Fuel 116 (2014) 103–108

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method

Krishan Ramluckan [a], Kandasamy G. Moodley [a], Faizal Bux [b,*]

[a] Department of Chemistry, Durban University of Technology, Durban 4000, South Africa
[b] Institute for Water and Waste Water Technology, Durban University of Technology, PO Box 1334, Durban 4000, South Africa

HIGHLIGHTS

- Thirteen solvents with varying characteristics were used in the extraction of algal biomass.
- Ethanol, chloroform and hexane produced average of >10% lipid extracts.
- Time-based trials showed optimum extraction efficiency at 3 h.
- Binary mixtures gave greatest extraction efficiency with 1:1 chloroform:ethanol.

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form 25 July 2013
Accepted 28 July 2013
Available online 14 August 2013

Keywords:
Microalgal biomass
Soxhlet extraction
Lipids
Binary mixtures
Biodiesel

ABSTRACT

The use of solvents for the extraction of lipids from algal biomass has been a method of choice for many years. The soxhlet extraction method was chosen because of its simplicity in operation, relative safety and potential for upscaling to industrial plant level. The source of algal biomass was a raceway pond. *Chlorella* sp. which is known to produce larger amounts of oil than other indigenous species was used for this investigation. Thirteen solvents spanning a range of polarities and solubilities were selected for this study. Extraction methodology involved the use of single solvents, selected binary solvent mixtures and time-based extractions which were varied from 1 to 5 h. Ultraviolet (UV) spectroscopy was used to determine chlorophyll content of the lipid extracts and gas chromatography was used for the identification and quantitation of the lipids. Analysis showed that ethanol, chloroform and hexane were generally more efficient in the extraction of lipids than the other solvents studied, producing lipid contents in excess of 10%. The time-based trials indicated that the optimum extraction time was 3 h for the solvents selected. The binary solvent mixture with the greatest extraction efficiency (i.e. >10% lipid extract) was obtained with the 1:1 mixture of chloroform:ethanol. Chlorophyll quantities varied for each solvent extract with chloroform and methanol producing the highest values at >1%. Chromatography was effective in identifying lipids used in the production of biodiesel.

1. Introduction

The gradual global depletion of fossil reserves has made it imperative for most countries to seek viable alternative sources of energy [1]. The continued use of petroleum based fuels is becoming unsustainable because of the diminishing fuel reserves worldwide. This, compounded by the environmental impact of carbon dioxide emissions, has prompted the search for more environmentally friendly and renewable fuel sources [2,3]. For these reasons, renewable and carbon neutral biofuels have grown in importance as environmental and economically sustainable fuels. First generation biofuels sources from edible oil such as soybean, palm and canola have a negative impact on food supplies, while second generation non-edible sources, exemplified by jatropha, require vast amounts of arable land. In the light of the above observations, algae based biofuels are considered to be a viable alternative since they do not impact on food supply. Furthermore, they can also be grown on any available land, water or saline [4,5]. Microalgae, like plants, use sunlight and the photosynthetic process to produce lipids, but they do so more efficiently [2]. Microalgae have therefore been regarded as a promising and potentially renewable fuel source that could replace fossil fuels [5].

Since the amount of lipids in microalgae is relatively small (on average between 15% B 30% depending on the algal species), it is crucial that the selected extraction procedure is efficient enough to extract the maximum quantity of lipids possible [6,7]. Lipids are made up of a diverse group of biological substances, some of

* Corresponding author. Tel.: +27 31 3732597; fax: +27 31 3732777.
E-mail address: faizalb@dut.ac.za (F. Bux).

0016-2361/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fuel.2013.07.118

which are polar while others are non-polar [8]. The extraction of lipids from microalgal biomass is thus a challenging task which is critical in the determination of the overall economics of biodiesel production [4]. Although lab-scale extraction of lipids is fairly routine, the variables affecting lipid extraction from microalgae are not well understood and make up-scaling for commercial production a greater challenge [9].

Solubility of lipids is an important criterion for the extraction of lipids. It depends heavily on the type of lipids present and the proportion of polar and non-polar lipids in the sample. Hence, several solvent systems may be considered depending on the type of sample and its components [10]. Several extraction routes may produce liquid fuels from microalgal biomass [11]. The type of organism and the permeability of its cell wall will govern the choice of solvent system for lipid extraction and the extraction efficiency of solvent mixtures [12]. Afi et al. reported presence of polysaccharide wall and tri laminar sheath (TLS) that were composed of highly aliphatic, non-hydrolyzable macromolecules (algaenan) in *Chlorella emersonii*. The resistant outer wall (sheath) was not present in *Chlorella vulgaris*. *Chlorella vulgaris* was reported to contain only a classical polysaccharide cell wall whereas, *Chlorella emersonii* contains both a classical cell wall as well as a resistant trilaminar outer wall (TLS). TLS are composed of solvent insoluble macromolecules with unusually high resistance to chemical degradation [13].

