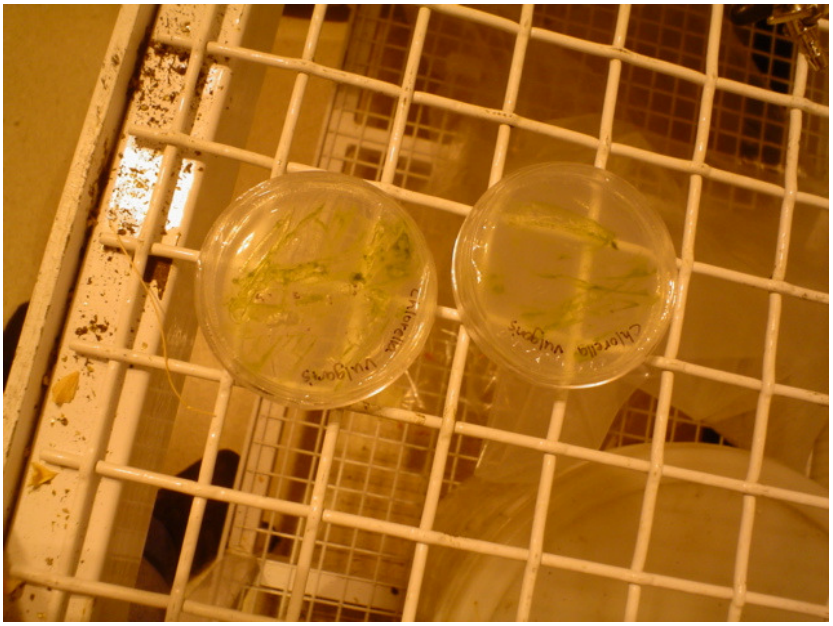

Figure 3.1. Streaked agar plates containing the *Chlorella sp* used in this study (× 0.4).

[7] Personal communication with Dr. Phil Novis, Landcare Research, New Zealand.

[8] The rbcL sequence of the *Chlorella* specie used in this study is presented in Appendix B.

3.2.2. Microalgae cultivation

The microalgae biomass was grown in batch cultures using BG 11 medium. A list of the chemical reagents and their concentrations in the growth medium is given in Table 3.1. All the reagents used in the preparation of the growth medium were of A.C.S. grade[9].

Table 3.1. Growth media reagents and concentrations.

Nutrient source	Concentration
$NaNO_3$	1.49 g l^{-1}
$MgSO_4$	74.9 mg l^{-1}
$CaCl_2.2H_2O$	36.0 mg l^{-1}
Citric acid	6.0 mg l^{-1}
Na-EDTA (pH 8.0, 0.25M)	11.2 μl l^{-1}
H_3BO_3	2.86 mg l^{-1}
$MnCl_2.4H_2O$	1.81 mg l^{-1}
$ZnSO_4.7H_2O$	0.222 mg l^{-1}
$Na_2MoO_4.2H_2O$	0.39 mg l^{-1}
$CuSO_4.5H_2O$	79.0 μg l^{-1}
$Co(NO_3)_2.6H_2O$	49.4 μg l^{-1}
1000x Fe ammonium citrate	1 ml l^{-1}
0.19M Na_2CO_3	1 ml l^{-1}
0.175M K_2HPO_4	1 ml l^{-1}

[9] The A.C.S. grade designates a high quality chemical for laboratory use, meaning the reagent meets all of the specifications of the American Chemical Society.

The growth media reagents listed in Table 3.1 were obtained from the collection of the Photosystem II lab, Biochemistry Dept., University of Otago, prepared from chemicals sourced from different suppliers. The cultures of the *Chlorella* biomass were grown autotropically at 25 ± 1°C in 5 l flasks in an environmental growth chamber with a light intensity of 80.15 μmol m^{-2} s^{-1} (LI-COR Photometer, Model LI-189). The light was provided by fluorescent tubes, with a 16 h/day photoperiod.

Continuous aeration and agitation of the batch cultures reactors was achieved by bubbling with air. The microalgae biomass was harvested after the stationary growth phase (8 days) observed for the *Chlorella* growth. The details, methods and results for the determination of the microalgae growth curve are presented in Appendix C.

3.2.3. Harvesting and storage

The microalgae cells were harvested by centrifugation using a Beckman-Avanti centrifuge J-25 operated at 2147 g for 8 min. The supernatant liquid was decanted from the tube leaving the *Chlorella* precipitate. The microalgae biomass was then collected in a container and stored in a deep freezer at -19°C to avoid any possible oxidation.

3.2.4. Characterisation of the microalgae biomass

To determine the size range of the microalgae cells used in this study, samples of the *Chlorella* cultures were extracted from the cultivation vessels prior to centrifugation and examined with a microscope equipped with an eyepiece micrometer.

Elemental analysis of the microalgae biomass was carried out to determine the percentage composition of carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and sulphur (S) in the *Chlorella* sample. The elemental composition enabled the empirical chemical formula, and the higher heating value (HHV), MJ/kg, of the microalgae biomass to be estimated.

The elemental analysis of the C, H, N and S was performed using a Carlo Erba EA 1108 Elemental Analyser. This analysis was based on the complete oxidation of the sample to convert all of the compounds in the biomass samples to combustion products. The harvested microalgae biomass was initially dried to a constant weight using a forced draft oven at 105°C for 12 h. The

dried samples were then combusted at 1020°C in the analyser containing a catalyst (copper and tungstic oxide). Helium, temporarily enriched with oxygen, was used as the carrier gas. These conditions ensured the complete oxidation of all the biomass substrates. The gas mixture was then passed over the catalyst layer and also through copper membranes, to aid the removal of excess oxygen and facilitate the reduction of nitrogen oxides to nitrogen. The resulting mixture was directed to a chromatographic column where the components CO_2, water (H_2O), sulphur dioxide (SO_4) and N_2 were separated and detected using a thermal conductivity detector which produced an output signal proportional to the concentration of the individual components of the mixture. Using this information, the elemental percentages were calculated based on the initial dry weight of the sample.

The percentage composition of oxygen in the biomass was also determined using the Carlo Erba analyser using a different type of combustion tube, which contained nickel-plated carbon and quartz turnings.

All elemental analyses were carried out at the Campbell Micro-analytical Laboratory, Department of Chemistry, University of Otago, NZ.

3.2.5. Biomass oil content determination

Freshly centrifuged *Chlorella* precipitate was dried to a constant weight in a forced draft oven at a temperature of 80°C for 8 h. This drying action was carried out to reduce the volume of the solvent used, and also any difficulties associated with handling the biomass (Nagle & Lemke, 1990). The dried biomass samples were pulverised in a mortar to reduce the average size. This action was also carried out to increase the active particle surface area on which the extraction solvents would act. The oil content of the dried microalgae was determined in triplicates using two types of extraction solvents: (i) butanol extraction and (ii) chloroform-methanol mixture.

3.2.5.1. The butanol method

Extraction using 1-butanol was carried out using the methods described by Nagle & Lemke (1990). This involved mixing 50.0 ± 0.1 g of the pulverised microalgae biomass with 150 g (188 ml) of 1-butanol (HPLC grade ≥99.7% purity, Sigma-Aldrich) in a sealed glass flask. The mixture was

constantly agitated, with the extraction maintained for 90 minutes at 90°C with a hotplate and stirring system (IKA RT 5 Power, IKA-Werke GmBH).

The biomass residues were then filtered from the solvent-oil mixture via vacuum filtration on a Büchner funnel through Advantec No.5C ash-less quantitative filter paper. The oil content in the mixture was further extracted from the butanol-oil mixture using hexane (reagent grade ≥95% purity, Sigma-Aldrich).The hexane layer containing the extracted lipids was separated using a separation funnel.

The oil product was obtained by drying to a constant weight in a vacuum. The microalgae oil was weighed using an analytical balance (Mettler Toledo), with an accuracy of ± 0.1 mg.

3.2.5.2. The chloroform-methanol method

The chloroform-methanol oil extraction was carried out as described by Zhu et al. (2002), which was adapted from the Bligh and Dyer (1959) method. This involved blending 50.0 ± 0.1 g of the ground biomass with 150 ml of the chloroform/methanol mixture (2:1,chloroform to methanol, v/v) (chloroform: ≥ 99.8% purity, Merck KGaA; and methanol: ≥ 99.95% purity, Romil) for 30 min, with the extraction temperature maintained at 35°C in continuously stirred flasks. The resulting mixture was cooled, and separation of the solvent phase was achieved by centrifuging the solution at 773 g for 15 min. Filtration of the solid residues was carried out, as described in section 3.2.5.1. The microalgae biomass residues precipitate were then re-extracted twice with the chloroform/methanol mixture for 5 min each. The extracted *Chlorella* lipids were obtained by evaporating the pooled solvent phases at 24°C with an absolute pressure of 13.3 kPa. A recovery of the extraction solvents was not considered in this study, with the chloroform/methanol mixture evaporate vented off in a fume cupboard. The extracted oil was then further purified with hexane. The oil extract was then dried to a constant weight in a vacuum. The hexane was not recovered for re-use and was vented off in a fume cupboard. The oil product was weighed and reported as the total lipid content per dry microalgae biomass.

The oil content of the microalgae biomass (on a basis of biomass dry weight, d.w.) was estimated using Eq. 3.1.

$$\text{Oil Content (g/g biomass)} = \text{Mass of the extracted oil} \Big/ \text{Mass (d.w.) of the microalgae biomass} \quad (3.1)$$

3.2.6. Characterisation of the microalgae oil

3.2.6.1. Specific gravity of the oil

The specific gravity (SG) of the extracted microalgae oil was determined at 25°C using pycnometers (specific gravity bottles) as described in the AOCS official method Cc10a-25 (AOCS, 2005). The micro-pycnometers (1 ml) used were fabricated by the Glass Blowing Unit, Division of Sciences, University of Otago, Dunedin NZ. Unlike the use of 25 or 50 ml specific gravity bottles commonly applied for SG determination, this volume was used in this study due to the small quantities of microalgae oil available for analysis.

Clean and dry specific gravity bottles (1 ml) were weighed to obtain their empty weight. Using a pipette, the pycnometer was carefully filled with recently distilled water, ensuring the entrapment of air bubbles was prevented. The stopper was inserted and the bottles were completely immersed in a thermostated water bath at 25.0 ± 0.1°C for the duration of 1 h. The bottles were removed from the bath and the external surface wiped dry. The pycnometer was then weighed with a balance (accuracy of ± 0.1 mg), with the water weight determined by subtracting the weight of the empty bottle. The process was repeated using the dried microalgae oil. The experiments were carried out in triplicates.

The SG of the microalgae oil at 25°C is calculated using Eq. 3.2.

$$\text{Oil SG at } 25°\text{C} = \frac{(\text{Mass, g of bottle and oil}) - (\text{Mass, g of bottle})}{\text{Mass, g of water}} \qquad (3.2)$$

The thermal expansion of the pycnometer was not expected to contribute errors to the specific gravity determination. This is due to the fact that the experiments were conducted using the same temperatures. The errors that could be associated with the pycnometer expansion were not considered in the calculation of the uncertainties of the measured SG of the *Chlorella* oil. Only the errors associated with the mass measurements of the pycnometer contents were used in the estimation of the uncertainties. The estimation of the uncertainties associated with the oil content determination and SG measurement are presented in Appendix I.

3.2.6.2. Fatty acid composition and molar mass of microalgae oil

The fatty acid composition of the extracted *Chlorella* oil was determined by gas chromatography (GC). This was carried out to provide information on the concentration of the component fatty acids, and an estimation of the molecular mass of the oil sample. The fatty acid profile of the triglycerides and that of the free fatty acids of the microalgae oil must be accounted for.

To achieve this, the triglycerides in the microalgae oil were initially hydrolysed to their constituent fatty acid salts. The cleavage of the triglyceride ester bonds was achieved under alkaline conditions. An esterification reaction was then used to convert the produced fatty acid salts and microalgae free fatty acids to fatty acid methyl esters (with methanol as the reacting alcohol). The concentration of the methyl esters obtained was representative of the fatty acid profile of the microalgae oil.

The fatty acid methyl esters for GC analysis were prepared via saponification followed by boron trifluoride/methanol esterification using methods described by Van Wijngaarden (1967). This involved mixing 150 mg of the dried microalgae oil with 2 ml of 0.5 N sodium methoxide (A.C.S. reagent, Fluka) in a 50 ml round bottom flask connected to a Graham condenser. The mixture was boiled under total reflux in a water bath for 3 min till the solution was completely homogenous. Through the condenser, 2 ml of boron trifluoride (BF_3)-methanol reagent[10] (containing 14% BF_3 in methanol, Aldrich) was added to the solution, which was boiled further for 2 min. Heptane (HPLC grade, 99% purity, Merck KGaA) (3 ml) was then introduced to the flask and boiling continued for 1 min. Saturated sodium chloride (NaCl) in methanol solution was added to bring the liquid level of the solution to the neck of the round bottom flask. Then 1 ml of the upper heptane layer was carefully pipetted into a stoppered glass tube. Any trace of water present in the solution was eliminated by the addition of anhydrous sodium sulphate. The solution was then stored in the freezer at -4°C, pending direct injection into the GC column.

The samples were run on an Agilent 6890A GC system, equipped with a 7683 series auto sampler and a flame ionisation detector (FID). Separation was performed on a BPX70® column (0.32 mm ID, 50 m length and 0.25 μm film thickness, SGE Analytical Science) with hydrogen as

[10] The BF_3-methanol reagent was stored in a refrigerator at 3±1°C.

the carrier gas (at a flow rate of 2 ml/min). The temperatures were as follows: injector, 250°C; detector, 250°C; oven, 205°C (programmed to start at 35°C, held at this temperature for 5 min and heated at a rate of 2.5°C/min to 205°C).

The reference FAME standard was FAMQ005 by AccuStandard (USA), diluted 1:10 in dichloromethane. The FAME reference (FAMQ005) was an external standard which were run on the GC using the same operating conditions as that for the FAME samples. This action was carried out to minimize any errors by ensuring the reference standard and FAME were analysed using the same GC conditions. A comprehensive list of the constituent FAME which makes up the reference standard is presented in Appendix J. The resulting data was then analysed using Chemstation® software by Agilent.

The percentage mass content of the fatty acids in the sample was calculated from the chromatograph using (Eq. 3.3):

$$X(\%) = \frac{A_{FA}}{(\sum A_{FA}) - A_{FAMQ}} \qquad (3.3)$$

where, X = the mass fraction of the fatty acid in the sample (%)

A_{FA} = the chromatograph peak area of the FAME

$\sum A_{FA}$ = the total peak area of FAME (C12:0-C24:0)

A_{FAMQ} = the peak area of the standard (FAMQ005)

The molar mass of the microalgae oil was then estimated using the fatty acid composition profile. The methods used here will be presented later in section 3.3.4.

3.2.6.3. Acid value and free fatty acid content

The acid value, and the free fatty acid content of the microalgae oil, was titrimetrically determined using the AOCS official method, Cd 3d-63 (AOCS, 2005). The acid value is the number of mg of KOH required to neutralise the free fatty acids in 1 g of the oil sample. These experiments were carried out in triplicates.

Phenolphthalein solution (1% phenolphthalein, 95% ethanol and 4% water), 2 ml, was added to 125 ml of a solvent mixture comprised of reagent grade isopropyl alcohol and toluene (1:1, v/v). The solvent mixture was neutralised with reagent grade 0.1N potassium hydroxide, KOH (Fluka chemicals) to a faint and permanent pink colour. 10.0 ± 0.1 g of the extracted *Chlorella* oil was weighed into a 250 ml Erlenmeyer flask. The solvent mixture (125 ml) was added to the flask containing the oil and the flask was agitated by magnetic stirrers until the oil was completely dissolved. The oil-solvent mixture was titrated with 0.1N KOH till the first permanent pink colour (of the same intensity with that of the neutralised solvent before the addition of the oil) was obtained. A blank titration was also carried out using 125 ml of the neutralised solvent mixture.

The titre values were recorded and the acid value was calculated using Eq. 3.4 (AOCS, 2005).

$$\text{Acid value, mg KOH/g of oil} = \frac{(\text{A - B}) \times 0.1 \times 56.1}{\text{W}} \qquad (3.4)$$

where, A = volume, ml of 0.1N KOH used in titration

B = volume, ml of the standard alkali used in titrating blank

0.1 = normality (N) of KOH (g equivalent/l)

56.1 = molar mass of KOH (g/mol)

W = mass, g of microalgae oil

The free fatty acids content (% FFA) was then calculated on the basis of the dry weight of the microalgae oil using Eq. 3.5 (AOCS, 2005):

$$\text{Free fatty acids content, \% FFA (on the basis of dry oil weight)} = \frac{\text{ml} \times 0.1 \times x \times 100}{10 \times 1000} \qquad (3.5)$$

Where, ml = is the titre value (A-B) in Eq. 3.4

10 = weight of microalgae oil (g)

0.1= normality, N of the KOH solution (g equivalent/l)

x = average molar mass of constituent fatty acids (g/mol)

3.3. Results and Discussions

3.3.1. Characterisation of the microalgae biomass

Using the graduated micrometer eyepiece, the *Chlorella* cells were determined to exhibit a size range of 2-8 μm. This size range was estimated from 13 different observations not all shown in this chapter. The cell magnification was performed using a 100× objective lens magnification (with one division= 1μm).

A magnified view of the cultivated *Chlorella* cells is shown in Figure 3.2.

The size range for the *Chlorella* cells in this study is similar to that (5-10 μm) obtained by Scragg et al. (2003) for *Chlorella vulgaris* samples.

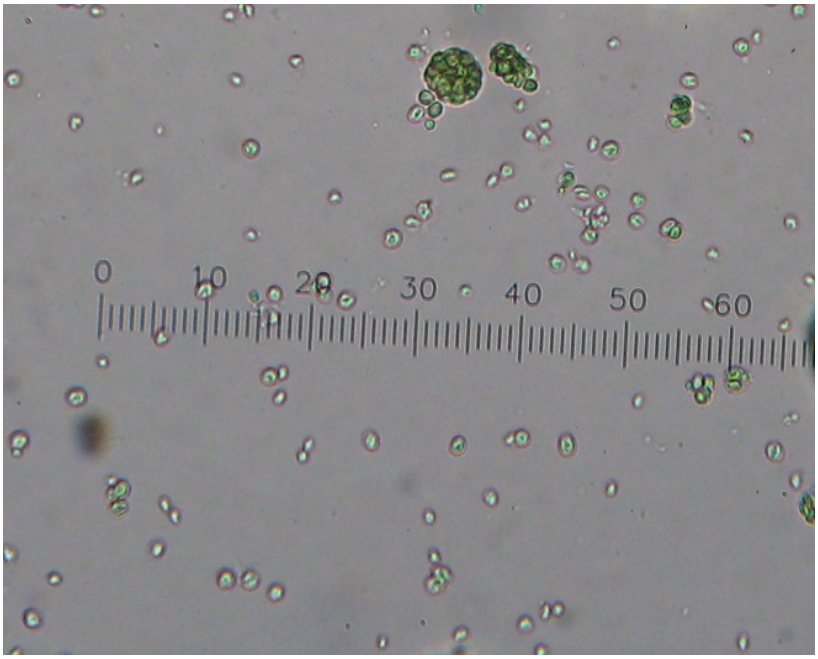

Figure 3.2. Magnified *Chlorella* cells used in this study. 1 division=1μm.

The results of the elemental analysis, and the empirical formula of the *Chlorella* biomass, are shown in Table 3.2. The percentage (%) compositions of the major elements in the microalgae biomass is represented on the basis of the dried and ash free microalgae biomass.

Table 3.2. Elemental composition and empirical formula of the *Chlorella* biomass samples

Parameter	Mass % composition of *Chlorella* biomass (ash free d.w.)
% Carbon, C	53.82
% Hydrogen, H	8.48
% Nitrogen, N	7.25
% Oxygen, O	29.95
% Sulphur, S	0.50
Empirical formula	$C_{287}H_{539}N_{33}O_{120}S$

Using the empirical formula shown in Table 3.2, the HHV (MJ/kg) of the microalgae biomass was estimated using the thermo-chemical equation for the estimation of the combustion enthalpy, put forward by Meraz et al. (2003) (Eq. 3.6).The equation is based on the use of the percentage mass composition of the biomass.

$$\text{HHV, MJ/kg} = \left(1 - \left(x/100\right)\right)\left(\begin{array}{l} -0.3708\,(\%\text{C}) - 1.1124\,(\%\text{H}) \\ +0.1391\,(\%\text{O}) - 0.3178\,(\%\text{N}) - 0.1391\,(\%\text{S}) \end{array}\right) \quad (3.6)$$

where, x is the biomass water content.

The HHV the microalgae biomass was calculated to be 26.09 MJ/kg. This biomass combustion enthalpy was taken to represent the gross energy (heat) recoverable from this feedstock.

The HHV of the *Chlorella* biomass used in this study was higher than the value, 25.1 MJ/kg, reported by Demirbaş (2009) for *Chlorella prothecoides* (both on the basis of a dry and ash free biomass).

Furthermore, the HHV determined for the *Chlorella* biomass used in this study was seen to be higher than that for dry woody biomass i.e. beech and spruce wood samples (17-20 MJ/kg) and for some lignite and brown coal samples (mined in Turkey) (19-25 MJ/kg), as reported by Bilgen et al. (2004).

3.3.2. Oil content of the microalgae biomass

The extracted oil, which represents the transesterifiable content of the microalgae oil, was measured using two types of extraction media: (a) 1-butanol, and (b) a chloroform-methanol mixture (2:1, v/v).

The selection of 1-butanol was based on previous work on lipid extraction and biodiesel production from *Chlorella* biomass by Nagle & Lemke (1990). That study showed that the use of 1-butanol provided the highest net extraction efficiency (90%) when compared with ethanol (73%) and with a 40% hexane/60% 2-propanol mixture by volume (78%).

The chloroform-methanol mixture was selected as the second test solvent, based on previous demonstrations of its application as an excellent solvent for the extraction of intracellular lipids (Folch et al., 1957; Bligh & Dyer, 1959; Zhu et al., 2002).

With the culture conditions used in this study, the neutral lipid contents (g microalgae oil/g of *Chlorella* biomass) and the measured standard error are shown in Table 3.3 for the extraction methods used.

It was observed that similar oil yields were obtained for with the use of 1-butanol and the chloroform –methanol extraction solvents for microalgae biomass.

Table 3.3. Oil content of the *Chlorella* biomass

	Butanol extraction	Chloroform-methanol extraction
Microalgae oil content (g/g dry microalgae biomass)	0.2754 ± 0.0017	0.2654 ± 0.0103

The oil content of the *Chlorella* biomass used in this study was higher than the estimates reported for *Chlorella vulgaris* of 14-22% of the microalgae dry matter, reported by Becker (1994). However, it was lower than the lipid content of dry *Chlorella* (63.4% and 77.1 %) reported by Milner (1948).

The reported neutral lipid contents in Table 3.3 refers to the extractable microalgae oil obtained using the extraction solvents investigated, and may not represent the entire lipid content available in the dried *Chlorella* samples.

It must, however, be noted that the biomass oil content obtained in this study is not only influenced by the considered microalgae specie, but is also highly dependent on the specific growth conditions. As mentioned in section 3.1, increases in the oil content of the biomass, and hence increases in the biodiesel production potential of the microalgae feedstock, could be achieved by manipulating the microalgae culture conditions, such as the culture nutrients and the light intensity (Hu et al., 2008).

The reported *Chlorella* oil content was representative of the specific culture conditions used in this study. In light of the primary goals of this thesis, manipulation of the cultivation process to increase the biomass oil content was not explored.

3.3.3. Specific gravity of the microalgae oil

The SG of the microalgae oil, which is the ratio of the weight of a unit volume of the microalgae oil at 25°C to the weight of an equal volume of water at the same temperature, was found to be 0.914 ± 0.001. This physical property of the extracted oil was used in Chapters 4 and 5 to monitor the progress of the conversion of the microalgae oil to biodiesel.

3.3.4. Fatty acid composition and molecular mass of extracted oil

Using the methods described in section 3.2.6.2, the conversion of the constituent triglycerides and free fatty acids of the microalgae oil to their respective FAME was achieved. The percentage mass composition of the principal fatty acids in the extracted microalgae oil was then estimated via GC analysis. GC profiles showing the microalgae FAME and standard is shown in Appendix K.

The result of the GC analysis, showing the percentage mass composition of the fatty acids in the *Chlorella* biomass oil, is shown in Table 3.4. The fatty acid profile is representative of the total triglyceride and free fatty acid content of the extracted *Chlorella* oil.

Table 3.4. Fatty acid composition of the *Chlorella* biomass

Fatty acid	Molecular mass (g/mol) (MM_{FA})	Mass % in sample	Molecular mass contribution (g/mol) (MM_c)
C16:0 (palmitic acid)	256.42	4.37	11.21
C16:1 (palmitoleic acid)	254.41	0.44	1.12
C18:1 (oleic acid)	282.46	61.81	174.59
C18:2 (linoleic acid)	280.45	19.94	55.92
C18:3 (linolenic acid)	278.43	12.22	34.02
C20:1(Eicosenoic acid)	310.52	1.22	3.79
Average molecular mass of constituent fatty acids (MM_{FA}):			280.65

The *Chlorella* oil was seen to be mainly composed of unsaturated fatty acids, with 62% (w/w)of the extracted oil composed of the mono-unsaturated oleic acid (C18:1).

The fatty acid profile obtained for the *Chlorella* biomass is similar to the typical ranges reported by Hu et al. (2008) for microalgae species of the division, Chlorophyta. The fatty acid profile obtained was, however, different from those reported by Demirbaş (2009) for *Chlorella* protothecoides, which was composed of 11% saturates, 24% monounsaturates and 63% polyunsaturates (w/w).

Compared to terrestrial plant sourced oils, the fatty acid profile of the *Chlorella* biomass in this study was observed to be similar to those of conventional vegetable sourced biomass (Srivastava & Prasad, 2000). However in contrast to oils from terrestrial plants, the fatty acid profile of the extracted *Chlorella* oil was seen to contain a very long fatty acid (C20:1), which is normally not found in higher plant oils. This was reported to be characteristic of photosynthetic microalgae (Hu et al., 2008).

The results for the fatty acid composition were further used to determine the average molecular mass of the *Chlorella* oil.

Since the neutral fraction of the *Chlorella* oil consists of different fatty acids, their contributions to the overall molecular mass of the microalgae lipid was used (as seen in the 'MM_c' column in Table 3.4) to estimate the mean molecular mass of the constituent lipid fatty acids (MM_{FA}).

The formation of the triglyceride molecule is facilitated by the combination of three fatty acid molecules and a molecule of glycerol, with the condensation of three molecules of water. The average molecular mass of the *Chlorella* microalgae oil (MM_{oil}) can thus be calculated using Eq. 3.7.

$$MM_{oil} = \left[3MM_{FA} + MM_{glycerol} \right] - 3MM_{water} \quad (3.7)$$

where, $MM_{glycerol}$ and MM_{water} represent the molar masses of glycerol and water respectively.

The average molecular weight of the *Chlorella* lipid was calculated to be 880 g/mol.

3.3.5. Acid value and free fatty acid content

The acid value determination for oils is important, since it provides information on the state of rancidity of the biomass oils (Van Gerpen et al., 2004). The acid value can be further used to estimate the free fatty acid content of the biomass oils.

With the biomass oils considered for the production of biodiesel using homogenous catalysts, the determination of the acid value and % FFA becomes increasingly important. This is due to the fact that the efficiencies and choice of suitable chemical transesterification catalysts is dependent on the % FFA of the biomass oil, as was reported in section 2.4.2.

With the results from the titration described in section 3.2.6.3, and using Eq. 3.4, the acid value of the microalgae oil was determined to be 10.21 ± 0.34 mg KOH/g *Chlorella* oil.

Using the estimated molar mass of 280.65 for the constituent fatty acids of the *Chlorella* biomass oil (as shown in Table 3.4) and the titre value in Eq. 3.4, the % FFA of the microalgae oil was determined to be 5.11 ± 0.17% (on the basis of the oil weight).

The calculated acid value and % FFA were higher than the values, 8.97 mg KOH/g oil and 2.1% (oil weight basis), reported for *Chlorella protothecoides* oil (Demirbaş, 2009; Miao & Wu, 2006).

In section 2.2 it was noted that for an efficient conversion of the biomass oils to biodiesel using alkaline catalysts, the acid value and FFA % of the feedstock oils should be below 1 mg KOH/g oil and 0.06% (w/w, on oil weight basis). Because the lipids extracted here do not satisfy these conditions, and because this thesis deals solely with the use of an inorganic chemical catalyst, acid catalysis was considered for the production of biodiesel from *Chlorella* oil in this study.

3.4. Chapter Conclusion

Chlorella sp. was selected for analysis in this study, due to the current widespread use of this microalgae genus in research and commercial applications.

Chlorella samples found locally in New Zealand were obtained and cultivated using BG 11 media. The microalgae samples were shown to be suitable for the proposed biodiesel production scheme, since it displayed a considerable content of neutral lipids i.e. 27.5% of the dry weight of the microalgae biomass.

The extracted lipids were similar to that of terrestrial biomass oils, with a fatty acid profile high in polyunsaturated fatty acids. The use of acid catalysts for the transesterification process was further proposed and will be applied solely in the following chapters (Chapters 4 and 5). This was due the high acid value and free fatty acid content (10.21 mg KOH/g *Chlorella* oil and 5.11% (oil weight basis) obtained respectively) for the *Chlorella* lipids.

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4. INVESTIGATING THE VARIABLES AFFECTING THE IN-SITU TRANSESTERIFICATION OF MICROALGAE LIPIDS.

4.1. Introduction: The In-situ Transesterification of Microalgae Lipids

As highlighted in section 2.10, the in-situ transesterification process was applied in this study for the direct conversion of the neutral lipids in the microalgae biomass to biodiesel. Biodiesel production using this process is facilitated by the reacting alcohol functioning simultaneously as both an extraction and transesterification reagent. The lipid extraction step required by the conventional biodiesel production method is eliminated in the in-situ method. The use of the in-situ method could therefore aid in process simplification, by reducing the number of process units compared with the conventional lipid extraction and transesterification process.

The use of the in-situ transesterification method for the production of biodiesel from other biomass has previously been detailed in the literature, as highlighted in section 2.10, but no investigations on its use for microalgae biomass was found.

This chapter reports on experimental investigations carried out on the application of the in-situ transesterification method for fatty acid methyl ester (FAME) production using *Chlorella* biomass. Some of the results in this chapter were published in the paper "Variables affecting the in-situ transesterification of microalgae (Ehimen et al., 2010)" presented in Appendix A (CD-ROM).

The primary motivation for the use of the in-situ transesterification process was its possible application to achieve a reduction in the overall energetic requirement of microalgae biodiesel production (compared with the conventional process). Potentially important reaction parameters that might influence the process costs were considered.

Accordingly, this chapter investigates the influences of the following parameters on the in-situ transesterification of microalgae lipids:

(i) the reacting alcohol volume (material costs input scale with alcohol volume),

(ii) the temperature (process heating requirement increases with temperature),

(iii) the reaction time (throughput scales inversely with the reaction time), and

(iv) process mixing (energy requirement for reaction agitation).

The effect of the moisture content of the microalgae biomass on the in-situ conversion process was also studied because this parameter affects the energy (and economic) requirement of the biomass production process, especially in regions where drying cannot be achieved using low cost sun drying or other solar heating methods. This is especially important since biomass drying is claimed to be one of the most important economic steps in the microalgae production process (Becker, 1994; Chen et al., 2009). With the microalgae biomass containing up to 80% moisture content after the harvesting process (i.e. via centrifugation) this investigation set out to evaluate the extent to which moisture containing microalgae biomass could be used in the in-situ transesterification process. This was carried out to examine potential reductions in the microalgae biomass drying requirement.

Three parameters were used in this chapter to monitor and quantify the conversion of the *Chlorella* biomass oils to FAME with the application of the different reaction variables. These are:

- the specific gravity of the purified biodiesel product,
- the mass of the purified biodiesel produced per biomass input i.e. biodiesel yield (g biodiesel/g microalgae biomass),
- the percentage conversion of the microalgae oil to FAME per unit time (i.e. conversion efficiency).

The use of these parameters in this study will be treated in more detail in the subsequent sections.

The conventional lipid extraction and transesterification of the *Chlorella* oil is also examined in this chapter to provide a basis for comparison of the conventional route with the in-situ biodiesel production method.

4.2. Materials and Methods

4.2.1. The in-situ transesterification set-up

The in-situ transesterification process was carried out using *Chlorella* biomass, and various volumes of the reacting alcohol (methanol) containing sulphuric acid (H_2SO_4) as the transesterification catalyst. Due to its relatively lower cost (compared to other alcohols) methanol was chosen as the reacting alcohol in this study. With the transesterification alcohol being an important reaction variable, the use of other reacting alcohols would lead to different results as presented in this study.

The choice of acidic catalysts was based on the fact that the *Chlorella* oil used in this study exhibited a high acid value and free fatty acid (FFA) content, as previously described in section 3.3.5. H_2SO_4 was selected as the acid catalyst, since it was shown by Al-Widyan & Al-Shyoukh (2002) that for oil feedstocks with high FFA contents, improved alkyl ester conversions were obtained using H_2SO_4 as a transesterification catalyst compared with hydrochloric acid (HCl). The use of H_2SO_4 as a catalyst for the conventional transesterification of microalgae oils was also demonstrated by Miao & Wu (2006). A fixed sulphuric acid quantity of 0.04 mol, which relates to a 100% weight ratio of the acid catalyst to microalgae oil content, was used throughout this study.

Except for the investigation of the influence of the biomass moisture content on the in-situ transesterification process, the *Chlorella* biomass used for the experimental study was dried to a constant weight using the methods described in section 3.2.5.

An overview of the major in-situ transesterification reaction, and product purification steps, used in this investigation is shown in Figure 4.1(a-e).

The methanol (reagent grade 99.95% purity, Romil) and acid catalyst H_2SO_4 (2.2 ml) (reagent grade 98% purity, Merck KGaA) were initially pre-mixed and then introduced into the reaction vessels (consisting of 50-200 ml Pyrex glass bottles) containing 15 g of the dried *Chlorella* biomass.

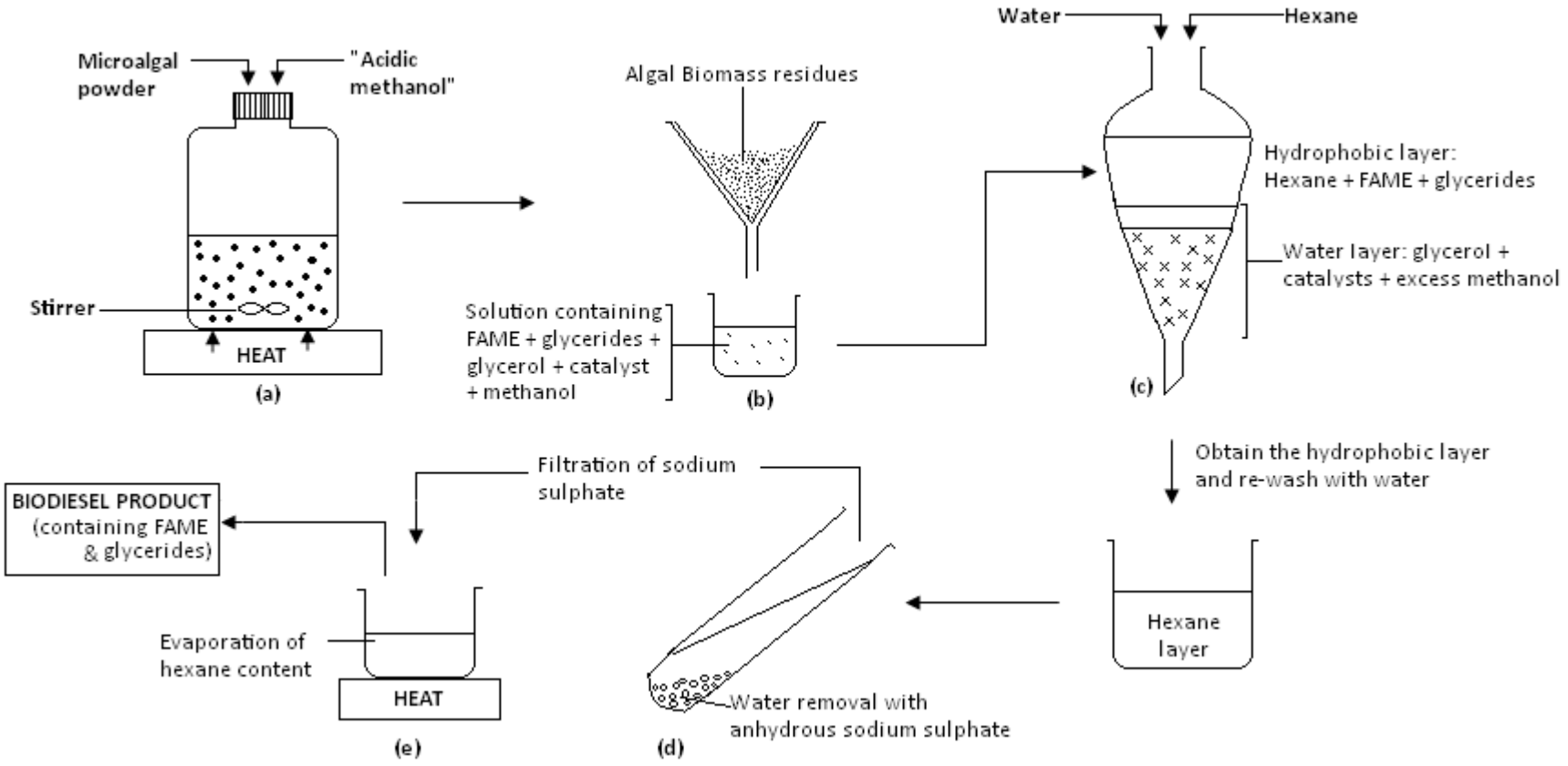

Figure 4.1 (a-e). Schematic representation of the in-situ transesterification and methyl esters purification steps used in this study.

The reaction mixtures were heated and maintained at the temperatures of interest for specified periods. The agitation (when required) of the reaction vessels was provided using Teflon covered magnetic stirrers (Figure 4.1(a)).

After the transesterification step (Figure 4.1(a)), the reaction vessel was allowed to stand for 1 h to enable its contents to settle. The reaction mixture was filtered and the residues washed twice by re-suspension in methanol (30 ml) for 10 min to recover any traces of FAME product left in the residues (Figure 4.1(b)). The filtrate was then transferred to a separating funnel where distilled water (50 ml) heated to 70°C was introduced to the filtrate via spraying using a syringe to facilitate the separation of the hydrophilic components of the mixture (Figure 4.1(c)). The lower level (containing glycerol) was then withdrawn from the separating funnel. Further extraction of the FAME and glycerides fraction was achieved by extracting the mixture three times for 15 min using 30 ml of hexane (reagent grade ≥ 95% purity, Sigma-Aldrich) (Figure 4.1(c)). The pooled hexane extracts were then washed with water (to remove left-over traces of the acidic catalyst and methanol), separated, and then dried over anhydrous sodium sulphate, Na_2SO_4 (Figure 4.1(d)).The FAME and remaining glycerides in the mixture were filtered and evaporated to obtain the biodiesel product in Figure 4.1(e). The biodiesel product refers to the mixture of the FAME and glycerides in this study. Due to the purification techniques used, a further separation of the unreacted glycerides from the biodiesel cannot be facilitated.

The glycerol co-product in the post transesterified mixture was not recovered in this study. With the application of multiple aqueous washes (i.e. for the extraction of the microalgae biodiesel), as well as the fact that some of the glycerol would entrained in the solid residue phase, the quantification of the exact glycerol produced by the reaction process would be difficult due to the losses encountered during the extraction and purification processes. Glycerol recovery was therefore not carried out in the experimental runs in this thesis. The glycerol product was therefore washed out of the biodiesel product (as described earlier) and the post extracted residues.

The FAME content of the biodiesel product was estimated using quantification methods as will be explained later in this chapter.

Triplicate experiments were carried out for each reaction parameter.

The dried purified biodiesel product was weighed using a Mettler balance (± 0.1 mg accuracy) and the result expressed as g biodiesel/g microalgae biomass. This biodiesel weight was later used to quantify the FAME yields (g FAME/g microalgae biomass) obtainable with the application of different reaction variables for the in-situ transesterification process.

The specific gravity (SG) of the dried purified biodiesel product was determined, using the same methods previously described in section 3.3.3 for the determination of the SG of microalgae oil.

The uncertainties in the measured SG for the processes were treated as described in Appendix I.

The SG of the purified biodiesel product is an important indicator in this study, being used to monitor the extent of the oil to FAME conversion process, as demonstrated previously by Al-Widyan & Al-Shyoukh (2002). With the forward reaction of the transesterification process resulting in FAME production and the process nearing completion, the SG of the purified reaction product (after the removal of the glycerol co-product) is expected to decrease until constant. This would signify an equilibrium conversion of the microalgae oil to the methyl esters.

A comparison of the SG of the untreated microalgae oil with that of the purified biodiesel product after the different reaction conditions was used to provide an easy and quick quantitative indicator of the progress of the in-situ transesterification process.

The measured SG and biodiesel yield was further applied in section 4.2.7 to quantify the percentage conversion of the microalgae oil to FAME, and the FAME yields (g FAME/g microalgae biomass) obtainable by in-situ transesterification with varied levels of the process parameters.

4.2.2. Effect of reacting alcohol volume and temperature on the in-situ transesterification process

HPLC grade methanol (99.9% purity) was used as the reacting alcohol in this study. Five levels of methanol volumes were used; 20.0, 40.0, 60.0, 80.0 and 100.0 ml for the in-situ transesterification of the microalgae biomass.

Dried *Chlorella* biomass (15 ± 0.1 g) was mixed with the various methanol volumes containing 2.2 ml of H_2SO_4 in screwed cap reaction vessels, as described in section 4.2.1. A minimum volume of 20.0 ml methanol was selected, since this was the minimum amount that was able to completely submerse 15 g of the microalgae powder.

The experiments involved heating the reaction mixtures in screwed cap vessels for 8 h, with each trial conducted at four different temperatures (23, 30, 60 and 90°C). Heating and continuous stirring of the reactors was provided by a hot plate with a magnetic stirrer. The lowest reaction temperature examined, 23°C, was selected because this was the local average temperature i.e. the process was not heated externally. To avoid temperature fluctuations with the 23°C investigations (i.e. during night time), the reaction vessels were fully immersed in a temperature controlled water bath set at the specified temperature.

Due to the expected build up of pressure in the reaction vessels when investigating temperatures greater than the normal boiling point of methanol (65°C), the experiments at 90°C were carried out in a fume cupboard with air pressure control. An air pressure setting of 300 kPa (absolute) was used to ensure the reacting mixture remained in a liquid state.

The biodiesel product at different variable levels was then obtained using the purification steps and weighed as previously highlighted in section 4.2.1, and their SGs determined as described in section 3.2.6.1.

4.2.3. Effect of reaction time on the in-situ transesterification process

At each of the four temperature levels examined in section 4.2.2, the in-situ transesterification of 15 g microalgae biomass was repeated in triplicates, with reaction times

of 0.25, 0.5, 1, 1.5, 2, 4, 8 and 12 h using a fixed reacting methanol volume of 60 ml containing 2.2 ml sulphuric acid.

This trial was carried out to provide greater insight on the progression of the transesterification process with time with respect to the reaction temperature.

The purification of the biodiesel product, the determination of the biodiesel yield and the measurement of its SG, were carried out as previously described in section 4.2.1.

4.2.4. Effect of microalgae biomass moisture content on the in-situ transesterification process

Chlorella biomass, initially dried in a draft oven at 80°C to a constant mass (after 8 h), as previously described in section 3.2.5, was designated to represent a 'dry' (0.0% moisture) sample, and was used as the basis for calculating the moisture content of the microalgae samples in this section.

To study the effect of moisture on the in-situ transesterification process, freshly harvested *Chlorella* paste, obtained after the centrifugation process (section 3.2.3), was air dried (fan aided) at 26 ± 1°C to obtain samples with biomass moisture contents of 72.5 ± 1.8, 56.0 ± 0.8, 40.9 ± 0.7, 31.7 ± 0.3, 19.5 ± 0.1 and 8.7 ± 0.1% (based on the dry sample weight). In addition, using a draft oven at 80°C, samples composed of 3.20 ± 0.04, 1.40 ± 0.01 and 0.70 ± 0.01% moisture content (dry-basis) were also obtained.

To ensure a uniform moisture content of the microalgae biomass, the samples were spread thinly on the drying plate (height < 2.0 mm) before introduction to the oven.

The in-situ transesterification process was then investigated in triplicates for different moisture levels, on the basis of a 15 g dry-weight reacting sample. For example, reacting sample weights of 25.88 ± 0.47, 23.40 ± 0.20 and 17.95 ± 0.13 g were used for the investigation of 72.5, 56.0 and 19.5% moisture levels respectively.

The in-situ process was then conducted using different reaction times (as in section 4.2.3), but up to 6 h, with the same reaction conditions (reacting methanol and catalyst) as in

section 4.2.3. The biodiesel purification and yield determination, as well as the measurement of the SG of the product, was then carried out as previously described in section 4.2.3.

4.2.5. Effect of stirring on the in-situ transesterification process

To investigate the effect of stirring on the in-situ transesterification of the microalgae biomass, the vessels containing the reacting mixture (dried *Chlorella* biomass, methanol and H_2SO_4) were subjected to four different agitation treatments: (1) continuously stirred, (2) stirred for only the first hour, (3) stirred intermittently, 1 h on and 1 h off, and (4) not stirred throughout the experiment.

The reaction stirring was carried out using Teflon covered magnetic stirrers. This involved the use of a mixing speed of 500 rpm, kept constant throughout the specified duration of the experiment. This speed was used since it was observed to facilitate a complete suspension of the particles in the reaction vessels.

For each treatment, transesterification was carried out as described earlier using 15 g dried biomass with 60 ml of methanol (containing 0.04 mol H_2SO_4). The experiments were conducted with the reaction time and temperature set at 8 h and 60°C respectively and carried out in triplicate runs.

The reaction products were then obtained after the specified period, purified, and the SGs determined as previously described.

4.2.6. The conventional transesterification of the microalgae lipids

The conventional acidic catalysed transesterification process was carried out using 5.00 ± 0.01 g of the extracted microalgae oil. This study investigated the use of three different reacting methanol volumes of 1.38 ± 0.01, 11.5 ± 0.1 and 72.35 ± 0.1 ml. H_2SO_4 (0.05 mol) was used as the reaction catalyst. The reasons for selecting these alcohol volumes will be presented in the results and discussion section of this chapter (section 4.3.6). The experiments were then conducted in triplicates.

The dried extracted oil (5 g) was weighed carefully and introduced to Erlenmeyer flasks as the reaction vessels. The various levels of methanol volumes (containing H_2SO_4) were then added to the reaction flasks. The transesterification process was then conducted with continuous stirring of the reacting mixture at 300 rpm provided by magnetic stirrers, and with the reaction temperature maintained at 60°C.

The experiments were conducted at reaction times of 0.25, 0.5, 1, 1.5, 2, 4, 8, and 12 h. The biodiesel product was purified, as in the processes shown in Figure 4.1(c-e), and its SG determined using the methods described in section 3.2.6.

4.2.7. Further quantification of the process monitoring scheme

To further examine the extent of *Chlorella* oil to FAME conversion by the in-situ transesterification process, a quantitative relationship between the measured SG and the percentage mass fraction of FAME in the biodiesel samples was established.

Using the fatty acid profile of the *Chlorella* oil presented Table 3.4, a FAME sample representative of a complete lipid conversion was prepared. This involved the preparation of a solution containing the different fatty acid methyl esters in the same proportions as in Table 3.4. This was used as the standard to provide a basis for calculating the percentage mass conversion of the microalgae oil to FAME. This method was based on the assumption that, upon completion of the transesterification reaction (i.e. 100% conversion), all the fatty acid components of the oil would be converted to FAME. Standards of palmitate (C16:0), palmitoleate (C16:1), oleate (C18:1), linoleate (C18:2), linolenate (C18:3) and eicosenoate (C20:1) methyl esters (Accustandard, USA) were then mixed in the proportions shown in Table 3.4.

The resulting solution was taken to represent a 100% FAME sample, the specific gravity of which was determined using a micro-pycnometer, as described in section 3.2.6.

Different purified biodiesel products obtained by application of the various experimental levels with dissimilar measured SGs (i.e. covering a range of SGs) were selected and subjected to a gas chromatographic (GC) analysis

The GC analysis involved dissolving 0.05g of the dried purified biodiesel product in 5 ml of methyl heptadecanoate solution (Sigma-Aldrich) (10 mg/ml) as the internal standard. Three μl (3 μl) of this solution was then injected into the GC using the same conditions as in section 3.2.6.

The percentage mass fraction of the methyl esters in the sample were calculated from the chromatograph using Eq. 4.1:

$$\text{FAME}(\%) = \frac{(\sum A_{FAME}) - A_{MHD}}{A_{MHD}} \times \frac{C_{MHD} \times V_{MHD}}{m_{sample}} \times 100 \qquad (4.1)$$

where, FAME = the mass fraction of the FAME in the sample (%)

$\sum A_{FAME}$ = the total chromatograph peak area from the FAME C12:0-C24:0

A_{MHD} = the peak area of methyl heptadecanoate

C_{MHD} = the concentration (mg/ml) of the methylheptadecanoate

V_{MHD} = the volume (ml) of the methyl heptadecanoate solution.

m_{sample} = the mass of the biodiesel sample (mg)

The mass fraction of the FAME species for each biodiesel sample was then acquired.

Using the selected range of purified biodiesel samples, a relationship between the measured SG and the FAME mass fraction in the biodiesel (%) was established. This was with the assumption that the unreacted *Chlorella* oil (SG, 0.914) represents 0% (oil to FAME) conversion and with the knowledge of the SG of the prepared 100% FAME sample

The resulting calibration curve was then used to quantitatively estimate the equilibrium oil to FAME mass conversion (%), based on the measured SG.

Furthermore, the FAME yields (g FAME/g microalgae biomass) were obtained by multiplying the mass FAME conversion (%) for the samples with the biodiesel product yield (g biodiesel/g microalgae biomass) measured for the investigations.

The FAME yields for the conventional transesterification process were estimated by using the measured lipid content of the microalgae biomass. This was based on the preceding extraction solvent used.

The method used in the estimation of the uncertainties associated with the determination of the percentage mass oil to FAME conversions from the measured SG are presented in detail in Appendix I.

4.3. Results and Discussion

4.3.1. Monitoring the transesterification process with the measured SG

Using the constant SG of the extracted oil (0.914) to indicate the start of the transesterification reaction, the conversion of the constituent microalgae oils to biodiesel was monitored. Here, lower asymptotic SG values of the purified transesterification products are indicative of an improved equilibrium production of FAME from the microalgae oil.

The microalgae oil has been shown to consist of ≈ 95% triglycerides and 5% free fatty acids. Equations 4.2-4.4 show that the transesterification reaction consists of a series of reversible steps in which the triglycerides are sequentially converted to di- and mono-glycerides, and eventually to glycerol, with one mole of fatty acid alkyl esters ($RCOOR^1$) liberated at each step.

$$\text{Trigyceride} + R^1\text{OH} \rightleftharpoons \text{Diglyceride} + RCOOR^1 \quad (4.2)$$

$$\text{Digyceride} + R^1\text{OH} \rightleftharpoons \text{Monoglyceride} + RCOOR^1 \quad (4.3)$$

$$\text{Monogyceride} + R^1\text{OH} \rightleftharpoons \text{Glycerol} + RCOOR^1 \quad (4.4)$$

With a fixed quantity of oil, and with methanol as the reacting alcohol (R^1OH), the equilibrium transesterification products range from a mixture of FAME, glycerides and glycerol (when the reaction is incomplete), to a FAME and glycerol mixture (on completion). Since the heavier glycerol product of the reaction is eliminated from the final product via purification steps, the SG of the purified biodiesel product (containing FAME and

glycerides) is expected to be less than that of the extracted oil. The measured SG would vary with the percentage composition of the FAME specie in the purified biodiesel product. For example, an increase in the lighter FAME concentration in the biodiesel product due to an improvement in the conversion efficiency would result in lower SGs.

4.3.2. Effect of alcohol volumes and temperature

A lipid content of 0.275 g/g *Chlorella* biomass, with an average molecular mass of the *Chlorella* oil (880 g /mol) was estimated for the *Chlorella* biomass (Chapter 3) used in this study. The stoichiometry of the transesterification reaction involves 3 mol of reacting alcohol per mol of oil.

Using the estimated oil content, molar mass and reaction stoichiometry, the methanol volumes examined in this study correspond to a reacting molar alcohol to oil ratio range of 105:1 – 525:1 (calculated using a methanol density of 0.7918 g/cm^3 at 20°C (Perry & Green, 1998). This range includes and exceeds that of the investigations of in-situ transesterification of sunflower oil by Siler-Marinkovic and Tomasevic (1998), of 100:1-300:1 methanol to oil ratio, described in section 2.10. A sample of the in-situ transesterification mixture prior to the separation step is shown in Figure 4.2.

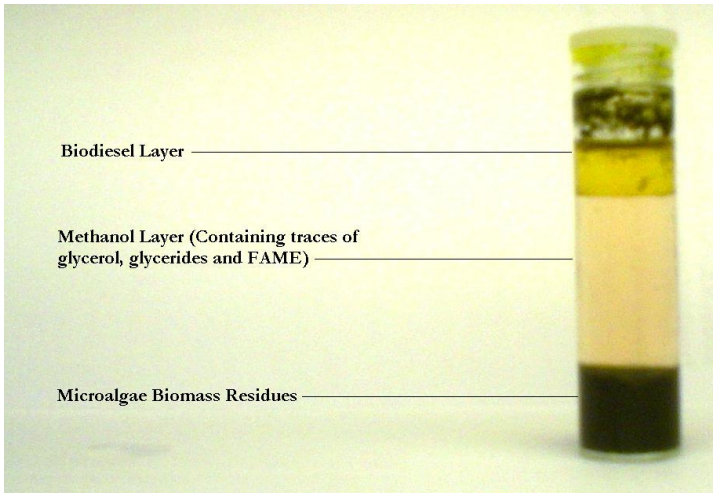

Figure 4.2. In-situ transesterified sample before separation and purification.

It must however be mentioned that the estimated solvent extracted lipids of the microalgae biomass was used in the planning of the experiments in this chapter. As mentioned in section 3.3.2, limitations arising from the specific extraction solvent used may not provide results which are representative of the total lipids available in the microalgae biomass. The calculated molar ratios of the reacting methanol to oil may therefore not completely represent the stoichiometry of the in-situ transesterification reactions under investigation. In the case that the available microalgae oils were underestimated, the reacting alcohol to oil stoichiometry would be less than that put forward in this chapter. This is not expected to affect the results obtained for the in-situ transesterification trials since the alcohol volumes used was based primarily on the amounts required to fully submerse the microalgae biomass. However, direct comparisons of the in-situ and conventional transesterification processes, as carried out later in this chapter would be restricted due to the errors associated with the extractable oil content of the microalgae biomass.

The SGs of the biodiesel products for the different reacting methanol volumes and temperatures, with the reaction conditions as described earlier in section 4.2.2, were determined. This is shown in Figure 4.3.

The results obtained indicate an improvement of the microalgae oil conversion to FAME with increasing temperature and increasing alcohol volume The lowest FAME equilibrium conversions (highest SGs) were achieved with the reacting molar ratios of the methanol to oil at 105:1 (i.e. a methanol volume of 20 ml) for all the temperature levels studied.

However, with the use of alcohol volumes over 60 ml for the in-situ transesterification of 15 g of microalgae biomass, no trends were obtained in the measured SG with the reaction temperatures greater than 60°C.

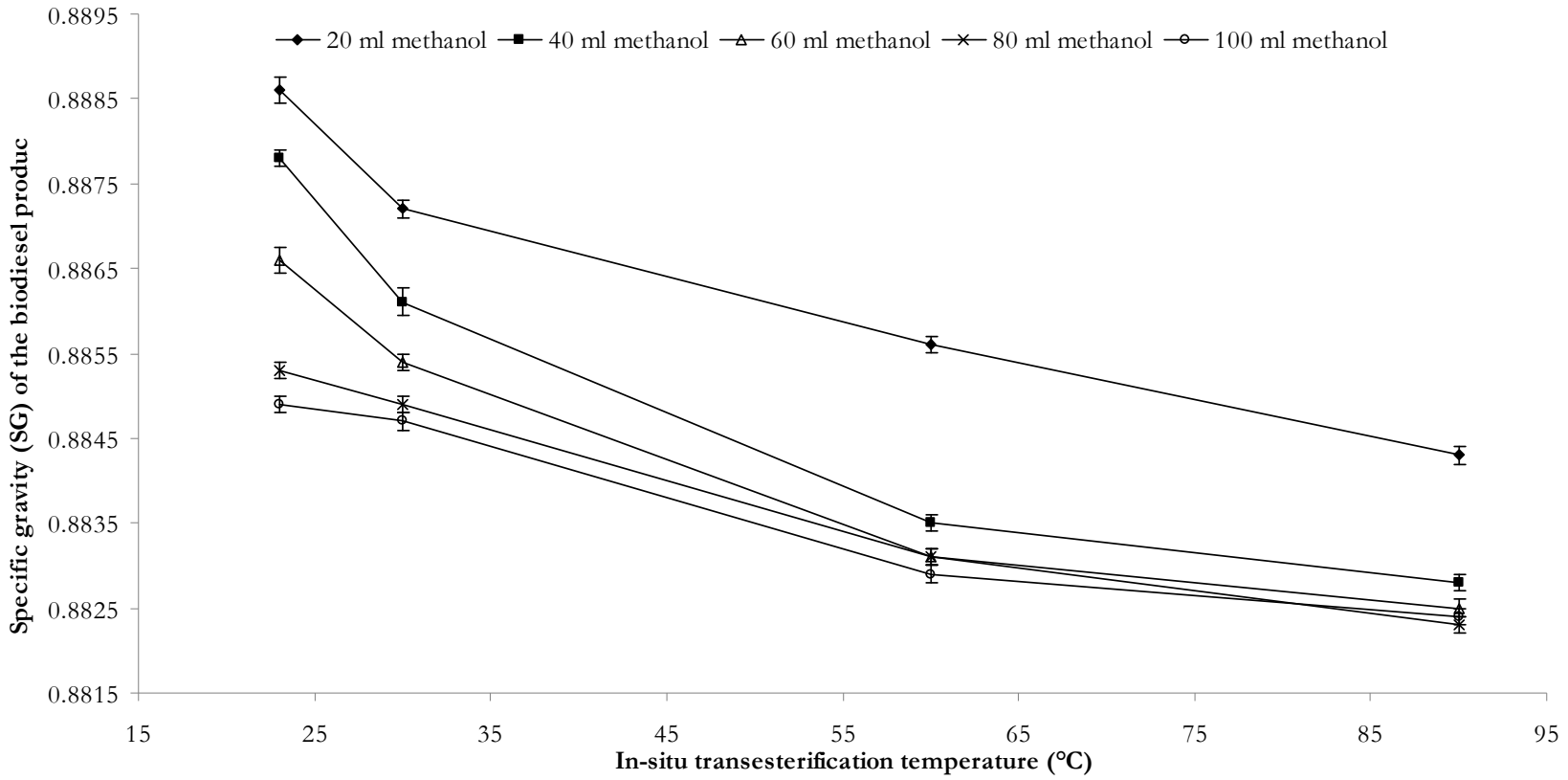

Figure 4.3. Effect of the alcohol volume and reaction temperature on the specific gravity of the biodiesel product after a reaction time of 8 h using 15 g microalgae biomass and 0.04 mol H_2SO_4 as the catalyst.

