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Factors affecting cellular lipid extraction from marine microalgae

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HIGHLIGHTS

- ▶ Investigate key factors affecting extraction efficiency and lipid characteristics.
- ▶ FFA was increased 3 times in extracted lipid for microalgae dried by solar.
- ▶ Chlorinated solvent systems resulted in higher extraction efficiencies than other solvent.
- ▶ Hexane had a poor extraction efficiency, but improved when polar solvents were added.
- ▶ The moisture content affected both lipid extraction efficiency and FFA content.

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ABSTRACT

Factors affecting intracellular lipid extraction from marine microalgae were investigated using various techniques. The biomass drying method and moisture content, and the solvent extraction system were the factors studied. Lipid was analytically classified into three categories i.e. neutral lipid, free fatty acid (FFA) and polar lipid using solid-phase extraction. Biomass drying methods (freeze-, oven- and solar drying) did not affect lipid yield, but the FFA content of the lipid was three times higher for solar dried biomass. Of the various lipid extraction methods tested, including sonication, homogenization, accelerated solvent extraction (ASE) and Soxhlet extraction, sonication was the least efficient compared to other methods when a partially miscible solvent system i.e. hexane–methanol was used. Chloroform–methanol solvent system had maximum lipid extraction efficiency (33%). A biomass moisture content up to 5% had no impact on lipid extraction efficiency, but higher moisture contents reduced lipid extraction and increased the FFA fraction.

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1. Introduction

Microalgae are currently subject to worldwide investigation as a promising sustainable and renewable energy source to meet future energy demand for liquid transportation fuels. Using microalgae to tap solar energy via photosynthesis is not a new concept; where an extensive study was conducted under the United States Aquatic Species Program [1]. Moreover, microalgae production represents a potential solution for the mitigation of climate change since 1 ton of algae results in the fixation of 1.83 tons of atmospheric CO₂ [2]. The yield of lipid–oil feedstock from microalgae has been estimated at 123 m³/hectare which is up to 20 times higher when compared to palm oil [2]. Chisti [2] estimated that if all US petroleum-based transport fuels were to be replaced by biodiesel, then palm oil plantation would require 61% of the total

cultivable land of the continental United States; whereas microalgae would need only 3%, assuming the microalgae contained 15% lipid oil as biomass dry weight.

Processing of harvested microalgal biomass to extract lipid–oil can be an energy intensive process as it requires separation and dewatering of microalgae, biomass drying and the subsequent extraction and purification of lipid–oil. The crude extract contains not only lipid–oils, but also carbohydrates, proteins and pigments. The lipids can be further classified into neutral lipids, free fatty acids, and polar lipids which include galacto- and phospholipids [3]. The lipid composition of various types of microalgae has been studied by several researchers, for example diatoms [3], *Nannochloropsis* [4] and *Phaeodactylum tricoratum* [5]. Lee et al. [6] studied various lipid extraction methods and solvent systems and compared relative extraction efficiencies. It was found that cell disruption with a micro-bead beater followed by extraction with chloroform:methanol (2:1 v/v) was an effective method for lipid extraction [7]. Addition of concentrated hydrochloric acid to a chloroform:methanol solvent system increased the total lipids extracted, especially phospholipids, in microalgae i.e. *Botryococcus*

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braunii, *Phaeodactylum tricornutum*, *Chlorosarcinopsis negevensis* and *Fragillaria construens* [8]. In addition to organic solvents, supercritical carbon dioxide has been used for the extraction of lipid from fungi [9,10]. However, to date, no study has reported on the effect of biomass moisture content, drying method, or solvent extraction system on the recovery and characteristics of extracted lipid from microalgae. Lipid composition with respect to neutral lipid, FFA and polar lipid content is of great significance during the transesterification reaction for biodiesel, where a higher neutral lipid content improves the overall yield of methyl esters from the transesterification reaction. If the FFA fraction exceeds 4%, then conventional homogeneous alkali catalysts react with FFA to form soap which impairs reaction efficiency and results in a poor separation of biodiesel from the reaction mixture. Similarly, the effects of polar lipids and associated reaction byproducts on the transesterification reaction have yet to be investigated in detail.