An optimum lipid extraction process for microalgal biodiesel production needs to be lipid specific (in order to minimize the co-extraction of non-lipid contaminants) and selective towards the required lipid fractions. The use of dry biomass may lead to a significant increase in energy costs since a drying step is required before the conversion step. Alba et al., have reported that a wet biomass-handling process, such as hydrothermal liquefaction (HTL) is more suited to the production of liquid fuels from wet microalgae. This is supported by the fact that it reduces the high energy cost for thermal drying and the need for removal of water [14]. Even though the classic chloroform-based lipid extraction protocol (Folch method) is effective for the majority of microalgal lipid analyses, an alternative organic method which is more user friendly would be more suited for up-scaling [9].

The search for a cost effective and efficient method for the production of biodiesel has been in the forefront of technology being developed since the late 1950s [6,7]. The first step in this process requires that a method or methods be developed for cell/cell wall disruption and the extraction of lipids with some degree of efficiency. Subsequent steps would involve the esterification of the lipids and production of biodiesel. Nevertheless the solvent extraction technique is probably one of the few methods that can be up-scaled for mass production of biodiesel with relative ease. However, optimization of this technique for up-scaling has not been comprehensively investigated. It was anticipated that any solvent/s that extracted the maximum quantity of lipids under optimized conditions would be considered for further investigation. The solvent chosen should also be reasonably inexpensive and non-toxic.

The Folch method or its variant, the Bligh and Dyer method, have been used extensively in the extraction and quantitation of lipids [15]. Although many solvents have been tested either individually or in combination for the extraction of lipids, there are no reports of a concerted study on the use of a range of solvents and solvent mixtures involving the soxhlet extraction technique.

Solvents used for the extraction of lipids tend not to discriminate when extracting compounds present in algae. This would imply that chlorophyll, carotenoids, pheophytins and associated degradation products would form part of the lipid extract and hence subsequently skew the results obtained for lipid quantities. In this study, an ultraviolet (UV) method was therefore used to determine the total amount of chlorophyll obtained after extraction by each of the solvents. Furthermore, a chromatographic method was optimized primarily for the identification and quantitation of lipids which are targeted for use in biodiesel production. Both these aspects are integral parts of the overall investigation which examines the use of a variety of solvents and their efficiency in the extraction of lipids from algal biomass via the soxhlet extraction method.

2. Materials and methods

2.1. Apparatus

A Büchi B 811 Soxhlet (Labortechnik, B. (1996) BÜCHI Universal Extraction System, Switzerland) apparatus was used for the extraction of lipids [16]. All solvents used were of HPLC grade with purity $\geqslant$99.5% (Sigma Aldrich, USA). The solvents from the sample extracts were evaporated using a rotary vacuum evaporator (BÜCHI Laboratorium-Technik AG, Switzerland). All weighing was carried out on a calibrated analytical balance (Boeco, Germany). Chlorophyll analysis was conducted using the Cary 50 UV–Vis spectrophotometer (Varian, Australia). Chlorophyll *a* and chlorophyll *b* standards (>95% purity) were used for calibration (Sigma Aldrich, USA). A gas chromatograph Shimadzu GC 2014 (Shimadzu Corporation, Kyoto, Japan) fitted with a FAME capillary column (SP 2380, 30 m $\times$ 0.25 mm I.D., 0.20 μm Supelco, USA) and a flame ionization detector (FID) was used for quantitative analysis. A mixed standard containing 10 mg/mL of a 37-component fatty acid methyl ester (FAME) standard was used for the calibration of the GC.

2.2. Sampling

The algal biomass used for this study was obtained from a resident laboratory raceway pond, of approximately 3000 litre capacity, designed specifically for the cultivation of algae. The propagation of the algae was carried out using BG-11 medium, modified with controlled carbon dioxide sequestration [17]. The biomass used for analytical work consisted of a mixed algal culture containing the predominant species *Chlorella* sp. which is indigenous to KwaZulu Natal in South Africa. The wet biomass was harvested, centrifuged and oven dried between 50 °C and 60 °C for approximately 24 h. Dried flakes of the biomass were obtained and pulverized using a grinder.