4.3.3. Effect of reaction time and temperature

To investigate the influence of reaction time and temperature, a methanol volume of 60 ml was used, since it was found (section 4.3.1) that no appreciable differences in the equilibrium FAME conversion were obtained with the use of higher alcohol volumes for reactions carried out at 60 and 90°C.

Figure 4.4 shows the changes in the measured SG of the purified biodiesel product after the in-situ transesterification process with time, at different temperatures (23, 30, 60 and 90°C).

For the samples investigated at room temperature (no process heating), asymptotic SG values were not reached within the time boundaries of this study. This indicates that some heat input would normally be required to improve the rate of conversion to FAME using the in-situ process.

After a reaction period of 8 h, the conversion of the oil to FAME (on the basis of the measured SG change) was seen to increase by 4.70 ± 0.05%, with an increase of the process temperature from 23 to 30°C.

Conducting the in-situ transesterification process at 90°C under the same experimental conditions, the lowest biodiesel SG were seen to be achieved with the shortest time.

Using a reacting molar ratio of methanol to oil of 315:1, and continuous stirring of the reaction at 500 rpm, a faster conversion phase was observed between 15 min and 2 h for the 15 g microalgae samples studied at 60 and 90°C, as shown in Figure 4.4.

This faster conversion phase is considered to be due to the emulsifying effects of the initial fatty acid esters formed after the mass transfer controlled phase, as demonstrated by Freedman et al., (1986). This trend was, however, not observed for the 60 and 90°C experiment runs, probably due to the fact that the elevated temperatures improved the initial miscibility of the reacting species, leading to a reduction in the reaction times, as shown in Figure 4.4.

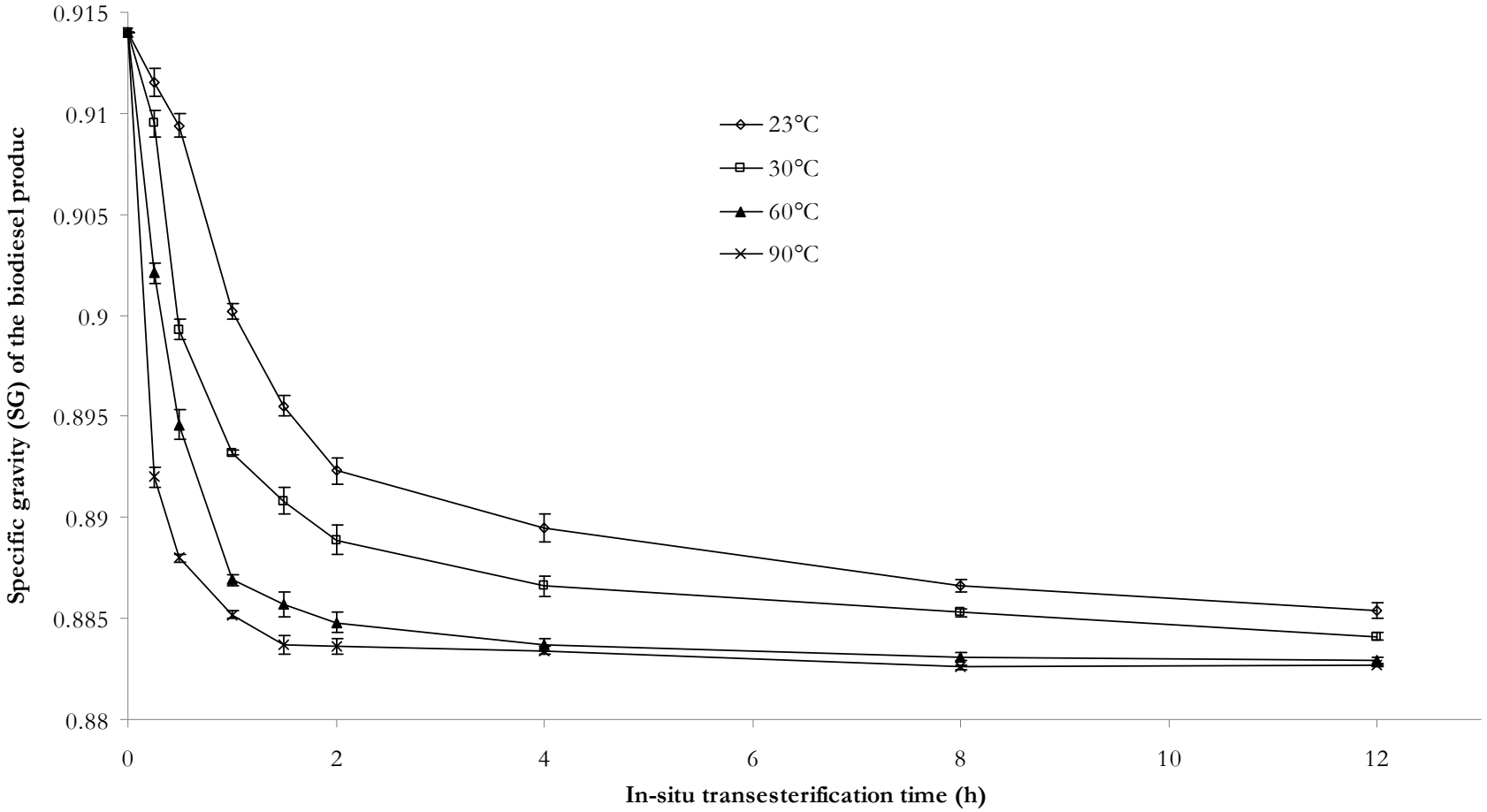

Figure 4.4. Influence of varying the reaction time on the biodiesel SG after the in-situ transesterification of 15g microalgae at reaction temperatures of 23, 30, 60 and 90°C using 60ml methanol and 0.04 mol H_2SO_4.

Within the experimental conditions, the measured biodiesel SGs were seen to reach similar asymptotic values after a reaction time of 2 and 4 h for temperatures of 60 and 90°C. Although faster conversion rates can be achieved by use of reaction temperatures greater than the normal boiling point of the reacting methanol (i.e. 90°C at 1 atm), the process heating and pressure requirement may inhibit the commercial use of such temperature levels. The use of a reaction temperature of 60°C may prove more beneficial when the total energy consumption and operation cost of the whole biodiesel conversion system is considered.

The correlation of the measured SG with the percentage mass fraction of FAME in the purified biodiesel will be examined later in section 4.3.7 .

4.3.4. Effect of moisture content

As presented in section 4.1, the influence of the microalgae moisture levels was important in this study, due to its potential impact on the overall biomass and fuel production costs. Also, as highlighted in section 2.4.2, FAME production via the acid catalysed transesterification might be negatively affected by increases in the biomass water content. This is due to the fact that the inorganic acids, with a strong affinity for water, would preferentially interact with water, than with the reacting alcohol (Loreto et al., 2005).

The different moisture content levels of the samples were used to represent a biomass sample, ranging from freshly harvested (after centrifugation), with a moisture content of 72.5 ± 1.8%, to completely dried. It must be noted, however, that the water content of the dried sample (0.0%), used as the basis for determining the moisture content of the microalgae samples, refers to free water and not the more tightly bound water.

The measured SG of the purified biodiesel yields obtained from the in-situ transesterification of the *Chlorella* biomass with different moisture levels and varied reaction time intervals are shown in Figure 4.5.

The general trends of the results obtained indicate that increases in the biomass moisture content had a negative effect on the measured specific gravity of the purified biodiesel product, with higher SG values seen with increasing biomass moisture content.

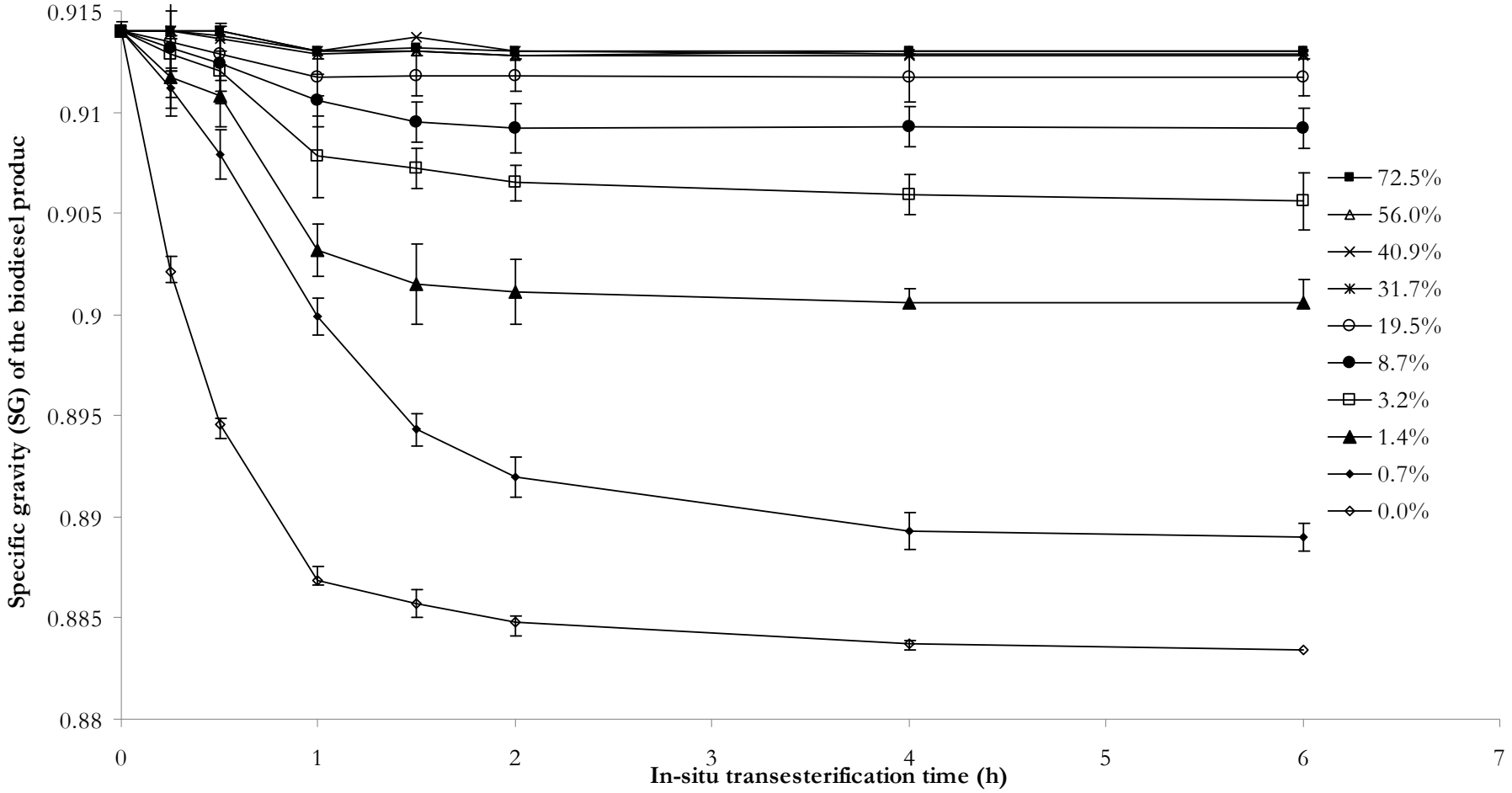

Figure 4.5. Influence of microalgae moisture content (dry basis) on the FAME conversion (using biodiesel SG as indicator) using 0.04 mol H_2SO_4 as catalyst and a fixed reaction alcohol volume of 60 ml and temperature of 60°C, respectively.

The measured biodiesel SG was shown to be similar, and almost totally inhibited, for the microalgae biomass samples with moisture contents greater than 31.7% (on a dry basis, i.e. containing >4.755 g water) or >114.85% (w/w) when compared with the microalgae oil weight of 4.14 g.

Hence, for the conditions investigated, these results indicate that the process would be inhibited in moisture containing microalgae samples with water levels greater than 115% of the reacting oil weight for the acid catalysed in-situ transesterification process.

Obvious differences in the SG of the biodiesel product were only obtained after a 73% removal of water from the freshly harvested samples, i.e. with the in-situ transesterification reaction using biomass samples with a moisture content of ≤ 19.5% (dry basis).

A reduction of the biomass moisture levels to 0.7% (dry basis), (i.e. a water mass content of 0.1 g), was observed to correspond to a change in the oil to biodiesel SG of 81.70% (± 0.19%) when compared to that of the completely dried biomass samples after a reaction time of 6 h.

Biomass drying cannot be overlooked in the in-situ transesterification process. A reduction in the measured SG of the biodiesel product was obtained with the use of the completely dried samples, compared with the use of any of the moisture containing samples, as seen in Figure 4.5.

4.3.5. Effect of process stirring

The effect of stirring on the in-situ transesterification of microalgae biomass was also examined, as a potential process energy reduction strategy. When the in-situ transesterification process was conducted without stirring, the equilibrium FAME content of the resulting biodiesel (indicated by the biodiesel SG) was reduced compared to that for the continuously stirred sample (Table 4.1).

This indicates that stirring is required to some extent to enhance the reaction progress, evidently by aiding the initial miscibility of the reacting species.

Table 4.1. Influence of stirring on the in-situ transesterification of microalgae lipids with 0.04 mol H_2SO_4 as the catalyst, methanol volume of 60 ml and reaction temperature at 60°C (8 h reaction time)

	Specific gravity of extracted biodiesel product
No stirring	0.9032 ± 0.0041
Stirring for 1 h	0.8859 ± 0.0018
Stirred intermittently (1 h off, 1 h on)	0.8845 ± 0.0023
Stirred continuously	0.8831 ± 0.0006

The stirring of the reaction vessels for only 1 h (from the start of the reaction) was investigated. This time was chosen since 87.0% (± 0.2%) of the asymptotic measured SG value was achieved after 1 h with continuous stirring under the experimental conditions as described in section 4.2.3 and shown in Figure 4.4. It was envisaged that, without further stirring, the equilibrium conversion might approach that of the continuously stirred system with the methyl esters produced acting as a dual solvent for the oil and alcohol, thereby facilitating further FAME production.

It should be noted that the SG of the biodiesel product for the trial without any stirring might have had a higher SG than indicated in Table 4.1 in the absence of any stirring at all. The reason is that all the samples were initially swirled by hand before being placed on the hot plate and stirring system. The purpose of this action was to prevent clumping and ensure that the biomass samples were adequately exposed to the 'acidic methanol' mixture. However, it could have also promoted some initial phase mixing of the reactants in the "not stirred" sample. The results in Table 4.1 may overstate the equilibrium conversion yields of the "unstirred" samples.

A comparison of the FAME conversion obtained with the different stirring schemes considered with the equilibrium oil to FAME conversions is presented later in section 4.3.7.1.

4.3.6. Conventional transesterification of the extracted microalgae lipids

The conventional acidic catalysed transesterification process was applied for the production of FAME from the extracted *Chlorella* oil to provide data for comparison with the in-situ transesterification process used in this thesis.

Three levels of methanol volume were used in the conventional transesterification process (i.e. 1.38, 11.5 and 72.35 ml). This corresponded to the use of molar ratios of methanol to *Chlorella* oil of 6:1, 50:1 and 315:1 respectively.

The reacting methanol/oil molar ratio of 6:1 was investigated since this quantity of excess alcohol has been reported previously in the literature to result in a maximum alkyl ester yields using acid catalysts for the transesterification of high FFA containing oils such as waste and microalgae oil (i.e. Miao & Wu, 2006; Al-Widyan & Al-Shyoukh, 2002). A molar ratio (methanol to *Chlorella* oil) of 50:1 was also used, based on reports of this value being used in commercial biodiesel production using feedstock oils with a high FFA content and acid catalysts (Zhang et al., 2003).

In addition, to facilitate a direct comparison with the in-situ transesterification process, a methanol to *Chlorella* oil ratio of 315:1 was applied to the conventional process (i.e. the same value employed in the in-situ transesterification trials using 60 ml methanol with 15 g dried biomass samples).

Figure 4.6 shows the influence of varying the alcohol volume, at a fixed temperature of 60°C, on the SG of the purified biodiesel yields after the conventional transesterification process with the reaction conditions as described in section 4.2.6.

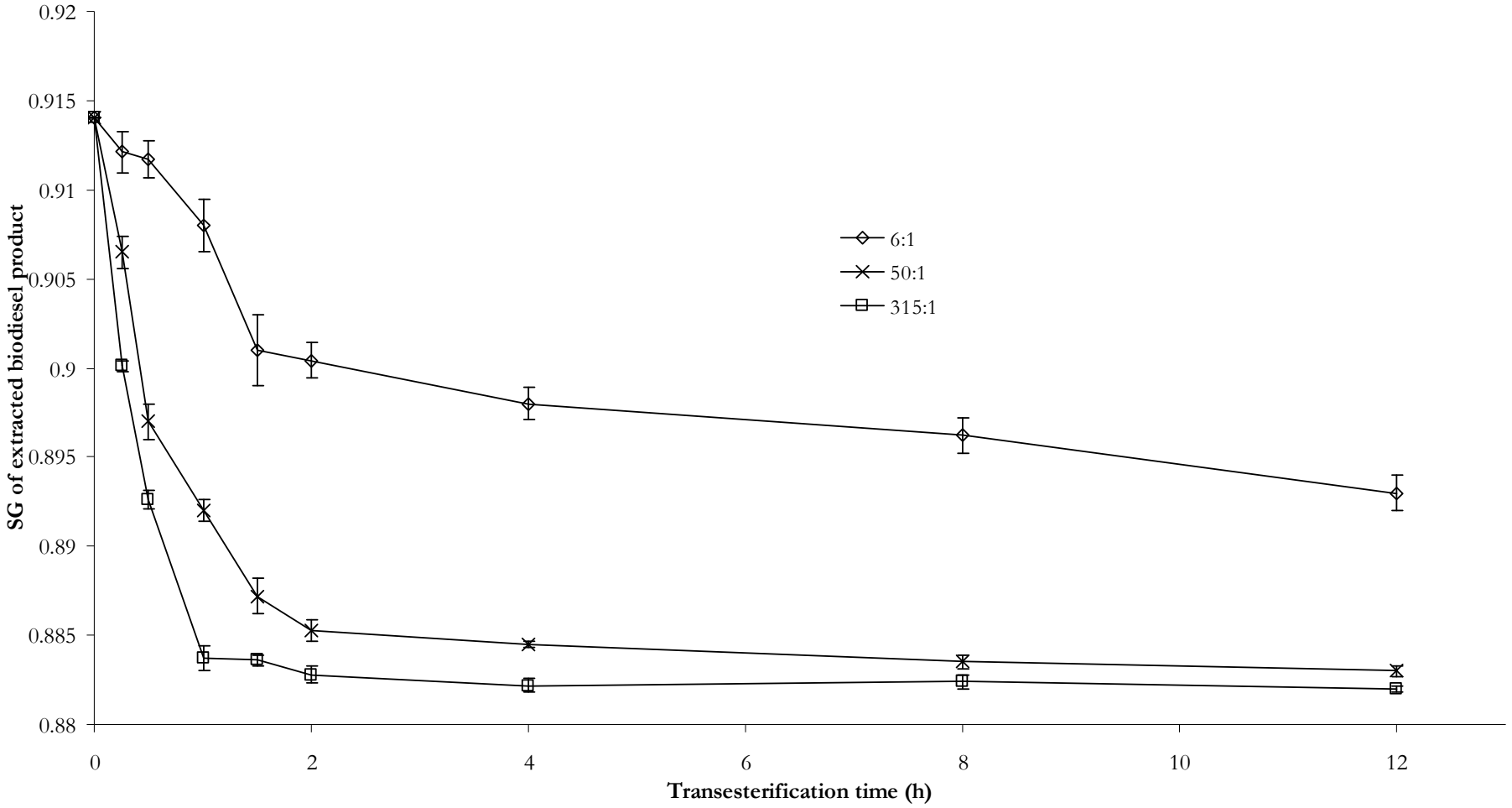

Figure 4.6. Influence of different reacting molar ratios of methanol to *Chlorella* oil, at different reaction times, on the SG of biodiesel produced by conventional transesterification (with 5g *Chlorella* oil, 0.05 mol H_2SO_4, and reaction temperature of 60°C.

With the transesterification reaction being a reversible reaction, an increase in the molar ratio of the alcohol to the *Chlorella* oil, would favour a shift of the reaction to the right, resulting in an increase in the equilibrium FAME conversion. This is indicated by the reduction of the measured SGs of the purified biodiesel product (a mixture of the initial triglycerides and FAME).

After a reaction time of 12 h, and at a reaction temperature of 60°C, the experiments using the molar ratios of methanol to oil of 6:1 for the conventional transesterification process can be seen to result in the lowest equilibrium FAME conversions.

This result is consistent with reports on the application of the conventional acid catalysed process by Freedman et al. (1984) using extracted soybean oil as the reaction feedstock. That study showed that unsatisfactory methyl ester yields were obtained for acid (H_2SO_4) catalysed transesterification reactions using reacting molar ratios of alcohol (methanol) to oil of 6:1 and 20:1 with a reaction temperature and time of 65°C and 3 h respectively. An improvement in the oil to ester conversion trends was however observed with the use of a molar ratio of methanol to oil of 30:1 for the transesterification process (Freedman et al. 1984). That result is consistent with this study which showed that a reduction in the measured SG of the purified biodiesel product obtained with the use of a 50:1 reacting methanol/oil molar ratio.

A further comparison of the in-situ transesterification experiments and the conventional process is made in section 4.3.7, on basis of the FAME yields.

4.3.7. Use of the SG to estimate the percentage equilibrium FAME conversion and yields

In most of the literature (i.e. Phan & Phan, 2008) the mass of the purified biodiesel product (a mixture of FAME product and unreacted glycerides) is used for the monitoring of the transesterification process. In this thesis the SG of the purified biodiesel product, coupled with the results of the percentage biodiesel FAME content from the GC analysis, was used to monitor the conversion of *Chlorella* oil to FAME. This was considered to be a

better approach because the purified biodiesel was expected to contain other chemical species (than FAME), as described in sections 4.3.1 and 4.3.6.

Since the SG of the biodiesel product is taken to correspond to a specific FAME concentration, a direct relationship between the measured SG and the mass fraction of the FAME in the biodiesel product was established.

As described in section 4.2.7, following the purification of the biodiesel products and measurement of their SGs, the percentage mass fraction of the total FAME species in the purified product was obtained via GC analysis.

The prepared 100% converted FAME sample was determined to have a SG of 0.878 ± 0.001. The 0% converted FAME sample (extracted microalgae oil) has a SG of 0.914. These two SG values provide a range of 0 - 100% mass oil to FAME conversion which was used in this study.

A calibration curve showing the relationship between the measured SG and the percentage mass fraction of FAME in the biodiesel product was obtained as shown in Figure 4.7.

A fitted relationship between the measured SG and estimated mass FAME conversion (%) was obtained, as seen in Figure 4.7. With an R^2 value of 0.998, the curve appears to be adequate for the prediction of the corresponding mass percentage FAME conversions using the measured SGs of the biodiesel product. The errors related with the estimated percentage mass FAME content in the biodiesel is presented in Appendix I[11].

The equilibrium mass percentage FAME content (%), which covers the range of the measured SG observed in the biodiesel product, was then estimated from the relationship shown in Figure 4.7.

[11] The MATLAB codes used in the estimation of the errors are included in the Appendix I (CD-ROM)

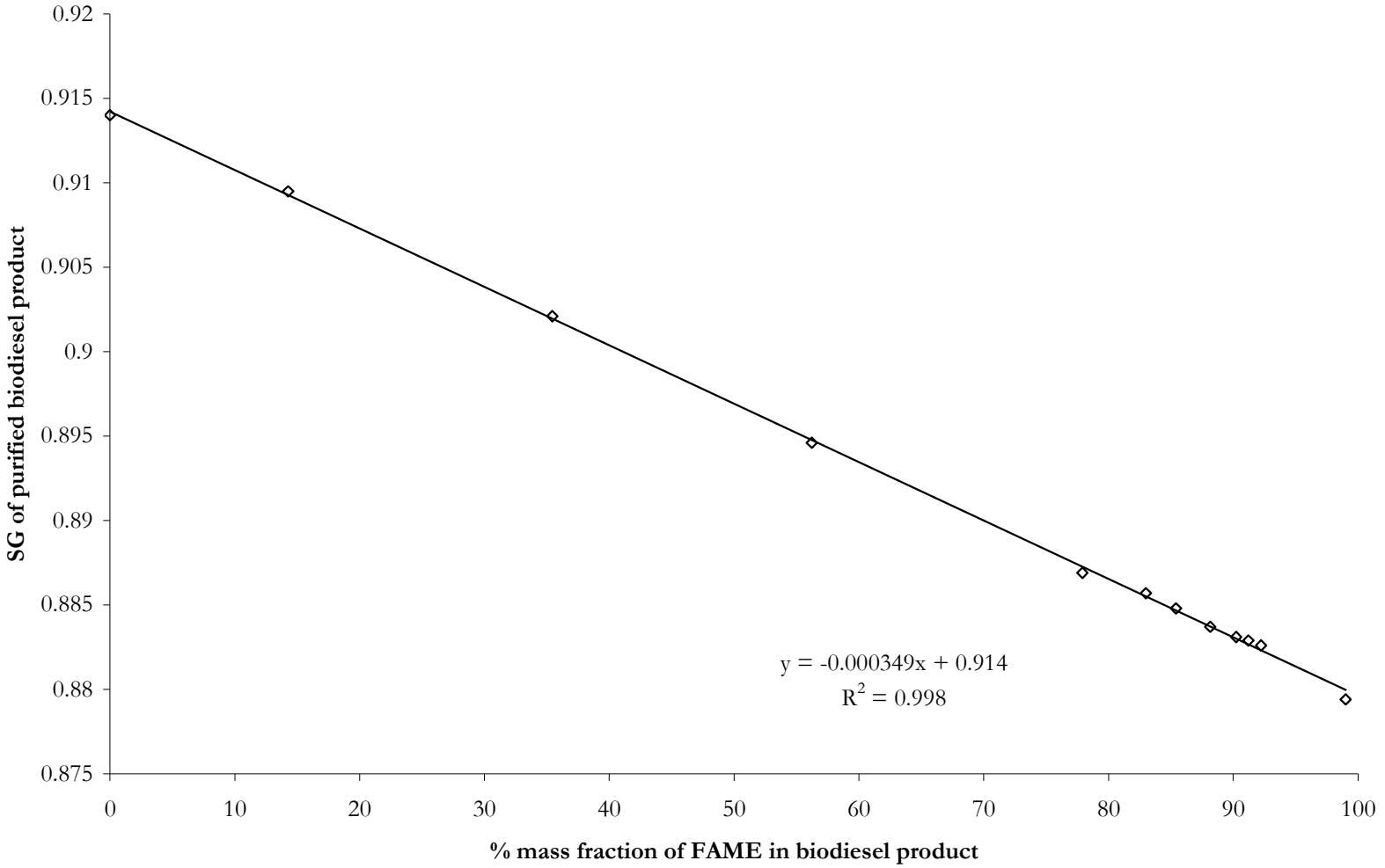

Figure 4.7. Calibration curve showing the relationship between the measured SG and the mass percentage FAME in biodiesel.

The mass percentage of FAME in the biodiesel product from the calibration curve was used to calculate the FAME yields from the different transesterification processes as described in 4.2.7. This is explored further in section 4.3.7.1.

4.3.7.1. Estimation of the FAME yields

To further compare the microalgae biomass to biodiesel conversion efficiencies using the conventional and in-situ processes, the mass of the dried purified biodiesel product obtained after both transesterification processes was recorded. The results were adjusted to g biodiesel/g dry microalgae input based on the estimated *Chlorella* oil content for the conventional process and the microalgae sample size used for the in-situ process.

The results obtained for some of the different investigated levels are shown under the column heading "biodiesel yield" presented in Table 4.2.

The uncertainties associated with the estimated FAME yields were obtained on the basis of the sum of the percentage error contributions of the measured SG, corresponding mass percentage FAME content in the biodiesel and biodiesel yields. The calculation used in this error estimation was presented in Appendix I.

Table 4.2. Estimated FAME yields obtained under different in-situ and conventional transesterification reaction conditions

Process description	Temp (°C)	Time (h)	Biomass moisture content (%)	Reacting methanol to oil ratio	Measured biodiesel SG	Mass percentage FAME in biodiesel (%)	Biodiesel yield (g biodiesel/g dry microalgae)	FAME yield (g FAME/g dry microalgae)
In-situ process (continuously stirred)								
	23	8	0.0	315:1	0.8866 ± 0.0007	78.51 ± 2.0	0.274 ± 0.005	0.215 ± 0.006
	35	8	0.0	315:1	0.8854 ± 0.0011	81.9 ± 3.2	0.275 ± 0.002	0.225 ± 0.007
	60	8	0.0	315:1	0.8831 ± 0.0012	88.5 ± 3.4	0.288 ± 0.003	0.255 ± 0.009
	90	8	0.0	315:1	0.8825 ± 0.0010	90.3 ± 2.9	0.287 ± 0.001	0.259 ± 0.008
	60	0.5	0.0	315:1	0.8946 ± 0.0037	55.6 ± 10.8	0.250 ± 0.002	0.139 ± 0.014
	60	1	0.0	315:1	0.8869 ± 0.0017	77.7 ± 4.9	0.269 ± 0.005	0.209 ± 0.011
	60	2	0.0	315:1	0.8848 ± 0.0038	83.7 ± 10.9	0.276 ± 0.003	0.231 ± 0.025
	60	4	0.0	315:1	0.8837 ± 0.0017	86.8 ± 4.9	0.284 ± 0.004	0.247 ± 0.013

60	6	0.0	315:1	0.8834 ± 0.0011	87.7 ± 3.1	0.283 ± 0.001	0.248 ± 0.008
60	6	72.5	315:1	0.9130 ± 0.0004	2.9 ± 1.2	0.210 ± 0.007	0.006 ± 0.001
60	6	40.9	315:1	0.9128 ± 0.0010	3.4 ± 2.9	0.230 ± 0.005	0.008 ± 0.001
60	6	19.5	315:1	0.9116 ± 0.0008	6.9 ± 2.3	0.235 ± 0.009	0.016 ± 0.001
60	6	8.7	315:1	0.9091 ± 0.0005	14.0 ± 1.4	0.260 ± 0.006	0.037 ± 0.001
60	6	1.4	315:1	0.9005 ± 0.0005	38.7 ± 1.4	0.281 ±0. 004	0.109 ± 0.002
In-situ process (not stirred)							
60	8	0.0	315:1	0.9032 ± 0.0047	30.9 ± 13.5	0.215 ± 0.006	0.067 ± 0.009
Conventional process							
60	8	-	6:1	0.8866 ± 0.0027	78.5 ± 7.7	0.274 ± 0.003	0.215 ± 0.017
60	8	-	50:1	0.8835 ± 0.0011	87.4 ± 3.1	0.270 ± 0.004	0.236 ± 0.008
60	8	-	315:1	0.8820 ± 0.0015	91.7 ± 4.3	0.269 ± 0.005	0.247 ± 0.012

The estimation of the biodiesel yields (g biodiesel/g microalgae) for the conventional process was based on the assumed use of 18.12 g of *Chlorella* biomass for the transesterification process. This was due to the fact that 5 ± 0.01 g of the *Chlorella* oil was used for the transesterification reaction, with lipid content (using butanol extraction) of 0.275 g/g biomass. This was diffent from the 15 g used for the in-situ experiments. 5 g of *Chlorella* oil was used for the conventional process mainly due to the convienience in obtaining this quantity for the experimental runs. This was not deemed to be an issue since the specific biodiesel and FAME yields were found to be independent of the reacting *Chlorella* biomass or oil masses (i.e. the reacting volumes could be arbitrarily selected). With different biomass sizes used for the conventional and in-situ processes respectively, the biodiesel yields (g biodiesel/ g microalgae) were therefore used to facilitate an even comparison of the results of the different transesterification processes, irrespective of differences in the sampling size.

The results of some of the FAME yields (g FAME/g microalgae biomass), and the estimated percentage FAME mass content in the purified biodiesel product obtained with the different variable settings used in this study, are also presented in Table 4.2. Depending on the extent of the percentage mass oil conversion to FAME, the biodiesel yields do not refer to pure FAME, since the biodiesel product also contains mono-, di- and triglycerides as highlighted in section 4.3.1. The biodiesel yield could be further used as an indication of the extent of microalgae lipid extraction using the in-situ transesterification process.

The results obtained in Table 4.2 show that using dried *Chlorella* biomass for the acid catalysed in-situ process[12], a 4.7 ± 0.1% improvement in the lipid extraction (and biodiesel production) from the *Chlorella* biomass was achieved. This was based on a comparison with the biomass oil content of 0.275 g previously estimated for solvent extraction. This implies that not all of the microalgae biomass oil is fully accounted for in the conventional lipid extraction process. This may be due to losses encountered during the oil extraction, or the limitations of the abstraction operation of the solvents used in this thesis.

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[12] With the use of 15 g dried microalgae biomass for the continuously stirred in-situ transesterification process with 60 ml methanol, 0.04 mol H_2SO_4 and a reaction time of 8 h.

The in-situ process was observed to have an overall FAME yield of 0.255 ± 0.009 g FAME/g dry *Chlorella* biomass at a reaction temperature, time and methanol/oil ratio of 60°C, 8h and 315:1 respectively. This was similar to the 0.247 ± 0.012 g FAME/g *Chlorella* biomass obtained for the conventional transesterification process under similar reaction conditions. Within the boundaries of this study, from the observed trends, the use of the acid catalysed in-situ transesterification process appears to not lead to increases in the FAME yields when compared to the conventional process under similar conditions.

The results in this study were different to those reported by Harrington & D'Arcy-Evans (1985) and Siler-Marinkovic & Tomasevic, (1998). Those authors demonstrated that the use of the acid catalysed in-situ process for sunflower seeds transesterification resulted in increased methyl ester yields when compared to the use of the conventional process. The increase in the FAME yield was proposed to be due to the acid environment provided by the catalyst enhancing the accessibility of the lipids in the oil bearing vesicles of the biomass (Harrington & D'Arcy-Evans, 1985; Siler-Marinkovic & Tomasevic, 1998). The difference in the FAME yields results in this study compared to those presented in Harrington & D'Arcy-Evans (1985) and Siler- Marinkovic & Tomasevic (1998) may be due to the biomass maceration carried out for the latter before conducting the transesterification process. This pre-treatment step could have increased the availability and subsequently, the conversion of the biomass oils to FAME.

The results in Table 4.2 also revealed that, for most of the different variable levels studied with the use of the dried (0.0% moisture) *Chlorella*, biodiesel yields were similar. The process stirring was also seen to be important process parameter, with the non-stirred in-situ reaction observed to result in a FAME yield (g FAME/g dry microalgae) 27% (w/w) that seen for the continuously stirred samples using the same reaction conditions. Biomass drying and stirring of the reaction are therefore important for the efficient conversion of the microalgae lipids using the in-situ transesterification process.The highest FAME yields of 0.259 ± 0.008 g FAME/g *Chlorella* biomass obtained within the boundaries of the study in this chapter were achieved using 15 g of dried *Chlorella* biomass for the in-situ process. This was with a reaction temperature, time and methanol volume of 90°C, 8 h and 60 ml respectively, and with 0.04 mol of H_2SO_4 as the catalyst.

4.4. Chapter Conclusion

This chapter studied the influence of reaction variables, that may have economic or energy implications, on the progress of the conversion of microalgae oil to FAME using the acid-catalysed in-situ transesterification process. These variables included the influence of reacting alcohol volumes, temperature, reaction time, biomass moisture content and stirring.

The results show that increases in the reaction temperature and alcohol volume favoured the production of FAME. However, within the experimental conditions, an increase in the reacting alcohol volume of more than 60 ml did not show observed trend changes in the percentage mass conversion of the oil to FAME, when using 15 g of *Chlorella* biomass.

The percentage mass content of the FAME in the biodiesel trends were shown to improve with more process stirring for the in-situ process. However, some savings may be achieved with the stirring energy since the intermittent stirring of the reactors was shown to produce only a small reduction in the percentage mass FAME conversion.

Biomass drying was shown to play an important role, with an increase in the moisture content of the biomass resulting in reductions of the equilibrium FAME yields (g FAME/g biodiesel). Extensive biomass drying may be needed prior to biodiesel production using the in-situ process. Schemes such as the continuous removal of the water from the reactor, i.e. via a water adsorption column connected to the transesterification reactor as described by Lucena et al. (2008), could be used to drive the forward reaction when microalgae biomass with high moisture content is used in the in-situ process.

Within the study boundaries, the application of the in-situ acid-catalysed process was observed to offer improved lipid extraction the microalgae biomass when compared with the use of the conventional process. However, the FAME yields (g FAME/g biodiesel) were shown to be similar. This influence of the different studied process parameters on the in-situ transesterification process rates would however be better investigated using a kinetic rate treatment. This approach was however not applied in this thesis mainly due to time constraints. It could further be investigated in future research.

Furthermore, the energetic cost of the recovery of the excess alcohol reactants used in the in-situ process might negatively affect the practicality of the use of this method for large scale FAME production. The use of excess alcohol also results in an increase in the downstream FAME purification requirement, with the biodiesel separation seen to be more difficult to achieve with increasing methanol volumes. Finding ways to reduce the large quantities of the reacting alcohol required for the in-situ transesterification process appears to be a major need that could be further investigated to improve the competitiveness of this method.

A further look at the possibility of reducing the reacting alcohol requirement for the in-situ transesterification process, and consequently the process energy requirement is examined in the next chapter.

4.5. References

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5. USE OF ULTRASOUND AND CO-SOLVENTS TO POTENTIALLY IMPROVE THE IN-SITU TRANSESTERIFICATION PROCESS

5.1. Introduction

This chapter investigates the possibility of reducing the reacting alcohol volumes required for in-situ transesterification to obtain high FAME yields. To achieve this goal, this chapter examines the effects of applying modifications to the in-situ transesterification process described previously. The process modifications investigated are: the use of low frequency ultrasound and the addition of co-solvents in the transesterification reaction.

Process agitation is required at the start of the transesterification reaction to enhance the mixing of the two initially immiscible liquid phases; the *Chlorella* oil and the alcohol-catalyst mixture. This action initiates the oil to fatty acid ester conversion process which occurs in the interfacial region between the two phases (Colluci et al., 2005). With the in-situ set-up as described in Chapter 4, mechanical agitation (using stirring bars at 500 rpm) was used to achieve phase mixing.

Here, ultrasound was considered for use for the in-situ process agitation. Based on the frequency level, ultrasound can be classified into: high frequency and low power ultrasound (2-10 MHz range), mid frequency ultrasound (100 kHz- 2 MHz), and low frequency and high power (20-100 kHz) (Rokhina et al, 2009). Ultrasound in the low frequency range will be of interest for use in the investigations presented in this chapter since it has been widely used for biodiesel production investigations in the literature (i.e. Stavarche et al., 2005).The integration of low frequency ultrasound in the conventional transesterification process has been increasingly studied (Armeta et al., 2007; Stavarche et al., 2005; Colluci et al., 2005). This is due to the possibility for increased mass transfer between the immiscible liquid phases. The oil-alcohol phase boundary was reported to be disrupted due to the collapse of ultrasonically induced cavitation bubbles (Stavarche et al., 2005). This

in turn was shown to lead to an accelerated alkyl ester product formation (Stavarche et al., 2005). The application of ultrasonic irradiation to improve transesterification yields and reduce reaction times have been reported in the literature for the conventional alkaline catalysed transesterification process (Armeta et al., 2007; Stavarche et al., 2005; Colluci et al., 2005; Georgogianni et al., 2008).

This chapter examines the potential improvements that could be achieved with the use of low frequency ultrasound for the acid catalysed in-situ transesterification process.

The investigations in this study were mainly motivated by the findings of Georgogianni et al. (2008). That work showed significant increases (>10%) in sunflower seed oil to methyl and ethyl esters conversion efficiencies (%, w/w) when low frequency ultrasound (24 kHz) was used compared to mechanical agitation under similar alkaline catalysed in-situ reaction conditions. Other advantages reported with the use of ultrasound for the transesterification process include shorter reaction times and reduced molar reacting ratios of alcohol to oil (Siatus et al., 2006).

No reports on the influence of low frequency ultrasonication on the acid catalysed in-situ transesterification process were found in the literature.

The primary aim of investigating ultrasound mixing for the in-situ process in this study was to assess the extent to which the reacting alcohol volumes could be reduced. The investigation was aimed at also providing useful information on how the percentage mass oil to FAME conversion efficiency and reaction times would be affected. The potential of improving microalgae biodiesel yields was also investigated, since cell disruption by ultrasound could result in increases in the oil yields from the microalgae biomass.

The use of co-solvents in the in-situ transesterification of microalgae biomass was also explored.

Large quantities of reacting alcohol were shown to be required for the in-situ process as demonstrated in Chapter 4. The recovery and re-use of the alcohol quantities in excess of the stoichiometric transesterification requirement would be necessary to reduce the process material inputs and wastes. In most industrial biodiesel production processes, the recovery of the excess reacting alcohol is usually accomplished with the use of distillation units. The

recovery of the excess methanol after the transesterification step could create a significant energetic cost.

Reducing the reacting alcohol volumes, and improving the overall process renewability, are primary aims of this thesis. To meet these aims, the integration of co-solvents in the FAME production process was proposed. It is expected that the added co-solvents would assist in the extraction of the biomass lipids in conjunction with the methanol by improving the diffusion of the microalgae lipids across the cell wall. This is facilitated by increasing the selectivity (and solubility) of the extraction media, hence providing greater availability of the oil for the transesterification process (Ranjan et al., 2010). The reduced methanol volumes, still available in quantities higher than that stoichiometrically required, would allow for the conversion of the microalgae lipids to FAME.

The integration of co-solvents for the acid catalysed in-situ transesterification of microalgae biomass was initially demonstrated by Johnson & Wen (2009).

The study of co-solvent use by Johnson & Wen (2009) examined biodiesel production from wet (80% moisture content, w/w) and freeze dried *Schizochytrium limacium* biomass. That study was mainly aimed at comparing the effect of co-solvent use (chloroform, hexane and petroleum ether) on biodiesel and FAME production compared with the conventional transesterification process using methanol alone. The study utilised a fixed volume of reacting methanol for all the experiments (Johnson & Wen, 2009). The results showed that for freeze dried microalgae, an increase in the crude biodiesel yields was obtained using the co-solvents in the in-situ process compared to the conventional method (Johnson & Wen, 2009). However, only the in-situ experiments using chloroform as a co-solvent were demonstrated to lead to higher mass percentage conversion of oil to FAME (w/w) of 72.8%, compared with 10.5% and 11.1 % shown with the use of hexane and petroleum ether respectively (Johnson & Wen, 2009).

This study further investigates the potential gains that could be obtained with the use of other common co-solvents on the in-situ transesterification of microalgae lipids.

The potential improvements that could be obtained from the combination of co-solvent use and ultrasound agitation for the in-situ process was also examined.

This chapter aims to address the following questions:

i. What effect would modifying the in-situ process to use low frequency ultrasound for process agitation, co-solvent addition, or a combination of both have on the conversion of the microalgae lipids to FAME?

ii. What other process improvements over the mechanically stirred system i.e. reaction time, biodiesel and FAME yields are achieved by the application of these schemes.

5.2. Materials and Methods

5.2.1. Ultrasound assisted in-situ transesterification

The in-situ transesterification of the *Chlorella* biomass samples was carried out using a batch process similar to that described previously in Chapter 4. This involved a fixed microalgae biomass input of 15.0 ± 0.1 g (dry weight), H_2SO_4 (0.04 mol) and a process temperature of 60°C.

The use of low frequency ultrasound for the agitation of the in-situ experiments was performed using a low frequency ultrasonicator of 24 kHz (UP200S, Hieslcher Ultrasonics GmbH) with a maximum power rating of 200 W. The set up and the safety procedures for the use of the ultrasound equipment were carried out as described in the handbook provided by the manufacturers.

A schematic representation of the laboratory set-up used in the investigations involving the use of low frequency ultrasound for the in-situ transesterification of the microalgae biomass is shown in Figure 5.1.

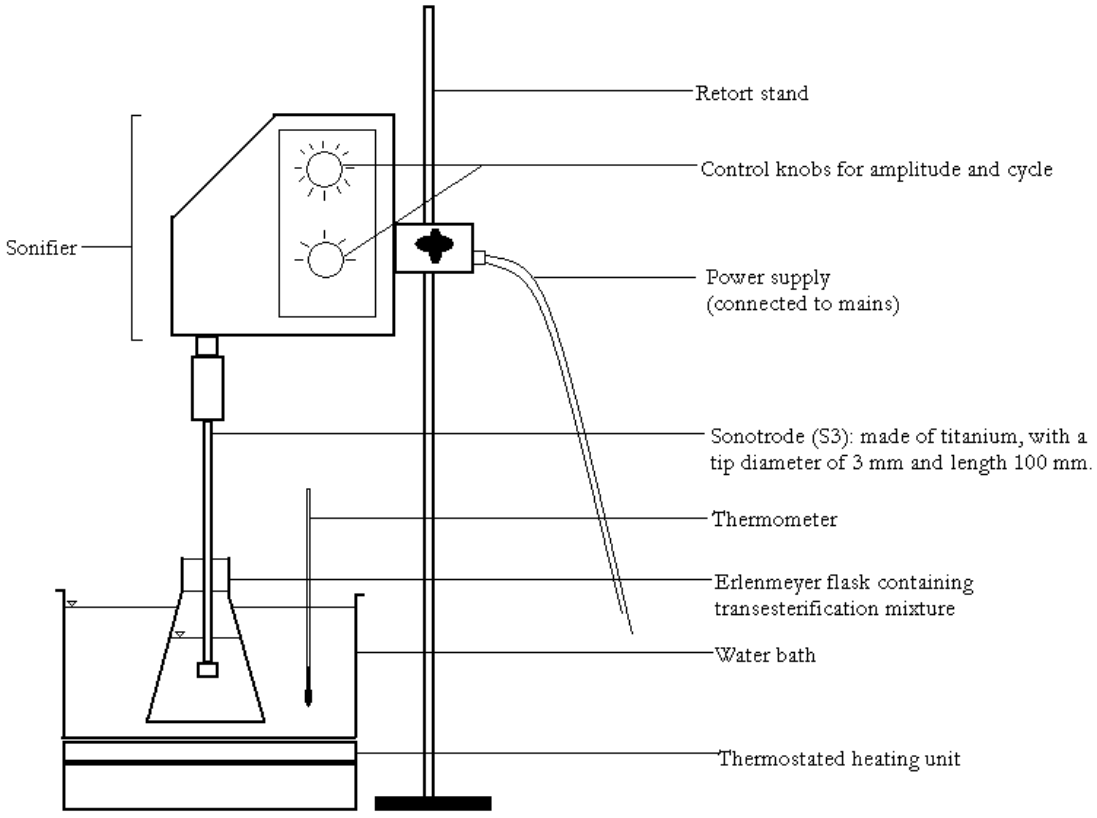

Figure 5.1. Schematic diagram of the ultrasound assisted in-situ transesterification process.

The ultrasound agitated reactor consists of an ultrasonic electric generator (sonifier) which converts the standard electricity line supply of 50 Hz to high frequency electrical power at 24 kHz. The high frequency electrical energy is then fed to a transducer, which converts it to mechanical vibrations of the same frequency.

The transducer consists of a piezoelectric element, which expands and contracts in response to the applied alternating voltage, causing it to vibrate in a longitudinal direction (Colucci et al., 2005). The resulting vibrations are transmitted to the tip of the sonotrode horn, immersed in the transesterification mixture.

The vibrations are transmitted to the reaction mixture, causing cavitations within the liquid medium. The cavitation process involves the continuous formation and collapse of microscopic bubbles. This leads to the creation of shockwaves within the surrounding

solution, thus facilitating cell disruption and enhanced mixing of the solution (Stavarache et al., 2005).

The sonotrode used in this study (S7, Hieslcher Ultrasonics GmbH) was made of titanium, with a diameter of 3 mm and a length of 10 cm. The ultrasonicator was set to a 100% power level and maintained for the duration of the transesterification process.

The ultrasonication set-up was used to investigate the effect of varying levels of reacting alcohol volumes on the biodiesel conversion process. The aim was to determine if the volume of alcohol required for the in-situ process could be reduced without negatively affecting the chemical conversion of *Chlorella* oil to FAME.

From the trends observed in section 4.3.1, it was shown that there were no improvements in the equilibrium FAME yields using the in-situ method, when the molar ratio of methanol to *Chlorella* oil in the biomass was more than 315:1, at a reaction temperature of 60°C. A reacting methanol/oil ratio of 315:1 was then chosen as the base level at which further improvements to the process alcohol requirement would be investigated in this chapter.

The study of the influence of low frequency ultrasound and various alcohol volumes on in-situ transesterification of *Chlorella* oil involved the use of methanol/Chlorella oil molar ratios of 105:1, 210:1, and 315:1. This corresponds to the use of 20, 40 and 60 ml methanol for the in-situ transesterification of 15 g *Chlorella* biomass. As in Chapter 4, 20 ml was chosen as the lowest methanol volume, since this was the minimum volume required to fully submerse the dried microalgae samples.

The reactor vessels (Erlenmeyer flasks) were maintained at the process temperature of 60°C by fully immersing them in a thermostated water bath (Figure 5.1). During the course of the experiments, the reaction temperature was monitored using a glass thermometer, as shown in Figure 5.1.

To investigate the effect of low frequency ultrasound agitation on the reaction time, the ultrasound assisted transesterification process was terminated after reaction times of 0.033, 0.066, 0.133, 0.25, 0.5, 1 and 2 h. This was carried out for each reacting methanol level. The minimum time of 0.033 h was selected since it was observed during the pre-

experimental trials that a complete and uniform suspension of the reactor contents was attained after an ultrasonication time of 70 s. After the specified reaction period, the ultrasonicator was switched off and the biodiesel product extracted as previously described in Chapter 4. The reactions were then conducted in triplicates for every level of investigation.

The *Chlorella* residues were obtained, as in section 4.2.1, by filtration. The biodiesel product was then purified with the same methods described in section 4.2.1. The biodiesel SG was determined, and the percentage mass content of FAME in the biodiesel was calculated at the different variable levels. The uncertainties associated with the percentage FAME content in the biomass was estimated same as used in Chapter 4 (presented in Appendix I).

The results obtained were then compared with those for the mechanical agitated system (with a stirring speed of 500 rpm), previously reported in Chapter 4.

5.2.2. In-situ transesterification with co-solvents

The minimum molar reacting ratio of alcohol to oil used in the in-situ transesterification experiments was mainly dependent on the volume of alcohol required to fully submerse the solid biomass samples. The reduction of the reacting alcohol volumes was then examined using co-solvents in the in-situ transesterification process. This involved the combination of different proportions of the selected co-solvents with the reacting methanol, to ensure complete submersion of the microalgae biomass in the liquid phase, while enabling reduced reacting alcohol to oil molar ratios.

For the in-situ transesterification of 15 g dried *Chlorella* biomass, three volumes (5, 10 and 15 ml) of the selected co-solvents (n-pentane (C_5H_{12}) and diethyl ether ($(C_2H_5)_2O$) were tested. The use of these co-solvent levels corresponded to the use of reacting molar ratios of methanol to *Chlorella* lipids of 79:1, 52:1 and 26:1 (i.e. 15, 10, and 5 ml methanol) respectively.

The criteria used in the screening and choice of the co-solvents, and the reasons for the experimental co-solvent volumes used in the transesterification process, are presented in section 5.3.2.

To determine the effect of co-solvents on the FAME production the results were compared with those previously obtained in Chapter 4. The in-situ experiments were conducted in triplicates using the same reaction conditions as when the reaction was carried out using methanol containing acid catalyst alone: 15 g of *Chlorella* biomass, reaction temperature of 60°C, and 0.04 mol H_2SO_4 acid catalyst was used.

Since the in-situ process was conducted at a temperature greater than the normal boiling points of the co-solvents used in this investigation (i.e. 36.1 and 34.6°C for n-pentane and diethyl ether respectively), the experiments were carried out in a pressure controlled cupboard as described in section 4.2.2. An absolute reaction pressure of 300 kPa acting on the reacting vessels was maintained for the experiment duration. This ensured that the co-solvents remained in a liquid phase throughout the transesterification process.

The in-situ transesterification reactors were mechanically agitated at 500 rpm using Teflon covered magnetic stirring bars. The reaction was terminated at pre-determined times of 0.25, 0.5, 1, 2, 4 and 8 h. After the transesterification reaction, the cupboard was depressurised by opening the vent. The bottle stoppers were then removed to facilitate the escape of the co-solvents in the vapour phase. This was carried out in the fume cupboard with the extractor fan on.

The biodiesel product was then extracted from the mixture, as in section 4.2.1, with additional steps included. A further separation step was applied which involved the centrifugation of the filtrate after the residue filtration at 7300 g for 7 min. This was carried out to hasten the formation of the two liquid phases in the filtered mixture compared to the use of gravity separation as used previously in Chapter 4. The biodiesel product was then purified by extraction with hexane.

The SG of the biodiesel product, mass content of FAME in the biodiesel samples (%) and the biodiesel and FAME yields (g/g microalgae biomass) were determined as previously described in section 4.2.7.

5.2.3. The use of ultrasound and co-solvents for the in-situ transesterification process

An additional investigation was carried out to see if coupling co-solvent use with ultrasound agitation would result in any improvements in the FAME conversion with the in-situ process.

Here, the in-situ process was conducted in triplicates using the different proportions of co-solvents to methanol with the same experimental methods described in section 5.2.2. However, instead of mechanically stirring the reactor contents, low frequency ultrasound mixing (24 kHz) was applied as in section 5.2.1.

Here, 15 g of the dried *Chlorella* biomass was used for the in-situ transesterification process. The process temperature was maintained at 60°C by immersing the vessels containing the reactants in a thermostatically-controlled water bath, as shown in Figure 5.1.

The in-situ transesterification experiments were then conducted in a pressurised fume cupboard as described earlier. The reaction was stopped after reaction periods of 0.033, 0.066, 0.133, 0.25, 0.5, 1, 2 and 4 h.

The methyl esters were then separated using the same purification processes as in section 5.2.2, and the SG of the biodiesel product and percentage FAME mass in the biodiesel samples (%) determined as earlier. The mass of the purified biodiesel product was also recorded and used to estimate the biodiesel and FAME yields (g/g microalgae biomass) as previously described.

An overview of the modifications applied to the in-situ transesterification set-up studied in this chapter is shown in Figure 5.2. The figure shows the different schemes that were applied for FAME production using ultrasonic stirring and/or co-solvents in the in-situ transesterification process.

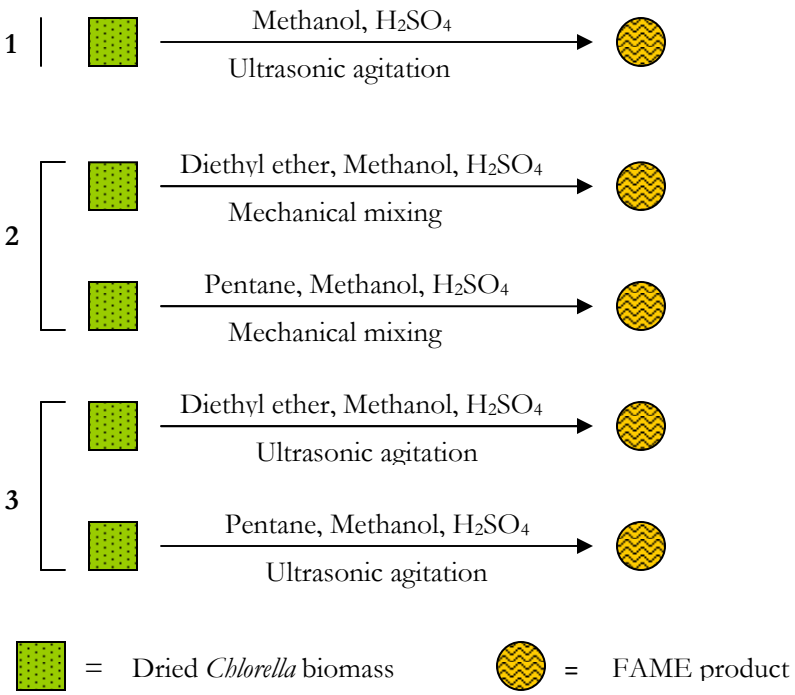

Figure 5.2. Modifications to the in-situ transesterification process studied in this chapter.

In Figure 5.2, the numbers 1, 2 and 3 refer to the different transesterification modifications applied as described in sections 5.2.1-5.2.3 respectively.

5.3. Results and Discussion

5.3.1. The use of low frequency ultrasound for the in-situ process

The results for the mass percentage FAME conversion obtained for the ultrasonic agitated in-situ experiments are shown in Figure 5.3, for three ratios of reacting methanol to oil and a range of reaction times. The reaction temperature was fixed at 60°C, with 0.04 mol H_2SO_4 used as the process catalyst.