2. Experimental

2.1. Chemicals and reagents

Hexane (HPLC grade) and acetone (HPLC grade) were purchased from Tedia Company Inc. Chloroform (ACS grade) was purchased from Merck. Ethyl Acetate (pesticide grade), diethyl ether (pesticide grade) and isopropanol (HPLC grade) was purchased from Fisher Scientific. Other solvents, including methanol (HPLC grade), absolute ethanol (ACS grade) and acetic acid (ACS Reagent) were purchased from Sigma Aldrich. The polar lipid, phosphatidylcholine, was obtained from Lipoid GmbH, Germany. Neutral lipid (tripalmitate) and FFA (palmitic acid) standard was purchased from Sigma Aldrich. Bond Elut Aminopropyl (500 mg, 3 mL) solid phase extraction columns with a stainless steel frit were purchased from Varian Inc. Thin layer chromatography (TLC) plates, comprising a silica gel matrix with a particle size of 25 μm , with aluminium support, were purchased from Sigma Aldrich, Singapore.

2.2. Microalgae cultivation

A strain of *Nannochloropsis* sp., which was isolated from Singapore's marine coastal waters, was used for the study. The microalgae was grown in a 200 L indoor raceway pond provided with artificial light at 70 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$, over a 12:12 h light/dark period, in enriched nutrient F1 medium. The raceway pond was inoculated with a 20% v/v of microalgae in exponential growth phase. When microalgae reached stationary phase, glycerol was added to the medium to final concentration of 5 g/L. The elevated carbon to nitrogen ratio is known to improve the lipid content of the microalgae (unpublished data). Pigment leaching in the microalgae was observed after addition of glycerol and microalgae solution turned to a yellowish green colour. After 7 days in the mixotrophic condition, microalgae biomass was harvested using a chemical coagulation and flocculation technique, using 100 mg/L of ferric chloride as the coagulant. After biomass settling, the aqueous layer was decanted. Biomass was washed three times with deion water to reduce salt content and further concentrated to 15% solids paste via centrifugation at 1790g for 5 min.

2.3. Drying methods

Microalgae paste was applied as thin film (thickness < 5 mm) [11] in petri-dishes and dried using three different methods i.e. freeze drying (16 h), oven drying (at 60 °C, 3 h) and solar drying (30–34 °C for 8 h). After drying, the biomass flakes were crushed in a porcelain mortar and pestle and the resultant powder was stored in a sealed container inside a desiccator at room temperature prior to extraction.

2.4. Moisture and iron analysis

About 0.1 g of the microalgae was dried in oven at 60 °C to constant weight [12] to determine the biomass moisture content. The iron content of the biomass was measured by heating 0.5 g of biomass at 105 °C for 2 h, followed by acid digestion of the residual ash. Acid digestion was carried out using the method described by [13]. After digestion, the mixture was cooled and filtered through a Whatman no. 41 filter paper and made up to volume in a 25 mL volumetric flask with Milli-Q water for ICP-OES analysis. The instrumental detection limit was 5 ppm.

2.5. Lipid extraction methods

Sonication, ASE, Soxhlet extraction, and homogenization were evaluated with respect to lipid extraction efficiency using two types of solvent systems i.e. a completely miscible solvent system of chloroform:methanol (2:1, v/v); and a partially miscible solvent system of hexane:methanol (3:2, v/v). The optimum extraction time was determined for each extraction method. Optimum extraction time for different methods was as follows: sonication 5 min; ASE 30 min; Soxhlet extraction 360 min; and homogenization 10 min. Individual extraction methods are summarized below:

2.5.1. Soxhlet extraction

About 2 g of dried biomass was placed in an extraction thimble (Whatman cellulose extraction thimbles 25 mm ID, 80 mm length) and placed into the Soxhlet extraction apparatus. 100 mL of solvent was used for extraction with a reflux period of 6 h.