2.3. Experimental

Pre-screening of the algal biomass for the presence of lipids was conducted using the Nile Red staining method [18,19]. The Büchi B 811 (Switzerland) soxhlet extraction system offers four modes of extraction, viz., soxhlet standard, soxhlet warm, hot extraction and continuous flow. Initial trials using the standard method and continuous flow method were conducted. The continuous flow method was chosen as it proved more efficient than the standard method for the samples analyzed (data not shown). At the end of the extraction process, which typically lasts a few hours, the solvent cup with the lipid extract is removed, the solvent evaporated and the mass of lipid extract remaining is measured [20].

All analyses were performed in triplicate as follows:

1 g sample of pre-dried and pulverised biomass (microalgae) sample was weighed accurately into a soxhlet glass sample tube. The sample tube was transferred to the extraction chamber in the soxhlet apparatus. A 100 mL aliquot of the extraction solvent was transferred into the solvent cup and placed on the heating plates. The cooling water supply to the condensers was opened

Table 1
Solvents used for soxhlet extractions showing their relevant properties in order of increasing polarity index.

	Solvent	Polarity index Units	Boiling point °C	Density @ 25 °C g/mL
1	Petroleum ether	0.1	35.0–60.0	0.640
2	Hexane	0.1	69.0	0.659
3	Cyclohexane	0.2	80.7	0.779
4	Isooctane	0.4	99.2	0.690
5	Toluene	2.4	110.0–111.0	0.865
6	Benzene	2.7	80.0	0.874
7	Diethyl ether	2.8	34.6	0.706
8	Dichloromethane	3.1	39.8–40.0	1.325
9	Isopropanol	3.9	82.0	0.785
10	Chloroform	4.1	60.5–61.5	1.492
11	Acetone	5.1	56.0	0.791
12	Methanol	5.1	64.7	0.792
13	Ethanol	5.2	78.0	0.789
	Water (for comparison only)	10.2	100.0	1.000

Data courtesy of aldrich handbook of fine chemicals, 2009–2010 [18].

to ensure continuous recycling of the solvent and temperature selected as per the Büchi manual for extraction in the continuous mode [16]. Boiling point temperatures were programmed using boiling points of the solvents shown in Table 1. The extractions were conducted for 3 h. On completion of the extraction process, the samples were left to cool for at least 15 min after which the solvent cups with the lipid extracts were removed. The extract was transferred quantitatively to pre-weighed round bottom flasks of 250 mL capacity. The solvent was removed using a vacuum rotavapor. The flasks were then transferred to a desiccator until cool and then reweighed. The optimum conditions for temperature, solvent volume and mass used for the soxhlet extraction were dictated by the manufacturer's manual for laboratory scale applications. The thirteen solvents used for extraction are shown in Table 1 while the results appear in Fig. 1.

Time-based extractions were conducted at hourly intervals from one to 5 h (Fig. 2). The binary ratios used for chloroform, ethanol and hexane are shown in Fig. 3. Extraction protocols were the same as those used for single solvent extractions.

Chlorophyll extraction was conducted by the same method used for lipid extraction. The total chlorophyll content was determined by UV spectroscopy (Varian, UV Cary 50). Calibration standards 1, 5 and 10 ppm were used for calibration of the instrument at wavelengths scanned in the range 300 –700 nm. The specific wavelengths for chlorophyll *a* and chlorophyll *b* were selected from the optima obtained from each scan. Standards were diluted with HPLC grade acetone prior to analysis. Acetone was used as a blank to establish the baseline for the range in which measurements were made.

Chromatographic using the GC 2014 fitted with an FID detector was used for the identification and quantification of the lipids present in the algal biomass. Optimization of the method produced the following parameters; a temperature program with initial temperature set at 60 °C held for 2 min and a ramp rate of 10 °C/min to 100 °C with zero hold time, a further ramp rate of 7 °C/min to a maximum temperature of 240 °C held for 1 min was used. The injector temperature and detector temperatures were set at 250 °C. The standard and sample injection volume was 1 ΦL. All samples were passed through a 0.45 Φm filter before injection into GC. A mixed standard containing 10 mg/mL of a 37-component fatty acid methyl ester (FAME) standard was optimized for the determination of the esters produced. The lipid extracts were esterified using a slightly modified method by D'Oca et al. [21]. The optimized measurement conditions for the temperature program method was used for the analysis of FAME produced from the esterification of sample extracts for chloroform, ethanol and hexane.