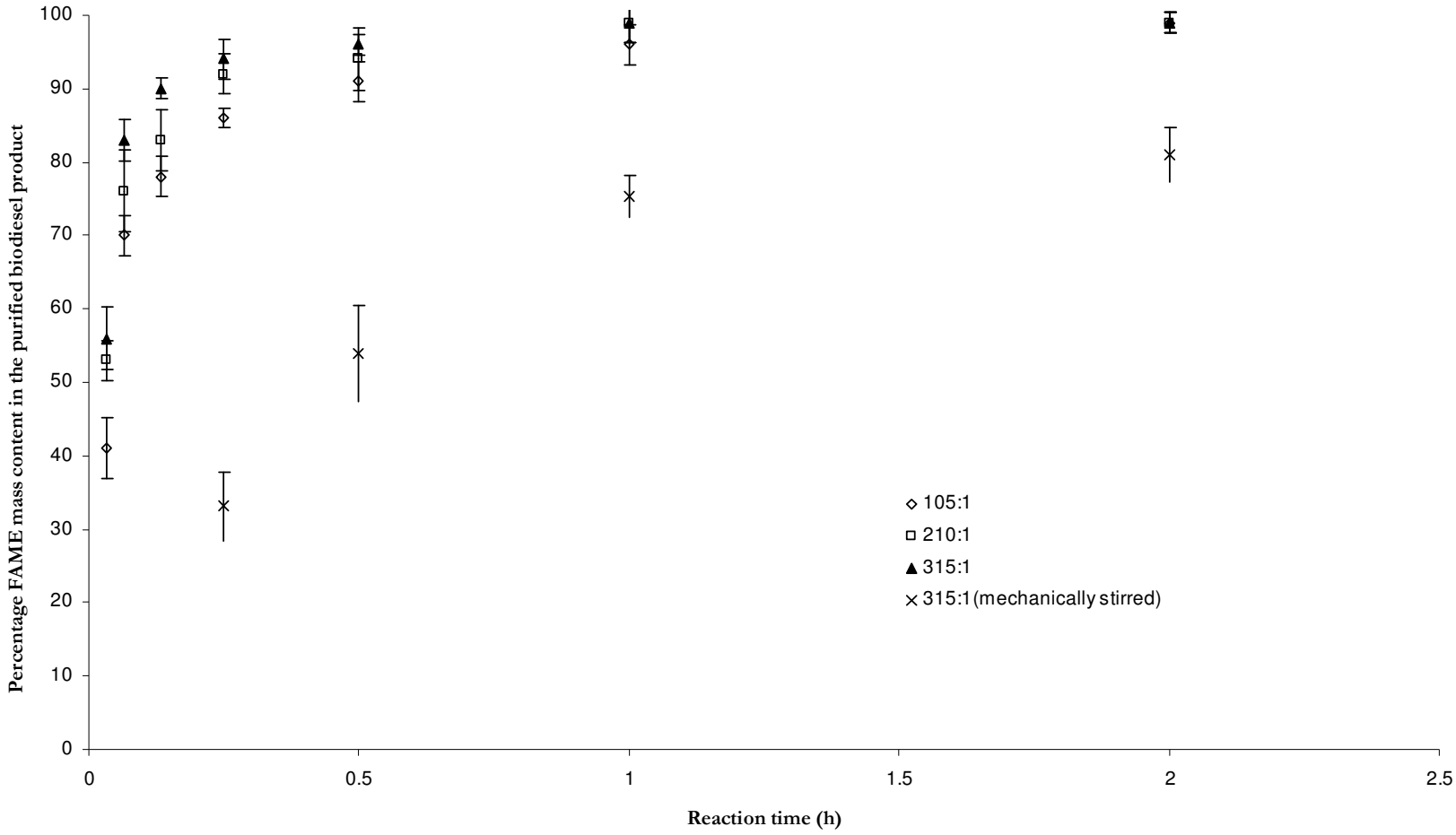

Figure 5.3. Percentage mass FAME conversion with reaction time (h) for the ultrasound assisted (24 kHz) process compared with the mechanically stirred transesterification (15 g *Chlorella* biomass, 0.04 mol H_2SO_4 and reaction temperature of 60°C).

It was observed that with the use of ultrasound agitation (24 kHz), a reaction time of 2 h was required to achieve a maximum equilibrium mass FAME conversion of 99.0 ± 1.4% for all the different levels of reacting alcohol. Also, a 91.0 ± 2.8, 94.0 ± 4.2 and 96.0 ± 1.4% of the percentage mass oil to FAME conversion was obtained after a reaction time of 0.5 h using methanol/*Chlorella* oil molar ratios of 105:1, 210:1 and 315:1 respectively.

These results indicate that, when low frequency ultrasound is used for the in-situ transesterification of *Chlorella* biomass, the use of the minimum methanol volume (20 ml) was sufficient for the conversion of the lipids to FAME with high efficiency.

Under similar reaction conditions, the ultrasound agitated in-situ set up was seen to lead to increases in the FAME mass content in the biodiesel (%, w/w) when compared with the mechanically agitated process trends (Figure 5.3). After a reaction time of 0.5 h, the percentage mass FAME in the biodiesel product for the mechanically stirred system was 55.6 ± 10.8 % with the use of a methanol/oil ratio of 315:1. This was less than the mass oil to FAME conversion of 91.0 ± 2.8% achieved for the ultrasonicated process with the use of a reduced molar ratio of methanol to oil of 105:1 with a similar reaction time.

After a reaction time of 1 h, with the use of a methanol/oil ratio of 315:1, the biodiesel yield obtained for the ultrasound assisted in-situ process was determined to be 0.295 ± 0.003 g biodiesel/g dry *Chlorella*. This biodiesel yield was higher than that obtained from the mechanically stirred in-situ experiment i.e. 0.269 ± 0.005 g/g *Chlorella* biomass under similar reaction conditions.

These results were attributed to improved availability of lipids from the microalgae biomass facilitated by the cellular disruption by low frequency ultrasound, as opposed to the passive diffusion of the lipids across the microalgae cell walls under mechanical stirring. The mechanical effects facilitated by the local high kinetic energies produced during the ultrasonication process, as well as from the solid biomass particles being impinged on one another, could have assisted in the increased disruption of the microalgae cell wall. This could consequently increase the availability of the cellular lipids for participation in the transesterification process. This may explain the increases in the purified biodiesel yields over the mechanically stirred in-situ transesterification process. This explanation was supported by the results of Raman et al. (2010), which showed improved lipid extraction

from microalgae (*Scenedesmus sp.*) when ultrasound was applied, compared with the use of extraction solvents alone. An increase in the extraction of cellular contents via the microalgae cell wall disruption due to the mechanical impact of decavitation was also described by Katoh & Yoshida (2009).

The increased percentage mass FAME conversions obtained by the ultrasound assisted process may be due to the vigorous mixing speed. The increased speed would facilitate an increased mass transfer between the microalgae lipids and the reacting methanol, when compared to the mechanically stirred process. The local turbulences associated with acoustic cavitation could therefore assist in enhancing the transport processes, leading to a reduction (or elimination) of mass transfer resistances in the reacting system (Gogate, 2008)

The formation and collapse of cavitation bubbles encountered during the ultrasonication process, apart from increasing the lipid extraction, may also contribute to the increased FAME content in the microalgae derived biodiesel. Bondy & Söllner (1935) and Chisti (2003) described the generation of high local pressures of ≈ 1000 atm and temperatures > 4000°C by the collapsing cavitation bubble. The local temperature in the vicinity of the formed or collapsed bubble was reported to change rapidly at a rate of > 110°C s^{-1} (Chisti, 2003). Due to these conditions, the dissociation of the reactant vapours trapped in the cavities could lead to the formation of free radicals of the reacting species during the cavitation process (Rokhina et al, 2009). These radicals may subsequently contribute to an acceleration of the formation of the transesterification products as achieved with the ultrasound assisted process.

The improvements in the percentage mass oil to FAME conversions and FAME yields observed with the use of low frequency ultrasound compared to mechanical stirring could therefore be due to the combination of both physical and chemical effects.

5.3.2. The influence of co-solvents on the in-situ transesterification process

5.3.2.1. Criteria for co-solvent selection

The selection of the co-solvents used in this study was based on the following four major criteria[13], highlighted below:

1. The co-solvent must be miscible with the reacting oil;
2. The co-solvent should ideally be immiscible with water;
3. The co-solvent must be chemically inert during the transesterification process; and
4. The solvent should be harmless or have a low toxicity.

The solvent miscibility with the reacting biomass oil was considered an important factor for the selection of the co-solvent since this would aid the oil extraction from the microalgae biomass.

Regarding the solvent immiscibility with water requirement, this would ensure that the solvent created an optimised environment for the transesterification process. It has already been shown that the presence of water in the transesterification (and esterification) process limits FAME production by favouring the backward formation of fatty acids as highlighted in 2.4.2. Thus sequestration of the microalgae lipids to be transesterified in a water free environment by the co-solvents should favour enhanced FAME production.

The use of chemically inert co-solvents was based on the assumption of their non-participation in the transesterification reactions, thus ensuring the purity, chemical and physical properties of the FAME products. Non-participation of the selected co-solvent in the transesterification process was also expected to ease FAME and the solvent recovery. Since it is anticipated that the co-solvents will be recovered from the methanol after the

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[13] Some of the criteria used in the selection of the process co-solvent were derived from a study by Xu & Mi (2010).

transesterification process, the energy costs associated with the use of co-solvents is important. This will be examined later in Chapter 8.

The toxicity of the selected co-solvent was also important in this study because the processes investigated in this study should be suitable for scaling up to an industrial level. Therefore, only solvents with low risk of adverse or detrimental effects on the health of the workers in the biodiesel production process were considered.

Two solvent candidates, n-pentane and diethyl ether $(C_2H_5)_2O$, were selected for this experiment, mainly due to their miscibility with oil and immiscibility with water (Perry & Green, 1998). Co-solvents such as chloroform, hexane, petroleum ether, toluene and dichloromethane were not used in this study since they have been previously studied for use in the in-situ transesterification of dry *Schizochytrium limacium* and *Spirulina* biomass by Johnson & Wen (2009) and Xu and Yi (2010) respectively.

The low toxicity of n-pentane and diethyl ether also contributed to their selection as potential co-solvents. McKee et al. (1998) carried out a toxicological assessment on n-pentane and demonstrated that it was not acutely toxic via oral or inhalation routes. That study also showed that it was not a skin or eye irritant, was not mutagenic and did not exhibit cumulative toxicity at levels up to 20 g/m^3. The non-toxicity of diethyl ether after human subjects were exposed to it was reported by Stevens et al. (1973). Safety issues must however be addressed with diethyl ether use for biodiesel production since it is a well known anaesthetic, although it is now rarely used in medicine. Prolonged inhalation of the vapour should be avoided since it could result in drowsiness and unconsciousness.

It is however still expected that utmost care would be exercised in the event that the selected co-solvents were used on an industrial scale. The release and accumulation of n-pentane into the local environment was reported to react with nitrogen oxides in polluted air to form ozone and other photochemical oxidants (EAUK, 2010). These can build up to concentrations that may be damaging to vegetation and human health. No information on the local or global environmental effects associated with diethyl ether emissions was found in the literature. However its release into the environment should also be restricted.

5.3.2.2. The influence of co-solvent use on the in-situ transesterification process

Tests of the in-situ transesterification of 15 g of the *Chlorella* biomass were carried out using molar alcohol to oil ratios of 79:1, 52:1 and 26:1. These was achieved by the combination of the reacting methanol with 5, 10 and 15 ml of the co-solvents (diethyl ether and n-pentane) respectively.

The results for the percentage mass FAME content in the biodiesel product, depending on the different types and level of co-solvents used, are shown in Figure 5.4.

The trends observed with the use of diethyl ether as an in-situ transesterification co-solvent was seen to mainly lead to an increase in the mass content of FAME in the biodiesel (%) for all levels of methanol/oil molar ratios and reaction times, compared with the use of the same level of n-pentane as co-solvent. This was with the exception of the trends observed for transesterification process carried out after a reaction time of 1 h, using a molar ratio of methanol to oil of 52:1, in which the percentage FAME conversions were seen to be higher using pentane over diethyl ether as the process co-solvent.

The maximum oil to FAME conversion of 79.9 ± 7.1% (w/w) was obtained with a methanol/oil ratio of 79:1 using diethyl ether as the co-solvent, after a reaction time of 8 h. This result was similar to the percentage mass FAME conversion of 77.8 ± 7.7% observed with the use of a reacting molar ratio of methanol to oil of 105:1 alone for the in-situ process. This percentage conversion was obtained using the measured SG (0.886) recorded for the triplicate in-situ runs carried out using 20ml methanol for the transesterification of 15 g dry Chlorella with a reaction time of 8h as shown previously in Figure 4.3. Thus the use of this diethyl ether co-solvent level, and its integration into the in-situ transesterification, assisted in achieving a reduction in the methanol process requirement without affecting the FAME conversion yield.

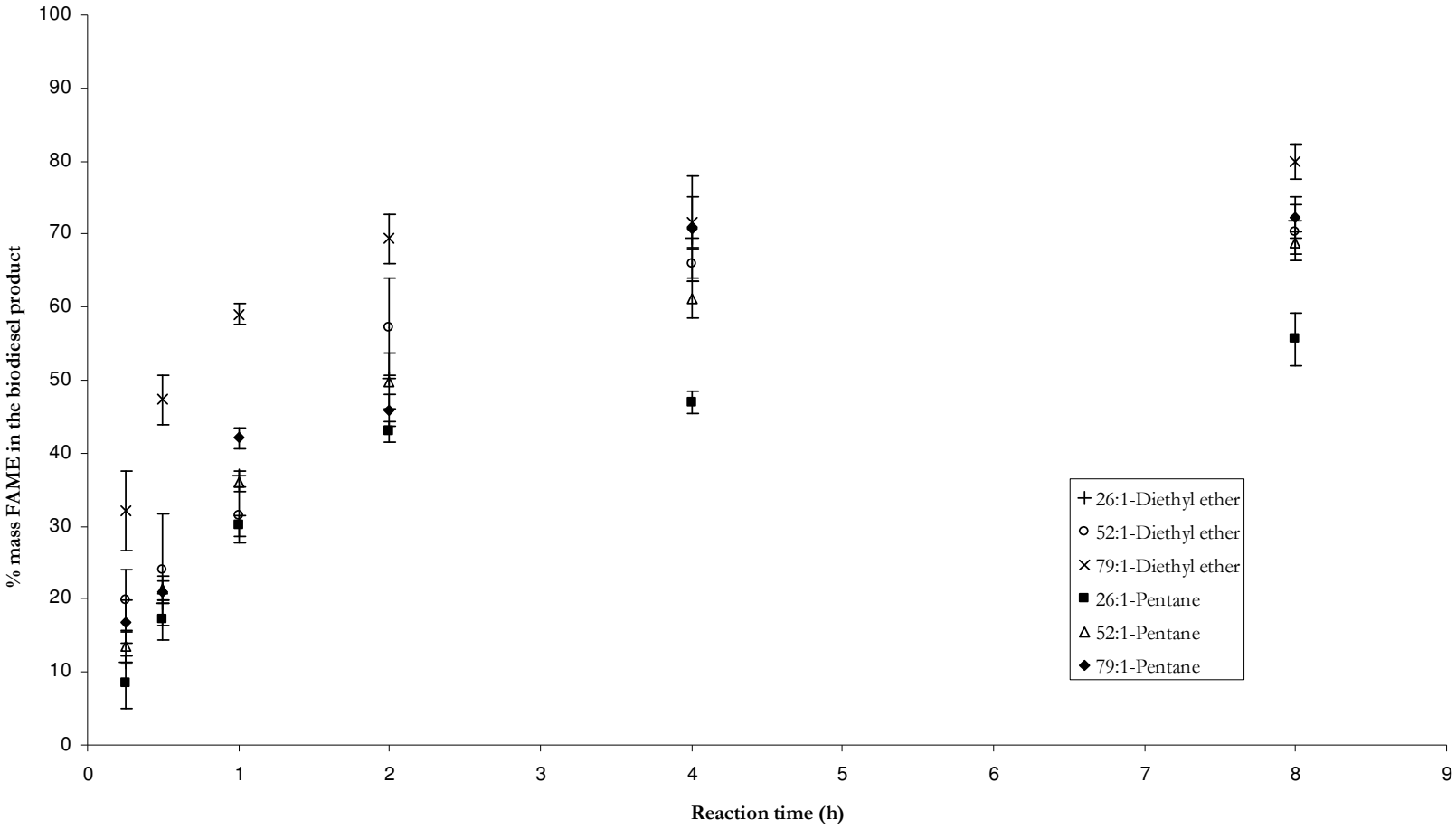

Figure 5.4. Percentage FAME content in biodiesel product (w/w) with time for the mechanically stirred in-situ process using diethyl ether and n-pentane as co-solvents (0.04 mol H_2SO_4 catalyst, reaction temperature of 60°C, reacting methanol/oil molar ratios of 26:1, 52:1 and 79:1).

5.3.3. Influence of both ultrasound and co-solvents in in-situ transesterification

As a result of the reduction in the reacting molar alcohol requirement obtained with the use of the modifications applied to the in-situ process (i.e. use of ultrasound and co solvents separately), the combination of these schemes was further explored.

Figure 5.5 shows the results of the percentage mass FAME content in the biodiesel samples obtained with the use of low frequency ultrasound with reacting molar ratios of methanol to oil of 26:1, 52:1 and 79:1.

With ultrasound agitation, a 99% mass conversion of the *Chlorella* oil to FAME was observed using n-pentane and diethyl ether, with an uncertainty of ± 2.6 and ± 3.6 % respectively. This involved the use of a reacting methanol/oil of 79:1 after a reaction time of 2 h. A similar percentage mass FAME content in the biodiesel samples was also achieved using a reacting methanol/oil molar ratio of 52:1 with diethyl ether as the co-solvent.

The combination of the ultrasound and co-solvent use was seen to result in improvements in the percentage mass FAME conversion and reaction times trends compared to the use of the reacting methanol alone for the mechanically stirred in-situ process.

With the use of a molar ratio of methanol to oil of 315:1, mechanical agitation at 500 rpm, and reaction conditions as in section 4.2.2, the percentage mass FAME conversion was 88.5 ± 3.4% after a reaction time of 8 h. This was 12% less than the average percentage mass FAME conversion obtained using a reduced methanol/oil molar ratio of 52:1 for the in-situ process with similar reaction conditions (with ultrasound stirring, diethyl ether as co-solvent, and a reaction time of 2 h).

A further examination of the biodiesel and FAME yields obtained from the integration of co-solvents under different agitation regimes and using varied molar ratios of methanol to oil for the in-situ transesterification of microalgae biomass is shown in Table 5.1.

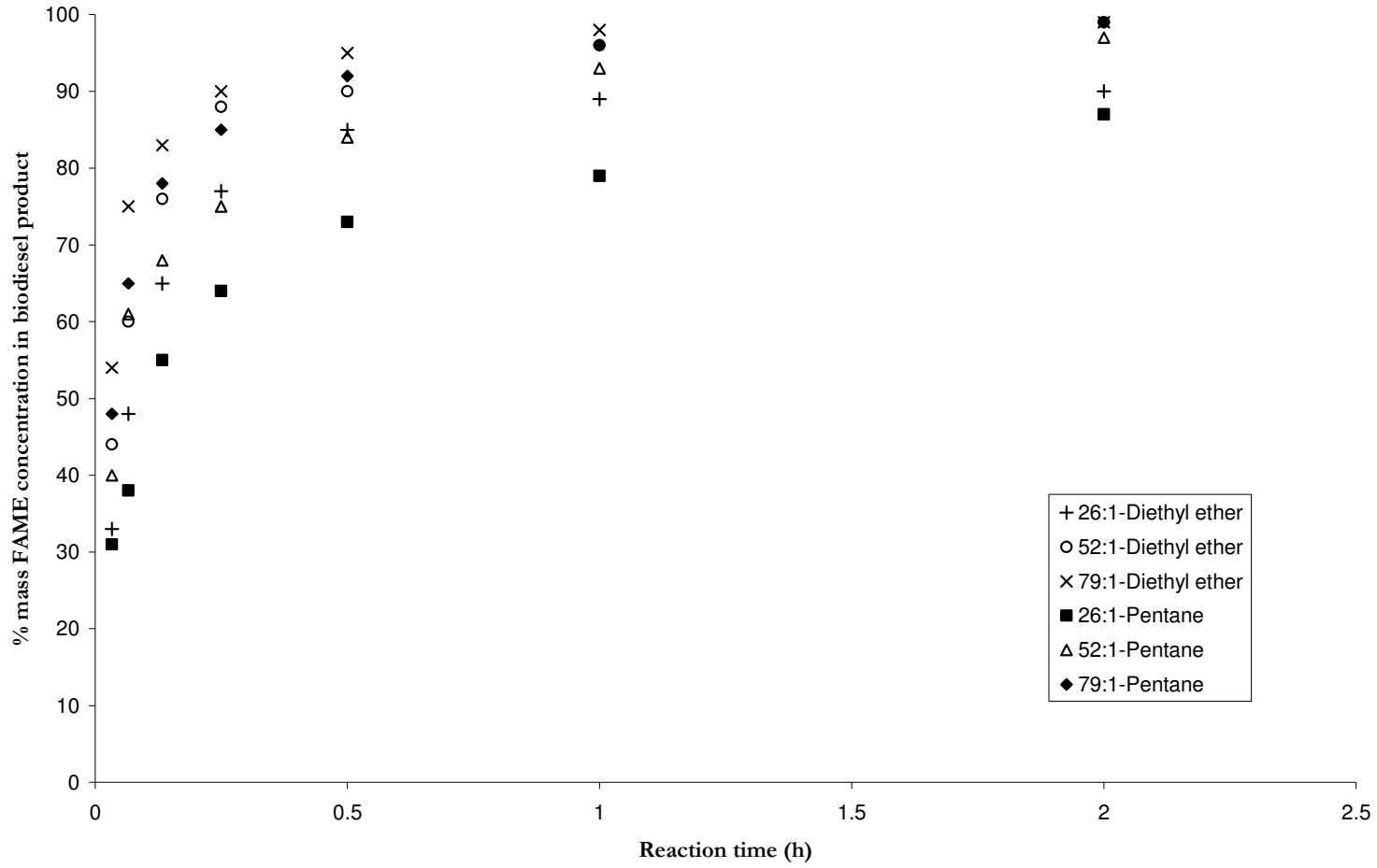

Figure 5.5. Mass FAME conversion (%) with time for the ultrasonicated in-situ process using diethyl ether and n-pentane as co-solvents (0.04 mol H_2SO_4 catalyst, reaction temperature of 60°C, molar methanol to oil ratios of 26:1, 52:1 and 79:1).

Table 5.1. Biodiesel and FAME yields obtained from the in-situ transesterification process using pentane and diethyl ether as co solvents (*Chlorella* biomass, 15 g: catalyst, 0.04 mol H_2SO_4; process temperature, 60°C)

Co-solvent	Reaction time (h)	Molar ratio of reacting methanol to oil	Biodiesel SG	% mass FAME conversion	Biodiesel Yield (g biodiesel/g dry *Chlorella*)	FAME yield (g FAME/g dry *Chlorella*)
In-situ process (mechanically stirred, 500 rpm)						
Diethyl ether	8	26:1	0.8890 ± 0.0040	71.6 ± 10.6	0.270 ± 0.004	0.193 ± 0.021
	8	52:1	0.8895 ± 0.0041	70.2 ± 11.4	0.275 ± 0.002	0.193 ± 0.022
	8	79:1	0.8861 ± 0.0022	79.9 ± 6.3	0.283 ± 0.001	0.226 ± 0.014
Pentane	8	26:1	0.8946 ± 0.0048	55.6 ± 14.1	0.268 ± 0.003	0.149 ± 0.021
	8	52:1	0.8900 ± 0.0006	68.8 ± 2.3	0.274 ± 0.001	0.188 ± 0.004
	8	79:1	0.8880 ± 0.0045	74.5 ± 12.5	0.277 ± 0.003	0.206 ± 0.026
In-situ process (ultrasonic agitation)						
Diethyl ether	2	26:1	0.8816 ± 0.0022	92.8 ± 6.3	0.285 ± 0.004	0.265 ± 0.017
	2	79:1	0.8782 ± 0.0011	99.9 ± 3.6	0.297 ± 0.002	0.297 ± 0.011
Pentane	2	26:1	0.8827 ± 0.0025	89.7 ± 7.1	0.290 ± 0.003	0.260 ± 0.019
	2	52:1	0.8790 ± 0.0006	99.9 ± 2.6	0.290 ± 0.004	0.290 ± 0.009
	2	79:1	0.8784 ± 0.0006	99.9 ± 2.6	0.293 ± 0.002	0.293 ± 0.005

The biodiesel yield for the mechanically agitated in-situ processes with co-solvents and reduced reacting methanol molar ratios were seen to be considerably lower than obtained using methanol alone as described earlier.

The use of low frequency ultrasound (24 kHz) was seen to improve the biodiesel and FAME yields trends, with the reasons for the enhancement proposed to be same as have been previously reported in section 5.3.1. With the use of ultrasound and co-solvents, it was found that the FAME yields from the in-situ transesterification process could be improved by 15-17% compared with the use of methanol and acidic catalysts alone, with a reduction in the molar ratios of the reacting methanol to oil reduced from 315:1 to 79:1

5.4. Chapter Conclusion

From the results obtained in this chapter it was shown that it is feasible to reduce the large alcohol volumes required for the in-situ process using low frequency ultrasound for the reaction agitation. Increases in the biodiesel and FAME yields were also obtained via this process modification. N-pentane and diethyl ether were selected as co-solvents for use in this study. Further reductions in the transesterification methanol requirement were attained with the integration of co-solvents in the in-situ transesterification process. With the use of mechanical agitation for process stirring, the percentage FAME biodiesel content (w/w) and FAME yields from the processes with co-solvents were observed to be less than that of the in-situ process using methanol alone. The co-solvent assisted process however used reduced reacting methanol levels than the latter process set-up. The combination of the ultrasonic agitation and co-solvent use was shown to increase the percentage mass oil to FAME conversion, biodiesel and estimated FAME yields trends with reduced reacting volumes of methanol used for the transesterification process.

Although the investigations in this chapter did not consider the recovery and re-use of the co-solvents, this is a major issue which must be considered for the application of these process modifications. This is since it would be economically and environmentally infeasible to emit these solvents directly to the environment. The use of recovery techniques such as distillation or membrane separation could be applied to recover the solvents from the post

transesterification mixture. Solvent recovery via distillation would be highlighted later in Chapter 8.

To further improve the energetics of the in-situ transesterification process using *Chlorella*, the potential for additional energy production from the post transesterified biomass residues will be investigated in this study. The generation of additional energy from this otherwise waste product could be used to offset some of the energy requirements of the in-situ transesterification process. An introduction to methane (CH_4) production from the microalgae residues, as well as the laboratory investigations on the recoverable energy potentials with the use of this feedstock is presented in Chapter 6.

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6. POTENTIAL METHANE RECOVERY FROM THE POST TRANSESTERIFICATION MICROALGAE RESIDUES (BATCH EXPERIMENTS)

6.1. Introduction

In addition to investigating the energetic improvements that could be attained in the overall "microalgae biomass to biodiesel" process via the use of the in-situ transesterification of the biomass lipids, this thesis also examined the recovery of energy from the microalgae residues, post transesterification. This route has the potential for generating energy that could be used to offset part of the energy requirements of the biodiesel production process. The use of the residual biomass for energy production would also serve as a waste treatment route, thereby reducing the load and environmental impacts of the otherwise waste product.

Methane (CH_4) production from the *Chlorella* residues (post transesterification) using the anaerobic digestion process was proposed in this study. To assess the potential benefits which could be obtained with this process, information on the optimum CH_4 yields from the residue feedstock must initially be provided.

As mentioned in section 2.12.2, a number of theoretical evaluation methods are available for the prediction the potential CH_4 production from biomass feedstocks (i.e. Buswell & Mueller, 1952; Boyle, 1977; Cowley & Wase, 1981). However, due to the limitations of such techniques i.e. overestimation of CH_4 yields (Hansen et al. 2004), a practical assessment was proposed for use in this study.

This chapter examines the application of batch anaerobic digestion tests to provide initial information on the maximum recoverable CH_4 yields from post transesterified *Chlorella* residues. Theoretical assessments of the maximum CH_4 yield from the post transesterified residues, and its HHV (MJ/kg microalgae residue), were also performed.

These were used as a preliminary basis to establish the potential for CH_4 production and energy recovery from the *Chlorella* residues.

This chapter also investigates the co-digestion of the microalgae residues with the glycerol quantities co-produced from the transesterification process. Glycerol is a by-product of the biodiesel production process (≈0.1 g glycerol formed with 1.0 g of biodiesel produced). It is usually upgraded and sold as feedstock to the chemical, cosmetic, pharmaceutical and food industries. This includes its use in the production of alkyd resins, absolute alcohol and personal care products such as soaps and laxatives (McCoy, 2006).

However the glycerol market has been faced with oversupply mainly due to an increase in global biodiesel production coupled with the shut-down of major glycerol refining and consumption industries (McCoy, 2006). This in turn has triggered a collapse of the price of this commodity. Yadzani & Gonzalez (2007) showed that while crude glycerol (80% mass concentration) production increased from 75.7 to 832.7 million l in the period 2004-2006, the price fell from 55 to 4 US cents/kg. Thus, glycerol, which was once considered a valuable transesterification co-product, is now regarded as a waste with a disposal cost attached to it (Yadzani & Gonzalez, 2007).

In addition, Haas et al. (2006) showed that the cost of biodiesel production has an inverse relationship with value of the glycerol co-product. As a consequence, conversion routes which could transform the glycerol co-product into alternative higher value products are increasingly being studied in order to improve the economics and overall viability of the biodiesel production process.

The use of glycerol as a primary or co-digestion feedstock in the anaerobic digestion process has been considered as a potential scheme to enhance the energy and cost value of this by-product (Yadzani & Gonzalez, 2007). This chapter investigates the use of glycerol as a co-digestate in the anaerobic digestion of the microalgae residues. Its influence on practical CH_4 yields was also examined.

This chapter presents a preliminary study, using batch anaerobic digestion tests, in order to answer the following questions:

i. How much useable energy (as CH_4) can be recovered (theoretically and practically) from the post-transesterified *Chlorella* residues via anaerobic digestion?

ii. What influences would the initial oil extraction steps (i.e. the type of extraction solvent) and the transesterification methods have on CH_4 yields?

iii. What practical CH_4 yields are obtainable on co-digesting the produced glycerol fraction with the residual microalgae biomass?

Some of the results presented in this chapter have been published in the paper "Energy recovery from lipid extracted, transesterified and glycerol co-digested microalgae biomass" (Ehimen et al, 2009) which is shown in Appendix A (CD-ROM).

6.2. Materials and Methods

6.2.1. Microalgae residues and biomass characterisation

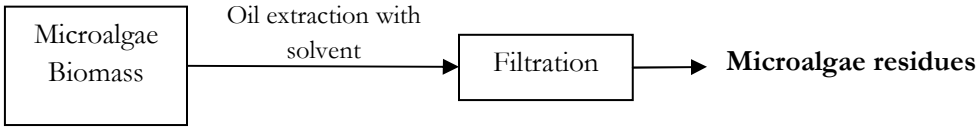
Methane (CH_4) production was examined using *Chlorella* biomass residues obtained using the conventional and in-situ transesterification processes as described earlier in Chapters 4 and 5.

A simplified overview the sampling points used in recovery of the biomass residues is shown in Figure 6.1 for both the conventional and in-situ transesterification methods.

The biomass retentate collected after the filtration of the in-situ transesterified mixture (as described in section 4.2.1) was continuously collected and pooled for use as the in-situ samples in this investigation.

The in-situ samples were obtained after conducting the in-situ process with dried *Chlorella* biomass using the following reaction conditions: methanol/oil ratio, 315:1; acid catalyst, 0.04 mol H_2SO_4; reaction time, 8 h; temperature, 60°C and mechanical stirring at 500 rpm.

Conventional Process:



In-situ Transesterification Process:

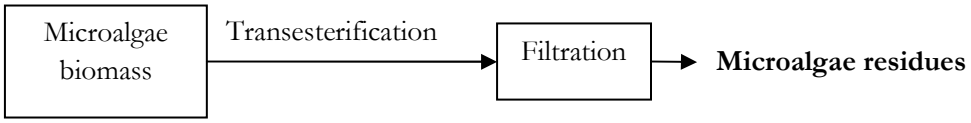


Figure 6.1. An overview of the microalgae residues recovery for the anaerobic digestion process.

Similarly, the *Chlorella* residues resulting after the lipid extraction process using a butanol and chloroform-methanol mixture (2:1, chloroform to methanol, v/v) were collected for use. The residues were labelled and represent the conventional samples.

The batches of the solvent extracted and transesterified *Chlorella* residues were deep frozen at -19°C and later thawed at room temperature (24 ± 2°C) in quantities required for the anaerobic digestion tests.

6.2.1.1. Total and volatile solids determination

Prior to their use in the anaerobic digestion experiments, the *Chlorella* residues were characterised on the basis of their total (TS) and volatile solids (VS). The TS (g/g sample) correspond to the sum of the organic (volatile) and inorganic (fixed) content of the samples. The VS (g/g sample) on the other hand refer to the organic fraction of biomass sample. The VS provides a rough estimation on the content of the sample which could be converted using biological processes. The TS and VS are important parameters in anaerobic digestion

studies since they are usually used as a basis in assessing the efficiency or degradability of samples.

The determination of the TS and VS of the *Chlorella* residues samples was carried out in duplicates using the standard methods described in APHA (2005).

Clean labelled porcelain evaporating dishes and covering glasses were heated in a muffle furnace at 550°C for 1 h. The heated ceramic wares were then cooled to room temperature (24 ± 2°C) in desiccators (containing granular anhydrous calcium chloride, CaCl). The dishes and covering watch glasses were weighed with an analytical balance (accuracy of 0.1 mg) prior to use, and their combined weight recorded as W_{dish}.

The pooled post-transesterified *Chlorella* residues were mixed thoroughly. The samples were carefully weighed (25 ± 0.1 g), and spread evenly in the evaporating dishes. The evaporating dishes (containing the samples) were covered with watch glasses (to prevent sample splattering), weighed and recorded as W_{sample}.

The samples were introduced into a forced draft oven where they were dried at 105°C for 12 h. After the drying period, the samples were extracted, cooled in desiccators and weighed. The residues were then re-heated for 1 h with the same oven temperature (105°C), cooled and re-weighed. The heating (for 1 h), cooling and desiccation were repeated till a weight change of < 4% (compared to the previous weighing) was observed. The final weight was recorded as W_{total}.

The evaporating dishes containing the dried samples were then transferred to a cool muffle furnace. The furnace was then heated to 550°C with the samples ignited for 2 h.

After the ignition time, the residues were carefully extracted from the furnace and transferred to desiccators to allow cooling to a room temperature (24 ± 2°C).

Close attention was paid to the desiccators during the cooling process, with the opening of the desiccators minimised to prevent the absorption of moist air by the dried samples. The residues were extracted from the desiccators and weighed.

A repetition of the sample ignition (30 min) at 550°C, cooling, desiccating and weighing was carried out until a weight change of < 4% was achieved. The final weight was recorded as $W_{volatile}$.

The total and volatile solids of the samples were calculated using Eqs. 6.1 and 6.2 respectively.

$$\text{Total solids (TS), g total solids/g sample} = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} \quad (6.1)$$

$$\text{Volatile solids (VS), g volatile solids/g sample} = \frac{W_{total} - W_{volatile}}{W_{sample} - W_{dish}} \quad (6.2)$$

where,

W_{dish} is the weight of the evaporating dish and watch glass (g);

W_{sample} is the weight of the wet sample and dish (g);

W_{total} is the weight of the residue dried at 105°C and the dish (g);

$W_{volatile}$ is the weight of the residue and dish after ignition at 550°C (g).

The uncertainties of the determined TS and VS of the residues were calculated from the percentage error contributions of the standard errors of the measurements (i.e. as in Eq. 6.3 for the TS measurements).

$$\frac{\Delta TS}{TS}\% = \sqrt{\left(\left(\frac{\Delta W_{Dish}}{W_{Dish}}\%\right)^2 + \left(\frac{\Delta W_S}{W_S}\%\right)^2 + \left(\frac{\Delta W_T}{W_T}\%\right)^2\right)} \quad (6.3)$$

where, $\Delta TS/TS$ = the percentage error associated with the estimated sample TS

$\Delta W_{Dish}/W_{Dish}$ = the percentage error associated with the mass of the dishes

$\Delta W_S/W_S$ = the percentage error associated with the mass of wet microalgae samples

$\Delta W_T/W_T$ = the percentage error associated with the mass of dried microalgae residues (at 105°C).

6.2.1.2. Elemental analysis of the Chlorella residues

An elemental analysis of the lipid extracted and post transesterified biomass residues was carried out to determine the percentage composition of C, H, O, N and S in the samples using a Carlo Erba EA 1108 Elemental Analyser. This was performed using the same methods as for the untreated *Chlorella* biomass, as previously described in section 3.2.4.

The results of the elemental analysis (i.e. empirical formula and the % elemental composition of the biomass residues) were then used in the theoretical methane production evaluations, presented in 6.2.2.

6.2.2. Theoretical methane potential and overall energy assessment of the *Chlorella* biomass residues

The theoretical CH_4 yield from the residual *Chlorella* biomass samples was estimated using two different methods:

(i) The digestion reaction proposed by Boyle (1977), which utilises the elemental composition of the biomass (Eq. 6.4):

$$C_aH_bO_cN_dS_e + \left[a - \frac{b}{8} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right]H_2O \longrightarrow \left[\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right]CH_4 + \left[\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} - \frac{e}{4}\right]CO_2 + dNH_3 + eH_2S \quad (6.4)$$

The estimation by Boyle (1977) was used in preference to the more commonly used Buswell equation (Buswell & Mueller, 1952), since the former equation incorporates the biomass nitrogen and sulphur content, which are not included in Buswell's equation.

From the results of elemental analysis of the biomass samples, an empirical formula for the *Chlorella* biomass residues was obtained. The subscripts for C, H, O, N and S were then used to assess the theoretical CH_4 production from the *Chlorella* residues using Eq. 6.4.

(ii) The use of the macromolecular composition of the biomass as described by Cowley & Wase (1981). This assessment is based on the theoretical CH_4 production of 1.04, 0.49 and 0.37 m^3 of CH_4 per kg of the biomass lipid, protein and carbohydrate content respectively.

The lipid, carbohydrate and protein fractions of the untreated and residual *Chlorella* samples were estimated from the results of the percentage elemental analysis of the biomass using the R-value concept and constituents' calculation method (Spoehr & Milner, 1949)[14].

The estimated macromolecular fractions for the residue samples were then used to assess the potential for CH_4 production from this biomass feedstock.

The knowledge of the lipid fraction of the biomass residues also served as a means of monitoring the extent of lipid removal (or its conversion to biodiesel) after employing the conventional or in-situ transesterification process. This was further used to highlight the efficiency of the preceding extraction or transesterification process.

In addition to the theoretical CH_4 estimation, the higher heating value (HHV) of the *Chlorella* residues, post transesterification, was calculated from the elemental percentage composition. This was carried out using the approximations reported by Meraz et al. (2003), presented earlier in Eq. 3.6. This was performed to assess the efficiency of the CH_4 recovery scheme on the basis of the enthalpy of combustion of the microalgae residues.

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[14] A detailed explanation of the R-value concept, including its use in this study is presented in Appendix D.

6.2.3. Practical anaerobic methane potential test

The CH_4 evaluation and measurement method described by Shanmugam & Horan (2009) was modified and utilised in this study as described in the subsequent sub-sections (6.2.3.1-6.2.3.3).

6.2.3.1. Anaerobic inoculum

An active anaerobic inoculum in granular form was obtained from Waste Solutions, Dunedin, New Zealand for use in this study. The inoculum was originally obtained from an up-flow anaerobic sludge bed (UASB) bioreactor used for the treatment of dairy industry process wastewater.

To characterise the inoculum samples, the TS and VS of the granular inoculum were determined using the methods described earlier in 6.2.1.1. Before the characterization, the inoculum granules were initially crushed to facilitate size reduction.

Prior to its use in the anaerobic digestion experiments, the inoculum (bacterial culture) was acclimatised to experimental operational conditions.

First 500 ml of the crushed granular inoculum (158.6 g total dry solids of the inoculum) was diluted to a total volume of 4 l with water in a closed reactor. To create an initial anaerobic environment in the vessel, the air in the space above the liquid phase was displaced using nitrogen gas. The vessel was constantly stirred under anaerobic conditions at 37.0 ± 1.0°C for 14 d with sucrose added at a rate of 1 g l^{-1} d^{-1}.

A modified mineral and trace metal solution, described by Shelton & Tiedje (1984) as shown in Table 6.1, was also added to the reactor at 10 ml/l vessel volume once. A buffer system was also provided once in the vessel with the addition of 350, 250 and 5000 mg/l of anhydrous di-potassium hydrogen orthophosphate (K_2HPO_4), potassium dihydrogen orthophosphate (KH_2PO_4), and sodium hydrogen carbonate ($NaHCO_3$) respectively.

After the acclimatisation period, to further adapt the bacterial culture for the degradation of the test microalgae biomass, 10.0 ± 0.1 g of powdered untreated microalgae was introduced to the system. The digestion of the biomass was maintained until CH_4 production ceased after 21 days. The process was further repeated with 5 g of the post transesterified *Chlorella* residues.

Table 6.1. Composition of the anaerobic digestion mineral and trace metal solution

Nutrient Source	Concentration (mg/l)
Ferrous sulphate hepta-hydrate, $FeSO_4.7H_20$	100
Manganese sulphate mono-hydrate, $MnSO_4.H_2O$	85
Cobalt (II) chloride hexa-hydrate, $CoCl_2.6H_2O$	170
Calcium chloride di-hydrate, $CaCl_2.2H_2O$	100
Zinc sulphate penta-hydrate, $ZnSO_4.5H_2O$	210
Copper sulphate penta-hydrate, $CuSO_4.5H_2O$	29
Perbromic acid, H_3BO_4	10
Sodium molybdate, $NaMO_4$	10
Sodium chloride, NaCl	1000
Sodium selenate, Na_2SeO_4	17
Nickel dichloride hexa-hydrate, $NiCl_2$-$6H_2O$	24

6.2.3.2. Experimental set-up

The collected filtered biomass samples obtained after the oil extraction and transesterification processes were used in the anaerobic CH_4 potential test.

The practical CH_4 production tests were carried out in duplicates using sealed 500 ml Duran® glass bottles (as the anaerobic reactors) equipped with tubes and gas opening valves. Microalgae residues (4.0 ± 0.1 g, on a dry mass basis) were weighed and inserted into the reactor bottles.

Figure 6.2 shows a schematic representation of the batch anaerobic experimental set-up.

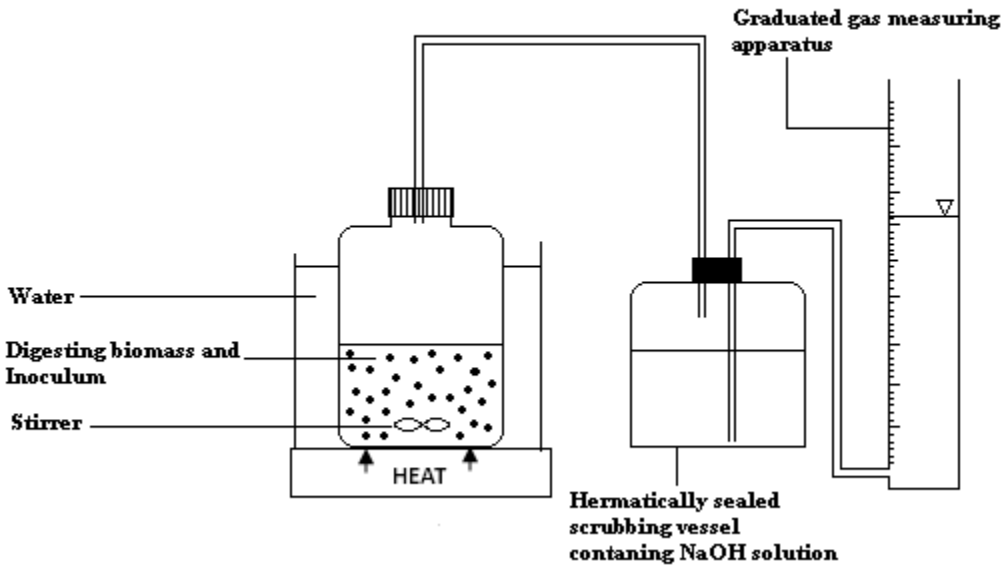

Figure 6.2. Schematic representation of the batch anaerobic reactors.

The prepared inoculum (section 6.2.3.1) was drawn from the acclimatisation vessel and carefully introduced to the reactor bottles to reach a target test volume of 400 ml.

The process pH range (6.7-6.9) of the reactors was maintained with the use of the buffer solutions in concentrations as described earlier described in section 6.2.3.1.

Batch reactors containing only the inoculum were prepared and used as a blank in this study. For the blanks, instead of the *Chlorella* residue, water was added to the reactor bottles to achieve the same volume as for the reactors containing the digesting residues. This was

carried out to investigate any potential additional production of CH_4 from the inoculum used.

Before sealing the batch reactors, residual oxygen was removed from the headspace of the vessels by flushing with pure nitrogen gas (N_2).

The vessels were transferred to a temperature controlled water bath at 37.0 ± 0.5°C, where the reactor bottles were fully immersed. Continuous mixing of the reactor contents was provided by the use of a magnetic stirrer (300 rpm). The reaction vessels were also agitated by shaking the bottles once a day to ensure complete re-suspension of the sediments and scum layers.

The CH_4 production was measured by the displacement of 5% molar sodium hydroxide (NaOH) solution from sealed glass bottles connected to the gas outlet of the batch reactor. The displaced NaOH solution was then measured using a measuring cylinder. NaOH solution was used as the displacement solution since it effectively absorbs the acidic gas contents i.e. carbon dioxide (CO_2) from the produced biogas after the anaerobic digestion process, leaving the CH_4 fraction (Guwy, 2004).

The displaced NaOH solution, collected and recorded at normal temperature and pressure, represents the CH_4 volume produced and was reported as ml CH_4/g (dry) biomass.

The practical CH_4 production from the *Chlorella* residues was obtained after accounting for CH_4 produced from the blank samples containing the inoculum and water.

The reaction conditions of the anaerobic vessels were maintained until the formation of CH_4 ceased. This was based on the criterion that the daily CH_4 production (ml CH_4) was less than 1% of the total CH_4 volume produced up to that time.

The total recorded CH_4 production was taken to represent a full (or almost complete) degradation of the *Chlorella* residues (Verein Deutscher Ingenieure, 2006).

6.2.3.3. Co-digestion of the *Chlorella* residues with glycerol

Glycerol solution (85 % purity, Merck KGaA) was considered for use in this study as a co-digestate. The purity of the glycerol solution which contained 12-16% water was chosen for use based on demonstrations in the literature i.e. Zhang et al. (2003) and Sheehan et al. (1998) which showed that crude glycerol produced in industrial biodiesel units with this purity are essentially methanol free.

The quantity of glycerol used in the anaerobic digestion process was based on the assumption of co-digesting the microalgae residues with the equivalent glycerol obtainable from the biomass sample via the transesterification process.

Eq. 6.5 was used in the calculation of the glycerol quantity for use as a co-digestate in the batch reactors. This equation assumed that all the microalgae oil was present as triglycerides.

$$\text{Equivalent glycerol production (g/g dry biomass transesterified)} = \frac{a \cdot M_{glycerol}}{M_{oil}} \quad (6.5)$$

where, a is the oil fraction of biomass;

$M_{glycerol}$ the molecular weight of glycerol;

$M_{oil:\ the}$ molecular weight of oil.

With a *Chlorella* oil fraction of 0.27, oil molecular weight of 880 g/mol, and a glycerol molecular weight of 92, this relates to 0.028 g glycerol produced/g (dry) microalgae biomass transesterified. This could also be represented as 0.028 g glycerol produced with 0.73g (dry) *Chlorella* residues

The anaerobic experimental process was carried out in duplicates as earlier described in section 6.2.3.2, but involved the use of 0.25 g of 85% glycerol (i.e. 0.21 g pure glycerol) and 5.85 g of the dried *Chlorella* residues in the reactor bottles.

For the study of the conventional transesterification samples, only the biomass residues obtained after extraction using 1-butanol were used. The reasons for the use of only the 1-butanol extracted samples will be highlighted in section 6.3.4.

6.3. Results and Discussion

6.3.1. Characterisation of the residual biomass samples and theoretical methane production

The TS and VS of the microalgae residues and inoculum are presented in Table 6.2.

Results from the microalgae biomass residues were obtained, based on the extraction and transesterification processes used: (i) conventional 1-butanol extracted; (ii) conventional chloroform-methanol extracted; and (iii) acid catalysed in-situ transesterified. These residual samples are denoted as 1-B, C-M and ACIST respectively in this study.

Table 6.2. Total (TS) and volatile solids (VS) of the samples

Sample	TS (g total solids/g sample)	VS(g volatile solids/g sample)
1-Butanol extracted *Chlorella* residues (1-B)	0.730 ± 0.008	0.670 ± 0.008
Chloroform-methanol extracted *Chlorella* residues (C-M)	0.750 ± 0.009	0.670 ± 0.008
In-situ transesterified *Chlorella* residues (ACIST)	0.680 ± 0.007	0.640 ± 0.007
Granular Inoculum	0.440 ± 0.006	0.250 ± 0.004

The results of the elemental analysis, empirical formula, and the calculated carbon to nitrogen (C/N) mass ratios of the microalgae residues samples are shown in Table 6.3.

Table 6.3. Elemental composition, empirical formula and mass carbon to nitrogen ratios of the *Chlorella* residue samples

Parameter (Ash free)	1-B	C-M	ACIST
% Carbon, C	47.45	46.63	44.77
% Hydrogen, H	7.05	7.17	7.46
% Nitrogen, N	9.89	10.13	9.39
% Oxygen, O	34.57	34.80	36.22
% Sulphur, S	1.04	1.28	2.15
Empirical formula	$C_{122}H_{216}N_{22}O_{67}S$	$C_{97}H_{179}N_{18}O_{54}S$	$C_{56}H_{110}N_{10}O_{33}S$
Carbon/Nitrogen (C/N) ratio	4.80	5.40	4.66

It was observed that the sulphur content (per d.w) of the ACIST samples was considerably higher than those recorded for the 1-B and C-M samples (Table 6.3). The ACIST sulphur levels were also higher than those of the untreated microalgae biomass as shown in Table 3.2.

Since the biomass residues were not subjected to a rinse step (with water), the increased sulphur levels of the ACIST samples may be due to the transesterification catalyst-H_2SO_4, which could have remained entrained in the microalgae residues after the filtration stage. The increased sulphur levels could lead to an increase in the H_2S fraction of the biogas produced using the ACIST samples. This could result in a drop of the digester pH levels and hamper the microbial gasification process (Sorensen, 2000).

The C/N ratios of the biomass samples enable a preliminary assessment to be made about the overall feasibility of the anaerobic digestion process. As highlighted in section 2.12.1.6, if the C/N mass ratio of the starting organic material is high (> 30), the nitrogen content may be insufficient to meet the growth protein demands of the anaerobic microbial population, thereby resulting in a diminished CH_4 production (Sorensen, 2000). Conversely, with a low C/N ratio (<15), there is potential for nitrogen build-up in the form of NH_3. The accumulation of ammonia could lead to an increase in the process pH levels with a corresponding toxic inhibition of the bacterial population.

The C/N ratio of the residual *Chlorella* biomass (4.70-5.40) was found to fall below the range of 15-30 recommended by Sorensen (2000) for complete anaerobic digestion and maximum CH_4 production. This could indicate that a complete degradation of the residues might not be achieved for the *Chlorella* residues, due to the possibility of "ammonia poisoning" of the bacteria in the digester.

The result of the calculated macromolecular fractions (lipid, protein and carbohydrate) of the residual *Chlorella* biomass using the R-value concept (presented in Appendix D) is shown in Table 6.4. The estimation was based on the assumption of ash and sulphur free dry biomass samples.

Table 6.4. Lipid, carbohydrate and protein composition of the dry residual and untreated *Chlorella* samples (ash and S free)

Macromolecular fraction (% ash free dry biomass, w/w)	1-B	C-M	ACIST	Untreated
Lipid	1.96	0.64	0.19	28.30
Carbohydrate	35.60	35.23	39.62	26.20
Protein	62.44	64.13	60.19	45.50

To serve as a basis for comparison, the macromolecular composition of the untreated *Chlorella* biomass (referred to as "untreated") was also investigated and shown in Table 6.4. The composition of the untreated samples was based on the elemental composition of the *Chlorella* samples presented earlier in Chapter 3.

Information on the efficiency of the lipid extraction and transesterification process could also be obtained from the estimation of the macromolecular composition of the residue samples.

A comparison of the calculated lipid fractions of the residues with the untreated *Chlorella* biomass samples (Table 6.4) shows a reduction of the biomass lipid fraction after the solvent extraction and transesterification processes.

The use of the acid catalysed in-situ transesterification method appears to result in the greatest stripping of the microalgae lipids. This could be further interpreted to indicate an improved lipid removal (compared to the 1-B and C-M extraction methods) with the use of the acid catalysed in-situ transesterification process. The findings from the theoretical estimation appear to confirm the observations and explanations regarding the improved extent of oil stripping of the in-situ process over the conventional transesterification method as described earlier in section 4.3.6.

The protein content of the biomass appears to remain largely unchanged after the extraction or transesterification steps, even for the ACIST samples where the use of inorganic acid catalysts was expected to cause denaturation and the possible loss of some of the proteins available in the microalgae residues.

6.3.2. Theoretical methane potential and HHV of the microalgae residues

Using the methods described by Boyle (1977) and Cowley & Wase (1981), the CH_4 production potential of the post lipid extracted and transesterified *Chlorella* residues, and that of the untreated microalgae biomass, was obtained, as described in section 6.2.2. The results are shown in Table 6.5.

Table 6.5. Theoretical CH_4 production estimates using the biomass elemental and macromolecular composition

Sample	CH_4 Production (m^3 CH_4/kg TS) (Based on the empirical formula)	CH_4 Production (m^3 CH_4/kg TS) (Based on the macromolecular fractions)
1-B	0.48	0.43
C-M	0.48	0.43
ACIST	0.46	0.42

The results indicate that the CH_4 production potential obtained from the macromolecular compositions are ≈10% lower than those calculated from the elemental composition of the *Chlorella* residue samples. Nevertheless, similar CH_4 production potentials were predicted for both solvent extracted samples, despite the differences in their estimated empirical formulae and macromolecular compositions.

The theoretical CH_4 estimation provides an approximation of the maximum methane yields achievable by the use of the anaerobic digestion process i.e. only gives an indication on the energy obtainable from the digestible fraction of the biomass. It however does not provide information on the overall energy efficiency of the anaerobic digestion process i.e. on the basis of the energy content of the microalgae residues.

The thermo-chemical equation (as shown in Eq. 3.6) was used for the estimation of the combustion enthalpy of the *Chlorella* residues.

The HHV of the lipid extracted and transesterified biomass residues was obtained and shown in Table 6.6.

Table 6.6. Higher heating values (HHV) of the *Chlorella* residues

	1-B	C-M	ACIST
Estimated higher heating value (MJ/kg)	22.32	22.36	21.76

In Section 6.3.3, the HHV of the samples (Table 6.6) is compared with the experimental CH_4 yields to examine the efficiency of the practical anaerobic CH_4 generation scheme using the *Chlorella* residues.

The results in Table 6.6 indicate that similar HHVs are obtainable for both solvent extracted samples. It must however be noted that there were only small differences in their estimated empirical formulae and in their macromolecular compositions.

6.3.3. Measured methane production potential for the microalgae residues

The averaged results of the methane (CH_4) production (ml CH_4/g TS biomass) obtained in duplicate for the untreated, 1-B, C-M and ACIST samples are shown in Figure 6.3.

Although the estimated theoretical CH_4 yields for the 1-B and C-M residues were similar (Table 6.5), the practical anaerobic digestion experiments show that CH_4 production trends from the C-M samples was lower than those of the 1-B samples. The C-M extracted samples exhibited a possible microbial inhibition (Figure 6.3), as described in Verein Deutscher Ingenieure (2006).

The increase and decrease in the average CH_4 production seen in Figure 6.3 for the 1-B sample and untreated samples at 5 and 12 days respectively was due to the difference in the recorded CH_4 yields from the duplicate runs. This deviation did not however affect the overall CH_4 yields, with the % error associated with the final CH_4 production being <1.4%.