2.5.2. Homogenization

About 1 g of dried biomass and 50 mL of solvent was placed in a 100 mL beaker. A Homogenizer DIAX 900 (Heidolph, Germany) was used for lipid extraction with a power and speed set at 810 W and 12000 rpm, respectively. Biomass homogenization was conducted for 10 min, after which biomass was separated by centrifugation at 1790g for 5 min.

2.5.3. Accelerated Solvent Extraction (ASE)

An ASE 200 Dionex extractor fitted with 33 mL volume stainless steel vessels was used for extraction. Filter discs made up of cellulose fiber (D28 filter, Dionex) were used to retain the biomass in the extraction cell. The ASE program was set at a temperature of 100 °C, pressure 1200 kPa with a 30 min extraction period. Extractions were performed using nine different solvent mixtures, as specified above. The extraction process comprised the following steps: (i) extraction vessels with biomass samples were loaded into the extractor; (ii) cells were filled with solvents up to a pressure of 1200 kPa; (iii) extraction vessels were heated for 5 min; (iv) a static extraction was performed for 30 min; (v) the vessel was rinsed using extraction solvent (10% vessel volume) and (vi) solvent was purged from the vessel with N_2 gas. The extracts were then retained in 60 mL collection vials for analysis.

2.5.4. Sonication

A Sartorius Labsonic (30 W and 50 Hz) sonicator operating at 90% power was used for biomass extraction. 1 g of microalgae and 50 mL of solvent were placed in a 100 mL beaker followed by sonication for 5 min with 30 s breaks at each minute, followed by biomass separated via centrifugation at 1790g for 5 min.

2.5.5. Purification of crude lipid extract

After extraction with chloroform:methanol, the supernatants were washed with Milli-Q water to remove water soluble impurities such as proteins and carbohydrates. The ratio of water: supernatant was 1:5 [14]. There were two clear layers present following

centrifugation at 1790g for 5 min. The bottom chloroform layer was separated and solvent was evaporated via an N₂ purge. Finally, the amount of lipid in the chloroform layer was determined gravimetrically and a portion kept at -4 °C for future analysis. For lipid extraction using the other solvent systems, the supernatants were evaporated in a rotary evaporator. The lipid was then re-dissolved in 10 mL chloroform:methanol (2:1) solvent and the aforementioned procedure were followed to remove water soluble impurities.

2.6. Solid phase extraction

Lipid classification was conducted using a solid phase extraction (SPE) technique with amino propyl columns (500 mg, 3 mL, with stainless steel frit), as described by Kaluzny et al. [15], with some modification to improve separation and recovery of lipid fractions. An SPE column was placed in the Vac Elut apparatus and a 10 kPa vacuum was applied to pull solvent through the column [15]. Initially, the column was activated via a double 4 mL hexane wash. After activation, approximately 20 mg of lipid, which was dissolved in 200 µL of chloroform, was applied immediately to the column and the entire lipid was retained following elution of chloroform. First, the neutral lipid (NL) fraction was collected by eluting 4 mL of chloroform:2-propanol (2:1) solvent mix, followed by free fatty acid (FFA) by eluting 4 mL of 2% acetic acid in diethyl ether, and finally the polar lipid (PL) fraction by eluting 6 mL of methanol through the column. The solvents were evaporated via an N₂ purge to determine the weight of each lipid fraction. A lipid standard containing NL (triolein), PL (phosphatidylcholine) and FFA (palmitic acid) at a 40:40:20 ratio was used to optimize the SPE method, and yielded more than a 90% recovery of each of the lipid fractions (unpublished data).