2.4. Calculation of quantities of lipids and chlorophyll

The mass of the total lipid extract (M_L) was determined from the difference in the flask mass before and after extraction. The value was expressed as a percentage of the original mass of biomass weighed (M_B). The exact amount of the lipid extract was determined after subtraction of the chlorophyll content. The chlorophyll content (C) was obtained from the standard calibration graphs for chlorophyll *a* and *b* and expressed as a percentage of the lipid extract obtained for each solvent or binary solvent mixture.

The quantity of lipid extract in the sample was calculated using the following general formula:

$$\% \text{ lipids} = (M_L \times 100)/M_B - C, \text{ where}$$

M_L = Mass of lipid extract
M_B = Mass of biomass weighed
C = Chlorophyll content

The quantitative analysis for chromatography using the internal standard method was conducted using the following [22]:

$$\text{Internal Response factor (IRF)} = \frac{\text{area}_{IS} \times \text{amount}_{SC}}{\text{amount}_{IS} \times \text{area}_{SC}}$$

where, IS = internal standard and SC = specific compound of interest.

$$\text{Amount of specific compound} = \frac{\text{amount}_{IS} \times \text{area}_{SC} \times \text{IRF}_{SC}}{\text{area}_{IS}}.$$

3. Results and discussion

3.1. Multisolvent lipid extraction

Table 1 shows the properties of the thirteen solvents used for the extraction of lipids from algal biomass. Since the principle of like dissolves like would govern the type of lipids extracted, di-

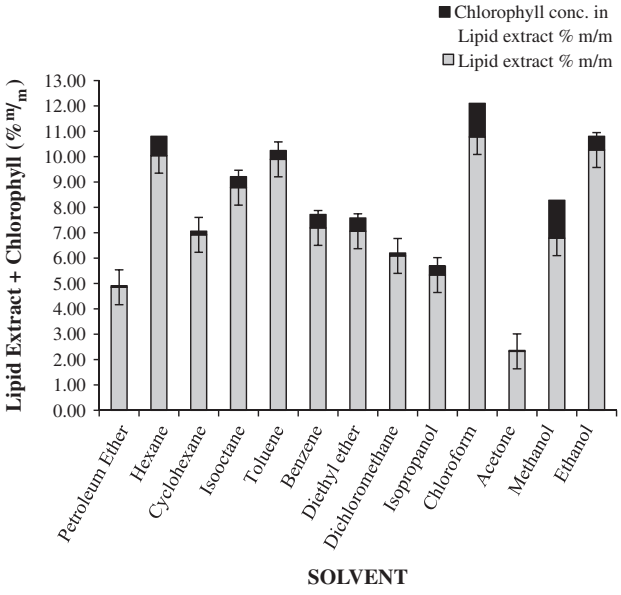

Fig. 1. Quantities of lipids and chlorophyll extracted by the soxhlet method using the thirteen solvents.

verse polarities of solvents were required for extraction [10]. The fact that different lipids have different polarities means that it is impractical to select a single organic solvent to extract them all. Therefore the total lipid content determined by solvent extraction depends on the nature of the organic solvent used to carry out the extraction. The total lipid content determined using one solvent may differ from that determined using another solvent [23]. The number and type of solvents chosen was purely random but accommodated polar, non-polar and intermediate polarities. This was done to ensure optimal lipid extraction. The polarity indices of the solvents are shown relative to that of water, hence the lower the indices relative to water, the greater the degree of non-polarity (Table 1).

The results (Fig. 1) shows the average of triplicate analyses conducted with each solvent. It also illustrates the amount of the lipid extracted in relation to the amount of chlorophyll for each solvent. The relative error for each solvent is also shown.

The highest values of the lipid extracts i.e. those above 10% was achieved by extraction with chloroform, ethanol, and hexane in an optimum time of 3 h. The remaining solvents gave lipid extracts of between 2% and 10%. Since chloroform, ethanol and hexane each proved to have the highest efficiencies for the extraction of lipids from the biomass, it may be concluded that a range of lipids varying from polar to non-polar were present in the algal biomass. It was also significant to note that acetone was the solvent with the lowest efficiency, extracting an average of 2.32% of lipids, when compared to chloroform which extracted the highest quantity of lipids, with an average value of 10.78% lipids. This also serves to confirm chloroform as the solvent of choice in reports by Bligh and Dyer and Folch and Christie, for the extraction of lipids [21]. A comparative study was conducted using the optimized chloroform:ethanol (1:1) and a modified Bligh and Dyer method using chloroform and methanol mixture (1:1) [8]. The results compared favourably with the Bligh and Dyer method which produced a slightly higher extract but with less than 1% difference between the two methods and a standard deviation of triplicate results for each method being <1. Chloroform, ethanol and hexane were selected for further trials owing to their extraction efficiency being greater than those of the other solvents tested.