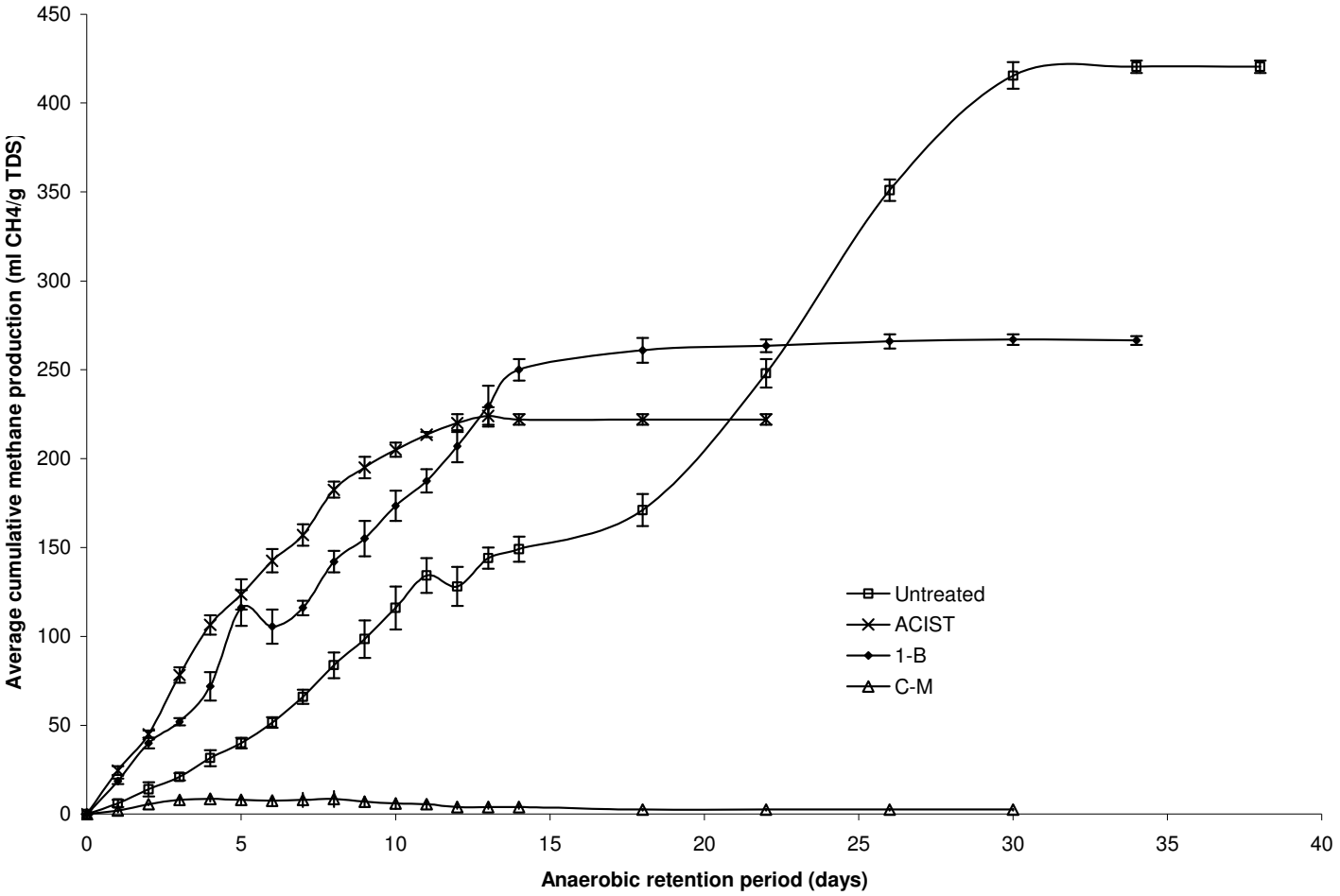

Figure 6.3. Cumulative CH_4 production from 1-butanol (1-B) and chloroform-methanol (C-M) extracted biomass, residues after the acid catalysed in-situ transesterification process and untreated *Chlorella* biomass.

After the filtration step to obtain the post transesterified (or lipid extracted) microalgae residues, the biomass was weighed. The microalgae residues were then dried to a constant weight as described in section 6.2.1.1. For the C-M samples, using the difference of the biomass residues before and after drying, the chloroform-methanol solvent entrained in the biomass was calculated to be 13.2 ± 0.7% of the weight of the dried microalgae residues. With 4 g (on the basis of TS) of the biomass residues added to the batch digester, this indicates that 0.418 g of chloroform was introduced into the digestion vessel.

It is most likely that the low rate of CH_4 production obtained for the C-M samples is due to the inhibitory effect of chloroform (that remained bound to the residual microalgae biomass after the filtration process) on the anaerobic bacterial cultures. This suggestion is consistent with demonstrations by Thiel (1969) which showed that the introduction of 8.0 μM chloroform had resulted in a 50% inhibition on methanogenesis by having a direct toxic effect on the methanogenic bacteria.

Methane production from the ACIST samples was shown to reach asymptotic values after a digestion period of 11-12 days. This was less than the >14 days required for the degradation of the 1-B extracted and untreated *Chlorella* samples (Figure 6.3).

The improvement in the digestion times recorded for the digestion of the ACIST samples may have been due to the initial pre-treatment facilitated by acid. The use of H_2SO_4 as the reaction catalyst may have improved the digestibility of the biomass macromolecules. The resulting microalgae residues after the in-situ process could be more susceptible to microbial digestion, hence the shorter retention periods. Although most of the acid catalyst was removed during the filtration process, it was titrimetrically determined (using 0.05 M NaOH) that 0.29 g H_2SO_4 was present in the post transesterified residues. The anticipated anaerobic process inhibition reported in section 6.3.1 resulting from H_2S accumulation in the batch reactors due to elevated sulphur levels in the ACIST samples was not fully explored during this study.

As shown in Figure 6.3, the practical CH_4 production trends from the *Chlorella* residues was seen to be lower than the theoretical yields estimated in Table 6.5. The CH_4 yields (m^3 CH_4/kg TS microalgae residue) from the batch anaerobic digestion of the 1-B and

ACIST *Chlorella* residues were approximately 60 and 50% of the maximum theoretical yields respectively.

With the HHV of CH_4 of 39.3 MJ/m^3, using the cumulative CH_4 yields obtained from the microalgae residues (Figure 6.3), a heat recovery of 10.5 ± 0.1 and 8.7 ± 0.1 MJ/kg TS residue from the 1-B and ACIST samples respectively was obtained by the anaerobic digestion route. This relates to an average biomass to CH_4 conversion efficiency of 47 and 40% for the 1-B and ACIST samples respectively, on the basis of the maximum HHV estimates of the microalgae residues as shown in Table 6.6. The untreated sample was seen to exhibit the highest CH_4 yields, with a 63% conversion efficiency achieved when compared to the maximum combustion enthalpy estimated for the microalgae sample.

The percentage conversion efficiency for the C-M sample was however not determined due to the process inhibition observed.

6.3.4. Co-digestion of the *Chlorella* residues with glycerol

Using Eq. 6.4 for the estimation of CH_4 production from glycerol ($C_3H_8O_3$), a theoretical production of 0.45 m^3 per kg glycerol digested was obtained. Since 0.25 g of 85% glycerol (i.e. 0.21 g glycerol) and 5.85 g TS *Chlorella* residues were used in the batch anaerobic study, the theoretical CH_4 production is 0.43 and 0.42 m^3 CH_4 per kg of co-digested 1-B-glycerol and ACIST-glycerol samples respectively.

Due to the CH_4 production inhibition seen with the use of the C-M samples seen in section 6.3.3, the C-M extracted residues were not considered further in this section.

As described earlier the HHV of the co-digested samples was estimated. For the digested glycerol fraction, a HHV of 16.13 MJ/kg for glycerol ($C_3H_8O_3$) reported by Fernando et al. (2007) was used. This corresponds to an estimated HHV of 22.10 and 21.56 MJ/kg for the co-digested 1-B-glycerol and ACIST-glycerol samples respectively.

The results of cumulative CH_4 production for the practical batch anaerobic test carried out for the co-digested samples are shown in Figure 6.4.

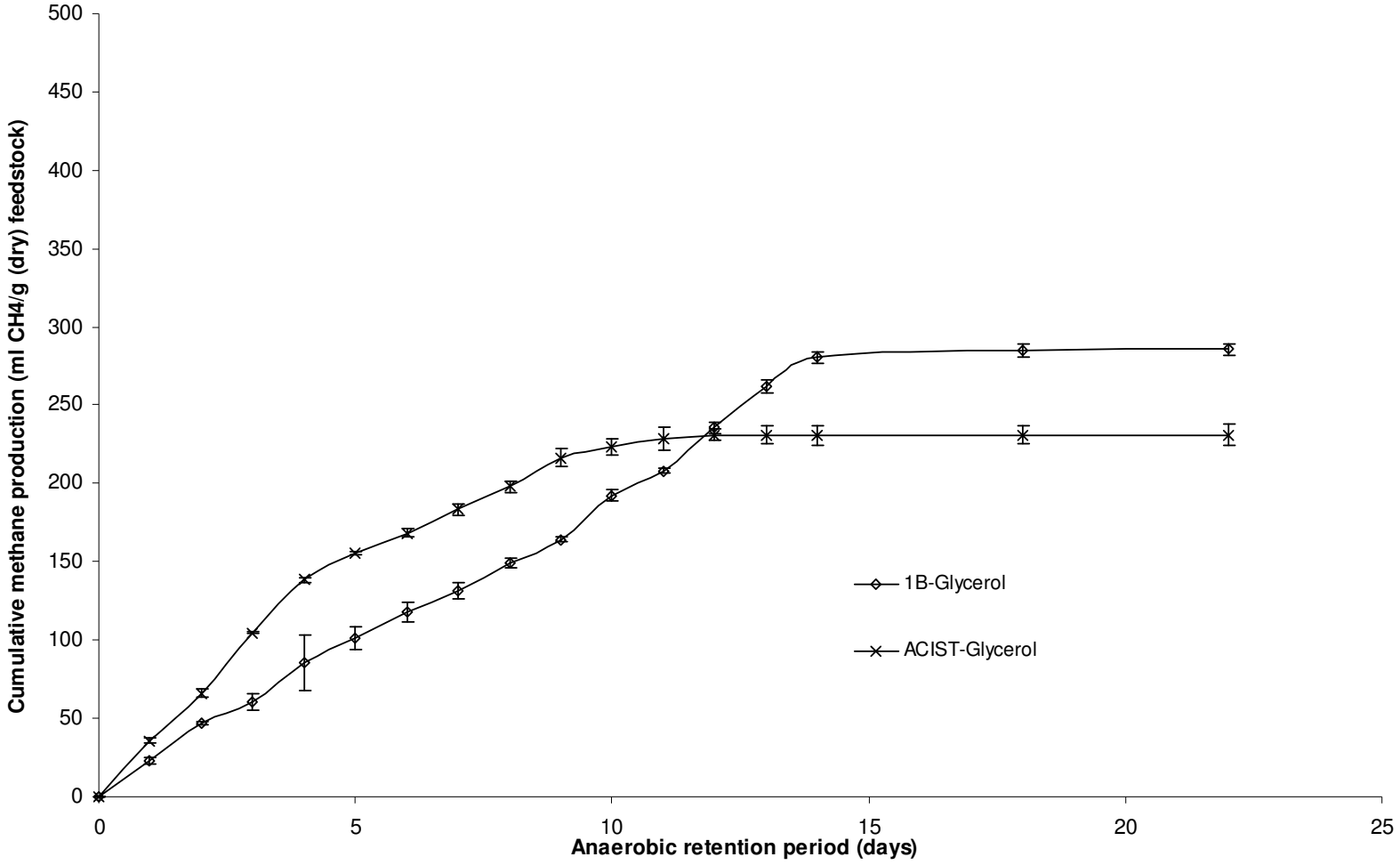

Figure 6.4. Average CH_4 production from the co-digestion of the lipid extracted (1-butanol) and in-situ transesterified microalgae residues with glycerol.

The co-digestion of the microalgae biomass residues and glycerol was found to improve CH_4 production, compared to when the microalgae residues were digested alone. An increase in the energy yield to 9.1 and 11.3 MJ/kg TS residue digested was obtained with the use of the glycerol co-product with the ACIST and 1-B samples respectively. The results indicate a 4-8% increase in CH_4 production from co-digestion with the glycerol quantities co-produced during the transesterification process. On the basis of the HHV of the digested samples, a microalgae residue-methane conversion efficiency of 51 and 42% was obtained for the co-digested 1-B-glycerol and ACIST-glycerol samples respectively. The observed increase in the anaerobic CH_4 production trends after the residue co-digestion may be attributable to the fact that the added glycerol is present in an easier to degrade liquid phase. However, to properly estimate the conversion efficiency of the microalgae residues-CH_4 process, an accounting of the CO_2 released during the digestion process should be carried out in addition to the knowledge of the residues elemental composition and CH_4 produced. This was not performed in this study since the CO_2 emitted in the batch processes were absorbed by the NaOH solution (used as a scrubbing unit).

6.4. Chapter Conclusion

This chapter examined the practical CH_4 production from *Chlorella* residues obtained following the oil extraction process or its transesterification to biodiesel. The purpose of the study was to provide preliminary information on the use of the residues for the production of additional energy, which could potentially improve the overall energetics or economics of biodiesel production from microalgae biomass.

A higher heating value of 21.8-22.4 MJ/kg TS *Chlorella* residues was estimated for the microalgae residues, depending on the preceding lipid extraction or transesterification route. The theoretical CH_4 yield of the anaerobic digestion process was estimated to be 0.42-0.46 m^3CH_4/kg TS for the ACIST samples. The theoretical CH_4 yields obtained for the 1-B and C-M samples were similar with 0.43-0.48 m^3 CH_4/kg TS sample. The results obtained from the laboratory-scale batch anaerobic CH_4 production tests were however less than the theoretical CH_4 estimates with an average recoverable energy of 8.7 and 10.5 MJ/kg TS residue recorded for the ACIST and 1-B samples respectively.

The type of solvent used in the oil extraction step (i.e. for the conventional transesterification process) appeared to have a major effect on CH_4 yield. The use of chloroform

as an extraction solvent was shown to result in the inhibition of the CH_4 production process. Therefore, where energy generation via the anaerobic digestion process is planned for the microalgae residues, investigations on the possible interference of the solvents with the microbial gasification process should be conducted prior to solvent selection. It must however be noted that if a biomass drying stage (i.e. as carried out in Chapter 3, at 105°C for 12 h) was performed on the post extracted residues, the problems encountered with the digestion of the chloroform-methanol samples may have been eliminated. This is since the solvents would have evaporated from the residue samples. However since this thesis was aimed at the use of the residues for the generation of additional energy carriers (CH_4), it was considered that conducting a residue drying step before the digestion process might negatively influence the overall process energetics. The filtered residues were therefore used as collected after the filtration step.

In cases where solvents may have inhibitory effects on the anaerobic process, a rinse step might be considered prior to the biological gasification process. This would assist in the removal of any traces of the toxic solvent in the biomass. The rinse step could however also reduce the calorific value of the biomass feedstock, where the rinsing process may strip unbound energy rich polar molecules from microalgae residues.

The co-digestion of the *Chlorella* residues with glycerol quantities equal to that co-produced during the transesterification of the biomass sample size was demonstrated to improve the anaerobic digestion efficiency and the CH_4 yields when compared with the use of the *Chlorella* residues alone.

Although this chapter involved an assumed use of a glycerol co-digestate quantity similar to that produced from the transesterification of a given mass of oil bearing microalgae, further investigations on the use of increased fractions of the glycerol co-feedstock is required. This would help to define the limits to which glycerol could be co-digested with the microalgae residues to enhance CH_4 production.

Information on the influence of using *Chlorella* residues at different concentrations in the digester would also prove helpful.

Although providing valuable information on the CH_4 production potentials from the microalgae residues, limitations are encountered with the use of a batch experimental set up since detailed information on the parameters described above cannot be obtained with batch tests.

The limitations of the anaerobic digestion batch tests are further explored in Chapter 7, using semi-continuously fed reactors. These tests are also important since they are representative of practical anaerobic digestion systems (i.e. large scale biogas plants).

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7. DETAILED INVESTIGATIONS OF THE VARIABLES AFFECTING THE ANAEROBIC DIGESTION OF THE MICROALGAE RESIDUES AFTER THE IN-SITU TRANSESTERIFICATION PROCESS

7.1. Introduction

As shown in Chapter 6, the use of batch anaerobic fermentation tests can provide valuable information regarding:

- The practical methane (CH_4) yields from the microalgae residues.
- The digestion period required for the conversion of the organic matter in the residues to CH_4.
- Factors which could potentially inhibit the anaerobic digestion process with the use of the microalgae residues.

The results presented in Chapter 6 were used to assess the influence of the preceding extraction or transesterification route on the digestibility of Chlorella residues. However, certain experimental limitations can be encountered with the batch experimental method.

Due to the design and time frame of the batch tests, valuable information on the substrate digestibility and process efficiency when the anaerobic digesters are fed continuously, as would be applied in practical systems, cannot be acquired. These include data such as the reactor stability, and the synergistic or inhibitory effects arising from different substrate loading concentrations. Detailed information on the influence of varying digestion times and the use of different levels of co-digestate cannot also be fully investigated using batch tests.

A continuous (or semi-continuous) experimental set up was therefore required to investigate useful process parameters, their interactions and their influence on the efficiency of the biomass to CH_4 production process. This test would also provide a basis for practical up-scaled continuous anaerobic digestion units aimed at CH_4 production using microalgae residues.

Previous laboratory studies on the anaerobic digestion of different species of freshly harvested untreated microalgae biomass using continuous reactors have been presented in the literature. These studies explored the limitations of applying the anaerobic process for CH_4 production from microalgae biomass. For example, due to the specific nature of the cell walls of some species, accessibility of the digestion enzymes to the internal cellular components may be limited (i.e. Golueke et al., 1957; Sialve et al., 2009).

However it was not expected that accessibility would be a major constraint for the anaerobic digestion of the *Chlorella* residues, following in situ transesterification, because the microalgae cell wall was expected to be disrupted. The enhanced accessibility to the cell contents could potentially improve the microbial degradation efficiency and conversion of the microalgae residues to CH_4 (Sialve et al., 2009). The extraction of the lipid fraction from the microalgae biomass results in a feedstock with a relatively high protein fraction, as presented earlier in Table 6.4. To improve the C/N ratio and digestibility of the residues, its co-digestion with available carbon rich substrates (i.e. glycerol) was proposed. With the availability of the glycerol by-product from the transesterification process, the co-digestion of the microalgae residues with glycerol was shown to increase the C/N ratio of the digestion feedstock and CH_4 production, as reported in Chapter 6. The use of glycerol as a process co-digestate will be further explored in this chapter.

Unlike the assumptions made in Chapter 6, which restricted the quantity of glycerol co-digested to only the equivalent amount produced during the transesterification process, the investigations in this chapter included increases in the glycerol co-digestate. The aim was to determine the influence of a wider C/N ratio range on the anaerobic digestion process. The potential use of increased glycerol levels arises because relatively cheap glycerol is available mainly from the production of biodiesel using conventional bio-oil feedstocks as presented in Yadzani & Gonzalez (2007).

It is expected that the biomass residues after biodiesel production from most conventional feedstocks (i.e. soybeans) would be used for livestock feed production, as is currently practiced in the industry. This implies that the glycerol co-product from those processes could be available for the proposed co-digestion with microalgae residues. This is however only applicable for scenarios where there are no competing demands for the glycerol product.

The aim of the semi-continuous anaerobic tests in this chapter was to obtain long-term operational data on CH_4 production from the *Chlorella* residues (post in situ transesterification), to help in defining the process conditions for optimising the CH_4 yield from the *Chlorella* residues.

Information such as the effect of the substrate loading limits and digestion time on the anaerobic process was also investigated using the semi-continuously fed tests. These parameters are important since they have a major influence on the size and cost (capital and operational) of the digesters. Increasing the substrate loading rates coupled with lower digestion retention times correspond to lower digester size and operational process requirements because more biomass would be treated with reduced times. The potential of reducing the digestion process heating requirement is also of interest. This was examined by studying the effects of process temperature variations on the CH_4 yield.

This chapter will focus solely on laboratory scale semi-continuously fed anaerobic digestion of the *Chlorella* residues obtained after the application of the acid catalysed in-situ transesterification process. Unlike Chapter 6, the residues obtained from the lipid extraction process was not studied since this thesis focuses on the potential improvements to the "microalgae-biodiesel" process obtainable via the in-situ transesterification process.

More specifically, this chapter aims to address the following questions regarding the use of the semi-continuous digestion of the *Chlorella* residues:

i. What influence would various combinations of substrate loading concentrations and retention periods have on the progression of the digestion process and CH_4 production?

ii. What ratio of glycerol co-digested with the microalgae residues would lead to optimum CH_4 production?

iii. What effects would temperature increases in the mesophilic operational range (25-40°C) have on the extent of the *Chlorella* residue digestion?

iv. How is the reactor stability and performance affected during the semi-continuous digestion process?

Parts of the results presented in this chapter has been reported in the paper" Anaerobic digestion of microalgae residues resulting from the biodiesel production process" (Ehimen et al., 2011) presented in Appendix A (CD-ROM)

7.2. Materials and Methods

7.2.1. Digestion substrates: Post transesterified *Chlorella* biomass and glycerol

The *Chlorella* residues obtained following the acid catalysed in-situ transesterification process was used as the principal digestion feedstock in this study.

As described earlier (section 6.2.1), the pooled *Chlorella* residues were obtained from the deep freezer where they were stored. The quantities required for the semi-continuous digestion experiments were thawed at room temperature (24 ± 2°C).

Same as in section 6.2.3.3, glycerol solution (85% mass purity, on the basis of weight) was used in this investigation. This was selected to be representative of the crude glycerol co-digestate likely to be available from an industrial biodiesel production process.

7.2.2. Inoculum adaptation

The inoculum used in the investigations in this chapter was the same as that described in section 6.2.3.1. Prior to the tests on semi-continuously fed anaerobic digestion, the inoculum was prepared and adapted for the digestion of the high protein microalgae samples using the same methods described in section 6.2.3.1.

The characterisation of the digestion substrates and inoculum on the basis of their volatile solids (VS) was carried out in duplicates as described in section 6.2.1.1 using standard methods (APHA, 2005).

As previously described in section 6.3.1, the VS of the microalgae residues, inoculum and glycerol were determined to be 0.946, 0.570 and 1.0 g VS/g total solids (TS) respectively.

7.2.3. Semi-continuously fed anaerobic digesters

7.2.3.1. Set up for investigating the influence of substrate concentration, hydraulic retention time and quantity of glycerol in the residue substrate

The influence of the variables; substrate concentration, hydraulic retention time and quantity of glycerol as a co-digestate for the anaerobic digestion of the *Chlorella* residues were investigated using duplicate experimental runs.

The hydraulic retention time (HRT) refers to the total length of time that the biomass sample remains in the digestion reactor. This can be calculated using:

$$\text{HRT (days)} = \frac{\text{Reactor volume } (m^3)}{\text{Substrate loading rate } (m^3/\text{days})} \quad (7.1)$$

The experiments were conducted using modified 2 l Erlenmeyer flasks as the anaerobic digestion reactors with a working volume of 1.5 l.

A picture and schematic diagram showing the semi-continuous digestion set-up is shown in Figure 7.1

Continuous stirring was provided using Teflon covered magnetic stirring bars operated at a speed of 300 rpm. The flasks containing the digesting samples were also shaken by hand once a day to facilitate the re-suspension of the scum layers and sediment in the reactor.

The anaerobic reactors were sealed air tight with silicon stoppers. The stoppers were equipped with three glass tubes to facilitate the removal of effluent, addition of fresh substrate and collection of the produced gas (Figure 7.1).

The daily withdrawal of the reactor effluents and the addition of residue feedstocks were carried out using syringes. The withdrawn digestate pH was recorded and used to assess the process pH of the digester.

The experiments were carried in out duplicates (repeated twice) with the anaerobic digestion temperature maintained at a fixed temperature of 35.0 ± 0.5°C throughout the reaction. The reaction temperature was controlled by completely immersing the reactors in thermostated water baths.

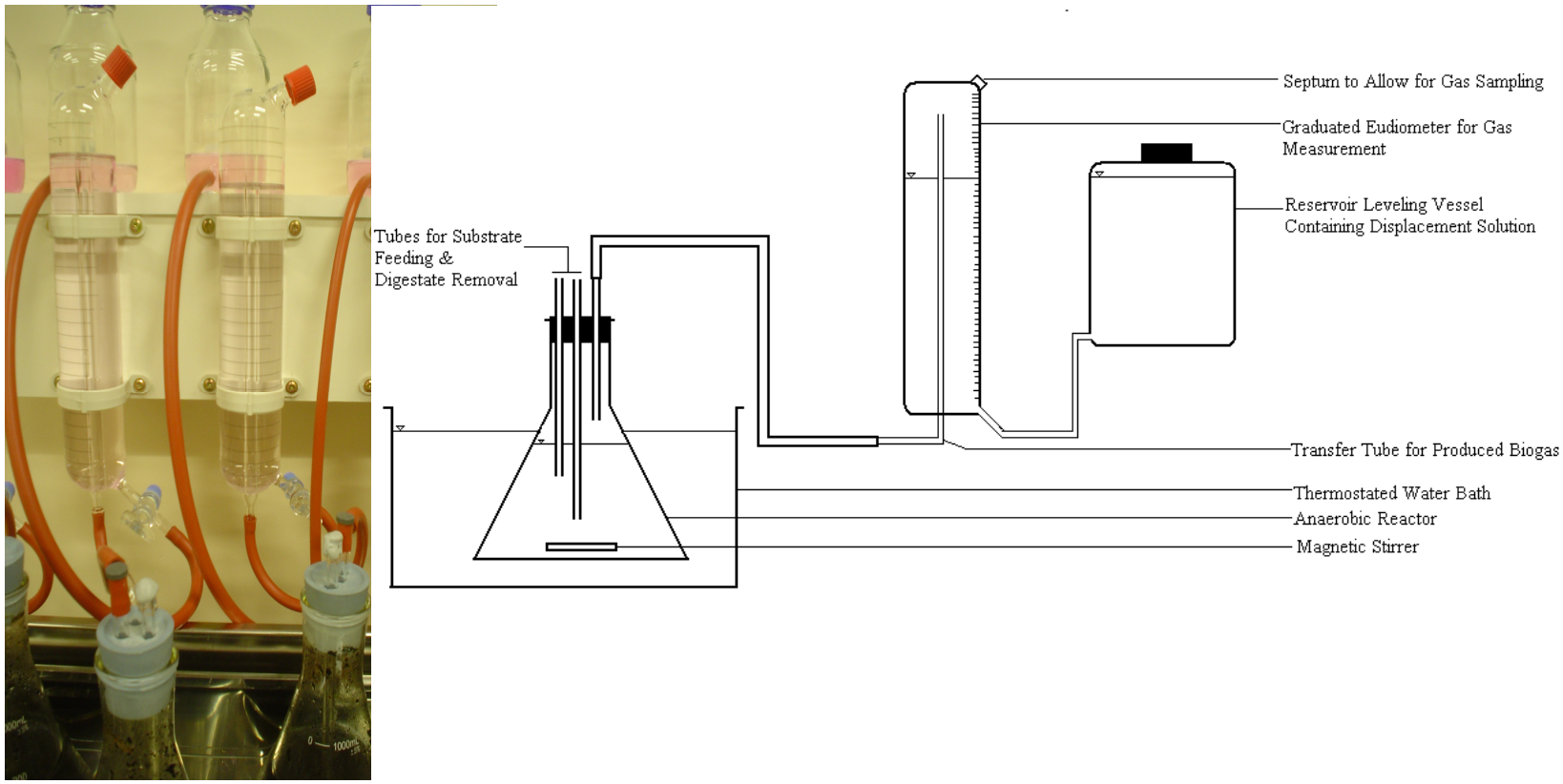


Figure 7.1. Pictorial (× 0.13) and labelled schematic representation of the semi-continuously fed reactors used to investigate the influence of retention time, substrate concentration and glycerol addition on the anaerobic digestion of *Chlorella* residues.

7.2.3.2. Set up to study the effect of process temperature on the anaerobic digestion process

This study examined the influence of varying the process temperature (in the mesophilic temperature range, 25-40°C) on CH_4 production from the *Chlorella* residues. For this experiment, a laboratory scaled continuously stirred tank reactor (CSTR) was constructed in the Physics Department mechanical workshop using Pyrex glass with stainless steel casings. The total reactor volume was 5 l with a working volume of 4 l. This experiment was conducted using duplicate runs repeated once.

A pictorial and schematic diagram of the laboratory scaled anaerobic digester used in this investigation is shown in Figure 7.2.

Process stirring was accomplished by the use of a mechanical stirrer operated at a speed of 310 rpm. This was carried out to ensure proper mixing of the substrates in the digestate, and a uniform temperature of the reactor contents.

The temperature of the digester was regulated using a hermetically sealed surrounding water jacket with the heated water supplied by a thermostated water bath (Figure 7.2).

Effluent removal and loading of the feed daily into the digester was accomplished by the use of a syringe system. For the continuous measurement of the process a pH and temperature probe (Mettler Toledo Inlab® Expert Pro) was inserted in the digester.

7.2.3.3. Measurement of methane produced

For all the semi-continuous reactors, the collection and measurement of the gas produced by the digesters was facilitated using eudiometer units (ISO/DIS 14853 (1999)) as described in Guwy (2004). The eudiometer units were connected to the gas collection tubes at the head of the reactors.

The eudiometer apparatus (shown in Figure 7.1) consists of a graduated (500 cm^3) gas collection tube with a rubber septum at the top of the equipment to allow for the extraction of the methane samples using a syringe. The unit was connected to a reservoir tank, containing the barrier solution that was open to the atmosphere to allow for pressure compensation.

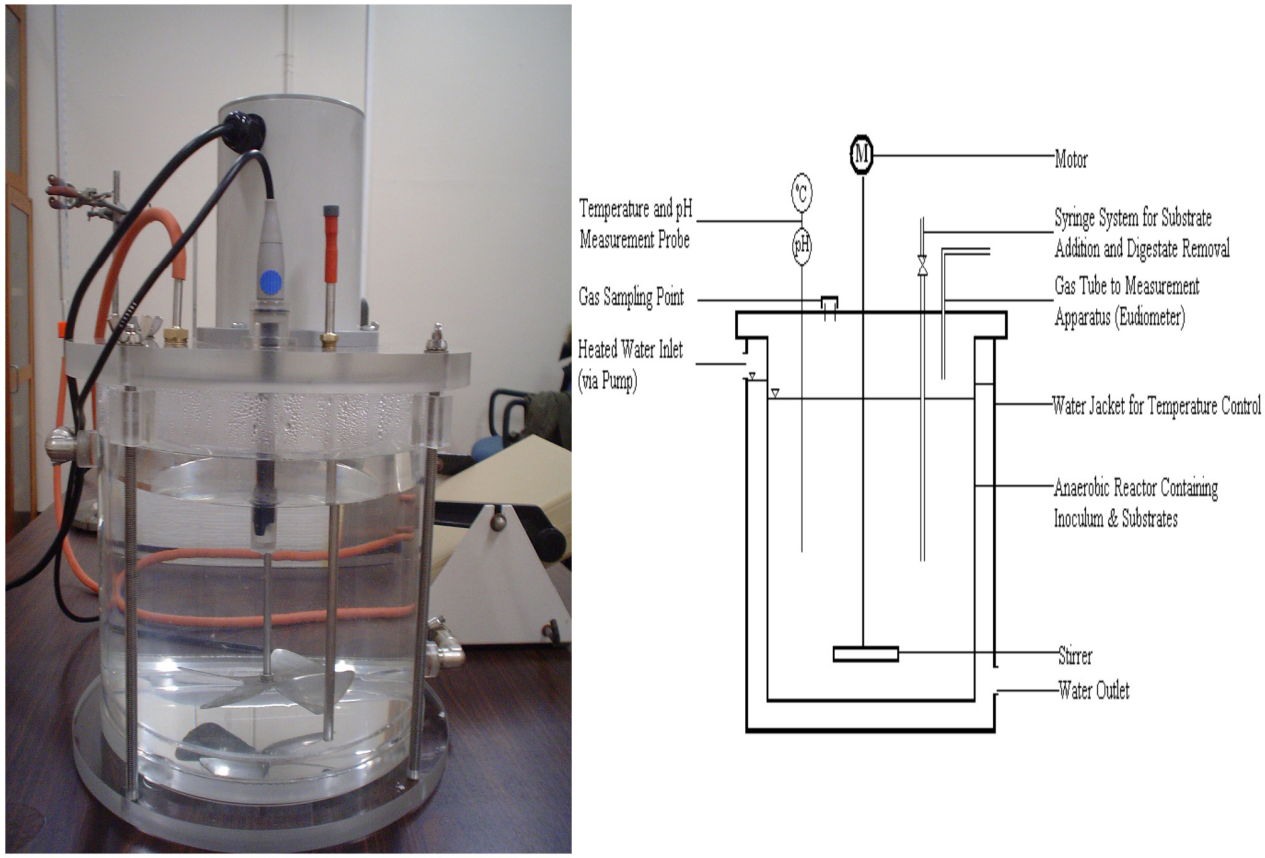


Figure 7.2. Pictorial (×0.11) and labelled schematic representation of the continuously stirred reactors used to investigate the influence of temperature on the anaerobic digestion of the *Chlorella* residues.

The gas produced in the anaerobic digesters passed from the headspace of the graduated apparatus displacing the barrier solution into the reservoir bottles. After the gas production was recorded, the reservoir bottles were lowered so the liquid level was the same as that in the eudiometer unit, thereby returning the system to atmospheric pressure.

The specific CH_4 production of the digested materials was determined by the use of a 5% molar sodium hydroxide (NaOH) solution in the eudiometers as the barrier solution.

To determine the percentage of CH_4 in the produced biogas (v/v), duplicate reactors were used in this study. Each duplicate reactor was equipped with a similar gas measurement setup, but with the displacement apparatus containing saturated sodium chloride (NaCl) solution to minimise the solubility of the carbon dioxide (CO_2) and other acid gas contents of the biogas. For all measurements, the CH_4 and biogas volumes determined were corrected to normal temperature and pressure.

7.2.4. Experimental design

7.2.4.1. Effect of varying retention times, substrate loading concentrations and *Chlorella* residues fraction of the digestion feedstock

To assess the influence of the hydraulic retention time (HRT), substrate concentration (SC) and the co-digestion of the microalgae residues with glycerol in different proportions, on the CH_4 yield, an empirical fitting technique, the response surface methodology (RSM) (Box et al., 2005) was used. Unlike other studies of the anaerobic digestion of untreated microalgae biomass (Golueke et al., 1957; Oswald & Golueke, 1960; Uziel, 1978; Samson & LeDuy 1982, 1983a, 1983b, 1986; Sanchez & Travieso, 1993; Chen & Oswald, 1998) which mainly investigated the response in the CH_4 production following a change in one anaerobic digestion parameter at a time, the use of a statistical design in this study facilitated a simultaneous examination of multiple factors. This illustrated their influence on the response separately, and showed the combined effect of their interaction with other experimental variables.

The RSM used for the investigation of the above factors in this study was the central composite design (CCD) method. This included the analysis of three different HRT (days) levels, six levels of SC (kg VS substrate/m^3) and five levels of Microalgae Residues (as a percentage of the total VS of the digested substrate, MR %) as the main variables. The digestion temperature

was kept constant at 35°C throughout the experiment. The different levels of these variables are shown in Table 7.1.

Table 7.1. Experimental design to investigate the influence of HRT, SC and MR % on methane production

Factor	Variable level					
HRT (days)	5	10	15			
SC (kg VS substrate/m^3)	5	10	20	30	40	50
MR % (% of VS substrate)	100	80	60	40	20	

The experimental design involved investigating CH_4 production using the 90 possible combinations (i.e. 3×6×5) of the variables.

The anaerobic digestion of 100% glycerol was not covered in this study due to the fact that glycerol was only considered for use as a co-digestate. Also, CH_4 production from glycerol has been previously demonstrated in the literature (i.e. Johnson & Taconi, 2007; Yadzani & Gonzalez, 2007; Siles López et al., 2009).

7.2.4.2. Effect of temperature changes

For the second set of experiments aimed at investigating the influence of mesophilic digestion temperatures (25°C < digestion temperature < 40°C) on CH_4 formation, the digesters were operated using a substrate concentration of 5 kg VS/m^3 only. A fixed HRT of 15 days was used with the substrate VS containing 40 and 60% of the microalgae residues.

The temperature investigations in this study were limited to the mesophilic temperature range primarily because the inoculum used was obtained from mesophilic digesters. Furthermore the use of the mesophilic digestion temperatures was considered to be a good starting point for the study of the microalgae residues digestion.

The temperatures used were attained after a gradual increase of the digester temperatures by 2.5 ± 0.5°C/week after two hydraulic cycles starting with a reaction temperature of 25°C.

The reason underlying the choice of the different variable levels in this study is presented in section 7.3.

The specific CH_4 yield (m^3 CH_4/kg VS substrate added) was used as the main response of interest for all the investigations in this chapter. It was used to represent the overall performance of the semi-continuously stirred digesters. The CH_4 yield was measured under a steady state condition, which was normally achieved after two hydraulic retention cycles.

7.2.5. Analytical procedures for monitoring the continuous anaerobic digester performance

Due to the large number of experimental tests and variables involved in this chapter study, the analysis of the anaerobic digestion process was mainly restricted to the relationships of the variables to the CH_4 yield. However, to further assess the performance of the digester, indicators such as the total volatile fatty acids (C_1-C_5) concentrations (VFA), and the total alkalinity of the digestate in steady state, were recorded. In addition, changes in the reaction pH, and its influence on the progress of the anaerobic digestion of the *Chlorella* residues, were briefly explored.

7.2.5.1. Determination of VFA concentration

The VFA (acetic, propionic, butyric and valeric acids) concentrations were determined using an Agilent 6850 series II gas chromatograph (GC) system. Twenty ml (20 ml) of the digestate were carefully withdrawn from close to the bottom of the reactor, using a syringe system described earlier in section 7.2.3. The samples of the digester effluents were transferred to a beaker where they were prepared for GC analysis.

The collected digestate was centrifuged at 7360 g for 5 mins to facilitate the separation of solid fractions in the samples. Ten ml (10 ml) of the centrifuged supernatant was carefully decanted into a beaker where 2 ml of 1N H_2SO_4 was added to the sample drop-wise using a pipette. The centrifuged effluents were then acidified (to a pH of ≈2) to inhibit further microbial degradation of the sample (APHA, 2005). 1 ml of the acidified samples was withdrawn for analysis using gas chromatography (GC).

The VFA analysis was carried out by a GC equipped with a 7683A auto-sampler and a flame ionisation detector (FID). A DB-FFAP column (30 m length, 0.25 mm ID and 0.25μm thickness) was used for the separation, with helium as the carrier gas. The injector and detector temperatures were at 250 and 300°C respectively, and the oven temperature was gradually increased from 100-250°C at the rate of 10°C/min. Five ml (5 ml) of the standard mixture containing 1 g/l of acetic, propionic, butyric and valeric acids (Merck KGaA chemicals) respectively was used as the chromatography standard.

The concentrations of the VFAs in the samples were then calculated from the chromatograph and presented as mg VFA/l digestate.

7.2.5.2. Determination of the process total alkalinity

The total alkalinity of the digestate represents the sum of the concentrations of bases in the digestate sample. It provides an indication of the buffering capacity of the digestion system. The total alkalinity expressed as mg $CaCO_3$/l digestate, was measured as in Callaghan et al. (2002) by titration to pH 4.5 with 0.05M sulphuric acid (H_2SO_4). This method was used since at the titration endpoint, all of the total alkalinity is consumed, with the number of moles of the acid added as a titrant equal to the moles of alkalinity of the sample. At this pH (4.2) all the alkaline compounds will be consumed by the acid. For example, at pH 10, the hydroxide groups, if present are converted to water; at pH 8.3, the carbonates are converted to bicarbonates and at pH 4.5 all the carbonates and bicarbonates are converted to carbonic acid (APHA, 2005). Below this pH (4.50, the sample is thus unable to neutralise the added H_2SO_4 any further, and a direct relationship in the reduction in pH with acid addition is observed (APHA, 2005).

The analysis involved drawing from the reactors a 10 ml sample of the digestate which was transferred into a clean beaker. The beaker content was stirred continuously with Teflon covered magnetic stirrers. The digestate pH was continuously monitored with the use of pH electrodes introduced to the beaker and completely immersed. Titration was then carried out with 0.05 M H_2SO_4 in the burette, dripped slowly to ensure a lowering of the pH by 0.1-0.2 pH units.

The number of moles of the H_2SO_4 of the titre was then calculated. With the overall reaction as (Eq. 7.2):

$$H_2SO_4 + CaCO_3 \rightarrow H_2O + CO_2 + CaSO_4 \quad (7.2)$$

Based on the reaction molar ratio of H_2SO_4 to $CaCO_3$, the molar concentration and mg of the $CaCO_3$ in the sample was calculated and used to represent its total alkalinity.

7.2.5.3. Determination of ammonia nitrogen

The ammonia nitrogen (NH_3-N) of the digester content was measured using the ASTM standard test method B (using ion selective electrodes) as presented in ASTM D 1426-08 (ASTM, 2008). This method was selected due to its accuracy in determining NH_3-N concentrations in the range of 0.5-1000 mg NH_3-N/l directly from the digestate. The measurements were carried out using the Orion 951201 ammonia electrode (Thermo-scientific instruments) with higher concentrations determined following dilution.

The use of the electrodes provided a quick, simple and accurate measurement of the ammonia ions available in the aqueous digestate. This method was ideal since the characteristic turbidity and colour of the digestate did not affect the ammonium determination. This was useful since a distillation of the samples was not required for the digestate samples, as would have been the case had other commonly applied titration methods been used.

Standard samples of ammonia solutions within the studied range (0.5-1000 mg NH_3-N/l) were prepared for the experiments. Furthermore, it was ensured that the digestate samples and the ammonia standard solutions used were of the same temperatures, to minimise sources of measurement errors.

The ammonia ionic strength adjuster (Cat. No. 951211, Thermo scientific), 0.5 ml, was added to both the 25 ml of freshly withdrawn digestate and prepared ammonia standards in beakers. This was added to ensure that the samples were of similar ionic strength.

The samples and the standards were then stirred continuously using Teflon stirring bars, with a stirring speed of 150 rpm. The electrodes, connected to the display meter (Thermo scientific Orion ISE meter) were fully immersed in the samples, ensuring no air bubbles were formed around the electrode membrane.

As a major precaution, the electrodes were rinsed with deionised water between measurements, and the electrodes were shaken to remove water and prevent sample carryover. The electrodes were stored in deionised water overnight.

The NH_3-N concentrations of the samples were then determined by a comparison with the standard.

7.2.5.4. Extent of VS destruction

The breakdown or destruction of the organic content of the sample (i.e. sample VS to CH_4) is representative of the efficiency of the anaerobic digestion process. A higher VS destruction corresponds to an improved digestibility of the biomass.

To determine the extent of the substrate VS destruction following the anaerobic digestion process (i.e. the anaerobic digestion efficiency), this study used the method proposed by Varel et al. (1977). This method utilises the molar concentrations of the CH_4 and CO_2 produced via the anaerobic digestion process. This estimation was based on the assumption that all of the carbon in the destroyed feedstock VS is converted to CH_4 and CO_2. By incorporating the mass carbon fraction of the digested biomass (w/w), the mass of VS destroyed can be obtained from Eq. 7.3.

$$\text{VS destruction (g/kg sample)} = (\text{mol } CH_4 \text{ / kg sample} + \text{ mol } CO_2\text{/kg sample}) \times \frac{12}{\text{Substrate carbon fraction}} \quad (7.3)$$

where,

mol CH_4 and CO_2 are the measured molar quantities of the respective gases in the produced biogas in moles (i.e. measured mass (g)/molar mass) per kg sample digested,

the substrate carbon fraction is the percentage mass fraction of carbon in the digested sample (w/w),

and, the constant, 12, represents the molar mass of carbon (C).

The anaerobic digestion efficiency (%) which is the percentage VS destruction per kilogram sample digestate was then estimated using the obtained VS destruction.

7.3. Results and Discussions

7.3.1. Analysis of methane yields obtained varying HRT, SC and MR% at a reaction temperature of 35°C

To investigate the interactions of the variables on the CH_4 yield, the MATLAB computing package (Version R2009a, the MathWorks Inc, Massachusetts, USA) was used for the regression analysis of the experimental data obtained in this study[15]. Experimental runs which resulted in no CH_4 production were not included in the analysis in this study.

An empirical relationship between the experimental parameters and the response (specific methane yield, m^3 CH_4/kg VS digested sample) was proposed.

Equation 7.4 shows the second order polynomial fit equation obtained for the CH_4 yield (Y) subject to the independent variables x_1 (HRT), x_2 (SC), and x_3 (MR %) investigated in this study.

$$Y = -0.3196 + 0.0798x_1 - 0.0015x_2 + 0.0023x_3 - 7.5852e^{-5}x_1x_2 - 2.4813e^{-6}x_2x_3 - 2.4423e^{-5}x_1x_3 - 0.0027x_1^2 + 3.6540e^{-6}x_2^2 - 2.1983e^{-5}x_3^2 \quad (7.4)$$

The significance levels of the regression coefficients in Eq. 7.4 are shown in Table 7.2.

[15] The data files for the regression analysis of the CH_4 yields are presented in Appendix H (CD-ROM).

Table 7.2. Significance levels of regression coefficients

Variable	Regression coefficient	Standard Deviation	t-value	Significance level (%)
Intercept	-0.3196	0.0177	2.0451	97.79
x_1 (HRT)	0.0798	0.0028	3.2267	99.91
x_2 (SC)	-0.0015	0.0005	0.3173	62.40
x_3 (MR %)	0.0024	0.0003	0.8326	79.62
x_1x_2	-7.5851e-5	3.0943e-5	0.2776	60.89
x_1x_3	-2.4422e-6	1.5707e-6	0.1760	56.96
x_2x_3	-2.4813e-6	3.6980e-6	0.0760	53.02
x_1^2	-0.0027	0.0001	2.2348	98.58
x_2^2	3.6540e-6	8.2234e-6	0.0503	51.99
x_3^2	-2.1983e-5	2.3367e-6	1.0652	85.49

* Degrees of freedom=77, $t_{critical}$ value=1.991, Root squared value (R^2) = 0.9790, Root mean square error (RMSE) = 0.01275

The statistical student t-value which is the ratio of the estimate to the standard error were used to determine the significance of each coefficient as put forward in Box et al., 2005.

The t-value of the experimental degrees of freedom (*n*-1, where *n* is the number of independent runs) was determined and used to represent the $t_{critical}$-value.

The significance level (%) of the variables was then adjudged by comparing the t-values obtained for the coefficients with the $t_{critical}$ value (1.9911), as seen in Table 7.2. The critical t-value was used as a benchmark to test the significance of the influence of the variables on the CH_4 yield. The t-values of the variable estimates must exceed the critical t- value in order for the null hypothesis to be rejected. Only test statistics with significance levels > 95% were considered to be important in this study. It was observed that x_1 (HRT) and x_1^2 had the most significant influence on the CH_4 yield from the *Chlorella* residues under the study conditions. The observed CH_4 increase due to increases in the retention times was attributed to the improved availability of the microalgae residues for digestion by the anaerobic bacteria in the digestion reactors.

The methane yields obtained from the experimental runs under the different process conditions investigated and the predicted values given by the derived model equation is shown in Table 7.3

Table 7.3. Observed and predicted specific methane yields at different process variables

Run no	HRT (days)	SC (kg VS/m^3)	MR (%)	Methane yield (m^3 CH_4/kg VS)	Predicted yield (m^3 CH_4/kg VS)	Residuals	% Residuals
1	5	5	100	0.021	0.016	-0.005	-23.81
2	5	10	100	0.013	0.006	-0.007	-53.85
3	10	5	100	0.201	0.209	0.008	3.98
4	10	10	100	0.143	0.197	0.054	37.76
5	10	20	100	0.130	0.173	0.043	33.08
6	10	30	100	0.123	0.150	0.027	21.95
7	10	40	100	0.109	0.127	0.018	16.51
8	10	50	100	0.075	0.105	0.03	40.00
9	15	5	100	0.245	0.267	0.022	8.98
10	15	10	100	0.208	0.253	0.045	21.63
11	15	20	100	0.185	0.225	0.04	21.62
12	15	30	100	0.152	0.198	0.046	30.26
13	15	40	100	0.139	0.171	0.032	23.02
14	15	50	100	0.116	0.146	0.03	25.86
15	5	5	80	0.040	0.048	0.008	20.00
16	5	10	80	0.019	0.038	0.019	100.00
17	5	20	80	0.007	0.018	0.011	157.14
18	10	5	80	0.214	0.242	0.028	13.08
19	10	10	80	0.216	0.230	0.014	6.48
20	10	20	80	0.200	0.206	0.006	3.00

21	10	30	80	0.169	0.183	0.014	8.28
22	10	40	80	0.132	0.161	0.029	21.97
23	10	50	80	0.103	0.140	0.037	35.92
24	15	5	80	0.277	0.299	0.022	7.94
25	15	10	80	0.239	0.285	0.046	19.25
26	15	20	80	0.231	0.258	0.027	11.69
27	15	30	80	0.211	0.231	0.02	9.48
28	15	40	80	0.186	0.205	0.019	10.22
29	15	50	80	0.133	0.180	0.047	35.34
30	5	5	60	0.054	0.063	0.009	16.67
31	5	10	60	0.045	0.053	0.008	17.78
32	5	20	60	0.003	0.034	0.031	1033.33
33	5	30	60	0.020	0.015	-0.005	-25.00
34	5	40	60	0.014	0.000	-0.014	-100.00
35	10	5	60	0.245	0.257	0.012	4.90
36	10	10	60	0.222	0.245	0.023	10.36
37	10	20	60	0.209	0.222	0.013	6.22
38	10	30	60	0.174	0.199	0.025	14.37
39	10	40	60	0.159	0.178	0.019	11.95
40	10	50	60	0.132	0.157	0.025	18.94
41	15	5	60	0.302	0.314	0.012	3.97
42	15	10	60	0.273	0.301	0.028	10.26
43	15	20	60	0.256	0.274	0.018	7.03
44	15	30	60	0.233	0.247	0.014	6.01
45	15	40	60	0.219	0.222	0.003	1.37
46	15	50	60	0.204	0.197	-0.007	-3.43

47	5	5	40	0.060	0.060	0	0.00
48	5	10	40	0.028	0.050	0.022	78.57
49	5	20	40	0.006	0.032	0.026	433.33
50	5	40	40	0.002	0.000	-0.002	-100.00
51	10	5	40	0.238	0.254	0.016	6.72
52	10	10	40	0.230	0.242	0.012	5.22
53	10	20	40	0.212	0.220	0.008	3.77
54	10	30	40	0.195	0.198	0.003	1.54
55	10	40	40	0.178	0.177	-0.001	-0.56
56	10	50	40	0.143	0.156	0.013	9.09
57	15	5	40	0.295	0.311	0.016	5.42
58	15	10	40	0.270	0.298	0.028	10.37
59	15	20	40	0.255	0.271	0.016	6.27
60	15	30	40	0.229	0.245	0.016	6.99
61	15	40	40	0.206	0.221	0.015	7.28
62	15	50	40	0.187	0.197	0.01	5.35
63	5	5	20	0.034	0.040	0.006	17.65
64	5	10	20	0.011	0.030	0.019	172.73
65	5	20	20	0.005)	0.012	0.007	140.00
66	5	30	20	0.007	0.000	-0.007	-100.00
67	10	5	20	0.267	0.233	-0.034	-12.73
68	10	10	20	0.232	0.222	-0.01	-4.31
69	10	20	20	0.214	0.200	-0.014	-6.54
70	10	30	20	0.186	0.178	-0.008	-4.30
71	10	40	20	0.150	0.158	0.008	5.33
72	10	50	20	0.117	0.138	0.021	17.95

73	15	5	20	0.291	0.291	0	0.00
74	15	10	20	0.253	0.277	0.024	9.49
75	15	20	20	0.225	0.252	0.027	12.00
76	15	30	20	0.218	0.227	0.009	4.13
77	15	40	20	0.197	0.202	0.005	2.54
78	15	50	20	0.154	0.179	0.025	16.23

As shown by the % residuals column (100×(predicted CH_4 yield − practical CH_4 yield/practical yield), the CH_4 yields for the investigations with HRT of 5 d were seen to be smaller than with the use of larger HRTs, these reduced yields led to the production of the relatively large absolute errors. The fit model was however deemed to be adequate for this study and it provides a useful indication of the relationship of the parameters on the CH_4 yield using microalgae residues.

Using Eq. 7.4, the CH_4 yields from anaerobic digestion process were graphically represented using response surface plots in which the individual and cumulative effects of the variables, as well as their interactions, on the CH_4 yields was described.

7.3.2. Influence of the different variable interactions on methane yields

Figures 7.3 – 7.5 show the response surface and contour plots of the CH_4 yield as a function of two of the independent variables with the third kept at constant levels.

Figure 7.3 shows the contour plot of CH_4 production (m^3 CH_4/kg VS substrate added) when varying the HRT and the substrate concentration while the microalgae fraction in the substrate is fixed at different levels i.e. 20-100 MR%.

The maximum retention time, 15 d, was selected for this study based on the findings of preliminary batch anaerobic digestion experiments demonstrated in section 6.3.3. This showed that 98.0 ± 1.0% of the optimum CH_4 yields were obtained after a digestion period of 12-14 d.

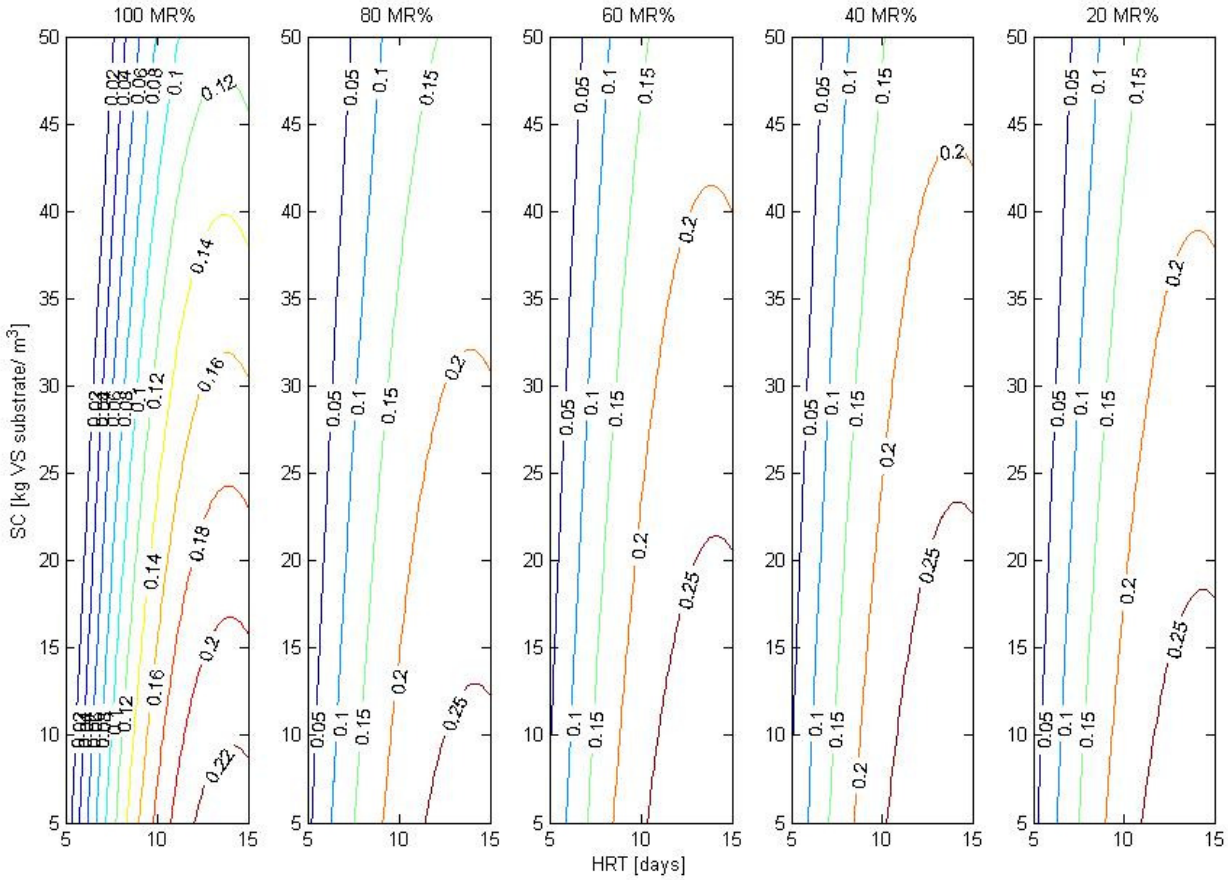


Figure 7.3. Contour plots of constant CH_4 yield (m^3 CH_4/kg VS substrate), showing the influence of varying SC and HRT with constant levels of MR % (20-100% microalgae residues in substrate) in the digester and an operating temperature at 35°C.

The possibility of reducing the digestion time requirement, by manipulating other process variables (i.e. SC and MR %), was one of the secondary aims of this study. This was due to the fact that this corresponds to a potential reduction in the process equipment and operational costs due to reduced digester sizes.

For all levels of MR%, it can be seen that an increase in the digestion time with a corresponding reduction in the loading concentrations of the substrate, led to an increase in the CH_4 yields (Figure 7.3).

However, the obtained trends show that digestion times of 11-15 d were required to obtain maximum CH_4 production from the *Chlorella* residues.

Increasing the fraction of the glycerol content (i.e. a decrease in the MR % as shown in Figure 7.3 from the left to the right diagram) of the process substrate was found to positively influence CH_4 production. This may be attributable to the improved digestibility of the substrates, since the liquid glycerol fraction would be more accessible to the fermentative bacterial mass (Siles López et al., 2009).

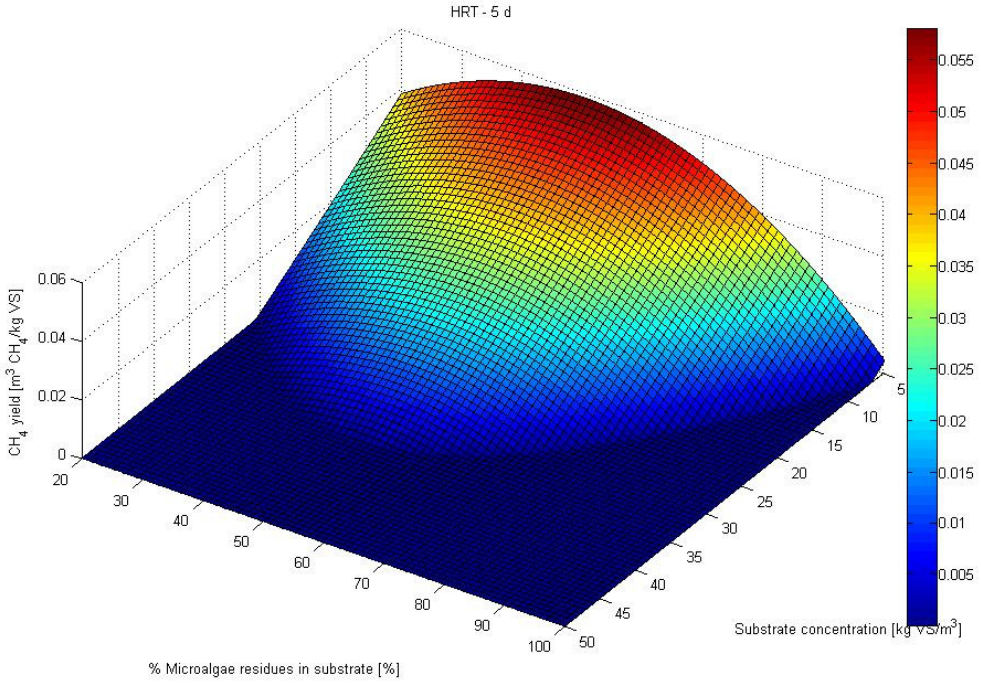
The varying fraction of microalgae residues with the glycerol co-product in the substrates used in this chapter was mainly used to investigate the effect of the feedstock C/N ratio on the anaerobic digestion process. The five levels of MR % (100, 80, 60, 40 and 20%) studied correspond to molar C/N ratios of 5.4, 6.56, 8.53, 12.44, and 24.17 respectively.