2.7. Thin layer chromatography

Thin layer chromatography (TLC) was used to equate separated lipid fractions against the neutral, FFA and polar lipid standards. The solvent system used for the elution was hexane:diethyl ether:formic acid (80:20:2, by volume) [16]. After drying the solvents, lipid spots on the TLC plates were visualized by subjecting plates to iodine vapour. The dark lipid spots were then identified

and their retention factor (R_f) values were calculated and compared against the standards.

$$R_f = (\text{distance travelled by compound}/\text{distance travelled by solvent front})$$

2.8. Transesterification of lipid

The lipid fractions obtained from the SPE were dissolved in 0.2 mL of chloroform and 1.6 mL of methanol, and then 0.4 mL of concentrated hydrochloric acid (37%) was added to lipid solution. The mixture was maintained at 80 °C for 2 h, so as to convert the lipid fraction to fatty acid methyl esters (FAME) i.e. biodiesel, and 2 mL of hexane was then added to extract FAME from the reaction mixture. The hexane layer was separated and analysed for FAME using a GCMS (GC-QP2010, Shimadzu, Japan). Each sample was spiked with methyl nonadecanoate as an internal standard. A capillary column DB-5MS, with a length of 30 m, a film thickness of 0.25 µm and an internal diameter of 0.25 µm was used for the analysis. Helium was used as the carrier gas, and the detector and injection temperature was set at 280 °C. The temperature program of the GC column was as follows: 50 °C held for 2 min; increased at a rate of 10 °C min⁻¹ to 150 °C and held for 2 min; at 185 °C held for 2 min; and at 300 °C held for 2 min.

2.9. Statistical analysis

Data was presented as means ± the standard deviation of the mean. One or two way analysis of variance (ANOVA) was performed to identify statistically significant differences in data sets at a 95% confidence level ($p < 0.05$). A Newman-Keuls (NK) multiple comparison test was performed for pair-wise comparisons after the null hypothesis was rejected by ANOVA.

3. Results and discussion

3.1. Effect of drying method

Fig. 1a shows the effect of drying method on total lipid yield, where it is evident that differences in yield were not significant (difference in mean value was $p > 0.05$). The NK test also showed