The solvents used were not expected to extract chlorophyll with the same efficiency as they would for lipids. This is illustrated by chloroform extracting the highest quantity of lipids and methanol extracting the highest quantity of chlorophyll (Fig. 1). Several researchers have used ethanol and acetone as solvents for chlorophyll suggesting that these solvents provide the best efficiency [24–28]. For the biomass sample used for this experiment, acetone showed the least efficiency in the extraction of both lipids and chlorophyll. Although methanol did not extract the highest quantity of lipids, it did extract the highest amount of chlorophyll when compared with the other twelve solvents. The work of Dere et al. (1998) support the fact that methanol is the best solvent for extraction of chlorophyll. They also noted that this is probably due to the type of algae and its cell wall structure [29]. The use of methanol is not encouraged since it is more harmful than ethanol and acetone. It has been shown that the use of methanol as an extraction solvent results in an unstable solution and leads to the formation of chlorophyll *a* degradation products [26].

3.2. Effect of time on extraction efficiency

Extraction of lipids with chloroform, ethanol and hexane were conducted by varying the extraction period with incremental increases from 1 to 5 h (Fig. 2). Normal extraction trends would be expected to approach a maximum as extraction time increases after which it would be expected to plateau as incremental extraction time increases. This trend, however, is not strictly followed by

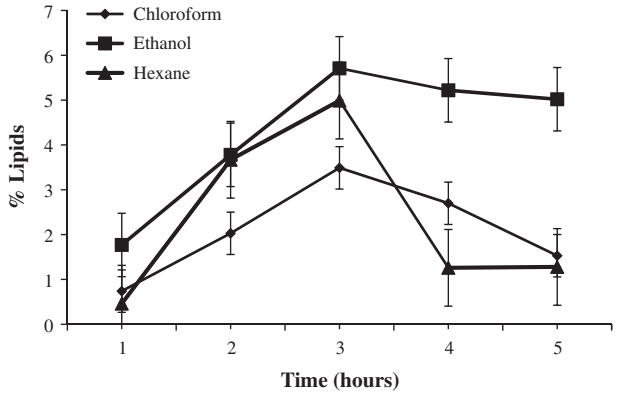

Fig. 2. Lipid concentrations obtained for chloroform, ethanol and hexane solvents with varying time.

the solvents tested. Ethanol simulates the expected trend to a certain degree, but hexane shows a sharp decrease after the 3 h maximum and chloroform shows a gradual decrease. The extraction behaviour of chloroform and hexane after 3 h could be attributed to the possible formation of volatile degradation products which may have caused the decrease in the amount of lipids extracted [23]. All three solvents show a decrease in extraction efficiency after 3 h, but in hexane the decrease is more pronounced probably indicating that greater degradation of lipids with increase in time of extraction [26].

Chloroform, ethanol and hexane show distinct optima at 3.49%, 5.71% and 4.99% respectively after 3 h of extraction. The sudden decrease in the content of the lipid extract could also be attributed to inconsistencies arising from the homogeneity of lipids in the algae. Nile red staining has shown that not all algal cells contain the same quantities of lipids [18,19]. However, repeated trials on the same sample showed similar trends in the extraction behaviour of the solvents thus reinforcing the fact that with extended time some form of degradation was taking place. This resulted in a gradual decrease in quantities of lipids extracted after 3 h. The extraction time of 3 h was thus taken as the optimum for the extraction of lipids.

Theoretical maximum values are not yet known for a cell's oil content, and oil content is highly specific to species and growth

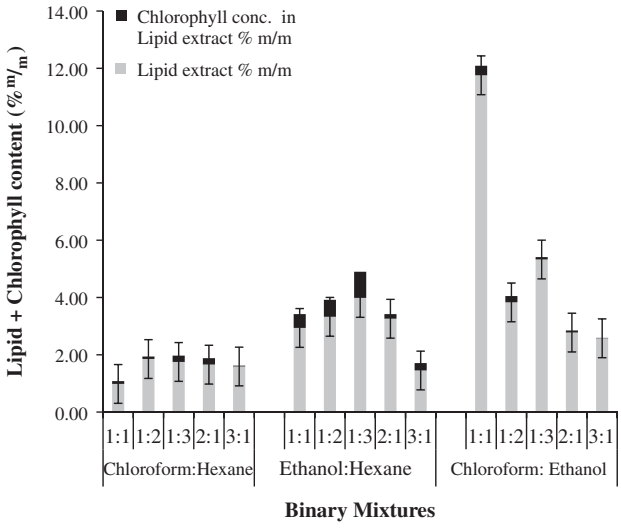

Fig. 3. Comparison of lipid content obtained by soxhlet extraction of algal biomass using binary mixtures of solvents.