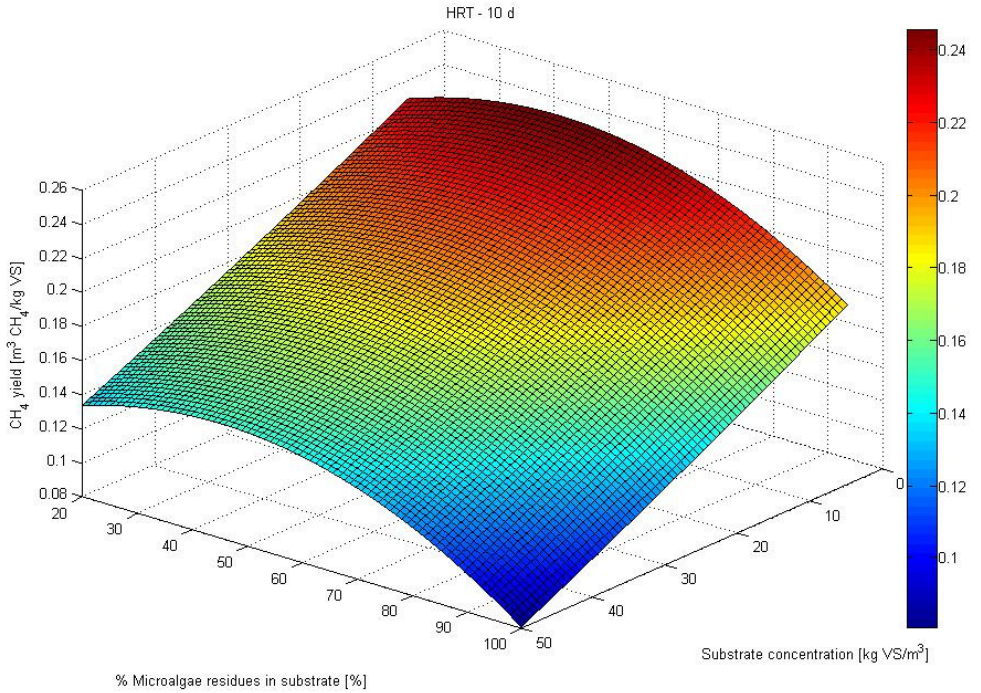
With a retention time of 15 d, an increase in the substrate molar C/N ratio from 5.4 to 12.44 was seen to improve the specific CH_4 yields by 20.0, 29.8, 30.0, 53.0, 48.0 and 61.0% at loading concentrations levels of 5, 10, 20, 30, 40 and 50 kg VS/m^3 respectively. Within the boundaries of this experimental study, no improvements on the CH_4 recovery were achieved with MR % levels < 40 (i.e. C/N ratios > 12.44) for all loading concentrations and anaerobic digestion times as shown in Figure 7.3.

The influence of the interaction of different levels of SC and MR % on CH_4 production from post transesterified microalgae residues using the semi-continuously stirred reactors is shown in Figure 7.4. The HRT of the described process was kept at constant levels of 5, 10 and 15 d respectively.

It can be seen from Figure 7.4 (HRT-5 d), that with a digestion time 5 d the reactor exhibited process inhibition (low CH_4 yield, < 0.06 m^3/kg VS sample digested) or complete

digester failure (i.e. zero CH_4 production) for all the loading concentrations investigated. As highlighted in Figure 7.4 (HRT: 10 and 15 d), increases in the digestion times lead to a corresponding increase in the CH_4 production from the residues.





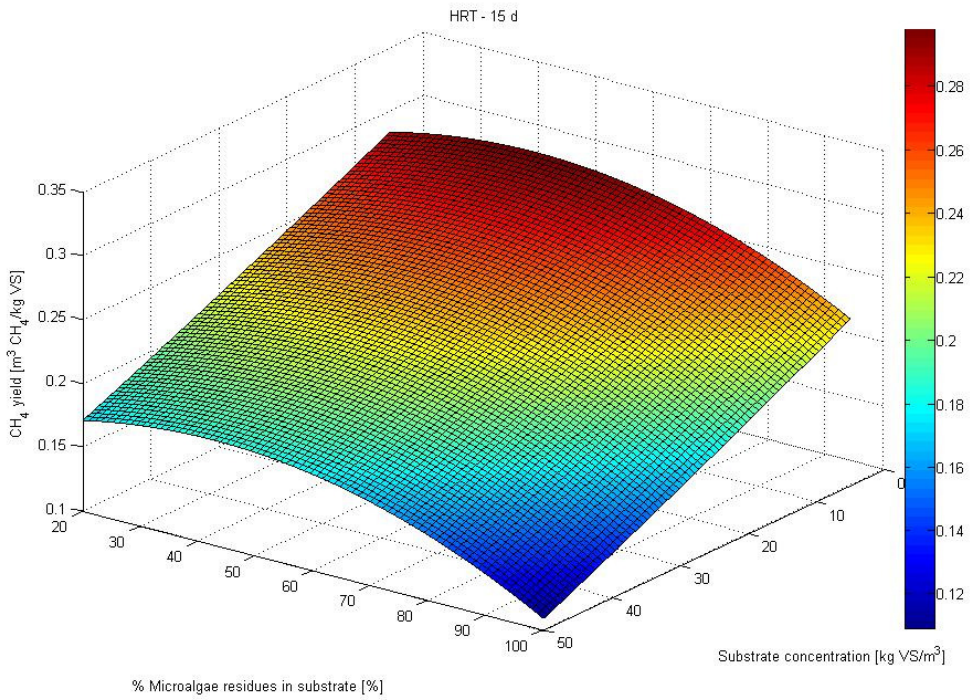


Figure 7.4. Contour plots showing the effect of varying the substrate MR % and SC on specific methane yield (m^3 CH_4/kg VS substrate added) at different constant levels of HRT (5, 10 and 15 d respectively) and with a process temperature of 35°C.

The inhibited CH_4 yields observed with a HRT of 5 d can be attributed to the increased washout the unreacted substrates and active micro-organisms (especially slow-growing bacteria) from the anaerobic digester (Chynoweth, 1987). Hence, the digestion of the *Chlorella* residues would improve with increased HRT due to the increased exposure of the substrates to the active bacteria in the digester.

Furthermore, Figure 7.4 shows that improvements in the specific CH_4 yields can be obtained with a decrease in the MR %, with a gradual decline subsequently noted after an "optimum" MR % value has been reached.

With a HRT of 15 d, the maximum specific CH_4 yield was obtained when the microalgae residues in the digested substrate was 40-50%, loading concentration < 10 kg VS/m^3.

Figure 7.5 shows the CH_4 yield contour plots for varying levels of the substrate MR % and retention times, with the SC kept constant at 5, 10, 20, 30, 40 and 50 kg VS/m^3 respectively.

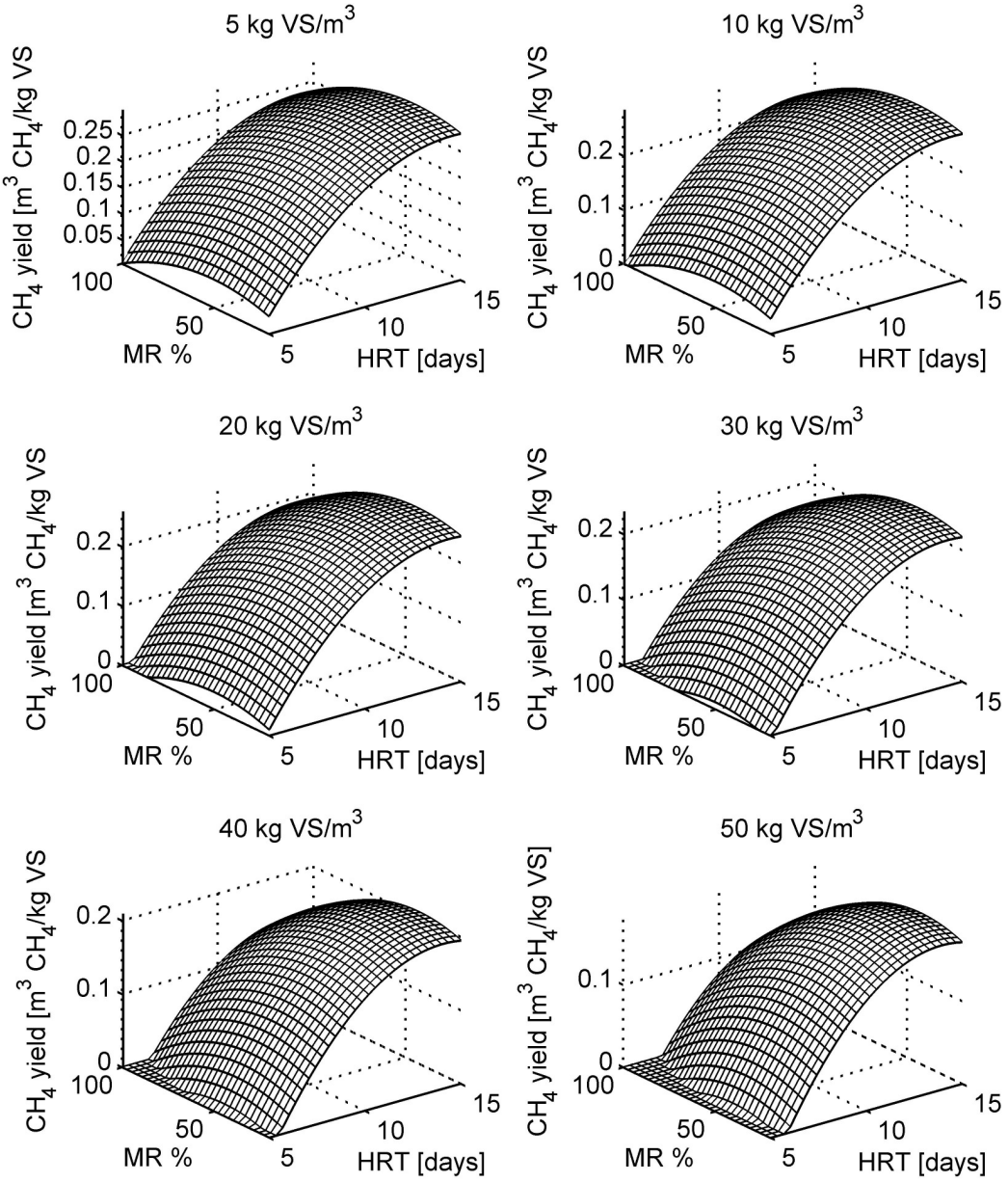


Figure 7.5. Response surface plots showing the influence of varying MR% and HRT on the methane yield (m^3 CH_4/kg VS substrate added) at fixed SC levels of 5-50 kg VS substrate/m^3 digester and an operating temperature of 35°C.

A decline in the specific CH_4 yields was obtained with an increase in the substrate loading rates for all the digestion times. This occurred for all fractions of the microalgae residues in the digested substrate. The highest CH_4 yields were obtained using the least SC, (i.e. 5 kg VS/m^3digester).

The reduction in the CH_4 production with increases in the SC could be attributable to organic overloading of the digester, resulting in the reduction or inhibition of the degradative capacity of the bacteria (Hill et al., 1987). With reduced retention times, as seen in Figure 7.5 an increase in the SC could result in imbalances in the bacterial population, leading to an increase in volatile acids and digester failure. This explanation will be addressed further when considering the influence of the volatile fatty acids in the digester on the CH_4 yield in section 7.3.5.1.

7.3.3. Influence of varying process temperatures on methane yield

Apart from the fact that the inoculum used for this study had been adapted for mesophilic digestion (40-55°C), as described in section 7.2.2, the temperature range was selected to enable the anaerobic digestion of the microalgae residues to minimise the digester heating demands.

Psychrophilic temperatures were not considered in this study because previous studies on its use for CH_4 production from untreated microalgae biomass showed that digestion using this temperature range resulted in lower CH_4 yields than at higher temperatures (Samson & LeDuy, 1986).

Table 7.4 shows a summary of the results obtained for the anaerobic digestion of post transesterified Chlorella biomass at 25, 30, 35 and 40°C at an organic loading concentration of 5kg VS/m3 and a hydraulic retention time of 15 d.

Table 7.4. Influence of different mesophilic temperatures on methane yield

	% Microalgae residues in substrate (% substrate VS)							
	40%				60%			
	Temperature (°C)				Temperature (°C)			
	25	30	35	40	25	30	35	40
Specific CH_4 yield (m^3 CH_4/kg VS)	0.192 ± 0.016	0.208 ± 0.012	0.295 ± 0.010	0.265 ± 0.013	0.188 ± 0.010	0.227 ± 0.011	0.302 ± 0.005	0.308 ± 0.007
% CH_4 in biogas (v/v)	62.0 ± 3.0	61.7 ± 1.9	65.3 ± 2.2	63.1 ± 2.8	64.5 ± 4.5	68.3 ± 1.6	67.9 ± 2.5	69.2 ± 0.9
Total alkalinity (mg $CaCO_3$/l)	12850	13150	14820	14550	12580	13200	16340	16200
VFA (mg acetate/l)	6005	5111	1533	2023	6850	4086	1135	989
pH	7.05	7.09	7.17	7.15	7.08	7.11	7.12	7.16

The loading concentration of 5 kg VS/$m^3$and a HRT of 15 d were used in this part of the investigation. This was selected because the specific CH_4 yields were found to be highest at these levels, as demonstrated earlier in section 7.3.2. To further examine any trends, the study examined the influence of temperature variations on the digestion of two different levels of microalgae residues (MR %) fractions (i.e. 40 and 60 %) in the digested biomass feedstock.

An increase in the specific CH_4 yield trend of the co-digested glycerol-microalgae biomass residues was seen with increases in the digester temperature from 25 to 35°C. Increasing the process temperature from 25 to 35°C resulted in a 53.65 and 60.64% increase of the specific CH_4 yield for the 40 and 60 MR % samples respectively. A further increase in temperature to 40°C was, however, shown to not exhibit any influence on the CH_4 production for both samples.

On the other hand, there were no significant trends observed for the % fraction of CH_4 in the biogas when the digester temperature was increased.

These results may however not fully characterise the influence of temperature on the anaerobic digestion of the microalgae residue substrates. The reason is that the inoculum used in

the experiments was initially adapted to a temperature level similar to the 'optimum' level (35°C) before the temperature level of the digester was reduced to 25°C with subsequent increases applied. The adaptation period could have favoured the selection of specific bacterial masses that thrived at this temperature.

7.3.4. Influence of the C/N ratios on biogas CH_4 content and anaerobic digestion efficiency

To further investigate the impact of co-digesting the microalgae residues with glycerol, the effect of C/N ratio on the percentage CH_4 content of the biogas (v/v) and VS destruction was studied. The substrate molar C/N ratios corresponded to the MR % as previously described in section 7.3.2. Due to the use of oxygen rich glycerol as the co-digestate in this study to improve the C/N ratio of the microalgae residues, the influence of glycerol on the biogas CH_4 fraction (v/v) was further explored. This was carried out to confirm if the anaerobic digestion CO_2 co-product would be available in increased quantities with increasing glycerol addition (i.e. increasing C/N ratio).

Figure 7.6 shows the CH_4 content in the biogas (%, v/v) obtained using a HRT of 15 d at a process temperature of 35°C. The experiments involved the use of substrate C/N ratios of 5.4-24.17 and loading concentrations of 5-50 kg VS/m^3 digester volume.

Within the experimental boundaries of this study, a reduction in the percentage CH_4 fraction of the biogas (v/v) was obtained with an increase in the C/N ratio of the co-digested biomass substrate with the different substrate loading rates.

The reduction in the concentration of CH_4 in the biogas (%, v/v) could be attributable to the relative ease with which the glycerol fraction of the biomass substrate was digested. Increases in the C/N ratio of the substrate, corresponded to an increase of the oxygen fraction of the feedstock i.e. from 36.22% (for the 100% MR samples) to 48.94% oxygen content (for 20 MR % samples) of the digestion feedstock (w/w). This could have led to an increase in the CO_2 fraction (v/v) resulting from the methanogenic process.

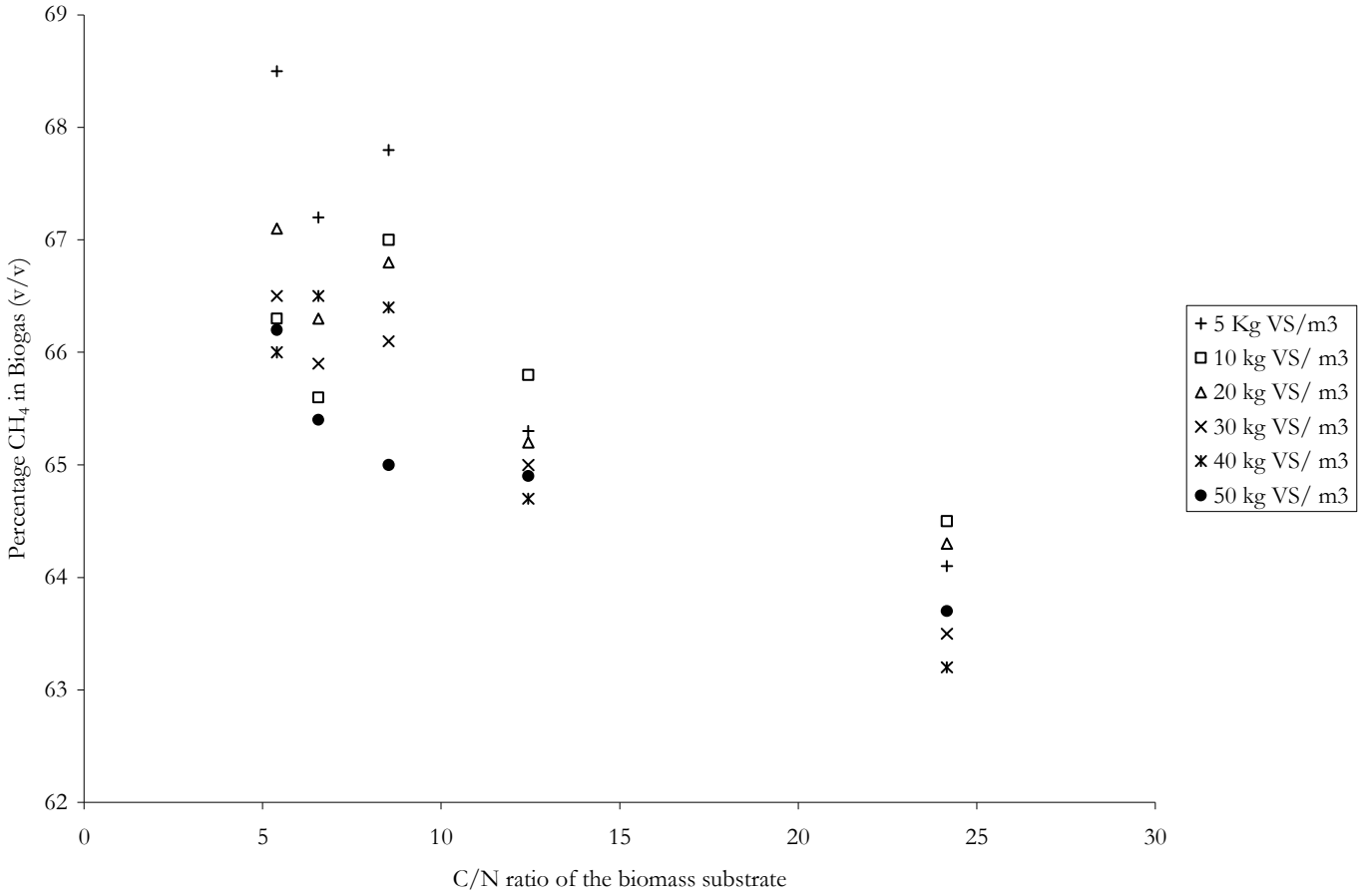


Figure 7.6. Influence of biomass C/N ratio on percentage CH_4 content in the biogas (v/v) from the microalgae residues at a HRT of 15 d and process temperature of 35°C.

The anaerobic digestion efficiency was estimated on the basis of the substrate VS destruction using Eq.7.3 and the molar quantities of CH_4 and CO_2 produced per kg sample digested. A biomass carbon content of 0.45 for the post transesterified *Chlorella* residues (as presented in section 6.3.1) and 0.39 for the glycerol was used in the equation. For the estimation of the percentage carbon fraction of the co-digested samples (i.e. containing the Chlorella residues and glycerol), the mass and empirical chemical formula of the respective digesting species was used.

An increase in the loading concentration coupled with a reduction of the HRT was found to result in a reduction in the anaerobic digestion efficiency. As described earlier in section 7.3.2, this may be due to the digester washout, facilitated by a reduction in the digestion times and increases in the substrate loading rate.

For example, with the HRT fixed at 15 d, Figure 7.7 shows the variation of the digestion efficiency with the studied C/N ratios and loading rates. The digestion efficiency was observed to increase by 37.1% with an increase in the C/N ratio of the *Chlorella* residues from 5.4 to 12.44.

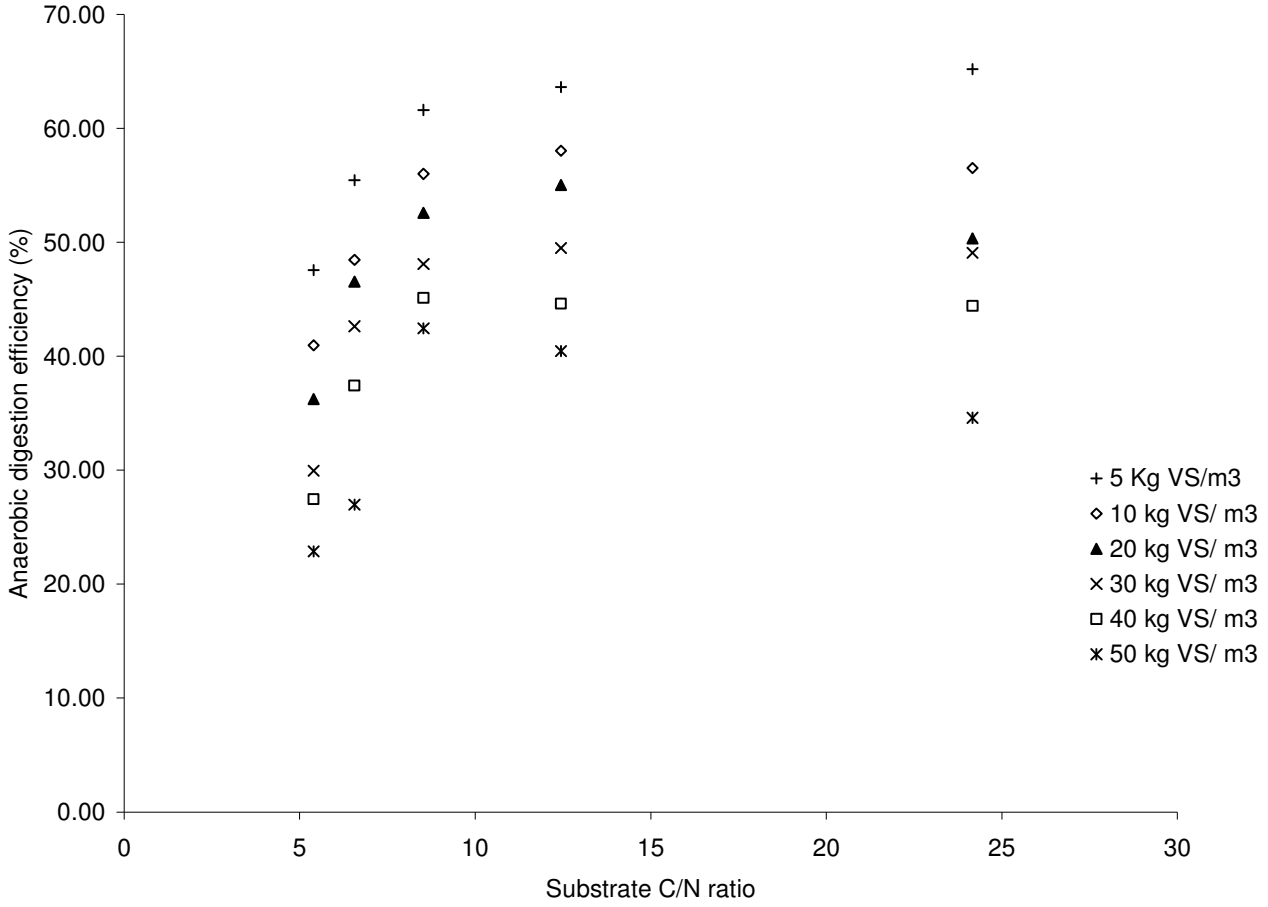


Figure 7.7. Influence of C/N ratio on the efficiency of the anaerobic digestion of co-digested microalgae residues with a retention period of 15 d and process temperature of 35°C.

7.3.5. Monitoring the digester performance

7.3.5.1. Volatile fatty acids

The literature has ample evidence that volatile fatty acids (VFAs) play an important role as intermediates in the CH_4 metabolic chain. Increases in the concentrations of these acids in anaerobic digesters are considered to have negative effects on the anaerobic digestion process via microbial stress (Hill et al., 1987). Hill et al. (1987) have also suggested VFAs provide a useful indicator of the effectiveness of anaerobic reactors).

Monitoring the different levels of VFAs (i.e. acetic, propionic, butyric and valeric acids) produced during the anaerobic digestion process could assist in predicting the performance of digesters. They could also aid in identifying underlying process problems, such as feedstock overloading. In conjunction with other process parameters, (i.e. the digester alkalinity and pH), observing the VFA levels could help in the control of slowly developing failures attributable to organic overloading (Switzenbaum et al., 1990).

With CH_4 production of primary importance in this study, the specific CH_4 yield (m^3 CH_4 /kg VS digested) was used to monitor the digester performance. The digester success or failure was based on the assumption that a volatile solids reduction of ≥50% indicates that the digester is "healthy" (Hill et al., 1987).

The specific CH_4 yield of 0.20 m^3 CH_4/kg VS digested, which corresponds to a 50% VS reduction (calculated using Eq. 7.3), was used as the indicator of a healthy digester in this study, with lesser yields signalling impending digester failure.

Irrespective of the influence of the retention times, loading concentration and %MR of the digested feedstock, it was seen that the specific CH_4 yield decreased with an increase in the total VFA (mg total VFA/l) (Figure 7.8).

Figure 7.8 shows the total digestate VFA concentrations recorded at different specific CH_4 yields for all the levels of MR %, SC and HRT and with a process temperature of 30°C.

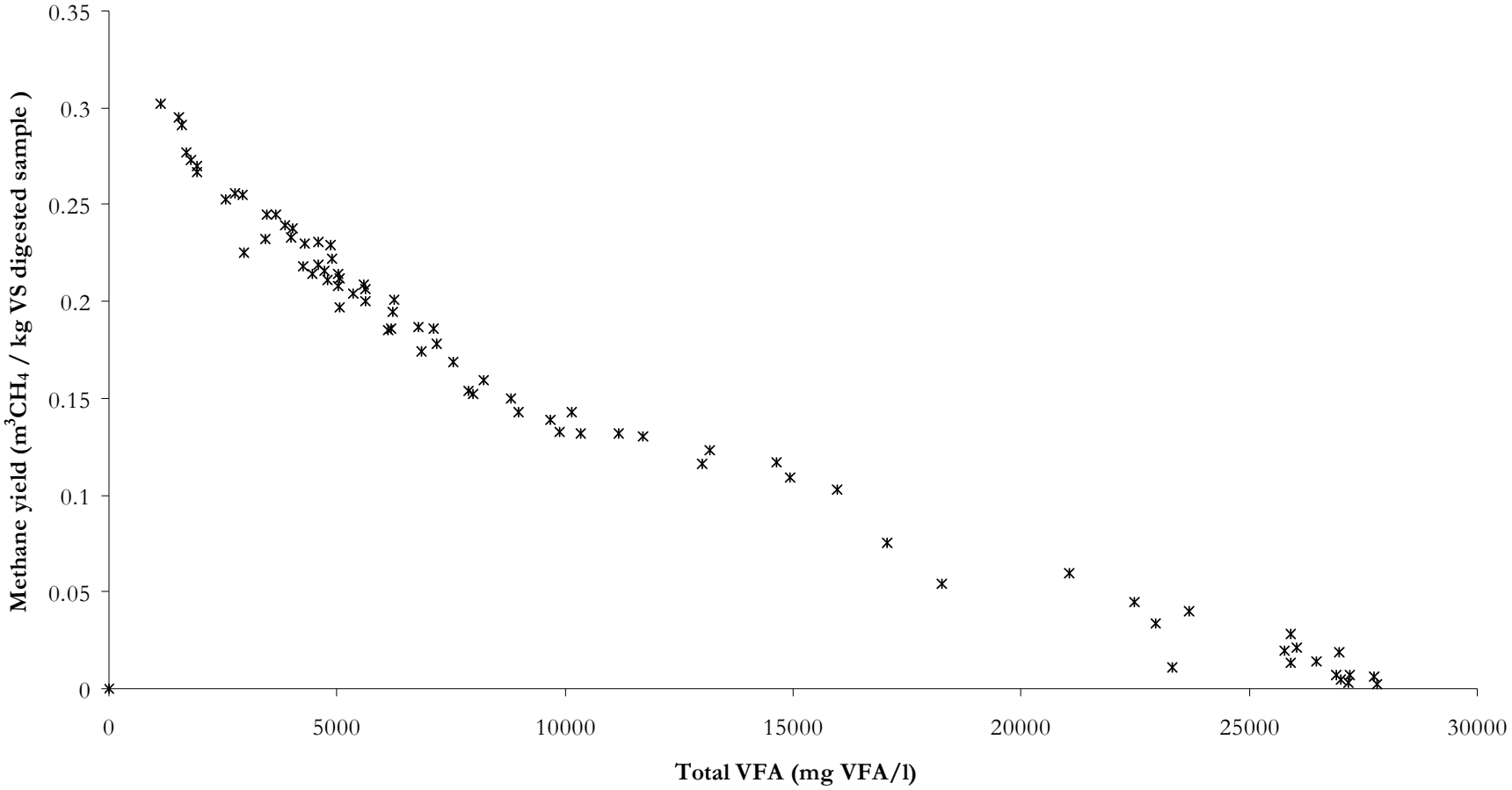


Figure 7.8. Observed relationship between total VFA concentration and specific methane yields for HRT (5-15 days), SC (5-50 kg VS/m^3), MR% (20-100%) and a process temperature of 35°C.

This build up of the VFA concentrations in the digesters may be due to an increase in the activity of the acidogenic phase bacteria, coupled with inhibition of the CH_4 forming bacteria. It may also be a result of a slower rate of consumption of the acid intermediates by the methanogenic process.

Our results demonstrated that high concentrations of VFAs (> 5000 mg total VFAs/l), indicated digester instability, was obtained for all the continuously stirred digesters operated with substrate loading concentrations > 40 kg/m^3 digester, regardless of the HRT, and the MR % in the digesting substrate.

A reduction in the HRT was also observed to contribute to VFA accumulation. This was attributed to a faster acid formation process relative to the CH_4 forming phase. This observation suggests that the methanogenic process is the rate limiting step for the anaerobic digestion of the post transesterified *Chlorella* residues.

Chromatographic analysis of the effluent VFAs revealed a variation in the concentration of acetic, propionic, butyric and valeric acids at different digestion conditions and specific CH_4 yields.

For all levels of MR % with a substrate loading of 5 kg VS/m^3 and a HRT of 10 and 15 d, it was seen that the predominant VFAs were acetic and propionic acids. Similar results were seen for the digesters with loading concentrations of 10 and 20 kg VS samples/m^3 digester with a HRT of 15 days. This was assumed to indicate active degradation of the biomass macromolecules (Samson & LeDuy, 1986).

At higher SCs, an increase in the butyric and valeric acid fraction of the total VFAs was observed.

Figure 7.9 shows the variation of the digestate butyric and valeric acids concentration with the CH_4 yield for the entire SC, HRT and MR % levels and an operating temperature of 35°C.

The butyric fraction of the total VFA was seen to increase from 0-2% in the high CH_4 producing digesters (with CH_4 yields of > 0.21 m^3 CH_4/kg VS sample) to ≈10% at the start of digester inhibition (with CH_4 yields <0.20 m^3 CH_4/kg VS sample).

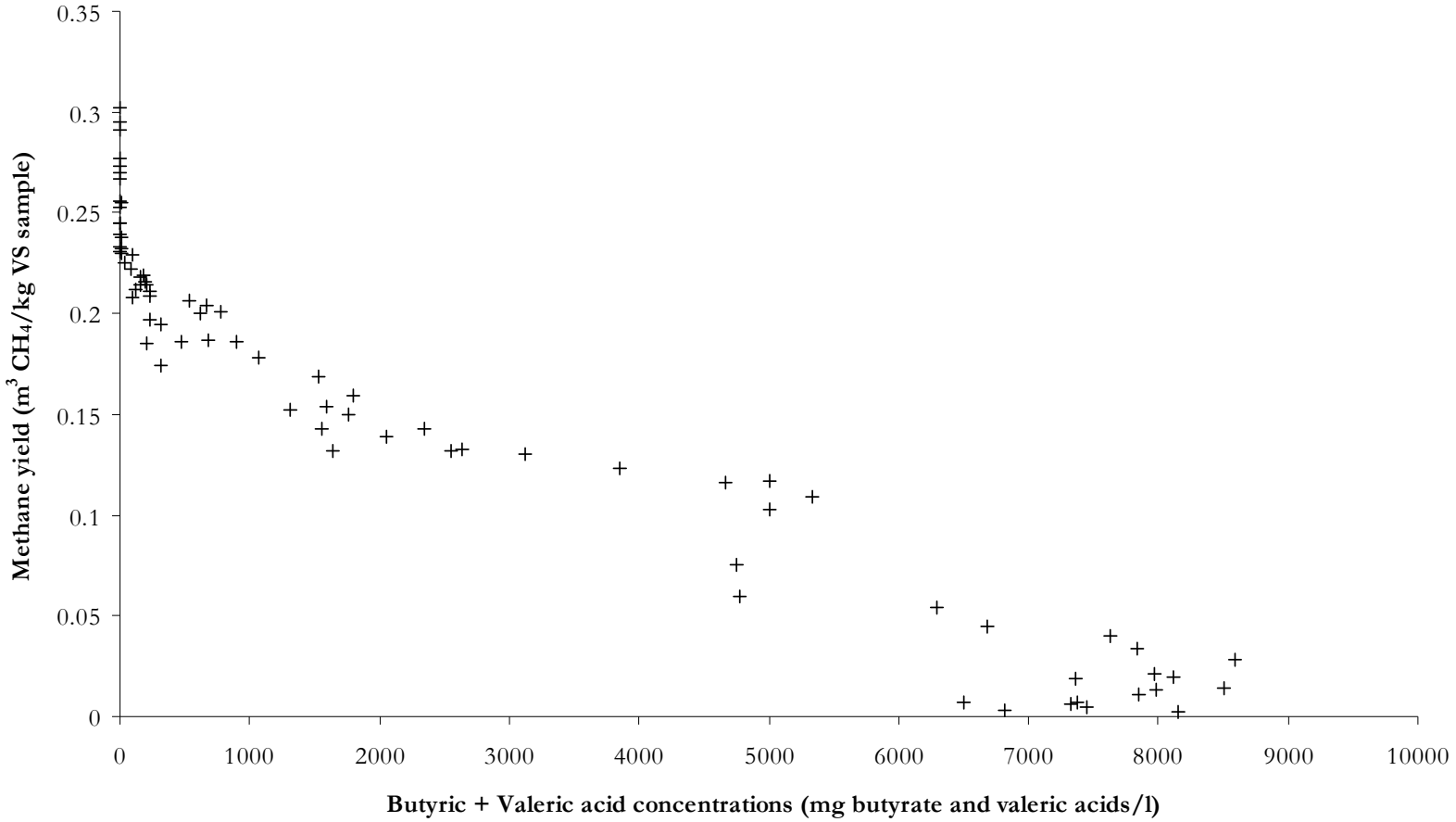


Figure 7.9. Variation in the digester butyric and valeric acids concentrations with specific methane yields at all the HRT, SC and MR% levels and at a digestion temperature of 35°C.

The accumulation of butyric and valeric acids in the reactors appears to exhibit inhibitory effects on the methanogenic process. This can be seen in the reduced specific CH_4 yields (shown in Figure 7.9) with almost complete digester failure at concentrations levels > 6500 mg butyric and valeric acids/l as shown in Figure 7.9.

The propionic to acetic acid ratios (P/A) are widely accepted in the literature as good indicators of anaerobic digester performance (Nordstedt & Thomas, 1985; Bolte et al., 1986). Hill et al. (1987) demonstrated that a P/A ratio > 1.4, and acetate levels > 800 mg/l, could serve as an indication of impending digester failure.

With the results obtained in this study, the observed variation of the P/A ratio with specific CH_4 yields (Figure 7.10), suggests that the digester performance is not adequately predicted by the P/A ratio. Poor digester performance was attained at P/A ratios as low as 0.6, whereas reasonable performance was obtained for P/A ratios as high as 1.2 as seen in Figure 7.10.

Based on these results, and taking into account that increasing butyric and valeric acid concentrations lead to process inhibition, it was proposed that the ratio of butyric and valeric acid to acetic acid (BV/A) be used as an indicator of the digester performance in this study.

Impending digester failure appears to be satisfactorily predicted at BV/A ratios of > 1.2 as is shown in Figure 7.11.

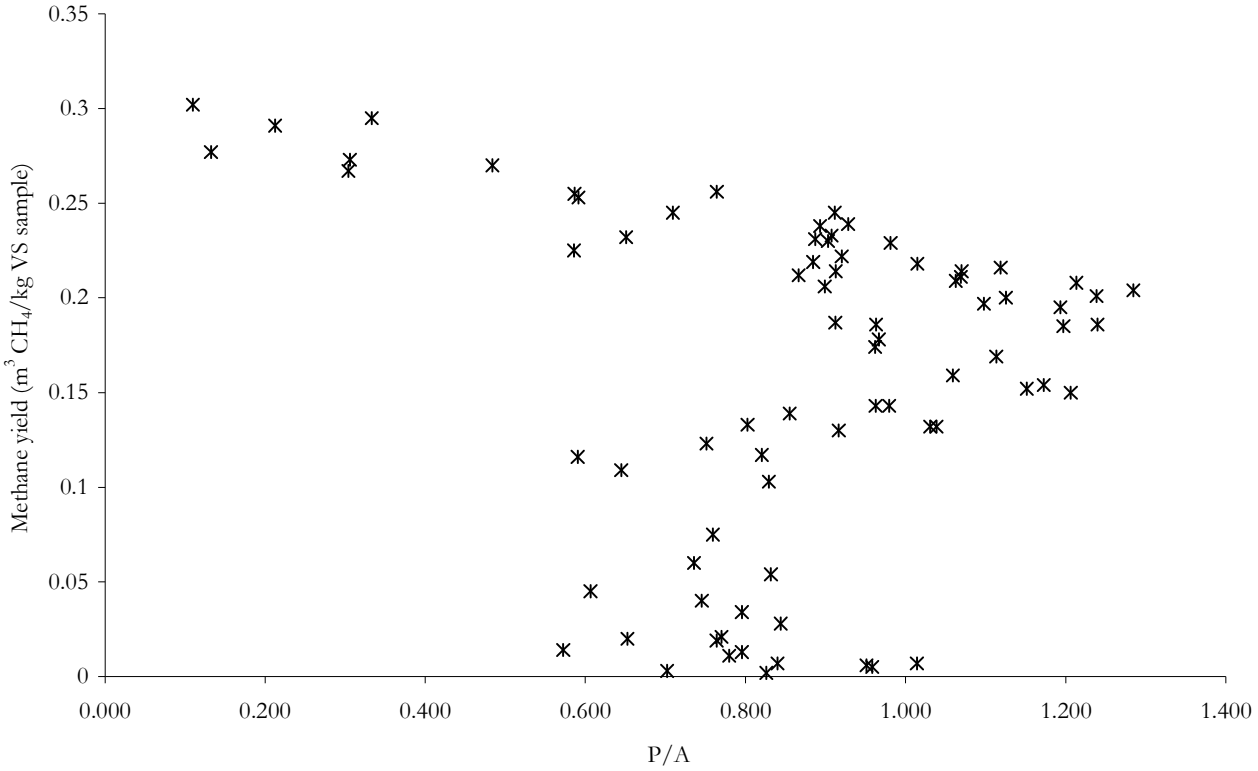


Figure 7.10. Relationship between the observed specific methane yield to the P/A (ratio of propionic acids to acetic acids) for all levels of HRT, MR % and SC and the process temperature at 35°C.

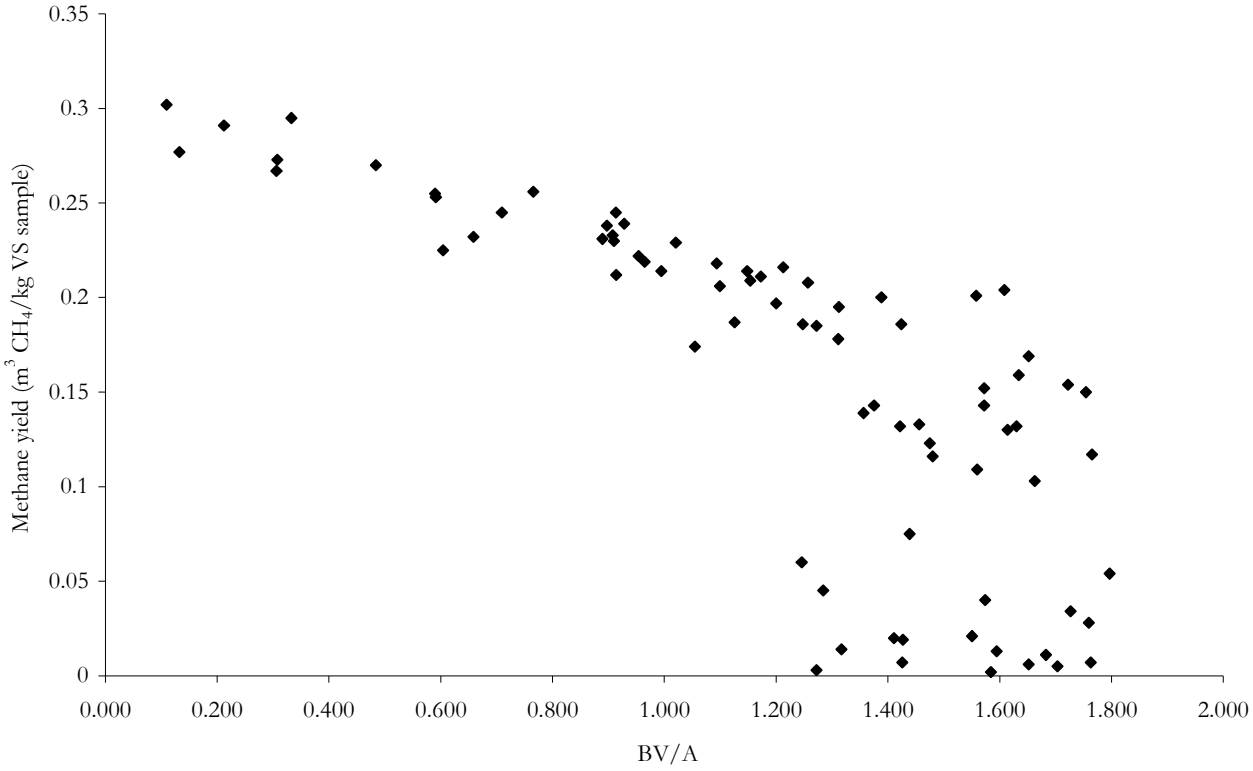


Figure 7.11. Relationship between specific CH_4 yield to BV/A (ratio of butyric + valeric acids to acetic acid) for all levels of HRT, SC and MR% and a process temperature of 35°C.

7.3.5.2. Process alkalinity

Coupling the process alkalinity with the total VFA concentrations was another criterion used to assess the performance of the anaerobic digestion process in this study. Using the process VFA to alkalinity ratio, three critical values were considered by Callaghan et al. (2002) for monitoring the CH_4 production process. These are:

< 0.4 (would ensure digester stability)

0.4-0.8 (some instability may occur)

≥ 0.8 (significant instability would be encountered)

With the specific CH_4 yield as the digester performance indicator, it can be seen from Figure 7.12, that these value of the total VFA/alkalinity ratio appear to adequately characterise the *Chlorella* biomass digestion.

The maximum and minimum CH_4 yields (0.295 and 0.002 m^3 CH_4/kg VS) were obtained with a total VFA/Alkalinity ratio of 0.103 and 4.059 respectively. The onset of the reactor instability indicated at a yield of 0.20m^3 CH_4/kg VS was seen to have a VFA/alkalinity ratio of 0.435 which is consistent with Callaghan's lower critical value.

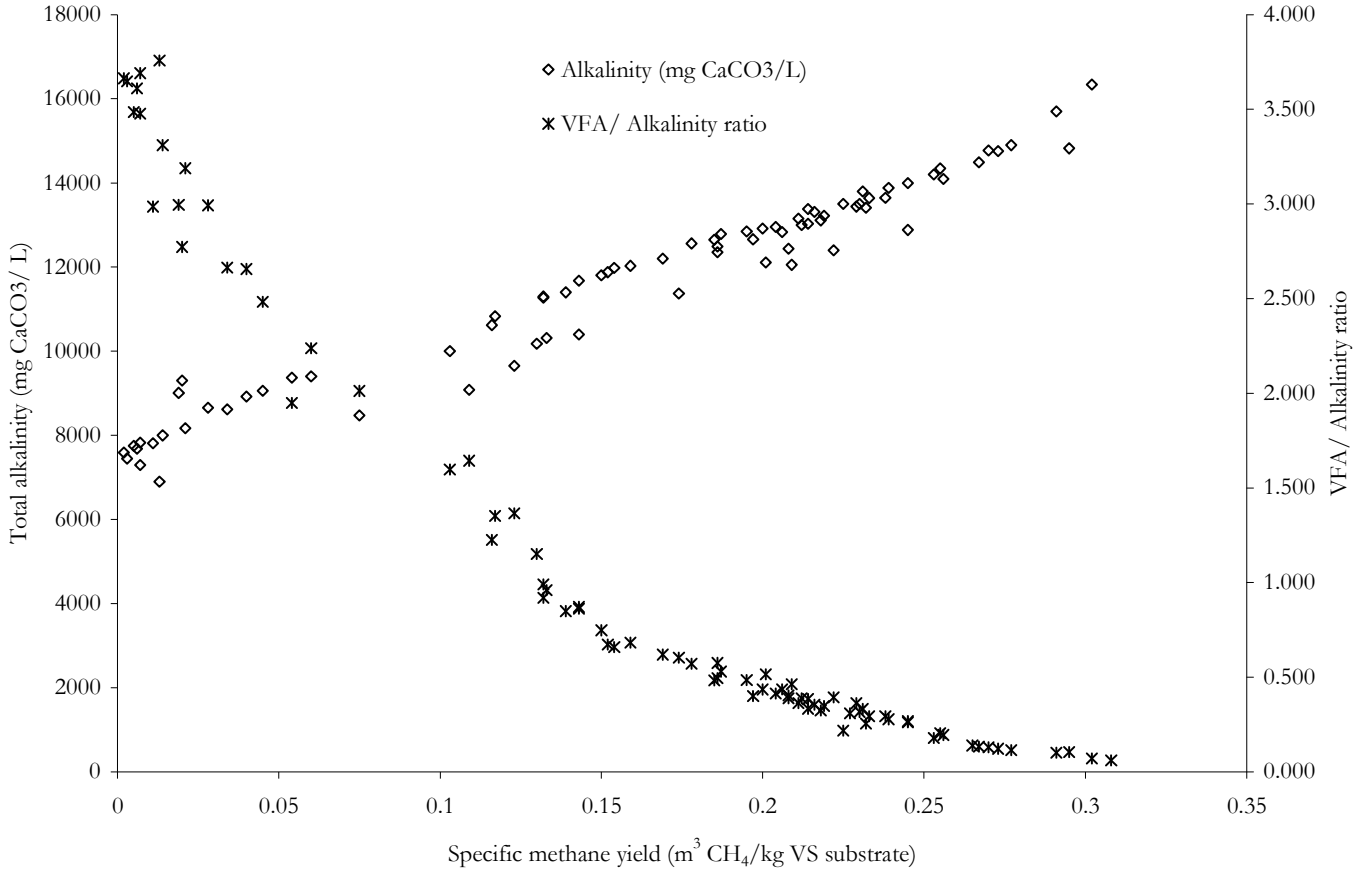


Figure 7.12. Relationship between total alkalinity levels and VFA/alkalinity ratio with specific CH_4 yields for all HRT, SC and MR % levels and with a digestion temperature of 35°C.

7.3.5.3. Process pH

Figure 7.13 shows the process pH measured with a change in the SC for different HRT and MR% with a process temperature of 35°C.

As highlighted in Figure 7.13, a stable pH range (6.6-7.32) was observed for most of the experimental runs in this study, indicating the digestion process has a high buffering capacity of the digestion process.

The buffer system was however shown not to adequately sustain high VFA accumulation in the digesters. This was mainly encountered during experimental runs involving large increases in the organic loading rates, and a reduction in the retention times, as seen, for example for the different substrate concentrations with a HRT of 5 d (Figure 7.13).

This led to a drop in the process pH to < 6.5, which resulted in an inhibition or complete disruption of the CH_4 production process, as indicated by the low specific CH_4 yields in those systems as shown in Figure 7.14.

Figure 7.14 shows the relationship between the measured digester pH and CH_4 for all HRT, MR% and SC levels with process temperature of 35°C.

Within the experimental limits of this study, a "healthy" CH_4 production from the anaerobic digestion of the microalgae residues was observed with the process pH of 6.8-7.4.

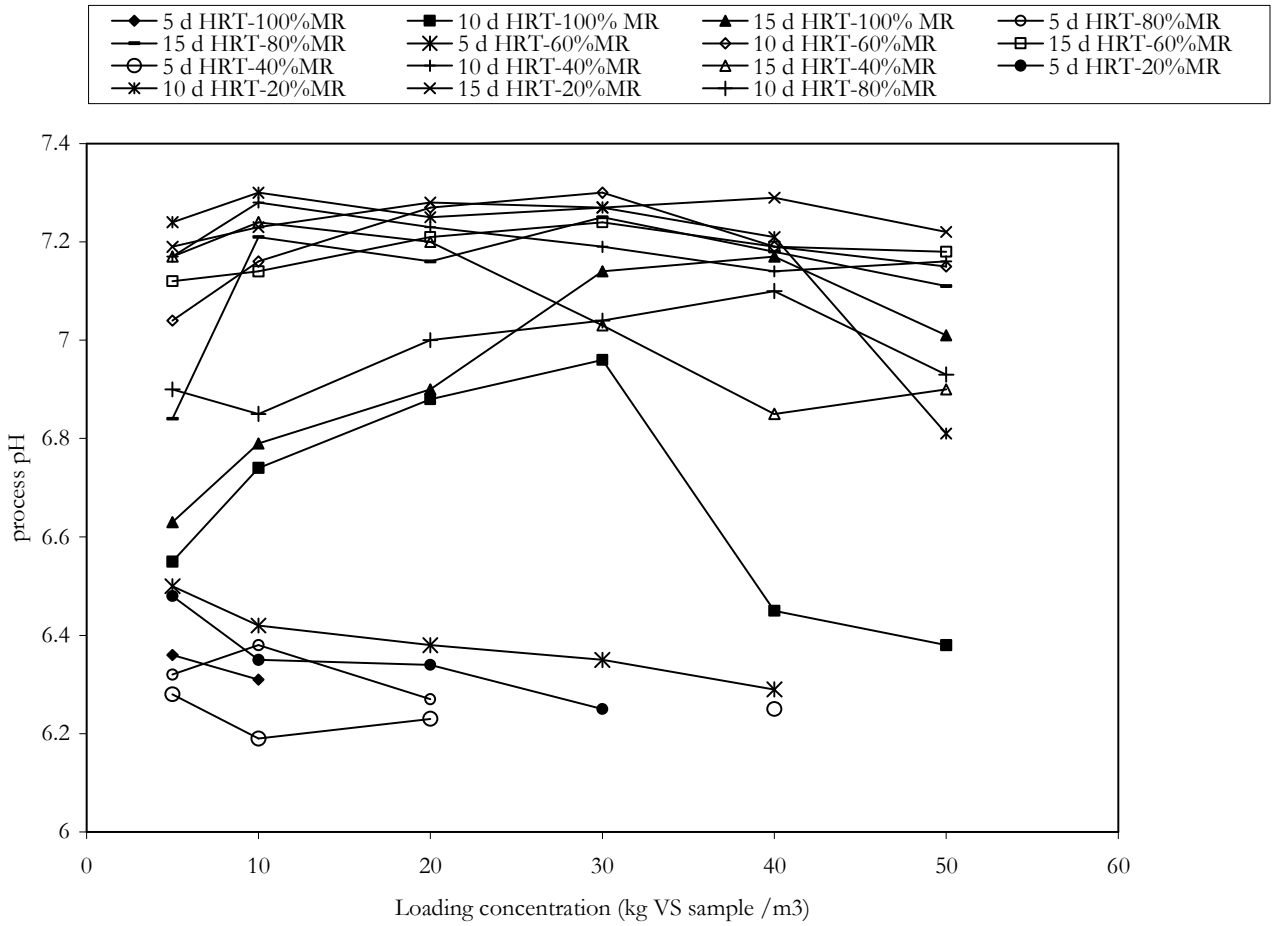


Figure 7.13. Measured process pH with varying loading concentrations for different HRT and MR % in the digested substrate for the anaerobic digestion runs at 35°C.

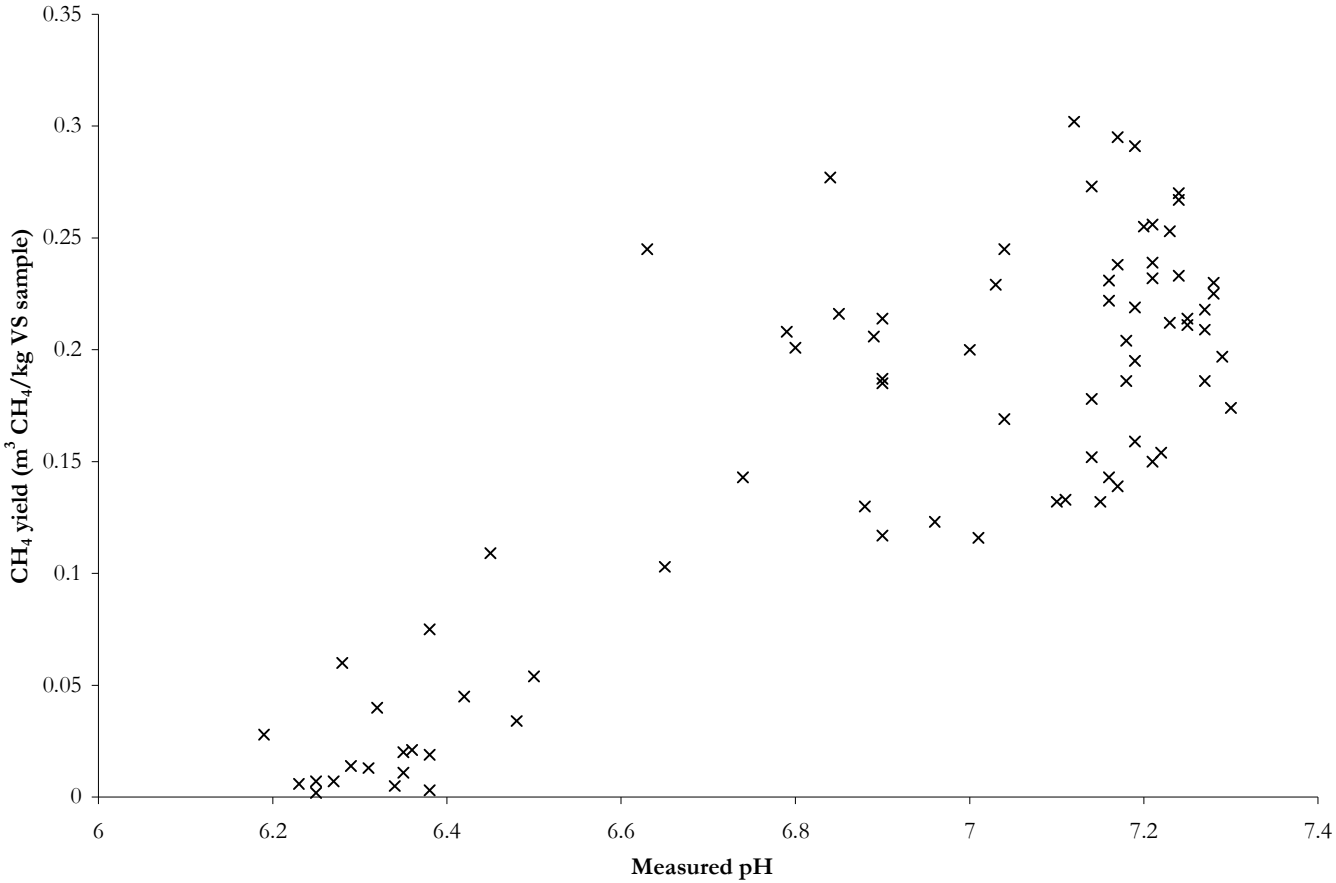


Figure 7.14. Relationship between measured digester pH and specific CH_4 yields for all MR %, SC and HRT levels and a process temperature of 35°C.

7.3.5.4. Process ammonia nitrogen (NH_3-N)

The high protein (60.19%) (as presented in Table 6.4) and corresponding nitrogen (9.39%) mass content (w/w) of the post transesterified *Chlorella* biomass residues used in the digestion process could result in the by-production of toxic ammonia concentrations as predicted by Boyle's equation (Boyle, 1977) (Eq. 6.4) (section 6.2.2).

Within the study experimental boundaries, it was observed that an increase in the HRT and loading concentrations of the substrate produced an increase in the total ammonia nitrogen of the digestate. This was attributed to more complete digestion of the microalgae biomass proteins with time, with an accompanying accumulation of ammonia in the liquid phase.

As expected, an increase in the substrate C/N ratio resulted in a reduction in the ammonia nitrogen levels of the digestate.

Inhibition of the anaerobic digestion process, due to passive diffusion of unionized free ammonia (NH_3) across the cell wall of the digestion bacteria, where its toxicity will be expressed (Sialve et al., 2009), was anticipated. It was expected that the acetoclastic methanogenic bacteria would be the most sensitive to free ammonia, as reported by Angelidaki & Ahring (1993).

However, the ammonia nitrogen concentrations in this study (880 – 4300 mg NH_3-N/l digestate) appear not to directly affect the process stability. To illustrate this point, the NH_3-N concentration of 3500 mg NH_3-N/l was obtained for the 80% MR sample digested with a HRT of 15 d and SC of 5 kg VS/m^3 which had a specific CH_4 yield of 0.245 m^3 CH_4/kg VS.

The influence of the process pH on the ammonia species proportion in the digester might have played a role in reducing the risk of ammonia inhibition. This is due to the fact that different forms of ammonia are present in the digestate depending on the process pH. The toxic free ammonia (NH_3) is the dominant species under alkaline conditions and the ionized ammonium (NH_4^+) in acidic environments.

The high VFA concentration in this study promoted a slightly acidic digester environment at an operational pH range of 6.2-7.2. This may have aided the protonation of the free NH_3 to the ionized ammonium species NH_4^+, by enhancing the shift of the chemical equilibrium (Eq. 7.5) to the right.

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad (7.5)$$

Hence, at the recorded pH levels, most of the ammonia nitrogen concentrations in this study may have been below toxic concentration levels. McCarty (1964) determined that ammonia inhibition in the anaerobic digester occurs only at concentrations of 1.5-3.0g N/l at a pH of over 7.4. The ammonia nitrogen concentrations toxicity for digestion processes at a pH < 7.2 was not found in the literature. A proper assessment of its effect on the anaerobic digestion of the microalgae residue could therefore not be made.

In addition, the digester acclimatisation period, and the inoculum and digestion temperature used in this study, might have helped reduce the toxic effects of NH_3.

The ammonia nitrogen concentrations in this study were observed to fall below the upper limits of the inhibition concentration range of 1.7-14 g N/l put forward by Angelidaki & Ahring (1993). This could imply that the ammonia nitrogen concentrations obtained in this study may not be toxic to the anaerobic digestion process.

7.4. Chapter Conclusion

The results presented in this chapter show that it is feasible to subject the microalgae biomass residues (post transesterification) to a semi-continuous anaerobic digestion process, with the CH_4 yields mainly influenced by the HRT of the residues in the digester.

Increases in the HRT were shown to affect the digestion process trends with periods longer than 5 d required for efficient anaerobic conversion of the biomass residues to CH_4.

When glycerol is available, its integration into the anaerobic digestion of microalgae residues has the potential to improve the overall energy recovered as CH_4 per unit of

microalgae biomass. Co-digesting the microalgae residues with glycerol in proportions of 60% microalgae residues to 40% glycerol was found to increase CH_4 production by 53%, compared with when the residues were digested alone. The substrate loading concentration was also found to be as an important process parameter with lower digester loading rates favouring higher specific CH_4 yields.

It must be noted that the results obtained in this experimental study are based on the use of the biomass residues after the application of the in-situ transesterification method using the freshwater microalgae specie, *Chlorella sp*, as the process feedstock. The results obtained should be treated as representative of the specific microalgae biomass and treatment method used. However, it is expected that similar CH_4 production results would be observed with the use of residues from other oil bearing microalgae species subjected to similar biodiesel production processes. The reason is that they are expected to also contain similar biomass protein contents as the *Chlorella* residues used in this study.

The results presented in this chapter, show that CH_4 production from the microalgae residues (post transesterification) could be used to meet part of the biodiesel process energy requirement. Alternatively, it could be sold as a secondary energy product to help reduce the overall process cost. This suggests that an improvement in the renewability of the overall 'microalgae biomass to biodiesel' process is feasible when CH_4 recovery from the residues is used.

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8. DESIGN AND ASSESSMENT OF THE TRANSESTERIFICATION PROCESSES AND ENERGY RECOVERY SCHEME

8.1. Introduction

Before any evaluations can be made on the potential energetic benefits of the transesterification processes, an assessment of the different systems must be carried out. This chapter provides a preliminary comparison of the different microalgae lipid transesterification processes using process models.

This chapter aims to achieve the following objectives:

i. Develop a simple large scale design basis for the in-situ transesterification processes using microalgae biomass (including all the modifications examined in this thesis).

ii. Establish a preliminary model of the energy recovery (anaerobic digestion) process using the microalgae residues as feedstock.

iii. Estimate the available process heat energy which could be used to meet some of the heat demands of the conversion processes.

iv. Compare the external energy requirement of the different modified transesterification processes.

The results obtained from the process model are used to determine the energy and material requirements of the various processes. These requirements were then used in simplified assessment of the renewability of the different microalgae biodiesel production options and energy recovery routes.

8.2. Process Modelling and Simulation

8.2.1. The process modelling software

The process simulation software Aspen Plus® version 11.1.1 developed by Aspen technology Inc., Cambridge, Mass., USA, was used in the design of the reaction and separation units of the transesterification methods. With limited detailed knowledge on the comprehensive design of the process units i.e. distillation columns, the use of this commercial software for the process modelling was useful, because the software provided a simple means of designing and calculating the energy demands of the processes investigated.

The commercial software was considered to be sufficient for the analysis in this thesis since it is widely used for demonstrations in the literature where only a simplified modelling and comparison of biodiesel production systems is required i.e. Haas et al. (2006), Sheehan et al (1998), Myint & El-Hawagi (2009) and Kasteren & Nisworo (2007).

The process model design approach involved the description of the reaction pathways and chemical components, and the selection of a suitable thermodynamic model for estimating the components physical and thermodynamic properties. The user defines the plant capacity and the process input and operational conditions i.e. flow rates, temperature and pressure[16].

To ensure that the modelled processes described in this chapter reflect practical biodiesel production, empirical data on oil yields and fatty acid methyl esters (FAME) production from microalgae (*Chlorella sp*) obtained from the laboratory experiments (Chapters 3-5) was used. In addition, assumptions made about the operation of downstream separation and purification processes for biodiesel production were based on current industrial practices as used in ASPEN assisted process models in the literature i.e. Sheehan et al. (1998) and Haas et al. (2006).

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[16] A detailed overview of the Aspen modelling software, the inputs into the process models and the mass and energy balances of the process units are presented in Appendix E (CD-ROM).

8.2.2. Preliminary design assumptions

A biodiesel production plant with a fixed biomass feedstock input of 1 t dry *Chlorella* biomass/ h was proposed. This input rate was selected to facilitate an even comparison of the transesterification routes evaluated in this study. The estimates of the energetic and process raw material requirements were consequently based on those required for the conversion of 1 t dry *Chlorella* biomass per hour. The determined plant capacity of 1 metric t biomass / h was also based on the premise of the practicability of the total areal requirement for the biomass production. Using a conservative microalgal biomass productivity of 20g (dry weight) m^2/day (72 MT/ ha/ annum) and a microalgal oil fraction of 0.27g oil/g dry biomass, this relates to a total primary cultivation area requirement (excluding processing and administrative structures) of 135 ha for the production of the process microalgae feedstock. This is with the assumption that all of the biomass oil is utilised for the transesterification process and a 90% 'oil to biodiesel' conversion efficiency. This land area compares favourably when considered against the use of more conventional oil sources i.e. rapeseeds which would require ≈ 17780 ha for primary oilseed production for the same plant capacity.

8.2.3. Process feedstock choice and specification

Microalgae (*Chlorella*) which is the lipid-bearing biomass feedstock for this study, is assumed to be dried (as in Chapter 4). The *Chlorella* oil has an oleic acid (C18:1) content of 61.8% (w/w), together with palmitic (4.37%), palmitoleic (0.44%), linolenic (19.94%), linoleic (12.22%) and eicosanoic (1.22%) acids (w/w) (section 3.2.6). The percentage of the free fatty acids (FFA) in the microalgae oil was 5.11% w/w (section 3.2.6.3). The esterification of these fatty acids, and the transesterification of the triglycerides which they form, results in FAME production. With oleic acid ($C_{18}H_{34}O_2$) as the principal fatty acid in the *Chlorella* oil, it and its corresponding triglyceride, triolein ($C_{57}H_{104}O_6$) were used as the model fatty acid and oil in this study. Since the thermal properties of the other fatty acids (and their triglycerides) are similar to that of oleic acid (Myint & El-Halwagi, 2009), it was expected that the results of the process models using oleic acid and triolein would be representative of the transesterification of the *Chlorella* lipids.The decision steps outlined by

Carlson (1996) were used for the selection of an appropriate physical property method for the process modelling using the Aspen Plus ®software, and for the estimation of any missing parameters.

The assumptions guiding the process modelling for the in-situ transesterification process are elaborated in section 8.2.4 and, the approaches used for the modified in-situ and conventional process models are presented in sections 8.2.5-8.2.8. Unlike previous ASPEN assisted conventional biodiesel production models (i.e. Zhang et al., 2003; Haas et al., 2006; Kasteren & Nisworo, 2007) where the process models were based on the extracted oil, the conventional process modelled in this study uses the microalgae biomass as the starting point. The aim was to use the same process boundaries for all the biodiesel production systems. The system boundaries employed here present a fair basis for comparing the different transesterification processes considered.

8.2.4. The in-situ transesterification process

8.2.4.1. Transesterification reaction, acidic catalyst and reacting alcohol

Sulphuric acid (H_2SO_4) and methanol (CH_3OH) were used as the process catalyst and reacting alcohol respectively. The transesterification reaction takes place in the continuous reactors, with the representative triglyceride, triolein ($C_{57}H_{104}O_6$) converted to methyl oleate esters ($C_{19}H_{36}O_2$) with glycerol ($C_3H_5(OH)_3$) as the reaction by-product as seen in Eq. 8.1.

$$C_{57}H_{104}O_6 + 3CH_3OH \xleftrightarrow{acidic-catalyst} 3C_{19}H_{36}O_2 + C_3H_5(OH)_3 \quad \Delta H = 6.5\times10^{-3}\ kJ \qquad (8.1)$$

Due to the presence of FFAs in the microalgae lipids, the conversion process in this study also accounted for the esterification reaction, with oleic acid as the representative FFA. The resulting esterification reaction with oleic acid converted to methyl oleate esters in the presence of methanol and acidic catalyst is shown in Eq. 8.2.

$$C_{18}H_{34}O_2 + CH_3OH \xleftrightarrow{acidic-catalyst} C_{19}H_{36}O_2 + H_2O \qquad \Delta H = 193.2\ kJ \qquad (8.2)$$

Both the transesterification and esterification reactions can be seen to be endothermic. The reaction enthalpies for the transesterification and esterification reactions were obtained from Kasteren & Nisworo (2007) and Camara & Aranda (2010) respectively. The esterification reaction was assumed to occur in the reactors to completion simultaneously with the transesterification reaction. This assumption was confirmed, as presented in Appendix D, using the acid value of the biodiesel product.

The continuous transesterification reactors were modelled on the basis of the reaction stoichiometry and percentage mass conversion of the microalgae lipids to FAME using the results of the in-situ transesterification trials as described earlier. From the results of the in-situ laboratory investigations, no appreciable improvement in the oil to FAME conversion was observed for reacting molar ratios of alcohol to *Chlorella* oil greater than 315:1. This was with the use of 0.04 mol H_2SO_4 as the transesterification process catalyst and a reaction time of 8 h (section 4.3.1). It was decided that the process models in this chapter would be based on a fixed process reaction temperature of 60°C. The use of this temperature level was deemed adequate since this setting was reported to be typical for most commercial biodiesel production facilities (Sheehan et al., 1998).The models used two reactors arranged in series as demonstrated by Haas et al. (2007) for the transesterification processes. For this process, the reactors were assumed to be agitated using mechanical stirrers which would ensure complete mixing and suspension of the reactor contents. The use of ultrasound agitation is considered in the following section.

The modelled transesterification reaction involved the use of a molar methanol to oil ratio of 315:1 which was shown to result in an 84% conversion of *Chlorella* oil to methyl esters in 4 h with the reaction temperature at 60°C. A 1:1 mass ratio of H_2SO_4 to the estimated *Chlorella* oil content was used as the process catalyst, similar to that employed in the laboratory investigations in Chapter 4.

The process flowchart and major stream tables of the in-situ biodiesel production route using *Chlorella* biomass as feedstock are shown in Figure 8.1 and Table 8.1 respectively.

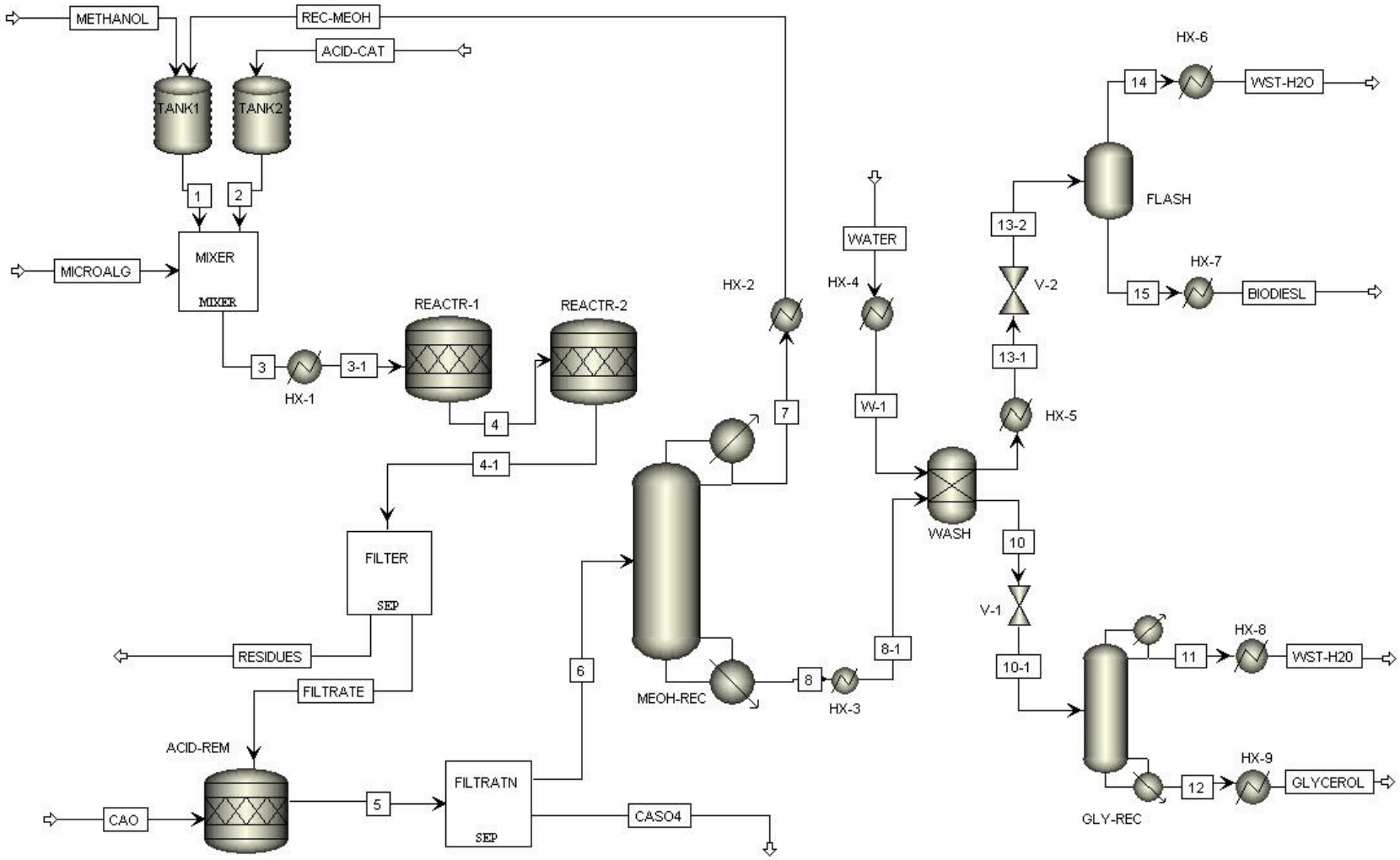

Figure 8.1. Flowsheet showing the process units and streams for the up-scaled in-situ transesterification of microalgae.

Table 8.1. Table showing components of major streams in the in-situ transesterification process

	8-1	10	13-1	ACID-CAT	BIO-DIESL	CAO	CASO4	GLYC-EROL	METH-ANOL	MICRO-ALG	REC-MEOH	RESI-DUES	WATER
Mass Flow, kg/h													
MICROALG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1000	0.00	712.00	0.00
MICROALG OIL	7.00	0.00	7.00	0.00	6.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
METHANOL	30.42	30.42	0.00	0.00	0.00	0.00	0.00	0.00	104.20	0.00	3011.16	43.19	0.00
WATER	52.60	98.20	10.91	0.00	0.53	0.00	0.00	2.08	0.00	0.00	28.82	0.00	56.51
FAME	282.56	0.00	282.56	0.00	281.88	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00
GLYCEROL	27.69	27.69	0.00	0.00	0.00	0.00	0.00	27.69	0.00	0.00	0.00	0.00	0.00
H2SO4	0.00	0.00	0.00	270.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
CAO	0.00	0.00	0.00	0.00	0.00	154.37	0.02	0.00	0.00	0.00	0.00	0.00	0.00
CASO4	0.00	0.00	0.00	0.00	0.00	0.00	374.73	0.00	0.00	0.00	0.00	0.00	0.00
FFA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Flow, kg/h	400.27	156.31	300.47	270.00	289.18	154.37	374.75	29.78	104.20	1000.00	3039.99	755.62	56.51
Temperature, C	70.00	70.37	150.00	20.00	30.00	20.00	60.00	30.00	20.00	20.00	30.00	60.00	20.00
Pressure, bar	1.00	1.00	1.00	1.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	1.00

Fresh (and subsequently recycled) methanol (3115.4 kg/ h) in stream 1 and acidic catalyst, H_2SO_4 (270 kg/ h) in stream 2 was pre-mixed (in MIXER) with the incoming dried *Chlorella* biomass (1000 kg/ h). The resulting mixture in stream 3 was heated in the heat exchanger HX-1to meet the desired the reaction temperature of 60°C. The heated mixture (stream 3-1) was transferred to the temperature controlled continuously stirred transesterification reactors (REACTR-1 & 2). The simplified reactors were assumed to be properly insulated, so heat losses from the reactors were not considered further. The concentrations and stream data of the major process streams are presented in Table 8.1.

Using the percentage mass conversion of *Chlorella* lipids to FAME of 84%, and a biodiesel yield (glycerides and FAME) of 0.288 kg/kg *Chlorella* (section 4.3.6), a combined percentage mass FAME conversion of 97.4% is obtained after both reactors (mechanical stirring).

8.2.4.2. Residues filtration

After the transesterification reactors, the resulting stream (4-1) contains a mixture of the methyl esters, "unreacted" oil, biomass residues, glycerol, H_2SO_4 catalyst, excess methanol and the water resulting from the esterification process. This stream was subjected to a physical solid-liquid separation process (FILTER unit) to extract the solid biomass residues. This was considered to be composed of a simple belt filtration unit, using a cloth filter. The simple filtration model in ASPEN software was used for the design of the separation unit.

The solid filtration model for this process was based on empirical data from the laboratory investigations for the biomass filtration, post transesterification. This involved using a material mass balance of the component streams in the modelling of the separation unit (FILTER), with the assumption that 100% of the solid residues are recovered along with traces of the liquid streams entrained in the biomass. From the laboratory experiments, it was observed that there was a 1.6 ± 0.4% increase in the weight of the vacuum filtered biomass residues (section 4.2.1) owing to the liquids held in the solid biomass phase. This was estimated based on the residues weight before and after drying to a constant weight at

105°C (as presented in section 6.3.3). The mass flows of the different components in the residue stream was obtained by multiplying their mass flows in the inlet stream (4-1) by the factors shown in Table 8.2, which were obtained from experiments.

Table 8.2. Ratio of components in the residues after the filtration unit,

Component	Fraction entrained in residues
Methanol	1.4×10^{-2}
FAME	1.4×10^{-3}
H_2SO_4	1.3×10^{-4}
Glycerol	1.4×10^{-4}
Water	4.3×10^{-5}

Using the ratios of the components in the stream (4-1), the fractions entrained in the residue stream were then defined. The solid biomass residues were separated as the stream – RESIDUES with the filtrate stream transferred to the acid catalyst removal step.

8.2.4.3. Acid catalyst removal

In the reactor (ACID-REM), the H_2SO_4 fraction was assumed to be completely removed from the filtrate stream via a neutralisation reaction. This was facilitated by the introduction of calcium oxide (CaO) to the reactor, resulting in the formation of calcium sulphate ($CaSO_4$) and water (H_2O) (Eq. 8.3), ACID-REM in Figure 2.1.

$$CaO_{(s)} + H_2SO_{4(l)} \longrightarrow CaSO_{4(s)} + H_2O_{(l)} \qquad \Delta H = -1143\,\text{kJ} \tag{8.3}$$

The low costing CaO (compared to other alkali oxides) was selected for use in this model in line with previous work by Zhang et al. (2003), which considered its preferential use in commercial biodiesel production systems.

The molar mass flow of the CaO utilised for the removal of the acid catalyst fraction was equivalent to the molar flow of the H_2SO_4 fraction in the incoming stream. After the neutralisation reaction, a solid-liquid separation process was carried out to facilitate the separation of the $CaSO_4$ from the liquid fraction of the stream. It must be mentioned that although the $CaSO_4$ production in the presence of water normally results in the formation of hydrates i.e. $CaSO_4.2H_2O$, this model only considered the anhydrous form, $CaSO_4$ as the filtered solid mass. This was carried out to allow for simplification of the process model as described in Zhang et al. (2003).

The acid removal step could also have been considered to be carried out in the same step with the residues removal, this was however not carried out in this study to facilitate a close monitoring of the process streams and to minimise any potential model problems.

8.2.4.4. Methanol recovery

Excess stoichiometric ratios of methanol to oil for the in-situ process were used to increase the percentage FAME conversion (w/w). The reacting alcohol level was also selected to ensure the complete immersion of the reacting microalgae biomass, thus ensuring full interaction between the reacting species.

The in-situ transesterification model was designed to incorporate a methanol recovery unit for the excess reacting methanol. This was carried out to reduce raw materials cost and processes wastes, and to improve the separation of the transesterification products in the subsequent stages. The recovered methanol was recycled back to the reactor units where it was mixed with fresh methanol.

To model the methanol recovery unit, the rigorous vapour liquid fractionation model (RADFRAC®) in the ASPEN modelling software was used Because of its reported suitability for two- and three- phase systems, as well as narrow and wide boiling systems[17]. This section will highlight only the important details used to model the distillation columns using the

[17] Information obtained from the ASPEN user guide for the modelling of distillation processes.

ASPEN software. This study used a simplified distillation set-up and does not to provide a comprehensive stage by stage analysis of the recovery columns, because this thesis is primarily concerned with the process energy requirement of the transesterification method investigated.

The distillation column was designed to ensure the methanol mass fraction (w/w) in the recovered distillate stream (i.e. at the top of the distillation column) was >99%.

Two key column parameters; the reflux ratio (RR) and the number of stages (N) of the distillation column were used as inputs in the distillation model.

The RR, which is the ratio of the liquid reflux from the condenser (stage 1) per unit quantity of the distillate product removed from the distillation column, has an influence on the energy requirement of the column. Increased RR values lead to a larger heating requirement with a corresponding reduction in the required number of distillation stages, N (Gilliland, 1940), and a reduction in the capital cost. However, increasing the column stages, N, reduced heating demands of the system only up to a point (Zhang et al., 2003). The column stages are also related to the required concentration of methanol at the top of the distillation column. The selection of a suitable RR and N for the distillation model is therefore important, since it provides a balance between operational and capital costs.

The estimation of these parameters was carried out using the simplified Gilliland empirical relation provided in the Aspen Plus® software. This was used to relate specified values of N to give RR, or specified values of RR to determine N.

The RR was specified as shown in Figure 8.2 using the ASPEN Gillard relation, and its relationship with the number of theoretical distillation stages determined.

An operational RR of 0.1 with N being 12 was selected for this study model since it was seen from Figure 8.2 that a further reduction of the RR (i.e. lower distillation heating demand) may not be achieved with the column stages > 12. The separation of the methanol component from the inlet stream (6) to the distillation column (Figure 8.1) was then carried out. It was considered that the additional heat energy required for the distillation process was supplied via the reboiler unit of the distillation column MEOH-REC.

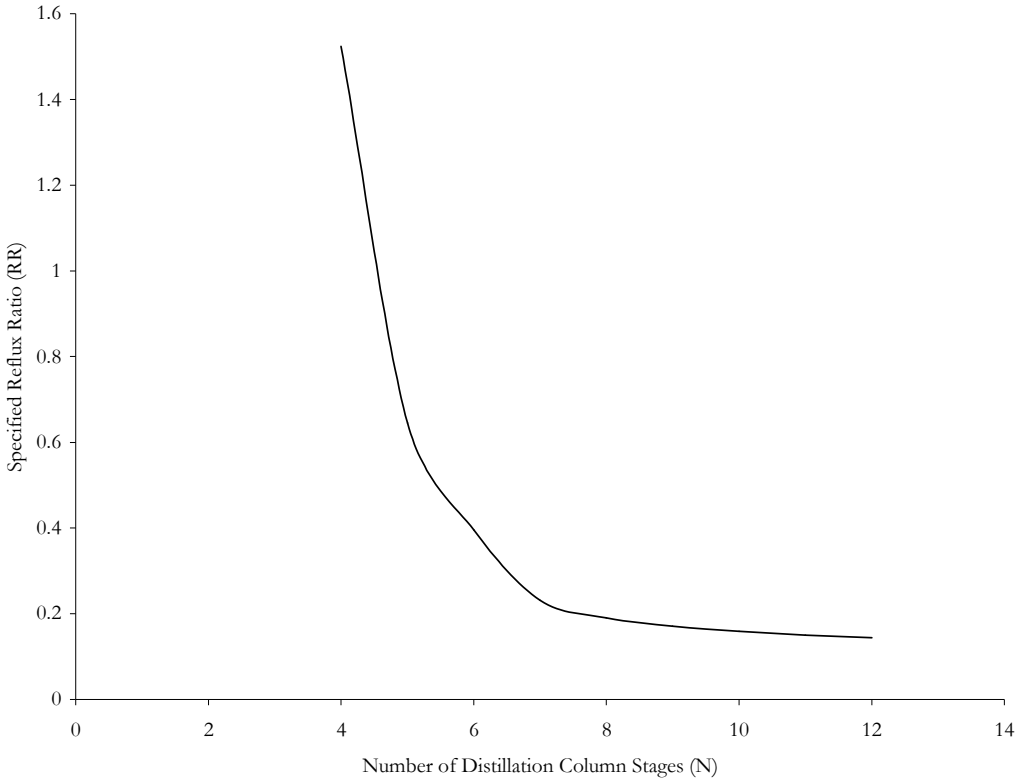

Figure 8.2. Relationship between specified reflux ratio (RR) and the number of distillation stages (N) for methanol recovery.

The distillate stream (7) primarily containing methanol with 99% purity (on a mass fraction basis) was sent for re-use in the transesterification reactors. The heat exchangers (HX-2 and HX-3) were used to recover the useful heat energy available in stream 7 and 8. The recovered heat was considered for use for the heating requirements of other process units. This will be explored further in section 8.3.1.1. The bottoms (stream 8) containing the biodiesel product was transferred to the water washing stage.

8.2.4.5. Water washing

The bottoms product of the distillation column (stream 8) was subjected to an ester washing process to enhance the formation of two distinct and easily separable layers (methyl esters and glycerol layers).

The water wash stage in this model (represented as WASH) was based on batch counter-current extraction column with the process specifications as presented in Sheenan et al. (1998). This involves the use of water heated to 70°C (using HX-4) in the wash column where the separation of the stream contents was carried out.

The mass flow rate of the process water input (WATER) for the wash process was equal to 20% of the mass flow of the FAME component in the incoming stream 8-1 (Sheenan et al., 1998). This was based on current biodiesel industrial practices (Sheenan et al., 1998). It was further assumed that a 100% recovery of the FAME and unreacted oil content in stream 8-1 was obtained after the wash. The methyl ester product and glycerides (stream 13-1) were accompanied by 10% of the wash water entrained. The heat exchanger (HX-5) was used to heat the contents of the stream 13-1 to the temperature of 150°C, with which it can be separated in the FLASH unit.

Stream 13-1 was transferred to a flash unit where a further reduction of its water content was carried out to improve the fuel characteristics of the biodiesel product. The valve, V-1 was considered for use to reduce the stream pressure to 0.1 bar to ensure that the flash temperatures were kept lower than the decomposition temperature of the methyl esters.

Stream 10 contained 100% of the glycerol fraction in stream 8-1, 90% of the wash water and any left over methanol. This stream is transferred to the glycerol recovery step (GLY-REC). The valve V-1 was used to reduce the pressure of stream 10 to 0.7 bar to ensure that the reboiler temperatures were less than that of glycerol decomposition.

8.2.4.6. Methyl ester purification

For this study, the European biodiesel standard as specified in EN 14214 (Table 8.3) was used to specify further purification requirements of the biodiesel product. Based on the minimum standard specification, it was decided that the biodiesel product, where possible, should contain a FAME mass fraction of ≥ 96.5 % (w/w). This was used to provide an appropriate means of comparing the different processes.

Table 8.3. European biodiesel standards specifications for biodiesel product

Component	European Biodiesel Standard Specification (EN 14214)
Methyl esters (%, w/w)	≥96.50
Glycerol (%, w/w)	≤0.20
Methanol (%, w/w)	≤0.20
Triglycerides (%, w/w)	≤0.20

A simple vacuum flash distillation process (FLASH) was used to upgrade Stream 13 to yield a FAME mass fraction of 97.5% (w/w).

The biodiesel extraction and purification could alternatively be carried out using drying agents as described earlier, or via physical stripping methods i.e. using membranes as in Wang et al. (2009). This study however did not examine those alternative methods, instead considered only current industrial practices.

The condensed water vapour (WST-H2O) was treated as a process wastewater stream. The WST-H2O stream contained a mass flow of unreacted microalgae oil, water and FAME of 0.24, 10.37 and 0.68 kg/hr respectively. The heat exchangers (HX-6 and HX-7) were used to recover useful heat from the distillate and bottoms streams respectively.

8.2.4.7. Glycerol recovery

The extent of the glycerol by-product purification depends on the proposed use of the glycerol product obtained after this step.

It was decided in this study that the glycerol purification would be carried out to achieve a commercial grade purity i.e. ≥ 90% (w/w), for sale as an industrial raw material (Zhang et al., 2003).

A RADFRAC® vacuum distillation routine of the Aspen Plus® software was used for modelling the column. N and RR inputs of 6 and 0.2 respectively were used to obtain a final

glycerol product with a glycerol mass purity of 93% from the bottoms stream (Stream 12). It was considered that the reboiler unit was used to provide the distillation heating requirements. Vacuum distillation was used to ensure that the temperature of the bottom stream was kept below the decomposition temperature for glycerol (150°C) (Haas et al., 2007). The distillation was carried out with an absolute operating pressure of 70 kPa, which ensured that the temperature of the bottom stream from the column was 145.9°C. The distillate waste stream composed of a mass flow of methanol and water at 30.41 and 96.12 kg/h respectively was treated as a process waste stream. The heat exchangers (HX-8 and HX-9) were used to recover useful heat from the distillate and bottoms streams respectively.

8.2.5. The ultrasound assisted in-situ transesterification process

The ultrasound assisted in-situ transesterification process was modelled out with the same process outline used for the in-situ model as in Figure 8.1. The process inputs, conversion efficiencies and operational units were as presented in section 5.2.1 and 5.3.1.

An overview of stream components for modelling the in-situ transesterification process using low frequency ultrasound agitation is shown in Table 8.4.

8.2.5.1. Process inputs and transesterification reaction

A microalgae mass input of 1 t *Chlorella* biomass (MICROALG) was used for this process, with a reacting methanol mass flow of 1063.72 kg/h (stream 1). This provided the same reacting molar alcohol to oil ratio of 105: 1 used in the laboratory experiments, described in section 5.2.1. The reacting acid catalyst, H_2SO_4 (stream 2), was kept constant at 100% of the estimated mass flow of the oil content of the *Chlorella* oil.

Table 8.4. Table showing components of major streams in the up-scaled ultrasound assisted in-situ transesterification process

	8-1	10	13-1	ACID-CAT	BIO-DIESL	CAO	CASO4	FILT-RATE	GLY-CEROL	METH-ANOL	MICR-OALG	REC-MEOH	RESI-DUES	WA-TER
Mass Flow, kg/h														
MICROALG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1000.00	0.00	705.00	0.00
OIL	1.01	0.00	1.01	0.00	1.00	0.00	0.00	1.01	0.00	0.00	0.00	0.00	0.00	0.00
METHANOL	10.17	10.17	0.00	0.00	0.00	0.00	0.00	1017.29	0.00	56.64	0.00	1007.09	14.44	0.00
WATER	52.18	100.17	11.13	0.00	2.61	0.00	0.00	24.02	2.18	0.00	0.00	23.06	0.00	59.12
FAME	295.60	0.00	295.60	0.00	295.58	0.00	0.00	295.60	0.00	0.00	0.00	0.00	0.41	0.00
GLYCEROL	29.01	29.01	0.00	0.00	0.00	0.00	0.00	29.01	29.01	0.00	0.00	0.00	0.00	0.00
H2SO4	0.00	0.00	0.00	279.00	0.00	0.00	0.00	278.96	0.00	0.00	0.00	0.00	0.04	0.00
CAO	0.00	0.00	0.00	0.00	0.00	159.52	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CASO4	0.00	0.00	0.00	0.00	0.00	0.00	387.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FFA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Flow, kg/h	387.97	139.35	307.74	279.00	299.19	159.52	387.24	1645.89	31.19	56.64	1000.00	1030.15	719.89	59.12
Temperature, C	70.00	70.04	121.51	20.00	30.00	20.00	60.00	60.00	30.00	20.00	20.00	30.00	60.00	20.00
Pressure, bar	1.00	1.00	1.00	1.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

The transesterification reaction was carried out using two continuous reactors in series as in section 8.2.4.1. Stream 3, containing the reaction feedstocks was heated to a temperature of 60°C and transferred to the reactors where this temperature was maintained. Unlike the mechanically stirred in-situ process in section 8.2.4, the reactor agitation was performed using low frequency ultrasound (24 kHz) connected to the transesterification reactors.

After a transesterification time of 1 h, a 94% mass conversion of the microalgae lipids to FAME was assumed to be achieved (section 5.3.1). This corresponds to an overall conversion of 99.6% (w/w) after both reactors, with a total biodiesel yield of 0.295 kg/kg dried *Chlorella* biomass.

8.2.5.2. Downstream processes and biodiesel upgrading

The *Chlorella* residues filtration and acid catalyst removal, post transesterification, were carried out as described in sections 8.2.4.2 and 8.2.4.3 respectively. The recovery of the excess methanol quantities was carried out as in 8.2.4.4 to achieve a ≥ 99% mass recovery of the methanol component in stream 6 (w/w). This was performed using a distillation column RR and N of 0.1 and 12 respectively.

The washing and purification of the FAME and glycerol fractions were then modelled with specifications as in section 8.2.4.5-8.2.4.7 . The final FAME mass purity in the BIODIESL stream was 98.9% (w/w). The final GLYCEROL stream contained a mass purity of 93% glycerol (w/w).

8.2.6. Integration of co-solvents in the in-situ transesterification process

As shown in Chapter 5, two types of co-solvents (diethyl ether and pentane) were used to potentially reduce the in-situ process reacting methanol requirement. However for the analysis in this chapter, only diethyl ether was considered for modelling the co-solvents assisted in-situ transesterification process. Diethyl ether was selected because the FAME

yield (g/g *Chlorella*) with the use of this solvent was higher than that observed with pentane use (sections 5.3.2.1 and 5.3.2.2).

The modelling for the continuous co-solvent assisted in-situ transesterification process was carried out using the same process outline as the mechanically stirred in-situ transesterification method. A pre-mixing unit for the diethyl ether inputs and the reacting methanol was however incorporated as shown in Figure 8.3.

The major components of the process streams when diethyl ether was used as the process co-solvent are shown in Table 8.5. The estimation of the diethyl ether component in the stream DIETET was based on the molar concentration of the reacting methanol. A reacting molar ratio of methanol to oil of 79:1 was selected (section 5.3.2.2). This was due to the fact that the highest percentage mass oil to FAME conversion was achieved at this alcohol level. The molar flow of the diethyl ether input (kmol/h) into the process was 0.13 times the molar flow of the reacting methanol (kmol/h). With a methanol mass flow of 781.31 kg/h this relates to a diethyl ether mass input of 232.11 kg/h into the mixer (MIXSOL).

The microalgae biomass inputs and acid catalysts were the same as the in-situ transesterification process in section 8.2.4.

The continuous transesterification process was assumed to be operated at a temperature of 60°C, with continuous stirring provided by the use of a mechanical stirring system. The transesterification reactors were operated at an elevated pressure of 300 kPa (absolute) to ensure that the reactor contents were in a liquid phase, because the temperature was higher than the normal boiling point of the diethyl ether co-solvent. After 4 h, a 70% mass conversion of the *Chlorella* lipids to FAME (w/w) was obtained (section 5.3.2.2), and a total percentage mass FAME conversion of 91% (w/w) was obtained after both reactors.

The resulting stream (4-1) was subjected to the biomass residues filtration and acid catalyst removal steps as in 8.2.4. The methanol and diethyl ether components in stream 6 were recovered using a distillation process as described in section 8.2.4.4. The specifications of the distillation column were for the recovery of 100% mass flow of the diethyl ether and ≥99% mass of the methanol component in the incoming stream 6.

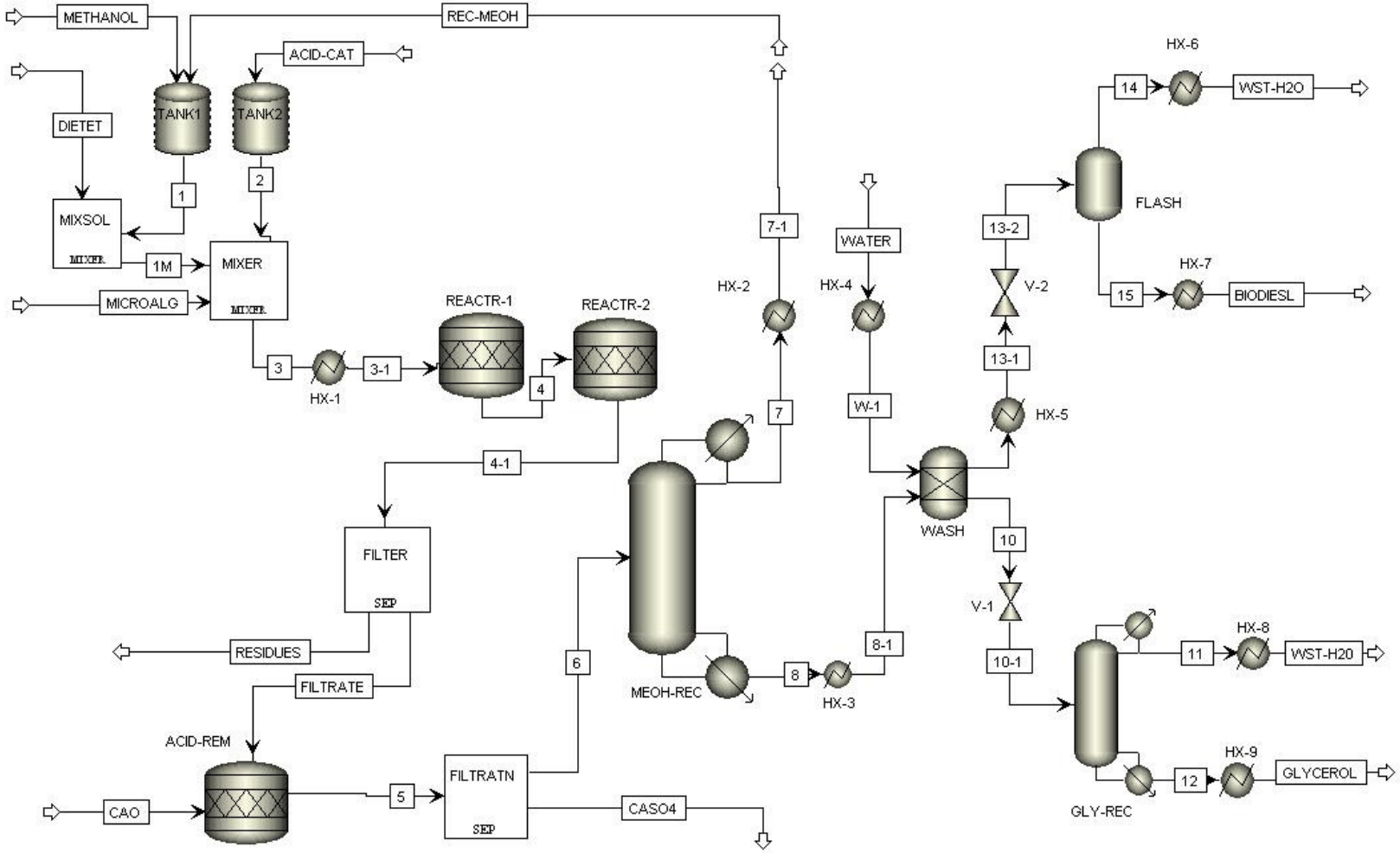

Figure 8.3. Flow sheet showing the process units and streams for the in-situ transesterification model for biodiesel production from microalgae with diethyl ether as a co-solvent.

Table 8.5. Table showing the major streams and their components for the in-situ transesterification process with diethyl ether used as a reaction co-solvent.

	8	10	13-1	ACID -CAT	BIO-DIESL	CAO	CASO4	DIE-TET	GLYC-EROL	METH-ANOL	MICR-OALG	REC-MEOH	RESI-DUES	WA-TER
Mass Flow kg/h														
MICROALG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1000.00	0.00	712.00	0.00
OIL	24.60	0.00	24.60	0.00	23.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
METHANOL	7.42	7.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.59	0.00	734.73	10.54	0.00
WATER	50.51	93.14	10.35	0.00	0.50	0.00	0.00	0.00	1.95	0.00	0.00	24.50	0.00	52.98
FAME	264.90	0.00	264.90	0.00	264.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.00
GLYCEROL	25.86	25.86	0.00	0.00	0.00	0.00	0.00	0.00	25.86	0.00	0.00	0.00	0.00	0.00
H2SO4	0.00	0.00	0.00	270.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
CAO	0.00	0.00	0.00	0.00	0.00	154.37	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CASO4	0.00	0.00	0.00	0.00	0.00	0.00	374.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FFA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DIETHYLE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	232.11	0.00	0.00	0.00	232.00	0.00	0.00
Total Flow kg/h	373.29	126.43	299.85	270.00	288.53	154.37	374.75	232.11	27.81	46.59	288.00	991.23	722.94	52.98
Temperature C	100.74	70.01	150.00	20.00	30.00	20.00	60.00	20.00	30.00	20.00	20.00	30.00	60.00	20.00
Pressure, bar	1.00	1.00	1.00	1.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00

Although not carried out in the experimental runs (Chapter 5), the recovery of the diethyl ether co-solvent was deemed important to facilitate its re-use in the transesterification process and a reduction in the process economic costs. The spot price of diethyl ether was reported to be 703 US$/ metric t (ICIS, 2010).

The biodiesel and glycerol were recovered using schemes similar to those described in sections 8.2.4.5-8.2.4.7. The final FAME purity in the BIODIESL stream was 91.6% (FAME mass fraction in the product), and the purity of the GLYCEROL stream was 93% glycerol (mass fraction of glycerol in the product stream).

8.2.7. The use of ultrasound and co-solvent for the in-situ transesterification process

This modified in-situ transesterification process was carried out using low frequency ultrasound for the reaction agitation with diethyl ether as a process co-solvent. The molar ratio of reacting methanol to oil was 26:1 as described in section 5.2.3.

The process model outline for the conversion of the microalgae biomass to biodiesel was similar to Figure 8.4, but with differences in the process inputs. Details of the major streams of the modelled in-situ transesterification process involving diethyl ether and ultrasonication are given in Table 8.6.

The continuous transesterification reactors were assumed to be the same as in section 8.2.4.1, with the process agitation provided by low frequency ultrasound (24 kHz). The reaction temperature was maintained at 60°C. With the use of diethyl ether, the percentage mass oil to FAME conversion was 80% (w/w) (section 5.3.3) after a reaction time of 1 h, corresponding to a total biodiesel yield of 0.295 kg/kg dry *Chlorella* biomass.

The methanol and solvents recovery, biomass residues filtration and biodiesel and glycerol upgrading were then carried out using processes similar to those in sections 8.2.4.2-8.2.4.7.

Table 8.6. Table showing the major streams and their components for the in-situ transesterification process with diethyl ether used as a reaction co-solvent and reactor agitation provided by low frequency ultrasound

	8	10	ACID-CAT	BIOD-IESL	CAO	CASO4	DIETET	FILT-RATE	GLYC-EROL	METH-ANOL	MICRO-ALG	REC-MEOH	RESI-DUES
Mass Flow, kg/h													
MICROALG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1000.00	0.00	705.00
OIL	11.20	0.00	0.00	10.84	0.00	0.00	0.00	11.20	0.00	0.00	0.00	0.00	0.00
METHANOL	2.29	2.29	0.00	0.00	0.00	0.00	0.00	229.26	0.00	36.54	0.00	226.86	3.26
WATER	52.05	94.53	0.00	0.54	0.00	0.00	0.00	31.22	2.10	0.00	0.00	30.27	0.00
FAME	285.38	0.00	0.00	284.73	0.00	0.00	0.00	285.38	0.00	0.00	0.00	0.00	0.39
GLYCEROL	27.95	27.95	0.00	0.00	0.00	0.00	0.00	27.95	27.95	0.00	0.00	0.00	0.00
H2SO4	0.00	0.00	279.00	0.00	0.00	0.00	0.00	278.96	0.00	0.00	0.00	0.00	0.04
CAO	0.00	0.00	0.00	0.00	159.52	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CASO4	0.00	0.00	0.00	0.00	0.00	387.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FFA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DIETHYLE	0.00	0.00	0.00	0.00	0.00	0.00	0.01	704.25	0.00	0.00	0.00	704.24	0.00
Total Flow, kg/h	378.87	124.77	279.00	296.11	159.52	387.24	0.01	1568.23	30.05	36.54	1000.00	961.36	708.69
Temperature, C	107.23	69.91	20.00	30.00	20.00	60.00	20.00	60.00	30.00	20.00	20.00	30.00	60.00
Pressure, bar	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	3.00

8.2.8. The conventional transesterification process

The process flowchart for the conventional transesterification route and the corresponding major stream flow data are shown in Figure 8.4 and Table 8.7 respectively.

8.2.8.1. Oil extraction & solvent recovery

The industrial scaled conventional transesterification process in this study covered the extraction of lipids from the microalgae biomass, and the transesterification process.

As reported in section 3.2.5, the use of butanol and a chloroform-methanol mixture were both examined for the extraction of the microalgae lipids. The results obtained showed similar oil contents for *Chlorella* biomass following extraction using both solvents.

Only butanol was used as the solvent in this study modelling. The reason is that where energy recovery (as CH_4) from the residues post transesterification is proposed, butanol use is preferred (section 6.3.3), because the chloroform remaining in the *Chlorella* residues appeared to inhibit the CH_4 generation process.

The choice of butanol as the process extraction solvent was also based on the fact that butanol could potentially be a renewable feedstock i.e. via acetone-butanol fermentation (Jones &Woods, 1986). Its use could further improve the overall 'greenness' of the biodiesel product by reducing fossil fuel derived process feedstocks. This is examined further in the next chapter (Chapter 9).

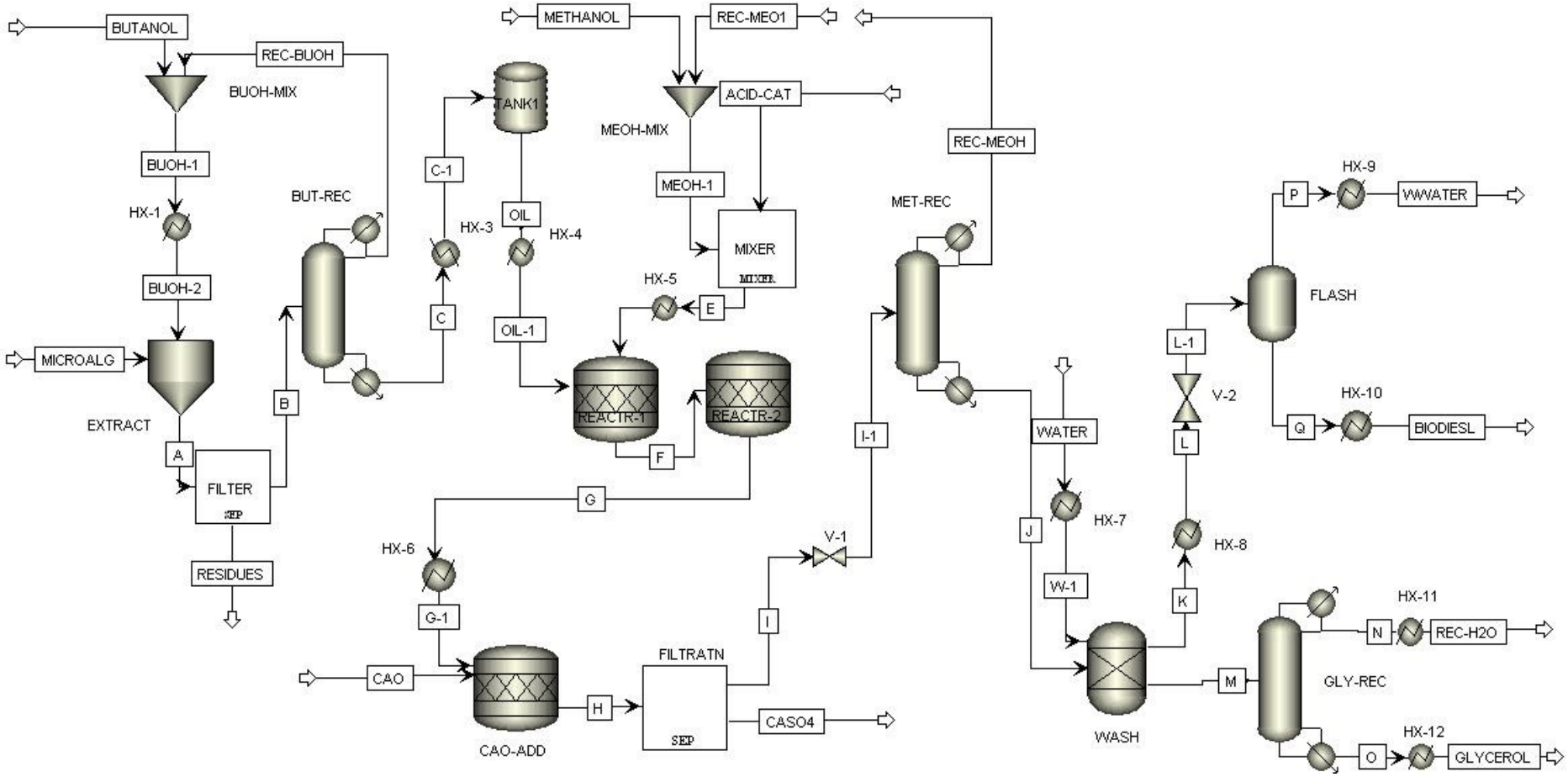

Figure 8.4. Flow sheet showing the process units and streams for the up-scaled conventional extraction and transesterification of microalgae oil.

Table 8.7. Table showing the major streams and their components for the acid catalysed conventional extraction and transesterification process with microalgae as the reaction feedstock

	ACID -CAT	B	BIO- DIESL	BUT- ANOL	G	GLYC- EROL	I	K	M	METH- ANOL	MICR- OALG	OIL	REC- MEOH	RESI- DUES
Mass Flow kg/h														
MICROALG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1000.00	0.00	0.00	725.00
OIL	0.00	237.00	8.17	0.00	8.47	0.00	8.47	8.47	0.00	0.00	0.00	234.62	0.00	26.33
METHANOL	0.00	0.00	0.00	0.00	398.51	0.00	398.51	0.00	3.99	30.02	0.00	0.00	394.52	0.00
WATER	0.00	0.00	0.45	0.00	18.35	1.77	66.11	9.66	86.96	0.00	0.00	0.00	17.58	0.00
FAME	0.00	0.00	239.85	0.00	240.45	0.00	240.45	240.45	0.00	0.00	0.00	0.00	0.00	0.00
GLYCEROL	0.00	0.00	0.00	0.00	23.52	23.52	23.52	0.00	23.52	0.00	0.00	0.00	0.00	0.00
H2SO4	260.00	0.00	0.00	0.00	260.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BUTANOL	0.00	2000.00	0.00	2.00	2.00	0.00	2.00	0.00	1.71	0.00	0.00	2.00	0.29	0.00
CAO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CASO4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FFA	0.00	12.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.65	0.00	1.41
Total Flow, kg/h	260.00	2249.65	248.48	2.00	951.30	25.29	739.06	258.58	116.18	30.02	1000.00	249.26	412.39	752.74
Temp, °C	20.00	84.44	30.00	20.00	60.00	30.00	30.00	81.38	81.38	20.00	20.00	30.00	48.70	84.44
Pressure, bar	1.00	1.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00

Dry microalgae biomass (1000 kg/h) was transferred to an extraction reactor (EXTRACT) with 2000 kg/h of preheated butanol (90°C) in stream BUOH-2, with continuous stirring throughout the extraction period. The heat exchanger HX-1 was used to heat the extraction solvent to the required extraction temperature of 90°C.

The extraction unit was based on the results of the laboratory lipid extraction experiments, which showed that 0.275 kg of microalgae lipids were stripped per kg of the dried *Chlorella* biomass using butanol. The resulting solid-liquid mixture (stream A) was subjected to a filtration process (FILTER), as in section 8.2.4.2, and the extracted microalgae residues collected. The filtrate (stream B) was sent to a distillation column to recover the butanol fraction.

The distillation column (BUT-REC) was used for the recovery of the 1-butanol solvent. Vacuum distillation was performed using the RADFRAC® model described earlier, with the RR and N at 0.2 and 6 respectively. A distillation column pressure of 10 kPa (absolute) was used to ensure that the bottom products were kept below 250°C, to prevent the decomposition of the triglycerides contents in the stream (Schawbo et al., 1988). Because butanol is a relatively expensive solvent[18], it was decided ≥99.9% of the mass butanol component of the incoming stream B (w/w) would be recycled. This was carried out to minimise process wastes. The recycled butanol (99.9 % mass purity, w/w) available in stream REC-BUOH was re-used in the extraction process.

The bottoms stream (C) containing the oil feedstocks were sent to a tank (TANK 1) for its use in the transesterification process. The heat exchanger HX-3 was used to recover some of the useful heat in stream C before the microalgae oil tank. The microalgae oil was then considered to be heated to the transesterification temperature of 60°C using HX-4. The use of only one heat exchanger between the bottoms of the distillation column (stream C) and the first of the continuous transesterification reactors (REACTR-1) could have been adequate for the recovery of the useful energy. However due to some convergence problems, the stream was split up as shown in Figure 8.4 as a simple means of solving this

[18] Compared to the use of methanol and chloroform. The 2010 first quarter spot prices of methanol, chloroform and butanol were 200, 500 and 1813 US$/metric-t respectively (ICIS, 2010).

issue. The stream data in the final stream entering the transesterification reactors were however not affected by the splitting of the stream.

8.2.8.2. Transesterification reaction

A 1:1 mass flow of H_2SO_4 to reacting oil was used as the reaction acidic catalyst. Prior to the transesterification reaction, fresh and recycled methanol were mixed in MEOH-MIX and mixed with H_2SO_4 (ACID-CAT) using MEOH-MIX. The resulting stream E was then heated to 60°C using HX-5. The microalgae oil feedstock (OIL) was also heated to 60°C (using HX-4) before it was transferred to the continuous transesterification reactors.

The continuous acid catalysed process used two transesterification reactors in series (REACTR-1 and 2) as described in section 8.2.4.1. The reaction however involved the use of a molar reacting methanol to oil ratio of 50:1. This was used since it was demonstrated in section 4.3.6 to result in an 81% mass conversion of *Chlorella* oil to methyl esters in 4 h at the reaction temperature at 60°C. This corresponds to a 96.4% mass conversion of *Chlorella* oil to FAME (w/w) after both reactors.

8.2.8.3. Methanol recovery

After the transesterification process, the heat in stream G was recovered using HX-6, and unreacted acidic catalyst component in the mixed product stream (G) was removed using CAO-ADD as described in section 8.2.4.3.

The methanol fraction in the filtrate stream (I) was subjected to a distillation process (using MET-REC) to recover the excess process methanol. Vacuum distillation with a RR and N of 0.1 and 8 respectively was used. The valve V-1 was used to reduce the pressures in stream I to the requirement of the distillation column. This involved fixing the column pressure at 50 kPa (absolute) to keep the temperature of the distillation column bottoms product below the decomposition temperature of glycerol (150°C).

8.2.8.4. Biodiesel purification

The separation of the biodiesel and glycerol fractions of the filtrate streams after the methanol recovery process was carried out using wash columns specifications similar to those in section 8.2.4.5. The methyl ester rich stream (L) containing 93.0% (w/w) FAME, was subjected to a flash vacuum process. This was carried out to reduce the water content and bring the fuel characteristics of the biodiesel product up to the selected biodiesel standards. The resulting methyl ester stream (BIODIESL) contained 96.5% FAME (w/w).

8.2.8.5. Glycerol purification

The glycerol upgrading process was performed using the ASPEN RADFRAC® routine with the conditions as described in section 8.2.4.7. This involved the use of a process RR and N of 1 and 6 respectively, and a column pressure of 70 kPa (absolute). A glycerol mass purity of 93.0% (w/w) in the stream GLYCEROL was obtained.

8.2.9. Scaling-up of the anaerobic digestion process for the microalgae residues

The methane (CH_4) production process was based on a simplified scaled up fermentation model using post transesterified *Chlorella* residues as feedstock.