conditions. Furthermore, lipid accumulation often corresponds with reduced biomass productivity thus high-growth requirement of production systems may necessitate species with lower lipid content and higher growth rates [27]. The relative errors are also indicated with the percentage lipid extract (Fig. 2). It should be noted that the biomass cultures used in this study were not optimized for optimum lipid production; hence yields from different batches of harvested biomass were not guaranteed to produce the same quantity of lipid extract. However, it was assumed that their behaviour towards solvents used in their extraction would be consistent. This explains the discrepancy in the lipid content between the batch used for the analyses shown in Figs. 1 and 2 for extractions with chloroform, ethanol and hexane.

3.3. Effect of binary solvents mixtures on extraction efficiency

A comparison of the various ratios of the three binary mixtures selected (Fig. 3) indicates that the 1:1 chloroform:hexane mixture extracted the least amount of lipids at 0.98% whereas the 1:1 chloroform:ethanol mixture recorded the highest quantity of lipids at 11.76%. The various ratios of chloroform:hexane show extraction levels below 2% hence offering lower efficiency than the chloroform:ethanol mixtures which varied from 2.5% to approximately 12%. Based on the principle of solvent extraction where "like dissolves like", the lower results produced by the chloroform:hexane mixture could indicate that the algae contained smaller quantities of non-polar lipids, whereas when using chloroform:ethanol mixtures the extraction results are indicative of larger quantities of polar and neutral lipids. For the soxhlet method used, it was found that polar and neutral lipids accounted for approximately 78% while non-polar lipids accounted for approximately 22% of the total lipids extracted. This is within the limits specified for algal oils [30]. The ethanol:hexane mixture of the 1:3 ratio only produced approximately 4% of lipids at its highest efficiency. It is interesting to note that when the extractions were performed using single solvents (Fig. 1), the optimum extraction was obtained using chloroform and ethanol with lipid extract values greater than 10%. When comparing this with the values obtained in Fig. 3, the highest lipid content was obtained using the chloroform:ethanol mixture (ratio 1:1) where an amount of 11.76% was extracted. The solvents show similar extraction efficiency to single solvents when they are in equal proportions, i.e. 1:1 ratio, but as the ratios are varied, solvent efficiencies deteriorate and lower efficiencies become evident. This may be attributed in part to the intermolecular forces (van der Waals forces) that exist between molecules of each of the solvents and the molecules of biomass presented for extraction. The extraction behaviour of the solvents when mixed depends not only on the result of intermolecular attractions, but also in discriminating between different types of polarities. Changes in viscosity will also affect the solubilities of the mixtures depending on their polarity and the van der Waals forces acting on them [25].

The best extraction efficiency produced by the 1:2 chloroform:hexane mixture yielded 1.85% of lipids; this was less than half of the maximum produced by the 1:3 ethanol:hexane mixture with 3.99% lipids and 6 times less than that produced by the 1:1 chloroform:ethanol mixture (Fig. 3). The 1:1 chloroform:ethanol mixture which produced 11.76% lipids was the highest lipid content of all the binary mixtures used. Since chloroform and ethanol have produced the highest yield for lipids in this study, the question arises about the safety of using these solvents on a regular basis. The toxicity of chloroform presents problems with prolonged exposure with regular use. A prolonged exposure limit (PEL) of 50 ppm dictates that it should be used in a well-ventilated area. Ethanol, however, presents little or no problems as it is derived from agricultural sources and is renewable [28]. At low concentrations, ethanol is rapidly metabolized by most living organisms without causing harm [31].

Table 2
Total FAME composition of biomass using selected solvents.

Solvent extract	% Lipid extract	Total FAME (wt.%)
Chloroform	7.26	76.60
Ethanol	9.40	51.87
Hexane	4.81	18.09

The highest chlorophyll extract was produced by the 1:3 ethanol:hexane mixture. Once again, it can be observed that there is no relationship between the quantities of lipids extracted and the quantities of chlorophyll obtained (Fig. 3). This may also be attributed to solvent behaviour resulting from varying degrees of molecular interaction between the mixtures. There is a dearth of evidence relating to the use of binary solvent extractions of chlorophyll. However, Hosikian et al. and Wasmund et al., show evidence that favour ethanol and methanol as the solvents of choice for the extraction of chlorophyll when the solvents are used individually [32,33]. Since the choice of binary mixtures in this study was governed by the optimum lipid yields produced by the solvents used, the three solvents chosen excluded methanol, hence the lack of evidence with this solvent. However, the binary extractions performed with ethanol and hexane produce larger quantities of chlorophyll than the other binary mixtures used [33].

4. Chromatography

The FAME standards chosen identify with those that are suitable for the production of biodiesel [34,35]. The SP 2380 column used is a highly polar cyanosiloxane column with good thermal stability. Changing the polarity of a phase does not change the order of elution of components within a given chain-length group, but it can affect the elution order relative to components of other chain lengths. The choice of column was therefore made after studying the choice of various researchers [23,27,36].

Table 2 illustrates the total fatty acid quantities from C12 to C22 obtained after esterification of the lipid extracts obtained from algal biomass. The chloroform extract produced more than 75% FAME. This was 25% more than the ethanol extract and about 58% more than the hexane extract. When comparing the amount of lipids extracted no correlation is shown between the amount of lipid extracted versus the amount of FAME produced. D'Oca et al. have reported similar trends except that choroform:methanol extracts were compared with ethanol extracts [21].

5. Conclusion

For single solvent extractions, chloroform, ethanol and hexane produced the highest lipid yields. Binary mixture of 1:1 chloroform:ethanol showed better efficiency producing a lipid quantity of 11.76% while the best single solvent, chloroform produced 10.78%. The use of solvents involving greater than binary combinations will be impractical for upscaling and would increase the costs of production of biodiesel. It was found that an extraction time of 3 h gave optimum yields of lipids. Thus the use of longer extraction times would lead to unwarranted increase in extraction costs. The soxhlet method proved to be a reliable, effective, efficient and a very amenable method for the extraction of lipids from algal biomass. The varied extraction efficiencies of the 13 solvents used correlates to their polarities and their abilities to disrupt the algal cells at their boiling points. The esterification method used needs to be tested for its efficiency in conversion of the lipids extracted. It did, however, show that a better conversion was achieved with the

chloroform extract when compared to ethanol and hexane. This limitation may also be due to the efficiency of the solvent in the extraction of lipids for conversion to biodiesel.

Acknowledgements

My sincere thanks to my colleagues in the Department of Chemistry and the Institute of Water and Waste Water for making analytical equipment and biomass available for analysis; special thanks to Mr. A. Guldhe, Dr. B. Singh and Mr. I. Rawat for their input; the Postgraduate Development and Support department at Durban University of Technology and the National Research foundation (NRF) for their financial support. The Authors would also like to thank the anonymous reviewers for their valuable comments that lead to the improvement of this paper.