The assumed daily loading rate into the anaerobic digesters was based on the mass flow of the microalgae residues (in the RESIDUES stream) of the transesterification processes described earlier. For example, for the mechanically stirred in-situ process, 712 kg /h (Table 8.1) of the microalgae residues is available for digestion following the conversion of 1 t *Chlorella* biomass to biodiesel. The modelling of the anaerobic digestion process was assumed to consist of a simple one stage semi-continuous digester. Here, the residues were added with the digestate removed periodically as described in Chapter 7. The reactor was assumed to be continuously stirred using a mechanical stirrer fitting the digester volume.

Figure 8.5 shows the process units for the anaerobic digestion of the microalgae residues.

The WATER stream was based on the water quantities required to dilute the biomass residues to the specified loading concentrations for the digestion process. The water and residues were mixed in the storage tank pending transfer to the anaerobic digester. It was further considered that the anaerobic digestion was a continuous process with most of the water in the digestate reused in the anaerobic reactor.

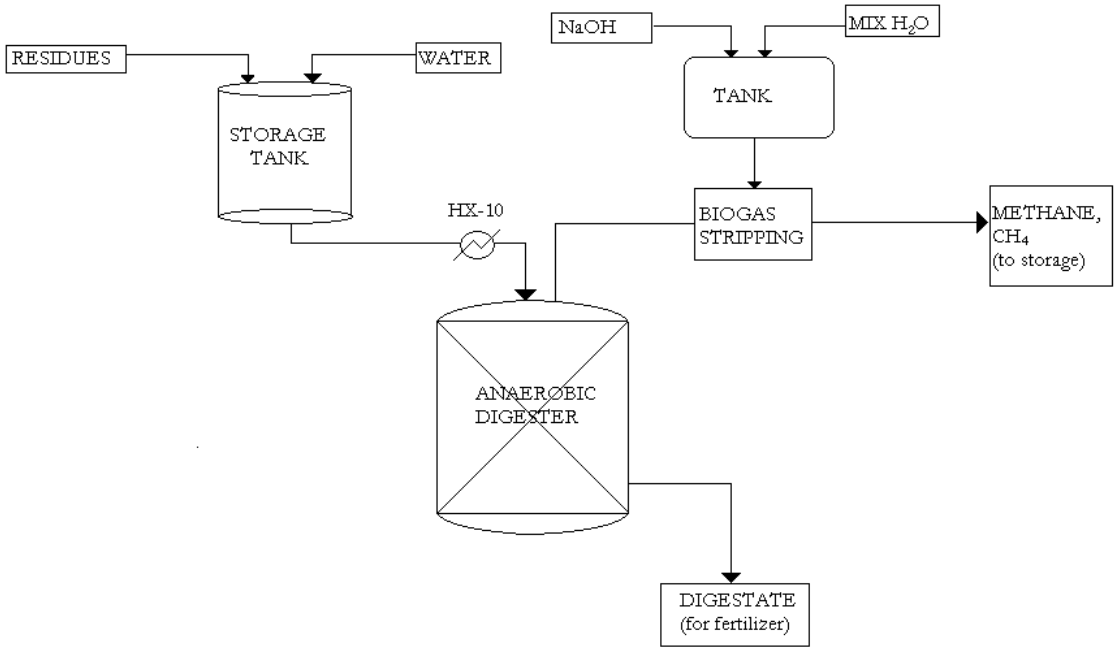

Figure 8.5. Overview of the modelled anaerobic digestion process.

The anaerobic digestion process was conducted using a mesophilic process temperature of 35°C, with a hydraulic retention time of 15 d as described in Chapter 7. The digester feedstock was heated to 35°C (using HX-10) and transferred to the reactors.

The size (volume) of the anaerobic digester used was dependent on the amount of organic matter fed daily and the retention time. Only the substrate concentrations of 5-20 kg

VS/m^3 were used in this chapter. Digester inhibition was shown using organic loading concentrations greater than 20 kg VS/m^3 as demonstrated earlier in section 7.3.5.The digester volumes required for the anaerobic digestion of the hourly produced biomass residues (705-725 kg dry residues/ h) are shown in Figure 8.6. The digester sizes were based using a fixed hydraulic retention time of 15 d. The digester volumes for the considered loading concentrations were calculated using Eq. 8.4.

$$\text{Digester volume}\,(m^3) = \left(\frac{\text{Mass of microalgae residues/h} \times \text{biomass VS}}{\text{Loading concentration(kg VS/}m^3\text{/ h)}}\right) \times \text{Retention time(days)} \quad (8.4)$$

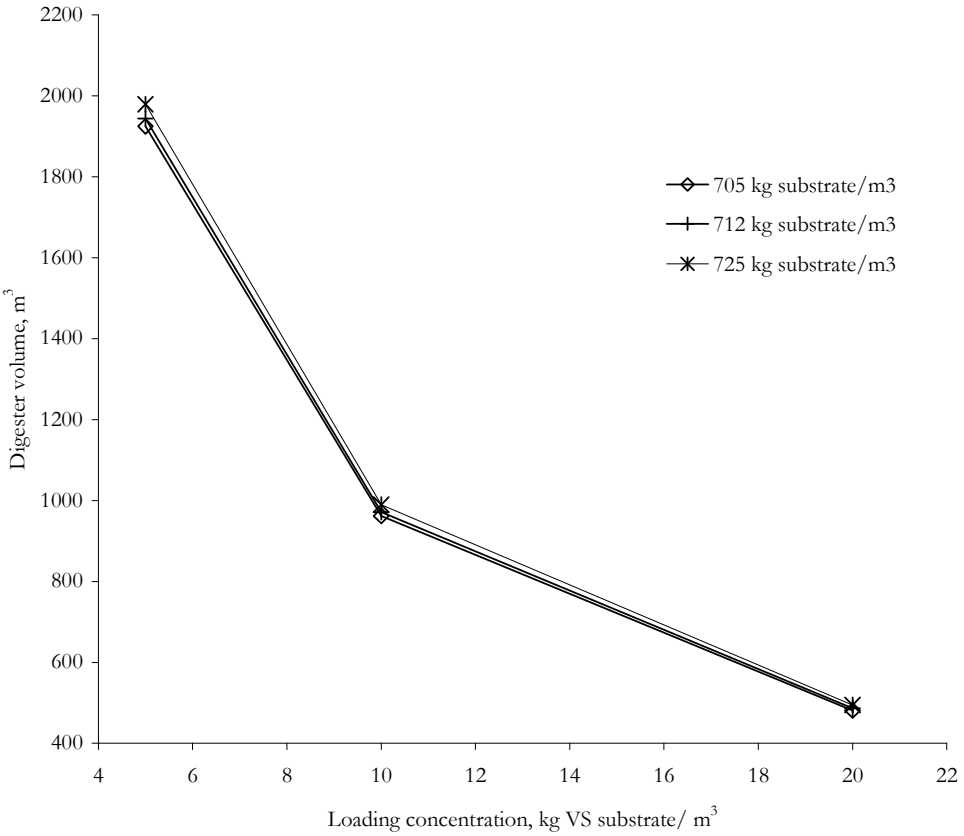

Figure 8.6. Digester volume (m^3) required for anaerobically digesting for 15 d on the basis of the loading concentration (kg VS microalgae residues/m^3).

The lowest digester size was obtained using of a loading concentration of 20 kg VS residues/m^3. Only this loading concentration level was further used in the anaerobic model assessment in this chapter.

The produced biogas was considered to be withdrawn from the digesters by pressure difference. The biogas was upgraded via scrubbing using 5% NaOH solution to obtain > 99% CH_4 (v/v) (Chapter 6). The regeneration of the NaOH solution or the treatment of the produced salts was however not further treated in this study. However, in practical applications, the CO_2 co-produced with the CH_4 during the anaerobic digestion process could be recovered from the NaOH scrubbing unit and used as a carbon source for the microalgae cultivation step. This could improve the biomass productivities as well as reduce the overall costs of the biomass growth stage (i.e. Chisti, 2007). An estimation of the quantity of CH_4 produced was obtained from the empirical CH_4 yields as demonstrated earlier in section 7.3.1. The CH_4 was stored using simple storage facilities i.e. using gas bags, balloons or tanks which do not require a significant energy input (GSES, 2005).

8.2.9.1. Energy demand of the anaerobic digestion process

The energy requirement for the anaerobic digestion process was considered to mainly arise from the digester heating. The process stirring also contributes to the process energy demand, although to a lesser extent (GSES, 2005). The electrical power required for the operation of supplementary processes i.e. for the feedstock addition and digestate removal, was not considered in this study since it was considered in GSES (2005) to be minimal.

Apart from the digestion feedstock heating, process heating was also carried out to compensate for any heat losses in the digester during the fermentation process. Due to heat loss from the digester, only ≈ 77% of the heat supplied was considered to be accessible for the reactor heating (GSES, 2005). This loss was included in the calculation for the quantity of heat to be delivered for the digestion process.

The heat requirement for the anaerobic digester was calculated by (Eq. 8.5)

$$\text{Heat demand}\,(\text{MJ/h}) = \text{Sample loading rate size (t/h)} \times c_{sample}\,(\text{KJ/kg/K}) \times \Delta T(\text{K}) \times 130\% \quad (8.5)$$

where, c_{sample} is the specific heat capacity of the digesting mixture and the factor 130% used to estimate the excess heat inputs due to potential heat losses from the digester.

ΔT (K) is the difference of the temperature of the digester from that of the fresh *Chlorella* residue, as seen in Eq. 8.6.

$$\Delta T\,(\text{K}) = T_{digester} - T_{fresh\ substrate} \quad (8.6)$$

As a rule of thumb, the specific heat capacity of water (4.18 KJ/kg/K at 20°C) was used as the specific heat of the digesting mixture (*Chlorella* residue and inoculum), as considered in GSES (2005).

Using Eq. 8.3, the heating requirement for the anaerobic digestion of the residues from the transesterification processes, using a loading rate of 20 kg VS/m^3 is given in Table 8.8.

Table 8.8. Table showing heat requirement for the anaerobic reactors

	705 kg residues/h	712 kg residues/ h	725 kg residues/ h
Heating demand, MJ/h	2614.6	2640.6	2688.8

Three methods could be applied to achieve the mixing of the digester contents: (a) sludge recirculation via pumping, (b) recirculation of the produced gas through the sludge, and (c) mechanical stirring of the reactor contents (Noone, 1990). A slow speed mechanical stirrer was used for the anaerobic digester model in this chapter due to its predominant use in practice (Noone, 1990). The size and type of the mixer was considered to be largely dependent on the dry matter content of the digester and its size (GSES, 2005).

A simplified estimation of the electrical power requirement for the fermentation process agitation was based on the use of an industrial slow speed mixer. With a dry matter content of ≈ 2% (w/w digester content) for the digester, the Flygt 4410 mixer (Flygt

Industries, UK)[19] with a rated power of 2.3 kW is suitable. This mixer was selected due to its suitability for the agitation of the proposed reactor volume, its widespread use in wastewater treatment applications[20].

8.3. Model Results and Analysis

This section presents the results of material and heating demands for the different conversion routes obtained from the process models. The external heat demand after process integration was used as a simple indicator of the process energy requirement. The process electrical power requirement for the operation of the pumps and valves in the process models were however not considered. This was due to the fact that it was considered that similar electrical requirements would be expected for the different process models. The electricity requirements for the process agitation are expected to differ, and its influence on the process energetics would be taken into account in the next chapter (Chapter 9). This section deals mainly with the net heat energy requirements of the different transesterification processes. The energy requirement of the different transesterification processes was thus carried out on the basis of their annual minimum utility heating demand. The economics (i.e. process and operational costs) of the transesterification processes was not covered in this study, as mentioned in Chapter 2.

The models presented in this chapter are relatively basic with regard to the level of the details of the individual process units. The simulation was primarily aimed at estimating the energetic and raw material demand of the conversion routes. The models are not intended to replace detailed engineering analysis which would be required in the design and construction of biodiesel production plants. The in-situ transesterification routes were seen to facilitate the microalgae-biodiesel conversion with the fewer processing steps compared with the

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[19] Mixer details obtained from Flygt ITT Industries. Slow Speed Mixers. Retrieved from http://www.flygt.co.uk/724490.pdf. Accessed on 14 May 2010.

[20] Personal communication with Dr. Sean Connaughton, Waste Solutions, Dunedin, NZ. 23 July 2009.

conventional method, because the oil extraction step and the accompanying solvent recovery unit were eliminated. This agrees with the discussions by Haas et al. (2007).An increase in the mass flow of the biodiesel product stream can also be observed for the different in-situ transesterification processes over that of the conventional process. The reasons behind the increases of the biodiesel yield have been previously reported in Chapters 4 and 5.

Regarding CH_4 production from the post transesterified residues, the estimated CH_4 yields (m^3/h) obtained from the digestion of the produced residues (kg/h) are given in Table 8.9. The CH_4 yields were calculated using Eq. 7.4 with a substrate retention period of 15 days and loading rate of 20 kg VS/m^3 digester. Due to the improved stripping of the microalgae lipids with the use of ultrasound for the in-situ process, the methane yield from the residues using this process was seen to be ≈1% less than for the mechanically stirred samples.

Although that equation represents the anaerobic digestion of the residues obtained following the acid catalysed in-situ transesterification process, it was also extended for use for the modified in-situ and conventional transesterification residues.

Table 8.9. Methane yields (m^3/h) from the residues after the application of various transesterification methods using 1 t of dried microalgae biomass

Process	CH_4 yield (m^3/h)
In-situ transesterification (mechanical stirring)	145.78
In-situ transesterification with ultrasound	144.35
In-situ transesterification with Diethyl ether (mechanical stirring)	145.78
In-situ transesterification with Diethyl ether and ultrasound	144.35
Conventional transesterification (mechanical stirring)	148.44

The conventional process post extracted residues were predicted to have the highest methane yields as seen in Table 8.9 due to the fact that the biomass contained a higher amount of lipids that the that of the in-situ process as previously described in section 4.3.6.

8.3.1.1. Process integration and energy comparison

Before the heating demands of the different transesterification routes are estimated, the extent to which the process heating and cooling could be met internally was explored. This involved recovering heat energy from process streams which can be taken as heat sources or supplying heat energy to process streams which can be taken as heat sinks. External utilities (i.e. pressurised steam and cooling water) were then considered to meet the shortfall of the available heat sources and sinks to meet the process heat and cooling demand. The estimated minimum external utility demand was then used as a preliminary indicator of the energy requirements of the various transesterification processes. The stream mass flow rates and enthalpy changes of the process streams obtained using the ASPEN models were used to estimate the maximum recoverable process heat.

The heat integration method, pinch analysis as demonstrated by Linnhoff & Flowers (1978), Linnhoff & Vredeveld (1984) and Kemp (2007) was applied to achieve this goal. This technique is applied for minimising energy consumption in industrial processes. The maximum possible process heat recoverable was calculated using pinch analysis which involves the calculation of the thermodynamically accessible heat energy which could be consumed by the modelled process. The process stream data i.e. heat sinks and sink streams and mass flow rates, the composite curves used in the determination of the minimum energy consumption targets of the respective processes, and the theoretical heat energy savings are described in detail in Appendix F [21].

Results for the minimum hot and cold utilities required (i.e. before and after the heat integration) for the various up-scaled in-situ and conventional processes are shown in Figure 8.7.

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[21] Details of the heat streams, hot and cold composite curves, as well as the grand composite curves used for the assessment for the different transesterification processes are presented in Appendix F (CD-ROM).

An additional 548.3 and 547 kW of hot and cold utilities respectively were found to be required by the mechanically stirred in-situ process compared to the similarly sized conventional biodiesel production unit. This was based on the assumption that the same biomass inputs (1 t microalgae/ h) were used for the transesterification processes. The increased heat demand for the in-situ process (93% compared to the conventional method) was due to the energy required to recover (via distillation) the higher methanol volumes used in the in-situ transesterification method.

A reduction in the utility heating requirement was obtained by the modifications applied to the mechanical stirred process. An 88.7% reduction of the process heating demand was obtained by the use of co-solvents and ultrasound, compared with the mechanically agitated in-situ process.

The process modifications appear to produce considerable reductions of the process heating demands due to the comparably reduced methanol quantities used in these processes. However, due to the differences in the mode of reactor agitation, the energy requirement for the process mixing must also be assessed to facilitate a proper comparison of the energy requirement for the different processes. This will be examined in the next chapter (Chapter 9).

It was observed that savings of the hot utility requirements of 8-30% were achievable for the different in-situ and conventional transesterification processes. Reductions of 8-17% for the total cold utility requirement were also achieved for the various in-situ and conventional transesterification processes after the application of pinch analysis.

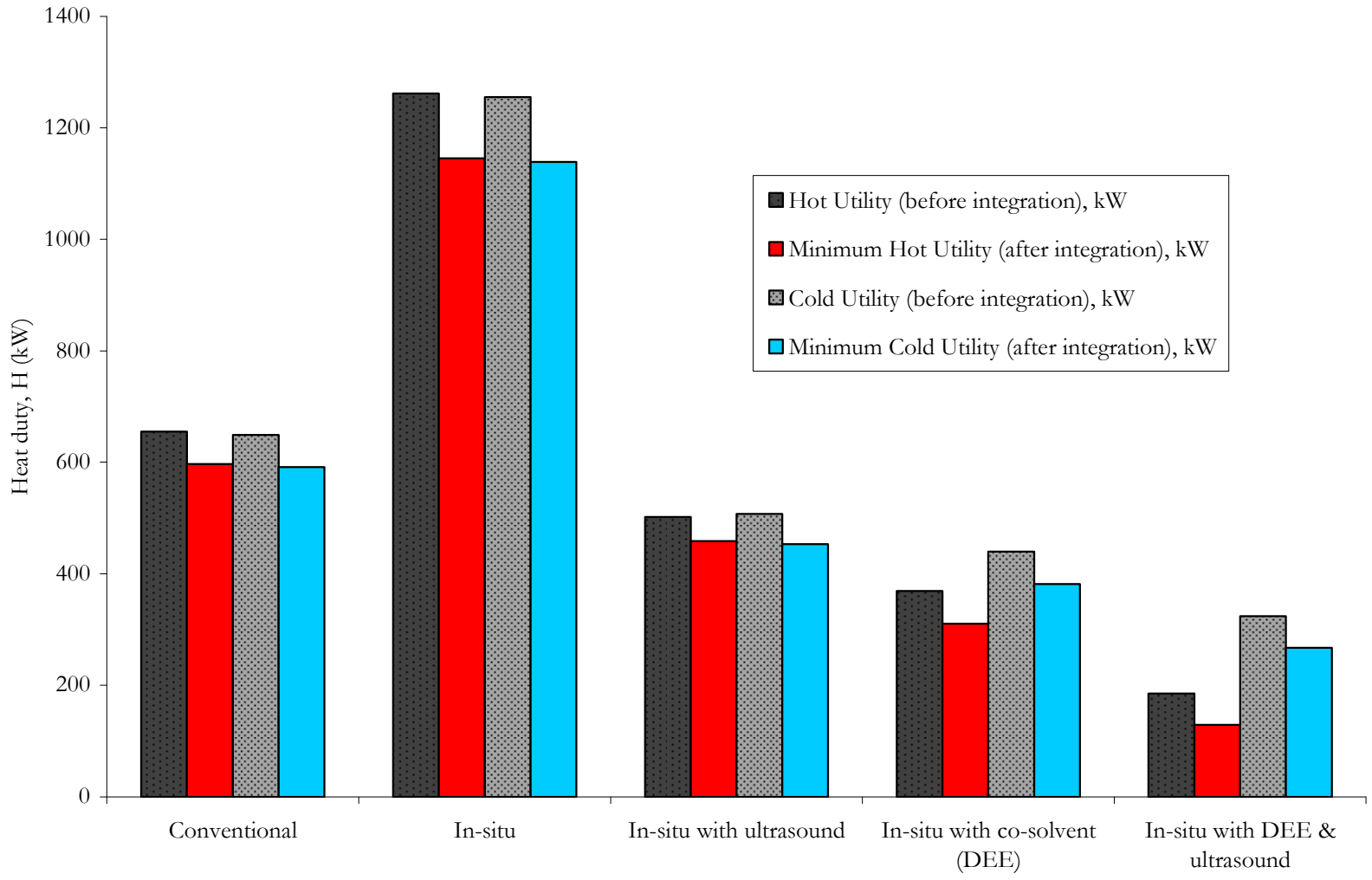

Figure 8.7. Heating and cooling utility requirements before and after heat integration using pinch analysis.

8.3.1.2. Integration of the anaerobic digestion process

It was considered that the anaerobic digestion process would be integrated with the transesterification processes to use the available surplus hot streams to preheat the digestion feedstock. Table 8.10 shows the minimum utility requirement for the transesterification processes when the anaerobic digestion process integrated.

Table 8.10. Minimum hot and cold utility requirement after integrating the transesterification and anaerobic digestion processes

Transesterification+anaerobic digestion process	Minimum Hot Utility, kW	Minimum Cold Utility, kW
In-situ process (mechanically stirred)	1145.3	412.32
In-situ with ultrasound	732.18	-
In-situ with diethyl ether (mechanically stirred)	662.4	-
In-situ with diethyl ether (ultrasound agitation)	587.68	-
Conventional process (mechanical stirring)	752.39	-

The combined processes (transesterification+anaerobic digestion) minimum heat requirements was then compared with the heating requirements of the anaerobic digestion process alone without heat integration as presented in Table 8.8. The results show that the heat requirements after process integration was only 43.37, 28.00, 25.09, 22.48 and 27.98% the heat inputs into the digestion process alone for the in-situ (mechanically stirred), in-situ (ultrasonicated), in-situ (with diethyl ether and mechanical stirring), in-situ (ultrasonicated with ether) and conventional processes respectively. The integration of the transesterification and the anaerobic digestion processes could therefore lead to savings in the process heating requirements.

The process heating and raw material requirement estimated for the modelled industrial transesterification processes is further used for the renewability assessment reported in the next chapter (Chapter 9).

8.4. Chapter Conclusions

The transesterification processes were modelled using commercial chemical engineering modelling software, ASPEN Plus®. This involved the use of a fixed microalgae input of 1 t-dry/h and reaction conditions similar to the laboratory experiments. The process models were used to estimate the energy and raw material requirement of the different transesterification processes. The heat demands for the transesterification processes obtained in this study are intended to be used only as a first indicator of the process energy requirement. The cooling demands for the respective processes in this chapter were however not further explored since the process cooling requirement could be met using process water. This was since a minimum target temperature of 30°C was considered in this study for the streams requiring cooling. However, in locations were this minimum cooling target would be too high, cooling towers could be incoporated to reduce the heat streams to the required temperatures. The mechanically stirred in-situ transesterification process was seen to have the highest heating requirement of the processes studied. The low frequency ultrasound agitated process using diethyl ether as a co-solvent required the least external heating inputs. The use of membrane technology instead of distillation methods for the recovery of the reaction alcohol, co-solvents and for the purification of the biodiesel yields could potentially reduce the energy requirements of the transesterification processes. This application of this method was however not covered in this study.

The sizing of the modelled transesterification processes in this study was based on an assumption of a practical scaled plant using 1 t dry biomass as the feedstock i.e. producing ≈2400 metric t FAME annually. This production capacity was less than current medium scaled industrial biodiesel plants of 8000 metric t biodiesel as described in the literature i.e. Haas et al (2006). An increase of the capacity of the microalgae transesterification process could provide possibly economic and energetic advantages, with reductions in the average

process heating and cooling requirements, as well as costs per unit of biodiesel produced obtainable with increasing plant capacities (Kasteren & Nisworo, 2007). A study by Kasteren & Nisworo (2007) showed that the recommended selling price for biodiesel via the supercritical transesterification using waste oils reduced over threefold from 641 US$/ metric t to 202 US$/ metric t when the plant biodiesel production capacity was increased from 8000 to 125000 metric t/ year. Process improvements due to economies of scale could therefore be attained by expanding the investigated microalgae biodiesel processes from the considered capacity. This was however not further explored in this study.

Simplified modelling of the anaerobic digestion process was carried out using empirical results obtained earlier. The main energy requirement i.e. for the reactor heating and stirring were estimated. The modelling results showed that the overall heating utility required was a minimum when the transesterification process was integrated with the anaerobic digestion route. It must be noted that the heat demand put forward for the anaerobic digestion process was based on current digester estimation practices (GSES, 2005). With the use of a well insulated anaerobic digester, it is expected that the heating demands for the methane production process would be lower than the estimates used in this chapter.

Although a validation of the modelled transesterification and anaerobic digestion of the microalgae biomass and residues would be useful, this could not be conducted in this study since there are currently no experimental large scale data for these processes using this feedstock.

One of the principal aims of this thesis was to determine whether or not the use of the in-situ transesterification and energy recovery (from microalgae residues) processes provide any potential energetic benefits over the conventional method. This aim will be explored in Chapter 9. The raw material and energy inputs and biodiesel and CH_4 products obtained for the process models are used for the renewability assessment of the different methods presented in Chapter 9.

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9. RENEWABILITY ASSESSMENT OF THE DIFFERENT TRANSESTERIFICATION PROCESSES IN THIS STUDY

9.1. Introduction

One of the aims of this study is to determine the potential advantages of the in-situ process for microalgae biodiesel production compared with the conventional process. To achieve this goal, quantification techniques are required to compare the different transesterification processes.

Comparisons based on the process economics (i.e. raw material, infrastructure and operational cost requirement) are commonly used in the literature for assessing biodiesel production systems (i.e. Haas et al., 2006; Zhang et al., 2003; Kasteren & Nisworo, 2007; Marchetti et al., 2008). Although reducing the processing and final costs of microalgae biodiesel was one of drivers of this study, economic assessments were not used in this thesis. Instead the influence of the transesterification method on biofuel product renewability was examined, based on the renewability criteria presented below.

The use of an energy balance calculation has been reported in the literature to assist in the evaluation of the renewability of biodiesel production (i.e. Wang et al., 1997; Sheehan et al., 1998; Hill et al., 2006). This assessment compares the energy of the biofuel product with the total fossil fuel energy consumed in its production. A ratio, MJ of unit biofuel produced/MJ total fossil fuel inputs for the production of a unit of biofuel, is then obtained. If the fossil fuel inputs into the conversion process exceed the energy content of the biofuel, the process is considered as non-renewable. The process is regarded as renewable when the biofuel energy value is greater than the sum of the fossil fuel energy inputs consumed. The boundaries of such studies vary and can be selected to cover the entire life cycle of the biomass and fuel production processes, or restricted to the biofuel conversion process alone (Hill et al., 2006).

The renewability analysis in this chapter is based on an assessment that quantifies the net gains (or losses) obtained from use of microalgae for biodiesel production. The renewability evaluation in this chapter was based on the process energy availability, or exergy, obtained using both the first and the second laws of thermodynamics.

The exergy (*B*) is the work that a system can produce if it is brought to physical, thermal and chemical equilibrium with its environment in a reversible manner. This corresponds to the maximum work extractable from a given system in a steady flow process without defying thermodynamic laws (Szargut et al., 1988).

The main objective of this chapter was to use the exergy based approach for the acquisition of a suitable indicator which would be used to quantify the renewability of different biodiesel production processes. This assessment was further used to evaluate the influence that the various modifications have on the renewability of the in-situ process. The potential improvement obtainable by integrating CH_4 production from the microalgae residues with the biodiesel production process was also assessed.

This chapter initially presents the principles underlying the assessment method selected, and proposes the renewability indicator to be used. The system boundaries for the renewability evaluation are defined, and the renewability of the biodiesel production processes examined in this thesis is evaluated.

9.2. Quantification of the Renewability Indicator

A process or resource can be regarded as renewable when regeneration mechanisms exist to return the resource to its original state i.e. a cyclic transformation is facilitated.

The production of biodiesel from microalgae, and its subsequent combustion in diesel engines, could be considered as a truly renewable resource, when the ideal process cycle shown in Figure 9.1 is feasible.

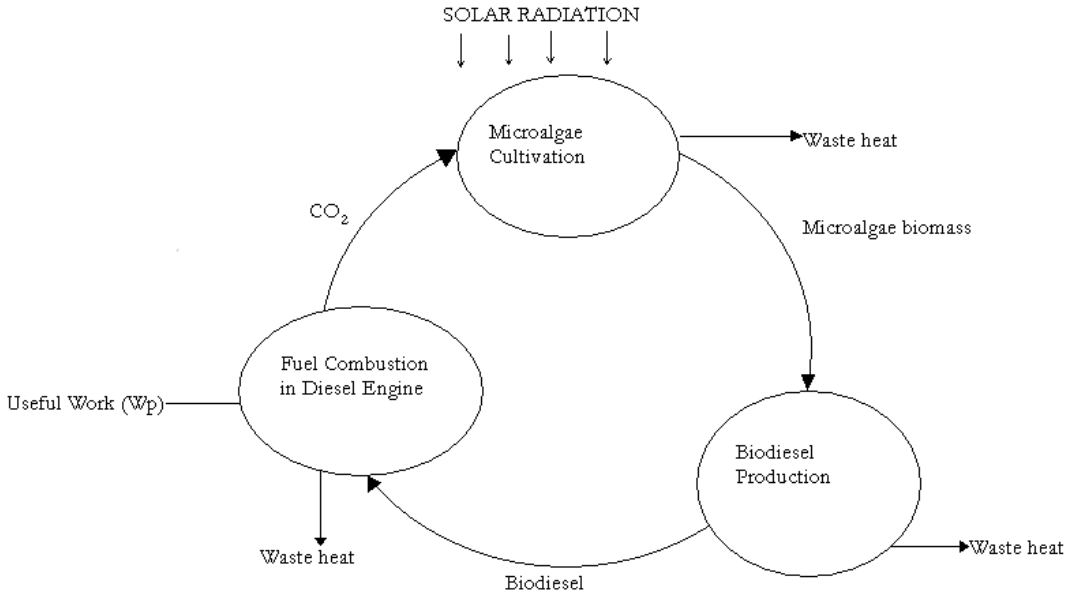

Figure 9.1. The ideal microalgae-biodiesel cycle.

In the ideal process, solar energy is harnessed by the thermochemical cycle which involves the following step-wise carbon transformations (Figure 9.1):

i. Microalgae biomass is naturally cultivated using water, atmospheric CO_2, nutrients, and the photosynthetic process driven solely by solar radiation.

ii. The lipid fraction of the microalgae biomass is obtained and converted to biodiesel without the need for fossil fuels.

iii. The biodiesel produced is combusted in diesel engines to produce useful work (Wp), with CO_2 and water as the process by-products.

For the ideal process, it is assumed that all of the chemical reactants and by-products are recyclable, with only low quality heat rejected by the microalgae-biodiesel cycle to the environment. Furthermore, all of the CO_2 released from the fuel combustion process and the organic process wastes after the biodiesel production process are assumed to be recycled into the microalgae production process.

The renewability of the microalgae biodiesel production process could however be altered when non-renewable resources are consumed in the biofuel processing units, or in the restoration of the environment to its initial state.

Resources classified as non-renewable resources (NRRs), are those which are consumed at a faster rate than they can be regenerated. The work required for the restoration of the environment to its initial state is greater than that produced from these resources (Berthiaume et al., 2001). Fossil fuels i.e. petroleum and coal are regarded as NRRs.

The direct use of these fossil fuels and their by-products (i.e. fertilisers), although increasing the microalgae biomass and biodiesel productivities, could potentially reduce the overall renewability of the conversion process as shown in Figure 9.2.

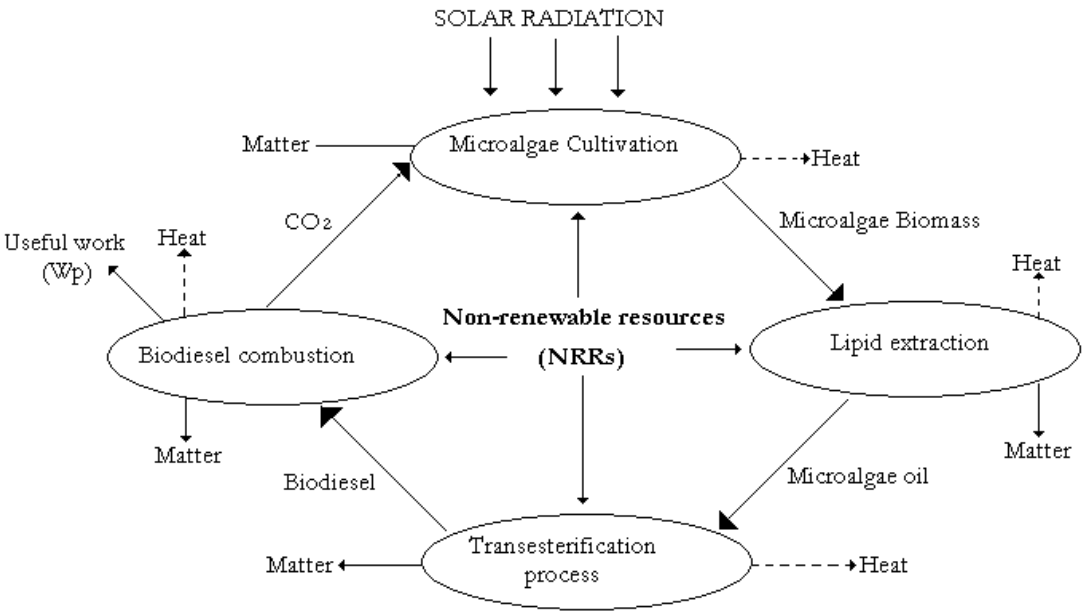

Figure 9.2. Microalgae-biodiesel process with non-renewable resources (NRRs) input.

The NRRs consumed during the biomass and the fuel production process must be accounted for to establish the renewability of the biodiesel production process.

The process exergy consumption approach, which is used in the renewability assessment in this thesis, is described in the following section (9.2.1).

9.2.1. Cumulative and net exergy consumption concept

Szargut et al. (1988) proposed the concept of cumulative exergy consumption (CExC) for the evaluation of production processes. The CExC is the sum of the exergy consumed for the production of the resources employed in all the steps of a process for the production of a considered product. This parameter is an estimate of the available work consumed by the process to obtain a product.

Berthiaume & Bouchard (1999) further extended the CExC concept to include the exergy content of the products resulting from a given process, using the cumulative net exergy (CNEx) consumed for the production of the products. The CNEx is the difference between the CExC and the exergy of the main product (E_p) of the process as shown in Eq. 9.1:

$$CNEx = CExC - E_p \quad (9.1)$$

where, E_p represents the exergy of the considered process product, MJ/kg.

The CNEx concept was illustrated by Berthiaume et al. (2001) using automotive gasoline as an example. To produce gasoline in a petroleum refinery, the cumulative exergy cost of all the resources (raw materials, electricity and fuels) consumed (CExC) to produce 1 kg of gasoline is 42.4 MJ (Berthiaume et al., 2001). If the gasoline product is stored, with the useful work available (E_p) from the gasoline product of 35.6 MJ/kg, a CNEx value of 6.8 MJ/kg is obtained (Berthiaume et al., 2001).

All the exergies consumed in the process, from the raw materials extraction to the exergy value of the final product are taken into account with the CNEx concept. The CNEx could be defined as the "minimum work required to restore a degraded NRR to its initial state by means of a series of ideal (reversible) transformations" (Berthiaume et al., 2001).

Waste generation mainly due to insufficient recycling of matter may also be encountered in the operation of large-scale biofuel production systems. Some work may be required for the treatment of these process wastes to avoid any potential detrimental effects they may have on the environment.

If the quantity of waste is relatively small and non-toxic, and where large land areas are available, such work may be provided by nature i.e. via aerobic degradation. On the other hand, when the waste is present in quantities which cannot be effectively treated using natural schemes, NRRs could be consumed to aid in achieving this remediation goal. To facilitate a proper assessment of the conversion processes, the net exergy requirement for the production of the resource without resulting in environmental damage must therefore be considered. This net exergy cost is regarded as the restoration work (Wr).

The Wr is the sum of the cumulative net exergy consumption during the production (CNExp) and waste treatment (CNExw) processes for the biofuel production (Eq.9.2) (Berthiaume et al., 2001).

$$Wr = CNExp + CNExw \quad (9.2)$$

As seen in the ideal microalgae-biodiesel cycle (Figure 9.1), useful work (Wp) may be produced from the product of a renewable cycle. However with NRRs consumed, the restoration of the process and its waste to its initial state, the net exergy consumption, Wr, must be taken into account in conjunction with the Wp when evaluating the extent of the overall process renewability. In this study, both Wr and Wp were used to establish a renewability indicator for the production of biodiesel from microalgae.

9.2.2. Renewability indicator

Berthiaume et al. (2001) proposed the use of the renewability indicator, Ir, to assess the renewability of production processes. The renewability indicator was defined as:

$$Ir = (Wp - Wr) / Wp \quad (9.3)$$

The process renewability can be evaluated on the basis of the Ir estimated for the different processes, i.e.:

- Ir = 1, is indicative of a fully renewable system, i.e. with Wr = 0, as for the ideal microalgae-biodiesel system.

- $0 < Ir < 1$, for a partially renewable system.

- Ir = 0, suggests a system in which the work produced by the biofuel and the restoration work required are equal. In such cases, the non-renewable resources could be considered for use directly instead of the produced biofuel.

- $Ir < 0$, for a process which consumes more restoration work than it produces. Such processes are considered as non-renewable, and are less efficient than similar processes where the non-renewable resources are used directly for the intended biofuel application.

This renewability indicator was applied to assess the microalgae transesterification processes examined and to compare the different processes studied.

9.3. Microalgae Cultivation, Harvesting and Biodiesel Production Processes

The processes involved in the microalgae cultivation, harvesting and biodiesel production used for the assessment in this chapter are shown in Figure 9.3.

The choice of the microalgae production and harvesting stages, and their exergetic requirement, are still contentious, as presented in Chapter 1. They depend on the specific microalgae species cultivated, the type of bioreactor used, the microalgae culture and the climatic conditions available.

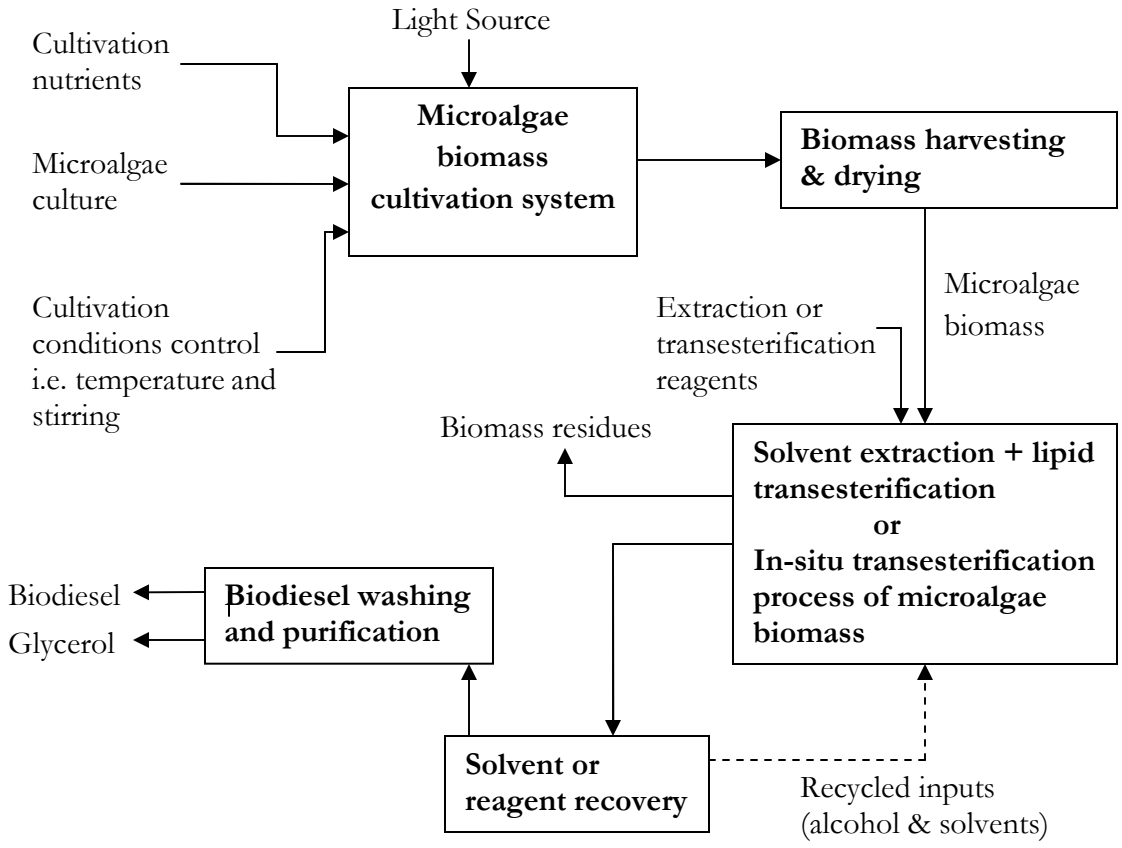

Figure 9.3. Simplified microalgae biomass cultivation and biodiesel production process.

The differences in the literature reports are mainly due to the developmental nature of microalgae technology aimed for cheap biofuel production. For example the cultivation of *Spirulina* in India was reported by Venkartaraman et al. (1980) with culture stirring provided with the use of hand held rubber brushes and with the biomass harvesting carried out via cloth filtration. The systems described by Venkartaraman et al. (1980) were however small scale, with the use of 2000 l cultivation reactors and the harvesting device designed for harvesting 670 l of *Spirulina* per m^2 cultivation area per hour. On the other hand, the use of open raceway ponds coupled with harvesting via sedimentation–centrifugation process (Sawayama et al., 1999; Hirano et al., 1998) for microalgae production was considered to lead to unfavourable process energetic requirements. The use of a raceway cultivation system, and harvesting via a sedimentation process followed by a continuous vacuum belt filter was

considered by Chisti (2008a) to reduce the energy requirement of the microalgae production process.

The analysis in Sawayama et al. (1999) was based on raceway microalgae production with 20.5% (w/w) oil content and a biomass productivity of 15 t dry microalgae/hectare/year. This biomass productivity is equal to 16% of the average practical annual productivities obtained for tropical raceway ponds over long periods (> 2 years) (Becker and Venkataraman, 1980). The considered biomass concentration in the microalgae broth was taken to be 0.5 kg dry microalgae/m^3, which is only 50% of the typical concentration achieved in a well operated raceway reactor (Pulz, 2001). The use of these assumptions alone was considered to be sufficient to result in doubling the energetic costs associated with the harvesting process (Chisti, 2008a).

Furthermore, Sawayama et al. (1999) and Hirano et al. (1998) considered the use of gravity separation followed by centrifugation for the biomass recovery from the culture. The centrifugation process has been demonstrated in the literature (i.e. Becker and Venkartaraman, 1980) to be an energy intensive harvesting scheme, and is thus considered to be impractical for the inexpensive recovery of the microalgae biomass. Since the microalgae are aimed for the production of a low value product, biodiesel, this harvesting method was considered to be unrealistic choice in this study. With higher lipid content and biomass productivities assumed for microalgae in this study for the production of cheap biodiesel, the cultivation and harvesting system presented in Chisti (2008a) (i.e. raceway systems with sedimentation-belt filtration harvesting) was considered to be more suited for use in this chapter.

The microalgae growth suggested by Chisti (2008a) and considered for use in this chapter is carried out using low cost open stirred raceway ponds i.e. as demonstrated by Becker & Ventkarman (1988). Most of the primary energy for these cultivation systems is from solar radiation with CO_2 provided by the atmosphere. Although higher process energy requirement for the microalgae cultivation and harvesting stages have been reported in the literature i.e. Clarens et al. (2010), the process energy demand of 9.07 MJ/ kg microalgae oil put forward by Chisti (2008a) for raceway cultivation systems was used in this chapter.

Although detailed reports on the energy requirement for the microalgae drying was not found in the literature, since this processing step potentially contributes a significant energy input (Chen et al, 2009), the energetic demand for the microalgae biomass drying was included in this study. This involved a simple assessment of the use of two drying methods (a) a conventional hot air dryer and (b) a heat pump dryer for the reduction of the moisture content of the post harvested biomass to levels suited for the transesterification process. Post harvest microalgae moisture content of 72.5% (w/w, on the basis of the dried biomass) as described in section 4.2.4 was used in this chapter.

The specific moisture extraction rate (SMER) which is the rate of water extracted from the product per unit energy consumed in the drying process (kg/kWh) was used to assess the effectiveness of the energy used in the biomass drying processes. During the drying process, the SMER value reduces with time as the humidity in the drier falls and as the moisture removal from the biomass becomes more difficult, due to hygroscopic retention of moisture in the product. This effect can be observed for wood drying using dehumidifiers as demonstrated in Sun et al. (1999), where the reported process SMERs clearly decreases towards the end of the drying process. Although factors such as the biomass thickness, the kiln air state and drying time could influence the SMER of the considered drying process, only reported average SMERs were used for the preliminary estimation of the biomass drying energy requirement.

A maximum SMER value of 1.55 kg moisture/kWh, based on the latent heat of water vaporisation at 100°C (Raghavan et al. 2005) was used to estimate the conventional dryer energy demand. This was used to represent best case scenarios for conventional dryers (Raghavan et al. 2005). For the biomass drying using a heat pump dryer, a SMER of 4 kg moisture/kWh electricity (Carrington, 2010) was considered in this study.

With the same biomass and cultivation processes considered to be applied for the production of a unit of microalgae biomass, the same energy inputs for the biomass production, harvesting and drying steps were used for all the different transesterification processes.

Details on the energy requirement and resources consumed in the biomass production process are presented in section 9.5.1.

The microalgae lipids are extracted and transesterified (conventional transesterification) or transesterified directly (in-situ transesterification) from the dried biomass. This involves the use of methanol as the reaction alcohol, and H_2SO_4 as the process catalyst as outlined in Chapter 8. For each processing option, the separation and purification of the biodiesel and glycerol fractions were assumed to be carried out as described in Chapter 8.

To estimate the process renewability in this chapter, the minimum restoration work (CNEx) required for the production of the non-renewable resources consumed in the different transesterification processes was obtained. This was then compared to the maximum useful work available from the main products of the considered processes.

9.4. System Boundaries

As thermodynamically required, the boundaries for the microalgae biodiesel production systems were selected in this study to start with the cultivation and harvesting of the microalgae biomass and end after the biodiesel and glycerol upgrading process. In cases where the anaerobic digestion of the microalgae residues was considered, the system boundaries were extended to the recovery of the CH_4 product.

The system inputs include microalgae cultures, CO_2, nutrients, electricity, fossil fuels and process chemicals. The system outputs are biodiesel, glycerol, calcium sulphate ($CaSO_4$), microalgae biomass residues, and process heat and material wastes. In cases where biological energy recovery is applied, the system outputs include CH_4, CO_2, and nutrient rich effluents.

A diagram showing the boundaries of the system and the various material and energy flows considered in this chapter is shown in Figure 9.4.

The material and energy inputs for the transesterification processes were considered on the basis of the requirements for the conversion of 1 t dry microalgae biomass/h. With

the process assumed to be in steady state, and the energy input for the recovery of the excess methanol and solvents accounted for, only the additional raw materials added to make up the required reacting quantities were used as process inputs.

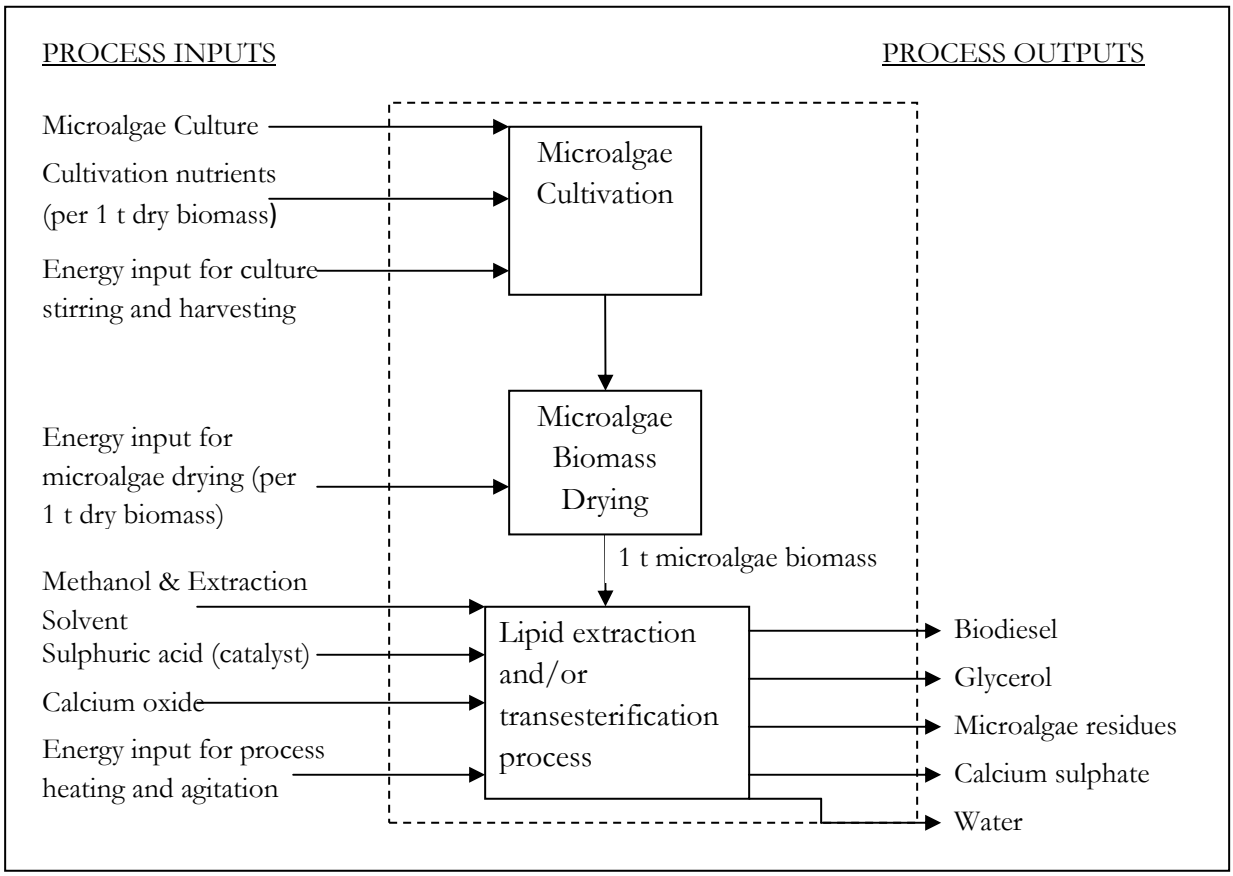

Figure 9.4. The system boundary (represented by the dashed lines) and material and energy flows considered in this study.

9.5. Renewability of Microalgae Biodiesel Using Different Transesterification Methods

9.5.1. Microalgae production, harvesting and biomass drying

The mean values for the major elements found in microalgae (expressed in percentage of ash free dry weight) are reported to be: Carbon (C), 53%; Hydrogen (H),8.0%; Oxygen (O),31.0%; Nitrogen (N),8.0%; and Phosphorus (P), 2.0% (Benefield & Randall,1980). This elemental composition was similar to those obtained for the *Chlorella* samples in this study (Table 3.2). The microalgae elemental mass percentage composition as presented by Benefield and Randall (1980) were used for the analysis in this chapter since it showed a similar elemental composition to the microalgae specie in this study, and furthermore could be used to estimate the process phosphorus requirement. The microalgae elements must be adequately supplied to the microalgae cultures (i.e. via fertilisers) to facilitate biomass synthesis and maintain productivity levels (Becker, 1994). Atmospheric CO_2 was considered to meet the culture carbon requirement, with the biomass H and O fractions obtained from the culture water. Only the major nutrients, N and P were considered in this chapter as process fertilisers for the biomass cultivation step.

Ammonia (NH_3) and urea ($CO\ [NH_2]_2$) are commonly used as N sources in microalgae cultivation systems (Becker, 1994). Only the use of NH_3 was considered in this thesis since it is relatively cheaper and is reported to be easier to handle than urea for application in microalgae cultivation systems (Neenan et al, 1986).

Triple super-phosphate (TSP) was used as the reference P fertilizer due to its widespread use in agricultural applications (Patzek, 2004).

Based on the microalgae elemental composition (w/w), the mass requirement for N and P in t/ha is obtained by multiplying the biomass productivities by 0.08 and 0.02 respectively. Using a microalgae areal productivity of 72 t (d.w) microalgae/ha (Goldman, 1980), with NH_3 the only N source in the cultivation media, the areal microalgae N mass requirement is 5.76 t/ha. The areal P input (using TSP) into the cultivation process was

estimated to be 1.44 t P/ha. This relates to a specific nutrient requirement of 80.0 and 20.0 kg of NH_3 and TSP respectively/t (d.w) microalgae produced.

Mixing of the microalgae cultures, pumping of the water and microalgae cultures, as well as the harvesting and concentration of the algal biomass (i.e. via centrifugation) are some of the processes which require electrical input for their operation (Becker, 1994; Becker and Venkatraraman, 1980; Neenan et al, 1986). As mentioned earlier, the process electricity demand presented in Chisti (2008a) was used in this chapter to assess the biomass production electricity requirement. The electrical energy requirement for biomass cultivation and harvesting was reported to be 8.77 and 0.30 MJ/kg microalgae oil respectively (Chisti, 2008a). This conservative electrical energy demand was 9 times less than that demonstrated in Becker and Venkatraraman (1980) for similar raceway cultivation systems, and is taken to represent the process electrical requirement in a present day scenario.

With an estimated extracted lipid content of 0.27g lipids/g *Chlorella* biomass, this relates to an electrical energy input of 2448.9 MJ/t dry *Chlorella* biomass produced for the microalgae cultivation and harvesting processes.

The renewability assessment carried out was initially based on electricity generated from typical fossil fuel generation systems. An analysis of the influence of electricity source (i.e. the use of hydro-electricity) on the overall process renewability was later conducted under the sensitivity analysis in section 9.5.8.1.

For the biomass drying stage, it was initially considered that fossil fuels combustion was used to generate the energy consumed in the conventional drying process. The use of heat pump dryers for the biomass drying and its influence on the renewability of the considered processes is subsequently explored in section 9.5.8.3. As highlighted earlier, a SMER of 1.55 kg moisture/ kWh was used the estimation of the energy demand for the biomass drying using conventional dryers. For the removal of 725 kg of moisture from the post harvested microalgae, this relates to an energy input of 467.74 kWh for the recovery of 1t dry biomass. In a fossil based scenario, it is initially assumed that diesel combustion is used to meet this energy requirement. This was carried out with an overly optimistic first law efficiency of 100% considered for the combustion process, even though best operating

boiler efficiencies of ≈ 80% is usually seen for direct fired heating system (Carrington, 1978). The analysis presented in section 9.5.2-9.5.7 was initially based on the use of separate heat and power (SHP) installations (i.e. electrical generators and/or onsite boilers) for providing the process electrical and heating requirement. The use of combined heat and power (CHP) systems as an efficient approach for power and thermal energy generation from a single fuel source has been discussed widely in the literature (i.e. Pilavachi, 2000; Graus et al., 2007). The use of CHP systems for the process electricity and heat generation and its influence on the process renewability was however not considered in this study due to the considered use of a 100% operating efficiency for heating fuel combustion in the SHP installations.

Using the general material and energy inputs for the biomass production steps, the evaluation of the different transesterification methods was carried out in the following sections (9.5.2-9.5.6). For the different processes, the cumulative exergy consumed for the production of the NRRs depleted in the different process (Wr) was initially estimated. The maximum useful work available from the process products (Wp) was then determined. The Wp and Wr were then used to estimate the renewability indicator (Eq. 9.3) in section 9.5.7.

Using the results of the renewability assessment, the critical issues affecting the in-situ transesterification process are presented in section 9.5.10.

9.5.2. Mechanically stirred in-situ transesterification

9.5.2.1. Cumulative exergy consumed for production of NRRs consumed in the mechanically stirred in-situ process

Using the process material and minimum heat demands presented in Chapter 8, the cumulative net exergy (CNEx) consumed for the production of the NRRs used in the mechanically stirred in-situ process is shown in Table 9.1. The process inputs (i.e. methanol, catalyst and CaO and outputs i.e. biodiesel, glycerol and $CaSO_4$) were based on the equivalent requirement for the in-situ transesterification of 1 t dried microalgae biomass. This was same as the hourly mass flow rates shown in Table 8.1.

For the stirring of the in-situ transesterification reactors, a mixing electrical energy requirement of 1.42 MJ /t biodiesel produced (Sorguven & Özilgen, 2010), was used in this study. A total hourly mass flow of biodiesel of 0.281 t was shown for the mechanically stirred in-situ transesterification process after the conversion of 1 t dry *Chlorella*. The stirring electrical energy requirement for this in-situ process therefore corresponds to 0.82 MJ for the stirring of the two transesterification reactors.

It was also initially considered in the analysis in sections 9.5.2-9.5.6 that the process heat demands would be supplied via diesel fuel combustion in boilers. The influence of the use of other common boiler fuels i.e. coal, natural gas and wood pellets on the process renewability is subsequently examined in section 9.5.8.3. Furthermore, the use of heat pump heating (using electricity) to meet the process heating demand was also explored in that section (9.5.8.3).

A maximum useful work (exergy) for the diesel heating fuel of 44.4 MJ/kg (Szargut & Morris, 1987) was used for in this analysis. The quantity of heating fuel (kg/t microalgae biomass consumed) required to meet the minimum heat demand (after process integration) of the different transesterification processes was estimated with the assumption of a 100% conversion of the fuel to heat.

The cumulative exergy values for the production of the NRRs consumed for the process raw material, heating fuel and electricity requirements(CNEx), considered to be representative of a present day scenario, were largely obtained from Szargut & Morris (1987). This analysis used the CNEx values from Szargut & Morris (1987) since they has been widely used in recent literature (i.e. Berthiaume et al, 2001; Yang et al, 2009; Sorguven & Özilgen, 2010) to determine the minimum net exergy cost for the NRRs production in a present day production scenario. The restoration work (Wr) calculated from the CNEx values was considered to correspond to the use of a conventional production scheme for the process raw materials and electricity.

The influence of altering the production scheme on the process renewability is considered in section 9.5.8.