References

[1] Pintoa AC, Guarieiroa LLN, Rezendea MJC, Ribeiroa NM, Torresb AE, Lopesc WA, et al. Biodiesel: an overview. J Braz Chem Soc 2005;16:1313–30.
[2] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
[3] Vasudevan PT, Briggs M. Biodiesel production—current state of the art and challenges. J Ind Microbiol Biotechnol 2008;35:421–30.
[4] Govindarajan L, Raut N, Alsaeed A. Novel solvent extraction for extraction of oil from algae biomass grown in desalination reject stream. J Algal Biomass Utilization 2009;1:18–28.
[5] Shen Ying, Pei Zhijian, Yuan Wenqiao, Mao Enrong. Effect of nitrogen and extraction method on algae lipid yield. Int J Agric Biol Eng 2009;2:51–7.
[6] Lewis T, Nichols PD, McMeekina TA. Evaluation of extraction methods for recovery of fatty acids from lipid producing microheterotrophs. J Microbiol Methods 2000;43:107–16.
[7] Matile P, Hörtensteiner S, Thomas H. Chlorophyll degradation. Plant Physiol Plant Mol Biol 1999;50:67–95.
[8] Manirakiza P. Comparative study on total lipid determination using soxhlet, Roese–Gottlieb, Bligh and Dyer, and modified Bligh and Dyer extraction methods. J Food Compos Anal 2001;14:93–100.
[9] Halim R, Gladman B, Danquah MK. Oil extraction from microalgae for biodiesel production. Bioresour Technol 2011;102:178–85.
[10] Extraction and Measurement of Total Lipids, Food Analytical Chemistry.
[11] Torri C, Garcia Alba L, Samorì C, Fabbri D, Brilman DWF. Hydrothermal treatment (HTT) of microalgae: detailed molecular characterization of HTT oil in view of HTT mechanism elucidation. Energy Fuels 2012;26:658–71.
[12] Lee J-Y, Yoo C, Jun S-Y, Ahn C-Y, Oh H-M. Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 2010;101:S75–7.
[13] Afi L, Metzger P, Largeau C, Connan J, Berkaloff C, Rousseau B. Bacterial degradation of green microalgae: incubation of*chlorella emersonii* and *chlorella vulgaris* with *pseudomonas oleovorans* and *flavobacterium aquatile*. pdf. Org Geochem 1996;25:117–30.
[14] Garcia Alba L, Torri C, Samorì C, van der Spek J, Fabbri D, Kersten SRA, et al. Hydrothermal treatment (HTT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. Energy Fuels 2012;26:642–57.
[15] Christie WW. Extraction of lipids from samples; 2009.
[16] Labortechnik B. BÜCHI universal extraction system. Switzerland; 1996.
[17] Stein JR. Handbook of phycological methods: culture methods and growth measurements. In: Handbook of phycological methods. Cambridge University Press; 1973. p. 448.
[18] Chen W, Zhang C, Song L, Sommerfeld M, Hu Q. A high throughput nile red method for quantitative measurement of neutral lipids in microalgae. J Microbiol Methods 2009;77:41–7.
[19] Fowler SD, Brown WJ, Warfel J, Greenspan P. Use of nile red for the rapid in situ quantitation of lipids on thin-layer chromatograms. J Lipid Res 1987;28:1225–32.
[20] McClements DJ. Food Emulsions: principles, practice and techniques. In: CRC Press: Boca Raton, FL; 2005.
[21] D'Oca MGM, Viêgas CV, Lemões JS, Miyasaki EK, Morón-Villarreyes JA, Primel EG, et al. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the chlorella pyrenoidosa. Biomass Bioenergy 2011;35:1533–8.
[22] Quantitation methods in Gas Chromatography, 1998. <www.animal.iut.ac.ir/images/stories/file/GCyields.pdf>.
[23] Phukan MM, Chutia RS, Konwar BK, Kataki R. Microalgae chlorella as a potential bio-energy feedstock. Appl Energy 2011;88:3307–12.
[24] Aminot A, Rey F. Standard procedure for the determination of chlorophyll a by spectroscopic methods. Denmark: ICES; 2000.
[25] Burke J. Solubility parameters: theory and application. The American Institute for Conservation; 1984.
[26] Ryckebosch E, Muylaert K, Foubert I. Optimization of an analytical procedure for extraction of lipids from microalgae. J Am Oil Chem Soc 2011;89:189–98.
[27] Weyer KM, Bush DR, Darzins A, Willson BD. Theoretical maximum algal oil production. Bioenergy Res 2009;3:204–13.
[28] Zhang Y, Dube MA, McLean DD, Kates M. Biodiesel production from waste cooking oil. 1. Process design and technological assessment. Bioresour Technol 2003;89:1–16.
[29] Dere S, Günes T, Sivaci R. Spectrophotometric determination of chlorophyll *a, b* and total carotenoid contents of some algae species using different solvents. J Bot 1998;22:13–7.
[30] Kale A. Extraction of neutral lipids by a two solvent method. In: Heliae development, LLC (Gilbert, AZ), United States; 2012. p. 1–45.
[31] Peretti S, Losordo T, Hobbs A. Algae to Biodiesel conversion and scale-up; 2007.
[32] Hosikian A, Lim S, Halim R, Danquah MK. Chlorophyll extraction from microalgae: a review on the process engineering aspects. Inter J Chem Eng 2010;2010:1–11.
[33] Wasmund N, Topp I, Schories D. Optimising the storage and extraction of chlorophyll samples. Oceanologia 2006;48:125–44.
[34] Karow R. Fatty acid composition of biodiesel; 2012.
[35] Knothe G. Analytical methods used in the production and fuel quality assessment of biodiesel. 2001 Am Soc Agric Eng 2007;44:193–200.
[36] Buchanan MD. GC analyses of free fatty acids, 2012. <http://www.sigmaaldrich.com/technical-documents/articles/reporter-us/gc-analyses-of-free.html>.