Table 9.1. Minimum restoration work required for the NRRs depleted in the in-situ transesterification of 1 t *Chlorella* biomass (mechanical stirring)

Process unit	NRRs	Unit	Quantity of NRRs	CNEx (MJ/unit)	Wr (MJ)
Microalgae biomass production					
	Ammonia	kg	80.00	30.9[22]	2472.00
	P_2O_5	kg	20.00	7.52[23]	150.4
	Electricity associated with cultivation & harvesting	MJ	2448.90	4.17[24]	10211.91
	Microalgae drying (diesel)	kg	37.93	53.20	2017.88
Biodiesel production					
	Methanol	kg	104.2	73.08[25]	7614.94
	H_2SO_4	kg	270.00	9.10[26]	2457.00
	CaO	kg	154.37	10.05[27]	1551.42
	Reactor stirring (electricity)	MJ	0.82	4.17	3.42
	Process heating (diesel)	kg	92.86	53.20[28]	4940.15
			Total Wr (MJ)		31419.28

[22] CNEx values based on ammonia gas production via steam reforming of natural gas (Szargut & Morris, 1987).

[23] CNEx value for P_2O_5 obtained from Berthiaume et al. (2001) based on the production energy values demonstrated in Wittmus et al. (1975).

[24] Typical CNEx value for electricity generated from fossil based fuels (Szargut & Morris, 1987).

[25] Methanol produced from crude oil (Szargut & Morris, 1987).

[26] CNEx of sulphuric acid based on H_2SO_4 production via the application of the Frasch process and sulphur combustion using sulphur from the ground as the process feedstock (Szargut et al, 1988).

[27] CNEx of calcium oxide based on CaO production via the mining, crushing and calcining of limestone as the process feedstock (Szargut et al, 1988)

[28] Typical diesel fuel from crude oil CNEx value (Szargut & Morris, 1987)

As described earlier, the quantity of methanol used in the mechanically stirred in-situ process was based on that demonstrated experimentally in Chapter 4. This involved the use of a reacting methanol to oil molar ratio of 315:1. The large methanol volume was also used to facilitate the complete submersion of the microalgae feedstock in the reactors. For the conversion of 1 t *Chlorella* biomass in this study, this corresponds to the use of 3.115 t methanol for the mechanically stirred process. The excess methanol than was stoichiometrically required was recovered via distillation methods as described in Chapter 8. Following the distillation process, 3.011 t of the recovered methanol was re-used in the in-situ reactors. Since the process was considered to be in steady state, only the 104.20kg methanol/h supplied to the process was considered as the reacting alcohol input.

9.5.2.2. Useful work available (Wp) from the products of the mechanical stirred in-situ transesterification process

The exergy content of the biodiesel and glycerol product was used to account for the useful work (Wp) obtainable from the transesterification process.

The sum of the minimum work available from the transesterification products was used to estimate the process Wp as described in section 9.2.1.

The minimum useful work of the FAME and unreacted oil content of the biodiesel product were treated separately to quantify the work available from the transesterification product.

The total useful work (Wp) available from the products derived from the in-situ transesterification (with mechanical agitation) of 1 t of microalgae is shown in Table 9.2.

Table 9.2. Useful work available from the in-situ transesterification products (per 1 t microalgae biomass converted) using a reacting ratio of methanol to oil of 315:1 (mechanical agitation)

Product	Exergy (MJ/kg)	Product quantity (kg)	Wp (MJ)
FAME	40.70	281.88	11472.52
Microalgae oil	40.15	6.76	271.41
Glycerol	18.52[29]	27.69	512.82
$CaSO_4$	0.06[29]	374.73	22.48
		Total Wp (MJ)	12278.71

The exergy content of the FAME and oil were obtained from the heat of combustion results (Appendix G[30]).

9.5.3. In-situ transesterification with ultrasound agitation

9.5.3.1. Net exergy consumed for restoration of NRRs used in the ultrasound agitated in-situ transesterification of 1 t microalgae biomass to its initial state.

For the low frequency ultrasonication of the in-situ mixture, the assessment in this section was based on the process electrical energy estimates obtained from the industry (Tyrrell, 2010).

Two 4 kW, 20 kHz ultrasonicator attached to the reactors were considered for the estimation of the agitation electrical energy requirement in this section. This was due to the

[29] Ayres & Ayres (1999).

[30] Appendix G provides the results of the fuel properties of the purified microalgae derived FAME.

fact that this commercially available ultrasonicator size was reported to be suited for biodiesel reactors with a flow rate of 1-3 m^3 transesterification mixture/h (Tyrrell, 2010).

This ultrasonicator size was also reported to be adequate for microalgae cell disruption for processes with a biomass flow rate of 0.1-0.8 m^3 microalgae/h (Tyrrell, 2010). Thus, the use of the considered ultrasonicator size seems adequate for the mass flow rates involved for the in-situ transesterification of 1 t of microalgae biomass using a molar methanol to oil ratio of 105:1.

The minimum work required to restore the NRRs depleted in the ultrasound agitated in-situ transesterification of 1 t *Chlorella* biomass is given in Table 9.3. This in-situ process was conducted using a reacting methanol to oil molar ratio of 105:1 as described in Chapter 5.The excess methanol was recovered using distillation methods and considered for re-use in the transesterification process.

Table 9.3. Minimum restoration work required for the NRRs depleted in the ultrasound agitated in-situ transesterification of 1 t microalgae biomass with a molar methanol to oil ratio of 105:1

Process unit	NRRs	Unit	Quantity of NRRs	CNEx (MJ/unit)	Wr (MJ)
Wr for microalgae production					14852.19
Biodiesel production					
	Methanol	kg	56.64	73.08	4139.25
	H_2SO_4	kg	279.00	9.10	2538.9
	CaO	kg	159.52	10.05	1603.18
	Reactor stirring (electricity)	MJ	28.80	4.17	120.10
	Process heating (diesel)	kg	37.20	53.20	1979.04
			Total Wr (MJ)		25232.66

9.5.3.2. Useful work available (Wp) from the products of the ultrasound agitated in-situ transesterification of 1 t *Chlorella* biomass

The mass flow of the main products following the application of the ultrasound assisted in-situ transesterification process for the conversion of 1 t *Chlorella* was used to estimate the Wp obtainable from the process.

The sum of the work available from the products (Wp) for the considered transesterification process is shown in Table 9.4.

Table 9.4. Useful work available (Wp) from the ultrasound agitated in-situ transesterification products (for 1 t microalgae biomass converted) using a reacting ratio of methanol to oil of 105:1

Product	Exergy (MJ/kg)	Product quantity (kg)	Wp (MJ)
FAME	40.70	295.58	12030.11
Microalgae oil	40.15	1.00	40.15
Glycerol	18.52	29.01	537.26
$CaSO_4$	0.06	387.22	23.23
		Total Wp (MJ)	12630.75

9.5.4. In-situ transesterification with diethyl ether used as a process co-solvent

9.5.4.1. Net exergy cost for the production of NRRs used in the mechanically agitated in-situ transesterification process using diethyl ether

The cumulative exergy consumed for the production of the materials and energy inputs of the mechanically stirred in-situ transesterification process incorporating the use of diethyl ether as a co-solvent are shown in Table 9.5.

Table 9.5. Restoration work required to restore the NRRs consumed in the mechanically stirred in-situ transesterification of 1 t microalgae biomass with diethyl ether as a co-solvent to its initial state.

Process unit	NRRs	Unit	Quantity of NRRs	CNEx (MJ/unit)	Wr (MJ)
Wr for microalgae biomass production					14852.19
Biodiesel production					
	Methanol	kg	46.59	73.08	3404.80
	H_2SO_4	kg	270.00	9.1	2457.00
	CaO	kg	154.37	10.05	1551.42
	Reactor stirring (electricity)	MJ	0.82	4.17	3.42
	Process heating (diesel)	kg	18.82	53.2	1001.22
			Total Wr (MJ)		23270.05

The energetic requirement for the stirring of the transesterification reactors was assumed to be same as for the mechanically stirred in-situ process in section 9.5.2.

Since the process was considered to be in steady state, and with the diethyl ether reactant completely recovered from the transesterification product stream as described earlier in section 8.2.6, the diethyl ether mass inputs were not considered in the accounting of the NRRs consumed in the process. This was used since the heat requirements for the co-solvent recovery was included in the process assessment. This analysis was on the basis of the resources required for the conversion of 1 t *Chlorella* biomass.

9.5.4.2. Useful work available (Wp) from the products of the mechanical stirred in-situ transesterification process

The sum of the useful work available from the main products of the mechanically stirred in-situ transesterification of 1t *Chlorella* biomass with a reacting methanol to oil ratio of 79:1 using diethyl-ether as a co-solvent is given in Table 9.6.

Table 9.6. Useful work (Wp) available from the main in-situ transesterification products (for 1 t microalgae biomass converted) using a reacting ratio of methanol to oil of 79:1 and diethyl ether as co-solvent

Product	Exergy (MJ/kg)	Product quantity (kg)	Wp (MJ)
FAME	40.70	264.24	10754.57
Microalgae oil	40.15	23.77	954.37
Glycerol	18.52	25.86	478.93
$CaSO_4$	0.06	374.73	22.48
		Total Wp (MJ)	12210.34

9.5.5. In-situ transesterification of microalgae with co-solvent (diethyl ether) and ultrasound agitation

9.5.5.1. Net exergy cost for production of NRRs used in the ultrasound agitated in-situ transesterification process using diethyl ether as a co-solvent.

The minimum work required to restore the degraded NRRs consumed in the in-situ transesterification of *Chlorella* biomass involving the use of diethyl ether as co-solvent with ultrasonic agitation to its initial state is presented in Table 9.7.

Table 9.7. Restoration work required for the NRRS degraded in the ultrasonically agitated in-situ transesterification of 1 t microalgae biomass with diethyl ether as a co-solvent

Process unit	NRRs	Unit	Quantity of NRRs	CNEx (MJ/unit)	Wr (MJ)
Wr for microalgae biomass production					14852.19
Biodiesel production					
	Methanol	kg	36.54	73.08	2670.34
	H_2SO_4	kg	279	9.1	2538.90
	CaO	kg	159.52	10.05	1603.18
	Reactor stirring (electricity)	MJ	28.8	4.17	120.10
	Process heating (diesel)	kg	10.46	53.2	556.47
			Total Wr (MJ)		22341.18

9.5.5.2. Useful work available (Wp) from the products of the ultrasound agitated in-situ transesterification of 1 t *Chlorella* biomass

Similarly, the product exergies for the transesterification of 1 t *Chlorella* biomass with a molar reacting ratio of methanol to oil of 26:1 is given in Table 9.8.

Table 9.8. Useful work available (Wp) from the transesterification products (per 1 t microalgae biomass) using ultrasound agitation and diethyl ether as co-solvent

Product	Exergy (MJ/kg)	Product quantity (kg)	Wp (MJ)
FAME	40.70	284.73	11588.51
Microalgae oil	40.15	10.83	434.82
Glycerol	18.52	27.95	517.63
$CaSO_4$	0.06	387.22	23.23
		Total Wp (MJ)	12564.19

9.5.6. Conventional extraction and transesterification of the microalgae biomass lipids

9.5.6.1. Cumulative exergy consumed for the production of the resources used in the extraction and transesterification of 1 t dry *Chlorella* biomass

For the conventional process, the material inputs included the extraction solvents required for the initial stripping of the oil feedstocks from the microalgae. The conventional transesterification process was assumed to be conducted with a reacting methanol to oil ratio of 50:1 as described in section 8.2.8.2. The process heat input was based on the minimum hot utility requirement after the application of process integration techniques.

The conventional transesterification process was also assessed on the basis of the exergy consumed for the production of the resources to facilitate the conversion of 1 t microalgae biomass.

The minimum restoration work required for the NRRs consumed in the extraction and transesterification of 1 t of dried *Chlorella* biomass to its initial environmental state is given in Table 9.9.

Table 9.9. Minimum work required to restore the NRRs depleted in the mechanically stirred conventional transesterification of 1 t microalgae biomass to its initial state.

Process unit	NRRs	Unit	Quantity of NRRs	CNEx (MJ/unit)	Wr (MJ)
Total Wr for microalgae production					14852.19
Biodiesel production					
	Methanol	kg	30.02	73.08	2193.86
	H_2SO_4	kg	260.00	9.10	2366.00
	Butanol	kg	2.00	60.13	120.26
	CaO	kg	148.67	10.05	1494.13
	Process stirring (electricity)	MJ	1.23	4.17	5.13
	Process heating (diesel)	kg	48.40	53.20	2574.88
			Total Wr (MJ)		23606.45

For the estimation of the cumulative exergy consumed for the butanol production, it was initially considered that the process butanol was derived from crude oil. This section used an exergy cost of 60.13 MJ/kg butanol produced[31] . This estimation is similar to crude oil derived ethanol as put forward by Sorguven & Özilgen, 2010. The influence of the use of butanol derived from fermentation processes on the transesterification process renewability would be examined later in the sensitivity analysis section (section 9.5.8).

[31] Butanol was initially considered to be synthesised via the acid catalysed hydration of propylene derived from the autothermic cracking of crude oil.

9.5.6.2. Useful work available (Wp) from the products of the ultrasound agitated in-situ transesterification of 1 t *Chlorella* biomass

The sum of the useful work (Wp) available from the main products of the conventional lipid extraction and transesterification process is presented in Table 9.10.

Table 9.10. Useful work (Wp) available from the conventional lipid extraction and transesterification products (for 1 t microalgae biomass converted) using a reacting molar methanol to oil ratio of 50:1

Product	Exergy (MJ/kg)	Product quantity (kg)	Wp (MJ)
FAME	40.70	239.85	9761.89
Microalgae oil	40.15	8.17	328.03
Glycerol	18.52	23.52	435.59
$CaSO_4$	0.06	360.90	21.65
		Total Wp	10547.16

9.5.7. Renewability evaluation of the different transesterification processes

Using the renewability indicator (Ir) as presented in section 9.2.2, Figure 9.5 compares the calculated Ir for the different transesterification processes assessed in sections 9.5.2-9.5.6. It must be noted that here the processes are considered to have their process heating supplied via diesel combustion and electricity using fossil fuel based sources. The renewability assessment provided is thus aimed at representing that of a present day production scenario.

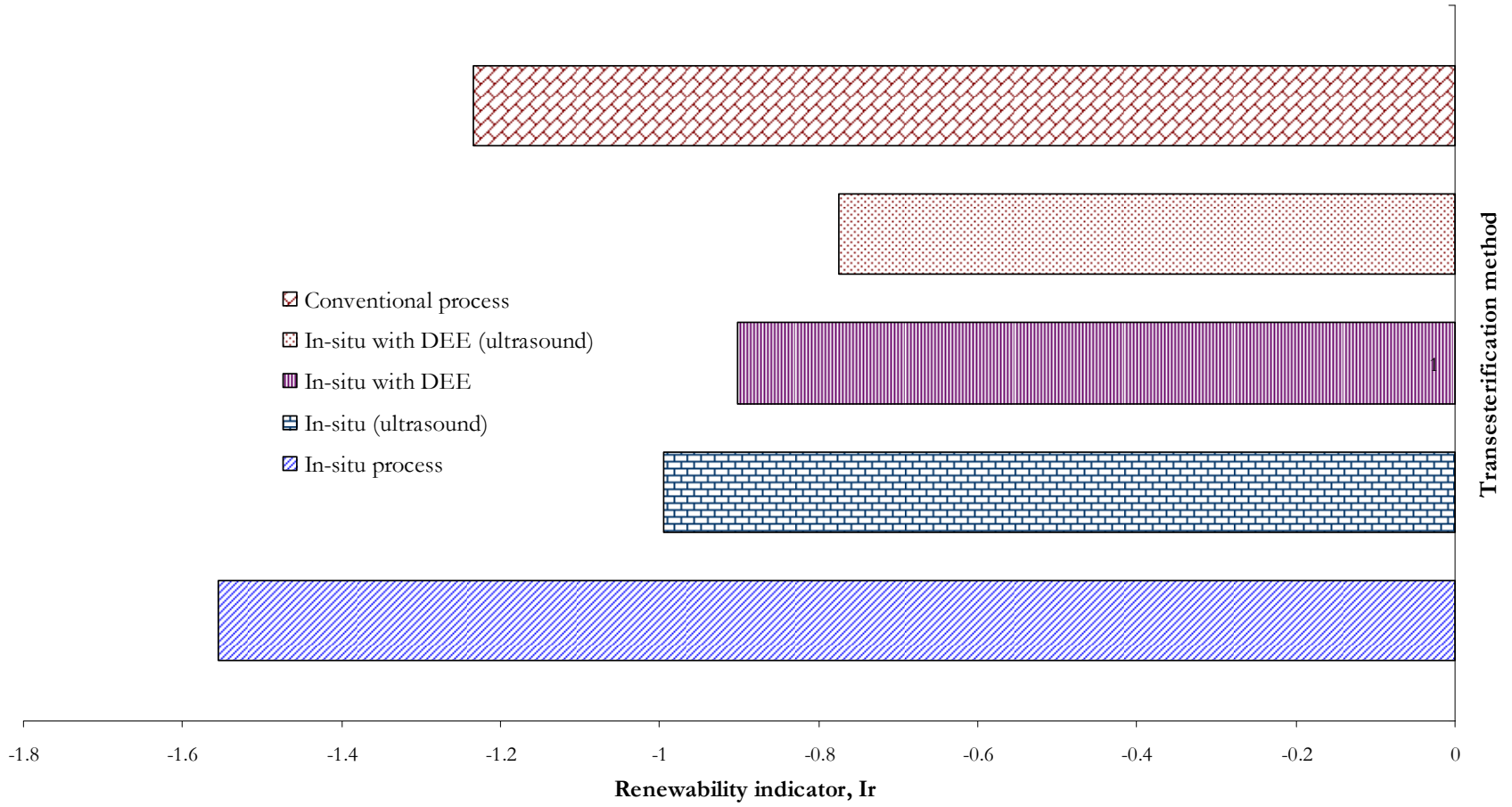

Figure 9.5. Renewability indicators for the different transesterification processes in a fossil fuel based scenario (per 1 t microalgae biomass converted).

From the results in Figure 9.5, it can be seen that for all the processes examined here, the estimated Ir was < 0. This means that more work was required for the restoration of the process to an equilibrium environmental state than was produced from the main products of the transesterification process. Moreover, this is the most favourable assessment, because the potential work outputs are the maximum possible and the work inputs are the minimum possible.

The application of the process modifications considered in this thesis to the in-situ transesterification process was observed to improve its renewability. The modified processes i.e. involving the use of low frequency ultrasound and co-solvents were seen to be more renewable than the use of the conventional method for microalgae FAME production under the process conditions of a present day scenario.

The use and recovery of large reacting alcohol quantities for the in-situ transesterification process appear to negatively affect the overall renewability of this method. The use of a reacting molar ratio of methanol to oil of 315:1 for the mechanically stirred in-situ process (including the heat requirement for the alcohol recovery) was shown to contribute ≈40% of the overall Wr calculated for this process.

The in-situ method involving the use of ultrasound and co-solvent aided in reducing the Wr, thus improving the transesterification process renewability. However that method was also seen to exhibit negative renewability (Ir) values mainly due to the source of the resources employed for the process electricity and raw materials. It was initially considered in this chapter that the resources consumed in the microalgae production and conversion was derived from fossil fuel based sources (i.e. methanol from crude oil). This assumption was based on a present day scenario were most of the industrial inputs are considered to be derived directly or indirectly from such sources. The influence of the source of these raw materials on the process renewability was further explored in section 9.5.8.

Changes in the electricity generation scheme and the choice of the fuel source for the process heat generation were also examined in section 9.5.8 since they could also influence the process Ir. The overall influence of these factors on the renewability of the different microalgae production and transesterification processes is presented in the following section.

9.5.8. Sensitivity analysis of Ir

9.5.8.1. Electricity generation scheme

The cumulative exergy consumed for the production of the process electricity inputs used in the renewability assessment in sections 9.5.2 - 9.5.8 was based on typical values for fossil fuels generated electricity of 4.17 MJ/MJ electricity as put forward by Szargut & Morris, 1987. Compared to other process inputs, the electricity requirement for the different biomass production and transesterification processes (from fossil fuel sources) were seen to contribute the most to the overall process Wr (≈30-46% of the total Wr). In scenarios where electricity is generated from alternative sources, the exergy consumed for the production of the process electricity would differ.

In locations with hydro-generated electricity (i.e. in New Zealand), the minimum restoration work (CNEx) required for the electricity production is 0.006 MJ/MJ of the generated hydro-electricity (Berthiaume et al., 2001). The cumulative exergy cost of 4.17 MJ/MJ electricity associated with fossil fuel based electricity generation was thus replaced with 0.006 MJ/MJ and Tables 9.1, 9.3, 9.5, 9.7, 9.9 were re-evaluated. The Wr and Ir of the different processes were then recalculated. Assuming the biomass production and transesterification electricity requirements were met using hydro-electricity, the changes in the calculated Ir are given in Table 9.11.

Table 9.11. Process renewability with the use of hydro-electricity

Process	Wr	Wp	Ir
In-situ (mechanical stirring)	21218.65	12279.23	-0.73
In-situ (ultrasound)	14915.60	12630.75	-0.18
In-situ with DEE (mechanical stirring)	13069.49	12210.34	-0.07
In-situ with DEE (ultrasound)	12024.09	12564.20	0.05
Conventional process (mechanical stirring)	13404.16	10547.16	-0.27

With the use of hydroelectricity, an improvement in the process renewability was observed for all processes compared with the use of fossil fuel generated electricity. Compared to the Wr estimated in 9.5.2-9.5.6 for the fossil fuel based generated electricity driven processes, percentage reductions in the total Wr of 32.50, 40.95, 43.90, 46.25 and 43.28 % were obtained for the in-situ (mechanical stirred), in-situ (ultrasonicated), in-situ (with diethyl ether), ultrasound agitated in-situ with diethyl ether, and conventional processes respectively using hydro-electricity.

The use of other sources i.e. wind or solar for electricity generation were not further considered, since it was expected that similar CNEx values as that seen for hydro-electricity would be obtained using these electricity sources for the considered processes.

With the use of hydro-generated electricity, an improvement in the renewability (compared with fossil fuel based electricity) of the all the considered process was seen, as shown in the Ir column in Table 9.11. This shows that it is essential to use a renewable electricity source to make the biomass production and transesterification process renewable, here, only the ultrasound agitated in-situ process with diethyl ether was deemed renewable.

9.5.8.2. Source of process raw materials (inputs)

The evaluation in sections 9.5.1 - 9.5.6 was based on the assumption that the process inputs were derived from fossil based sources. Under that scenario, the cumulative exergy consumed for the production of the process raw materials was seen to influence the process renewability. For example, the exergy consumed for the methanol used for the different transesterification process was seen to contribute 10.18-25.93% of the total estimated Wr. The influence of using alternatively sourced raw material inputs for the transesterification processes was considered in this section, here, the use of reacting alcohols from biomass fermentation was examined.

The large scale production and use of ethanol via biomass fermentation has been widely detailed in the literature (i.e. Lin & Tanaka, 2006; Hattori & Morita, 2010). The use of ethanol as a reacting alcohol in the transesterification process has also been demonstrated in

the literature (i.e. Al-Widyan & Al-Shyoukh, 2002). The use of ethanol as the reacting transesterification alcohol is considered in this section in order to investigate the influence of fermentation sourced alcohols on the process renewabilities. The cumulative exergy cost (CNEx) required for the production of ethanol via the fermentation of sugars of 26.40 MJ/kg ethanol (Sorguven & Özilgen, 2010) was used in this analysis. The transesterification process using ethanol was further considered to result in the same main product yields and useful work available from the main transesterification products as obtained with the use of methanol as the reaction alcohol.

The same assumption of using biomass derived ethanol was also applied to the conventional process to facilitate an even comparison with the in-situ processes.

Butanol was used as the extraction solvent in this study because it can be produced in large quantities via fermentation with various biomass materials i.e. starch, sugars and non-cellulosic substrates (including algae biomass) as feedstocks (Jones & Woods, 1986).

Instead of the use of butanol derived from propylene, as previously evaluated in section 9.5.6, the use of butanol derived from fermentation processes on the conventional extraction and transesterification process was examined. The process butanol solvent was assumed to be produced using the acetone-butanol-ethanol (ABE) fermentation process. A conservative exergy cost of 26.40 MJ/kg butanol produced was considered for use in this section. This was based on cumulative exergy consumed for the production of fermentation derived alcohols, as presented by Sorguven & Özilgen, 2010.

The minimum restoration work required for the production of the reacting alcohol, as presented in Tables 9.1, 9.3, 9.5, 9.7 and 9.9, were replaced with those described in this section. The Ir of the different processes was then recalculated. This evaluation also considered that the process electricity requirement was met using hydro-generated electricity as in section 9.5.8.1.

The influence of the use of the alcohols obtained from biomass fermentation on the Wr of the processes and their corresponding Ir is shown in Figure 9.6. The sum of the useful work (Wp) available from the main products of the different transesterification processes is also shown in Figure 9.6.

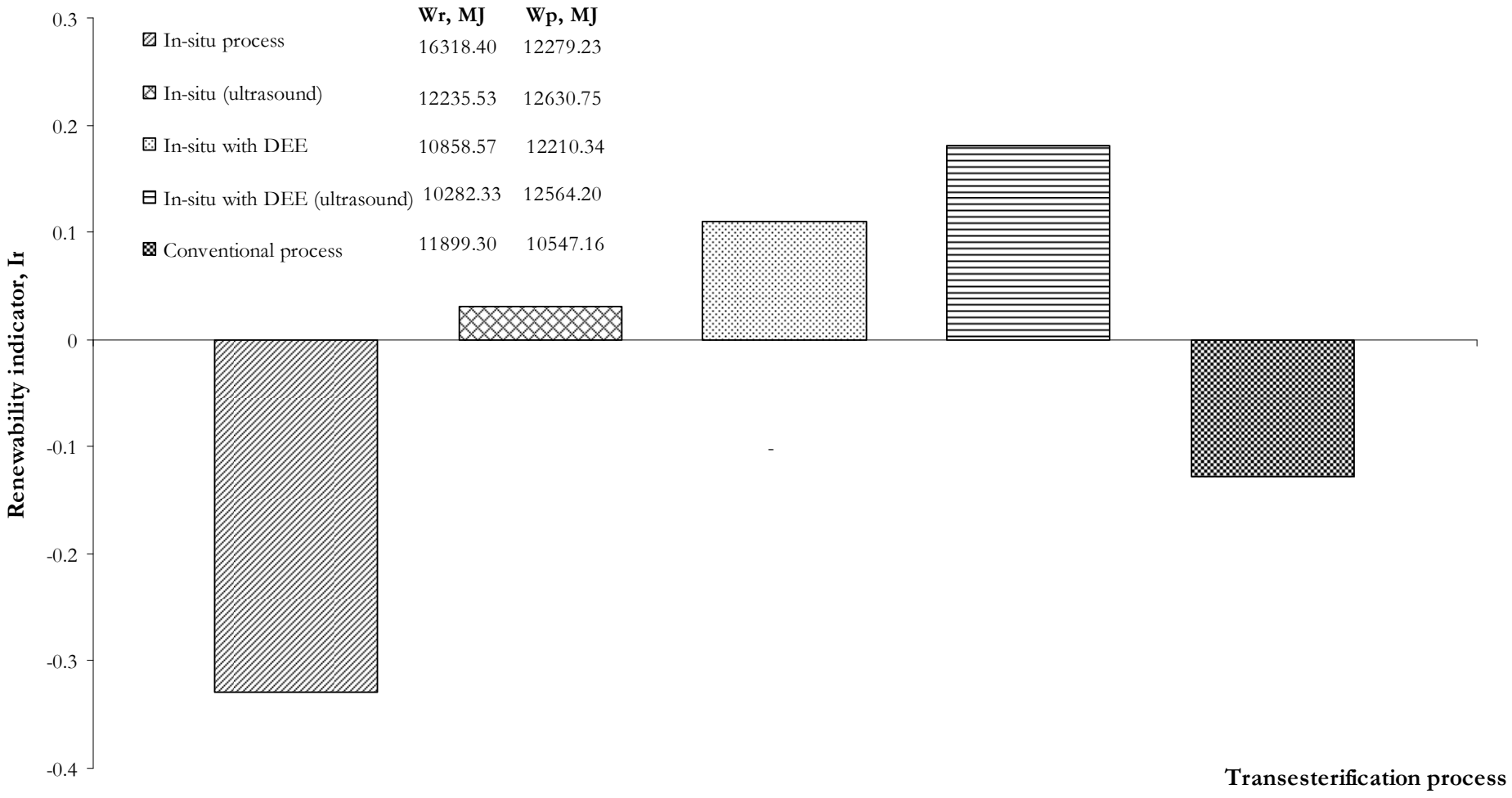

Figure 9.6. Influence of the use of alcohols and hydro-electricity on the process renewability.

With the use of fermentation derived alcohols and hydro-electricity for the microalgae production and its conversion to biodiesel, it was observed that all the modified in-situ transesterification processes (i.e. using ultrasound agitation and/or co-solvents) could be deemed renewable. The ultrasound agitated in-situ process using diethyl ether as a co-solvent was however seen to be superior to all the considered transesterification processes with regards to the process renewability.

The mechanically stirred in-situ transesterification process involving the use of reacting methanol to oil molar ratios of 315:1 was shown to still be non-renewable with the use of hydro-electricity and fermentation alcohols for the conversion process. The overall process heat demands (for the biomass drying and recovery of the stoichiometrically excess methanol) were seen to contribute to the minimum restoration work calculated for the process. With the use of diesel fuel to meet the process heating needs, the exergy cost associated with the process heating contributed 42.64% of the total process Wr.

The influence of the choice of the process heating fuels was thus carried out in the following section (9.5.8.3) to explore if any improvement in the process renewability can be achieved. The use electrically driven heat pumps to meet the heat requirement of the different processes was also considered in section 9.5.8.3.

9.5.8.3. Choice of process heating fuels or technology

The use of petro-diesel fuels for the operation of boilers was initially considered to meet the minimum process heating requirement. The influence of the choice of the boiler fuels on the process renewabilities will be carried out in this section. This section investigates the effect of the use of the common fossil fuel derived industrial boiler fuels; coal and natural gas on the estimated Ir. The use of wood pellets to meet the process heating demands was also explored. It was also considered that the process heat is supplied using heat pumps as have been well demonstrated for the heating of water and other fluids (i.e. Carrington & Knopp, 1983; Anderson et al., 1985)

The useful work available using coal and natural gas of 29.00 and 50.75 MJ/kg respectively as presented in Szargut & Morris (1987) were used in this section. The influence of coal and natural gas on the process renewability was investigated due to their widespread use for industrial heat generation. The quantity of heating fuel (kg/t microalgae biomass consumed) required to meet the minimum heat demand (after process integration) of the different transesterification processes was estimated with the assumption of a 100% conversion of the fuel to heat. The cumulative exergy cost associated with the production of the heating fuels of 30.44 and 57.87 MJ/kg consumed was used for coal and natural gas respectively (Szargut & Morris, 1987).

The average useful work available (Wp) value of 20.0 MJ/kg for dry softwood (Bilgen et al., 2004) was used for to represent the exergy content of the wood pellets.

A conservative cumulative exergy cost (CNEx) of 6.4 MJ/kg wood pellets was used for the process renewability evaluation. This CNEx value associated with the wood pellet production was presented in Patzek & Pimentel (2005) and Theka & Obernbergera (2004). This estimated value incorporates the exergy costs for the production of the NRRs consumed in the biomass cultivation, wood felling and collection, the wood chipping and drying process. The wood drying process was considered to be performed using best available technology tube bundle dryers with wood residues used as the process heating fuel (Theka & Obernbergera, 2004).

For the heat pump process heating, a conservative Carnot efficiency of 40% (Carrington, 2010) was considered for use to estimate the electricity inputs with the use of this method. The process was then assumed to be driven using hydro-electricity.

Furthermore, as highlighted in section 9.3, the use of drying technologies such as heat pump drying (Catton et al., 2010), which could reduce the estimated exergy cost for the restoration of the NRRs degraded in the biomass drying process was also considered in this section. This was carried out using a SMER of 4 kg moisture/ kWh (Carrington, 2010) as previously mentioned in section 9.3.

The estimated Ir with the use of the different process heating fuels is shown in Figure 9.7

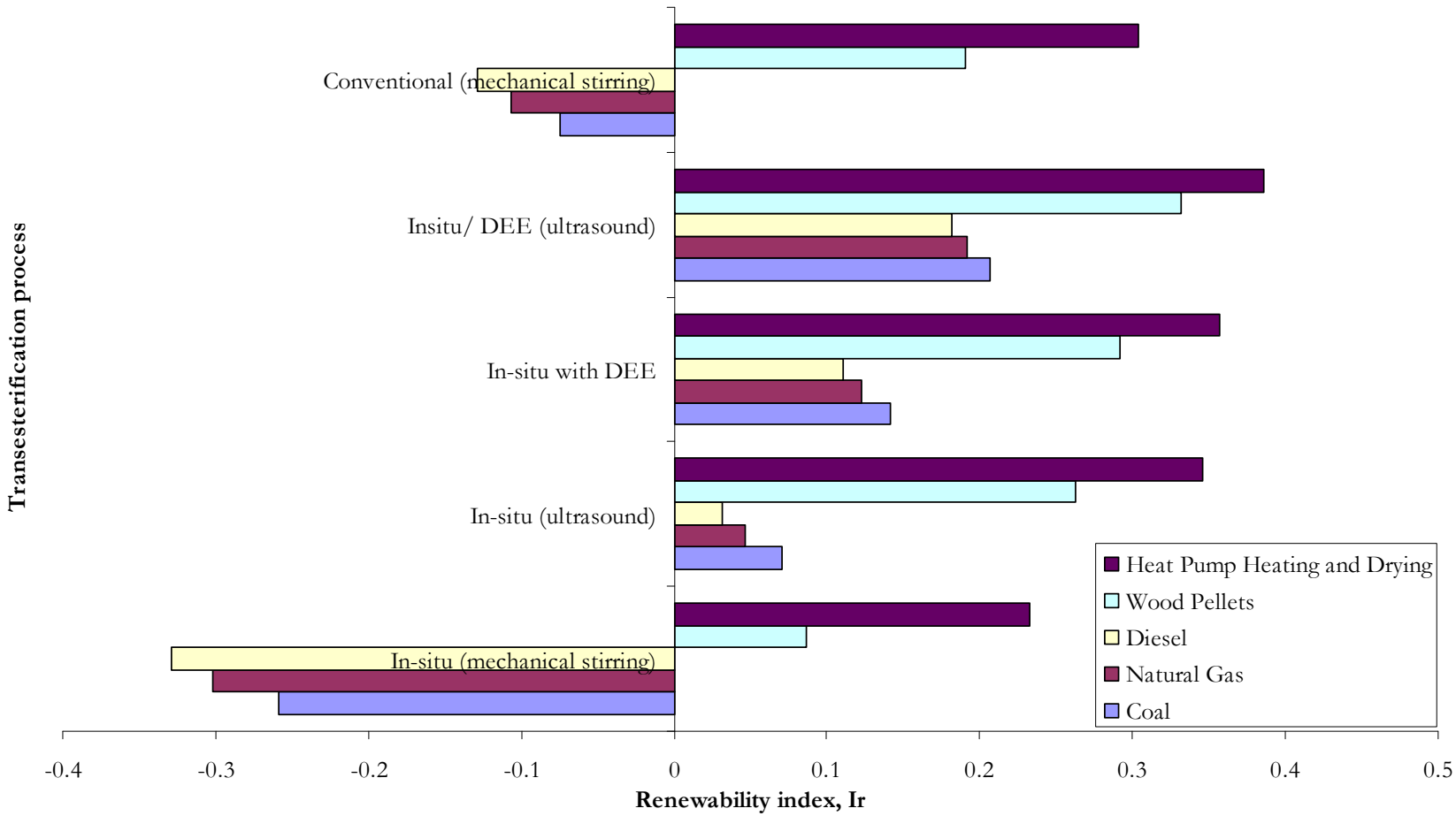

Figure 9.7. Influence of choice of heating fuel on the renewability of the different transesterification processes.

The process renewabilities presented in Figure 9.7 were estimated on the basis of the use of hydro-generated electricity and fermentation derived reacting alcohol for the biomass and biofuel production process. The electricity and heat generation was assumed to be carried out using separate heat and power (SHP) systems with a 100% operating efficiency. The heating fuels considered in this section was used to meet both the heat demands of the biomass drying process, as well as that of the process heating.

The use of heat pumps for the biomass drying and process heating was seen to result in the best improvements for the renewability indicators (Ir) of all the transesterification processes compared to the Irs obtained using other fossil fuel based heating fuels and wood pellets as the heat source. The total Wr for the different processes using wood pellets as a heating fuel was shown to be reduced by 8.1-16.0% for all the considered processes with the use of the heat pump drying and heating technology (operated with hydro-electricity). The modified ultrasound agitated in-situ process using diethyl ether was seen to be the most renewable of the considered transesterification processes.

The results in Figure 9.7 show that the type of heating fuel or technology used to meet the heat requirement of the biomass drying and process heating affects the renewability of the process. The mechanically stirred in-situ process which was previously un-renewable with fossil based fuels was seen to be renewable with the use of heat pump drying and heating, as well as using wood pellets for the process heating.

Although the use of heat pump heating and drying is not as commercially applied compared to the conventional boiler and drying systems, the use of this technology holds a lot of promise for the long term viability of biodiesel production from microalgae.

Comparing the process Ir of the different fossil fuel derived heating fuels, the use of coal was observed to lead to increased renewabilities when compared to diesel fuel and natural gas. This was attributed to the current ease of coal extraction and processing compared to the other fossil fuels.

9.5.9. Co-generation of methane from the microalgae biomass post transesterification

The assessment carried out in sections 9.5.2- 9.5.8, showed the process renewability on the basis of the cumulative net exergy consumed for the NRRs used for the biomass production and transesterification processes alone. In this study, the microalgae residues are considered to be subjected to the anaerobic digestion process to facilitate a recovery of useful energy as CH_4 and also a reduction of the organic waste load. The CH_4 production process was integrated with the biodiesel production process.

The system boundary analysed in this section had the similar process to that presented in Figure 9.4 but with the anaerobic digestion of the microalgae residues contained within the boundaries. The CH_4 product of the digestion process was considered as a process output instead of the microalgae residues. The maximum work available from the produced CH_4 (per 1 t microalgae transesterified) was thus included in the calculation of the process Wp. Nutrient rich digestate was also produced as a process output with the co-generation of biodiesel and CH_4 from the microalgae biomass.

The cumulative exergy consumed for the restoration of the degraded NRRs used for the combined transesterification and anaerobic digestion process to its initial environmental state was estimated using the process material and energetic requirements described in section 8.2.9. This was on the basis of the inputs required for the transesterification and subsequent digestion of the residues obtained using 1 t *Chlorella* biomass. This evaluation involved the use of fermentation alcohols, hydro-electricity and heat pump technology to facilitate the biomass drying and process heating for the different transesterification and anaerobic digestion processes.

The minimum work required (Wr) for restoration of the process NRRs, and the sum of the useful work available from the main process products (Wp) are given in Figure 9.8. The calculated Irs for the different transesterification processes with CH_4 recovery are also shown in Figure 9.8

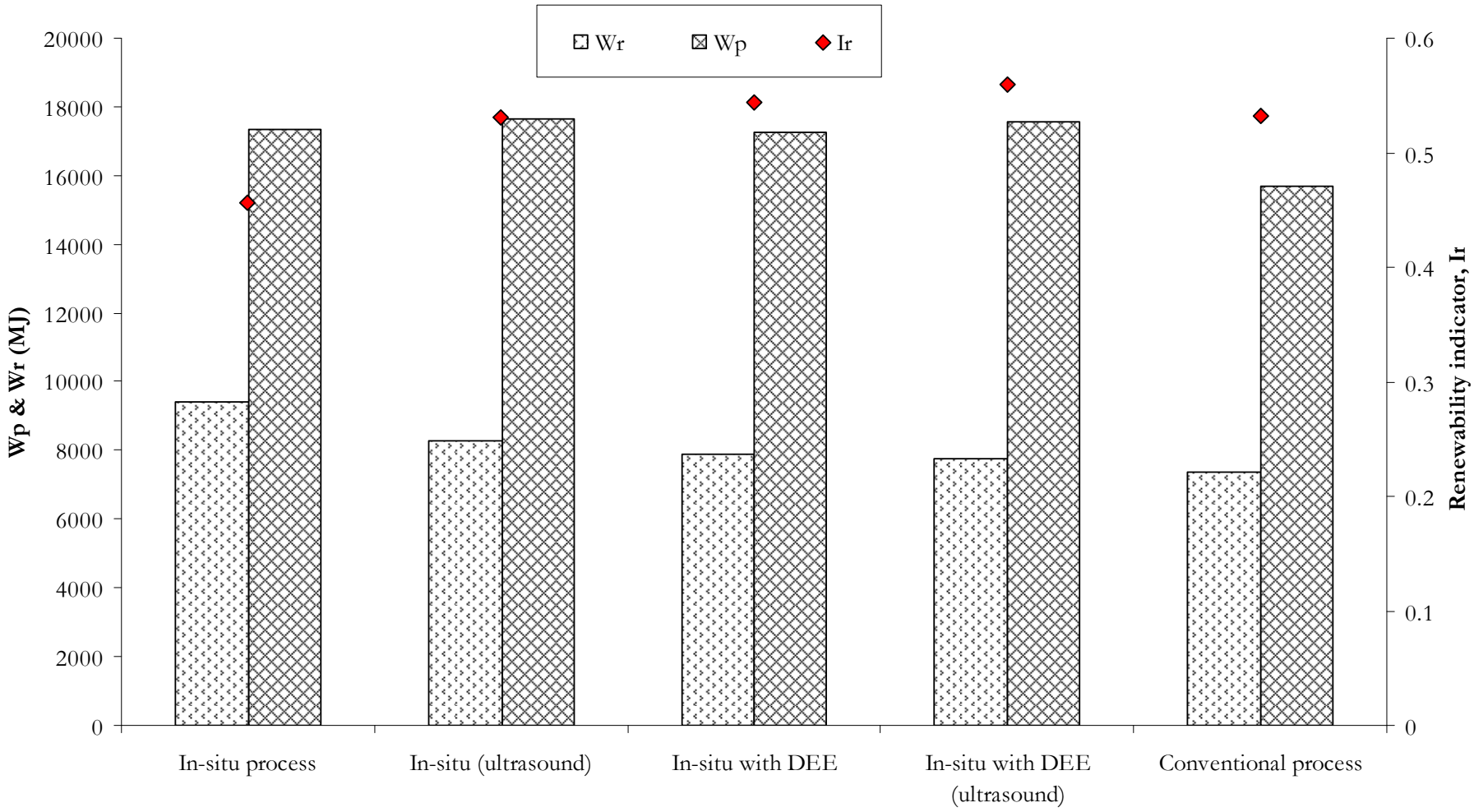

Figure 9.8. Comparison of the renewability indicators for the transesterification processes with methane recovery from the microalgae residues.

With CH_4 production from the digestion of the biomass residues integrated with the different transesterification process, the sum of the useful work available from the process products (Wp) were seen to be 39.79-48.74% higher than when the transesterification process was conducted alone. The higher Wp (MJ) values resulted in improved renewability indexes for all the considered processes as seen in Figure 9.8.

With energy recovery (in the form of CH_4) from the biomass residues, the in-situ process with ultrasound agitation and diethyl ether was adjudged to be the most renewable of the considered transesterification investigations, with the mechanically stirred in-situ process least.

The results of the renewability assessment assisted in revealing critical process issues which affect the renewability of FAME production using the in-situ transesterification process. These issues are presented in section 9.5.10.

9.5.10. Critical process issues affecting the renewability of the in-situ process

The source and energy required for the recovery of the excess reacting methanol volumes required to complete submerge the microalgae biomass for the in-situ transesterification process has been shown to be a major factor influencing the process renewability. With the methanol considered to be sourced from fossil fuels, and using fossil heating fuels, the mechanically stirred in-situ process was shown to be non-renewable, with the work required to restore the process to its initial environmental state greater than the work available from the transesterification products. The use of a reacting alcohol derived from biomass fermentation, and the supply of the process heat requirement using heat pumps (or wood pellets) are schemes which could be applied to improve the process renewabilities. The type of drying technology used for drying the post harvested microalgae biomass was also seen to be an important parameter affecting the renewability of the studied processes. The renewability of the considered process was seen to be improved with the use of heat pump drying (using hydro-electricity) compared with the use of conventional dryers operated using fossil fuels and wood pellets.

The source of the process electricity was also shown to influence the renewability of the in-situ process. For the mechanically stirred in-situ process, the use of fossil based electricity for the transesterification process contributed 32.55% of the total process Wr. The use of hydro-generated electricity for the same process was shown to contribute only 0.07% to the total process Wr. The source of the process electrical inputs is thus important when considering the overall process renewability.

9.6. Chapter Conclusion

To facilitate a comparison of the different transesterification processes in this study, the renewability of the processes were evaluated. The process renewability was adjudged by comparing the minimum restoration work consumed for the production of the non-renewable resources depleted by the conversion process with the maximum work obtained from the main process products.

The results obtained showed that in a present day fossil fuel based scenario, all the processes were deemed to be non-renewable. Here, the ultrasound agitated in-situ transesterification process with diethyl ether as co-solvent was shown to be less non-renewable than the other conversion processes.

Altering the electricity generation scheme from a fossil based source to the use of 100% hydro-electricity for the biomass production and transesterification methods led to an improvement in the process renewability for all the investigated routes. All of the processes considered were shown to be non-renewable here, with the exception of the ultrasound agitated stirred in-situ process using diethyl ether as co-solvent.

An improvement in the process renewability (with all the considered modified in-situ processes exhibiting positive Ir values) was obtained with the use of alcohols sourced from biomass fermentation. The use of heat pumps to supply the process heat and facilitate the biomass drying was shown to increase the process renewability compared to the use of commonly used fossil heating fuels and wood pellets. The mechanically stirred in-situ process was shown to be renewable with the use of fermentation derived alcohols for the

transesterification process, with the process heat and biomass drying supplied using heat pumps and wood pellets. Although not covered in this study, the use of combined heat and power (CHP) could potentially improve the renewability of the considered processes, since less NRRs would be consumed for the process electricity and heat generation.

The integration of the transesterification process with the anaerobic digestion process was also examined. The use of the digestion process for CH_4 recovery and waste treatment was observed to provide an increase in the useful work available from the process products due to the CH_4 produced. All the considered processes were shown to be renewable with the use of this scheme, with the ultrasound agitated in-situ process the most renewable, and the mechanically stirred process least. Best operating conditions of the use of hydro-electricity, biomass derived alcohols, and heat pump heating and drying were used for the estimation of the renewability conditions.

Recycling the nutrient rich digestate after the anaerobic digestion process into the microalgae cultivation unit is another scheme which could be used to further increase the overall renewability of microalgae biodiesel production using the in-situ process. The cultivation nutrients could also be supplied using the anaerobically digested residues as was demonstrated by Levine et al. (2010) for *Neochloris oleoabundans* cultivation aimed for biodiesel production. In that case, the digestate residues would be considered as the major nutrient supply without the need for the use of in-organic fertilisers as have been reported in this chapter. Post-treated organic industrial and municipal wastes could therefore be considered for use for the microalgae cultivation step.

Generally, the applied modifications to the in-situ transesterification process (i.e. use of low frequency ultrasound and co-solvent) were demonstrated to exhibit improvements in process renewability compared to the mechanically stirred in-situ process.

Although the results of the work in this thesis show promise with the use of the in-situ process for microalgae biodiesel production, further investigations which may be carried out to improve the use of this feedstock and process are presented in Chapter 10.

A general discussion of the difficulties and limitations encountered with this study are also be highlighted in Chapter 10.

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10. STUDY LIMITATIONS AND FUTURE RESEARCH RECOMMENDATIONS

10.1. Overall Conclusions

Locally obtained *Chlorella* samples were obtained, laboratory cultivated and used as a feedstock for fatty methyl ester production using the in-situ transesterification process. The *Chlorella* samples were shown to have a neutral lipid content of 0.275 g/ g dry microalgae biomass with a free fatty acid content of 5.11 (on an oil weight basis).

The influence of the reaction variables: reacting alcohol to oil molar ratios, temperature, time, biomass moisture content and stirring on the acid catalysed in-situ transesterification process was investigated. The results obtained showed that increases in the reaction temperature and alcohol to oil molar ratios favoured FAME production. The percentage methyl ester conversion trends were also seen to increase with process stirring and a reduction in the reacting biomass moisture content. To further reduce the reacting alcohol volumes used in the in-situ process, the application of modifications i.e ultrasonic agitation or/and co-solvent use were explored. The combination of the low frequency ultrasound stirring and co-solvent integration in the in-situ process was shown to further increase the percentage FAME conversion, biodiesel and FAME yields with reduced alcohol to oil molar ratios.

The generation of additional energy carriers (CH_4) from the post transesterified microalgae residues was considered using the anaerobic digestion process. Batch and semi-continuous laboratory experiments were used to quantify CH_4 yields from this otherwise waste resource. The co-digestion of the residues with glycerol quantities co-produced during the transesterification process was also carried out. CH_4 yields of 222-268 ml CH_4 /g and 231-286 ml CH_4/g were recorded for the residues and co-digested samples respectively.

Using process modelling tools, the laboratory investigated processes were upscaled and a renewability analysis performed. . In a present day scenario (with the use of fossil fuel sources for the production of the process raw materials, such as for methanol and sulphuric acid production, and electricity), all the transesterification processes were shown to be non-renewable. The influence of the choice of the electricity generation scheme, raw material source and the type of heating fuels (including heating and drying technology) on the process renewability was also examined. The process renewability of the in-situ transesterification of microalgae lipids to biodiesel was found to significantly improve with the use of renewable electricity, reacting alcohols from biomass fermentation and heat pump technology to facilitate the biomass drying and process heating

Before discussions on opportunities for future research on the production of petro-diesel replacements using microalgae lipids are presented, limitations encountered during the course of this study which may influence the study results will be presented in the following section (Section 10.2).

10.2. Study Limitations

The major limitation encountered with this study was the restrictive microalgae biomass quantities available for the experimental trials. Due to the cultivation method (batch set up) utilised for the microalgae production, only small quantities were available for the laboratory investigations. Despite the continuous[32] pooling of the harvested biomass samples, at any given time, the available microalgae quantities were still insufficient to facilitate a wider range of investigations.

Other limitations or difficulties experienced during the investigations in this study include:

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[32] The microalgae biomass was cultivated using a batch set-up (5 L flasks) for the duration of the experimental investigations, pooled and stored in a deep freezer.

10.2.1. Microalgae production and lipid content

The microalgae cultivation was carried out using batch cultivation systems with culture conditions (temperature, light intensity and culture nutrients) as described in section 3.2.2. The estimated lipid content of the microalgae biomass is thus representative of the particular cultivation conditions used in this study. The use of a continuous biomass cultivation system and alterations in the culture conditions may result in microalgae samples with different lipid content, as detailed by Hu et al, 2008. This cultivation route and its influence on the biomass oils were, however, not explored further.

The samples used for the lipid content determination experiments were obtained from pooled harvested microalgae samples which were collected and stored in a deep freezer to allow for sufficient biomass quantities required for the experiments. The lipid content (g lipids/g dried microalgae biomass) was thus treated as a mean of the lipid yields of the *Chlorella sp* (under specific culture conditions). It may therefore not be particularly representative of an individual microalgae batch harvest.

10.2.2. Transesterification processes

The reacting molar ratios of methanol to oil used in this study were based on the estimated neutral lipid content of the *Chlorella sp*. As highlighted from the result of higher biodiesel yields obtained after the application of the in-situ transesterification process, these lipid contents represent only the extractable lipid obtained by the use of specific extraction solvents and methods.

This meant that the actual reacting molar ratios of methanol to oil used for the in-situ transesterification processes could be lower than stated. However due to the limitations faced with the elucidation of the absolute neutral lipid content of the microalgae biomass, the estimated neutral lipid content was used as a basis for the determination of the stoichiometry of the transesterification processes in this study.

Although utmost care was taken in the purification of the transesterification products, due to potential losses that may be encountered during the washing steps, the biodiesel and FAME yields (g/g dried microalgae biomass) reported for the different processes may have been underestimated.

As mentioned in Chapter 4, the dried *Chlorella* biomass used in the transesterification process was on the basis of the elimination of the free biomass water and not the more tightly bound cellular water. This level of drying was used since it would most likely be reflective of what would be practically available for use if microalgae biomass is to be applied for biodiesel production. The use of more dehydrated microalgae biomass samples may influence the progress of the transesterification process differently. Furthermore, as highlighted in Section 4.4, a kinetic treatment of the in-situ data would be helpful in providing a clearer picture on the influence of the in-situ transesterification variables on the process.

10.2.3. Anaerobic digestion trials

The adaptation of the digestion inoculum initially at a process temperature of 35°C could have influenced the results of the anaerobic digestion trials on the effect of reaction temperatures on the methane yields from the post transesterified microalgae residues.

The experimental trials did not consider the recovery of the glycerol co-product from the transesterification process due to the fact that the material losses from the aqueous washes applied might reduce the glycerol quantities recoverable. The experiments were designed to obtain residues with the least entrained glycerol possible, facilitated by multiple washes. With glycerol from external sources used for the anaerobic experiments to account for that produced by the transesterification process. The use of reagent grade glycerol as a co-digestion feedstock for the anaerobic process to represent the glycerol by-product of the microalgae transesterification might not be truly representative of this feedstock obtained in practice.

10.2.4. Process modelling and renewability assessment

Due to a lack of data on the commercial production of oils or biodiesel from microalgae, the assumptions used in the modelling of the processes were mainly based on the experimental results obtained in this study. Current industrial biodiesel production practices, as described in the literature, were also used in the process modelling. These assumptions may however not be representative of best technology scenarios. For example, the extent of reacting methanol recovered for all the processes was based on maximised reactant recovery which may not be achievable in practice. In practice, a balance between energetic cost input and the price of methanol would most likely influence the extent of the alcohol recovery.

This project was also limited in that it did not attempt to optimise the design and operation of the industrial distillation units, which could have been helpful in the modelling of the distillation columns in this study. Commercial software (ASPEN plus) was used to meet this goal.

Because this thesis did not deal with microalgae cultivation, the biomass production step used in the renewability evaluation of the different processes considered the same inputs for the biomass cultivation stage for all the processes (Chisti, 2008a). This assumption was used to present a simplified best case biomass production and harvesting system, and may therefore not be representative of the *Chlorella sp* studied in this thesis.

10.3. Further Research Recommendations

This study provided preliminary experimental information on the application of the in-situ transesterification process for biodiesel production using microalgae biomass. Also the use of the microalgae biomass residues for the production of CH_4, which could serve as an additional energy production route, was investigated. From the generalised renewability evaluation results in this thesis, although positive renewabilities were seen with the use of the modified in-situ process, more work is required on the production of petro-diesel

replacements using microalgae lipids. However due to the constraints of the present project, the additional directions which could have been explored could not be undertaken. This section highlights further research which could be carried out in this research area.

Further research on the influence of the microalgae biomass lipid content and productivities on the renewability and overall cost of the in-situ scheme over the transesterification method is required. This may provide information to the preferred transesterification method to be used at different levels of microalgae lipid contents.

The influence of the choice of cultivation systems (i.e. batch or continuous) and conditions such as light intensity, process temperatures on the lipid content should be studied to highlight optimum conditions leading to maximum biomass and lipid productivities. The integration of genetic manipulation techniques could also be explored as a useful tool to facilitate the development of strains with improved productivities and lipid yields.

A more comprehensive assessment of the biomass production and transesterification scheme NRR consumption should be carried out for particular microalgae species. This investigation should include an examination of the effect of the degree of the drying of the harvested microalgae on the conventional lipid extraction process, which was not considered in this thesis. The assessment in this study used an assumption of the same energy input into the biomass cultivation and harvesting stage, which might not be true in practice.

Regarding the conversion of microalgae lipids via the application of the in-situ process, the use of high moisture containing biomass samples immediately after the harvesting process could be further examined. This could involve an experimental set up with the integration of a water adsorption apparatus i.e. as demonstrated by Lucena et al. (2008) which could facilitate the constant removal of water from the transesterification reactants. The efficient use of high moisture containing biomass for the in-situ process could then potentially improve the calculated renewability index for the overall biomass production and in-situ transesterification process. The practical application of novel drying technologies, for example the use of heat pump drying for microalgae biomass as reported by Catton et al. (2010) may be further explored to improve the use of this feedstock and conversion method.

More research into the design of in-situ reactors could be carried out to facilitate a reduction of the process reacting alcohol requirement and potentially improve the biomass-biofuel conversion and the renewability. Studies on the influence of replacing the distillation units with other separation technologies i.e. membrane separation on the process energetics could also be carried out.

More research is still required for the microalgae production and harvesting steps with the proposal of low cost and energy requiring technologies, vital to the eventual practicality of commercial microalgae biofuel production systems.

The co-digestion of glycerol with the microalgae residues was demonstrated to result in improvements in the CH_4 yield from the digested feedstock (compared to when the post transesterified residues were digested alone). However, the practicality of glycerol use in the anaerobic process should be further assessed to see if greater economic gains are obtainable from the CH_4 produced over the revenue generated from its sale as a chemical feedstock. With the increasing production of biodiesel globally, this recommended investigation could also include the evaluation of the costs or available quantities in which crude glycerol would be preferentially utilised for the anaerobic digestion process.

The ideal utilisation route of the CH_4 generated from the microalgae residues post transesterification could also examined. This could include an economic assessment to see if it should be used onsite to meet some of the process electrical and heating requirement or preferentially upgraded and sold for use as a domestic heating or transport fuel or for use in CHP systems. The influence of the use of thermophilic digestion temperatures for CH_4 production from the microalgae residues could also be explored. This would provide information on the improvements that could be achieved with an increase in the process temperature.

The use of the in-situ transesterification method for biodiesel production from microalgae in this study was compared with the conventional conversion process solely on the basis of a simplified renewability analysis in this thesis. Other comparisons on the use of this method could be further examined. The economics of microalgae derived biodiesel conversion processes were not covered in this thesis, mainly due to a lack of the

demonstrations and conflicting reports in the literature. More research into the capital and operational costs involved with the various transesterification methods is required to provide information on the economics and practicality of biodiesel from microalgae.

Further, the use of microalgae biomass for the production of petro-diesel replacements using alternative conversion methods such as the use of the Fischer Tropsch process for "green diesel" production from microalgae should be investigated. A comprehensive renewability assessment of the application of such technologies should also be carried out to see how they compare with transesterification methods.

10.4. References

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