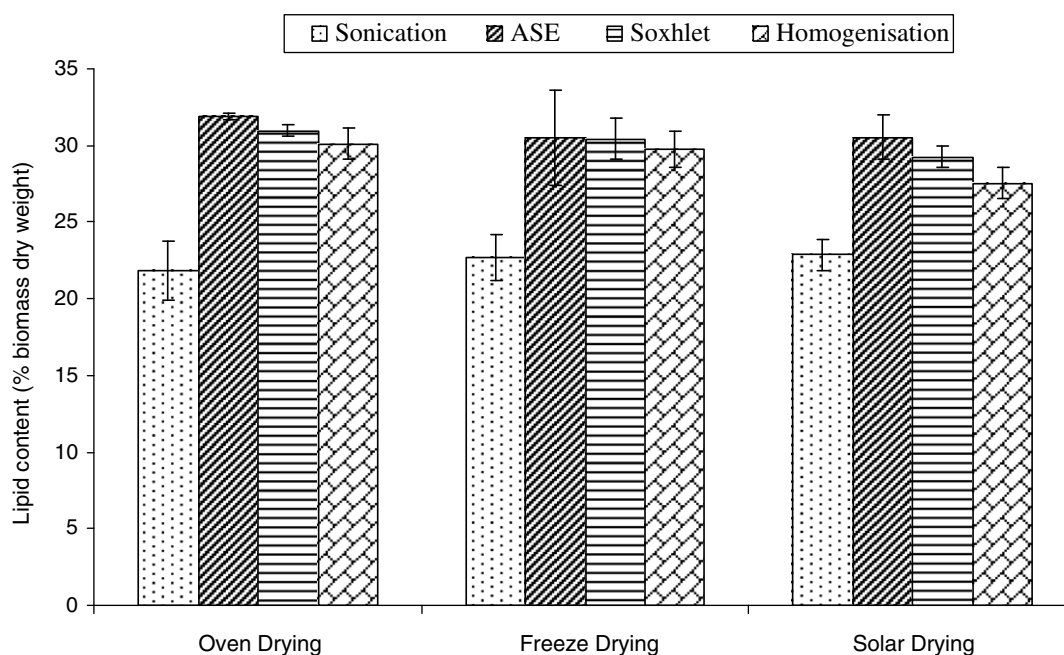


Fig. 1a. Effect of biomass drying and lipid extraction method on lipid recovery using a hexane:methanol (3:2, v/v) solvent system.

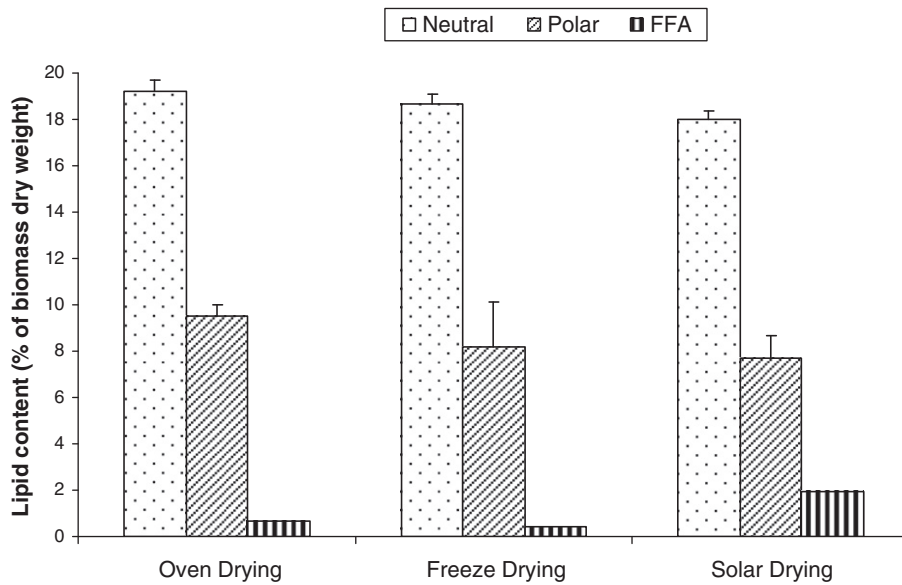


Fig. 1b. Effect of biomass drying method on recovery of microalgal lipid fractions.

no significant differences between any pair of the drying methods studied. However, Fig. 1b shows that the type of drying method had a significant effect on the amount of FFA in the lipid ($p < 0.05$), where lipid extracted from freeze-dried microalgae had the least amount of FFA (4.0 mg of FFA/g of microalgae dry biomass, equivalent to 0.4% of dry biomass), followed by oven-dried biomass (6.8 mg of FFA/g of dry biomass), where solar dried biomass had about 5 times more FFA in the lipid (19 mg of FFA/g of dry biomass). The long exposure time of biomass used for solar drying i.e. 8 h likely enhanced the oxidation of triglyceride molecules, thus releasing more FFA from the glycerol. Harvesting of microalgae prior to extraction was conducted using ferric-chloride

coagulation. The pH of the medium was above 7.2; hence ferric hydroxide flocs formed during the agitation where microalgae biomass was entrapped in the gravity settled flocs. The iron content of the biomass was as high as 9.7% prior to washing, but reduced to 1.5% after washing three times with DI water. Transition metal ions (e.g., Fe, Co, Cu) are effective catalysts for the free radical oxidation of lipids [17], and it is likely that the iron content of algae induced the oxidation of triglyceride molecules to increase FFA content in the lipid. For solar dried biomass, lipid oxidation could have been further enhanced by microbial degradation, light and UV irradiation, where it is known that chlorophyll serve as a sensitizer to promote photo-oxidation of lipid [17].

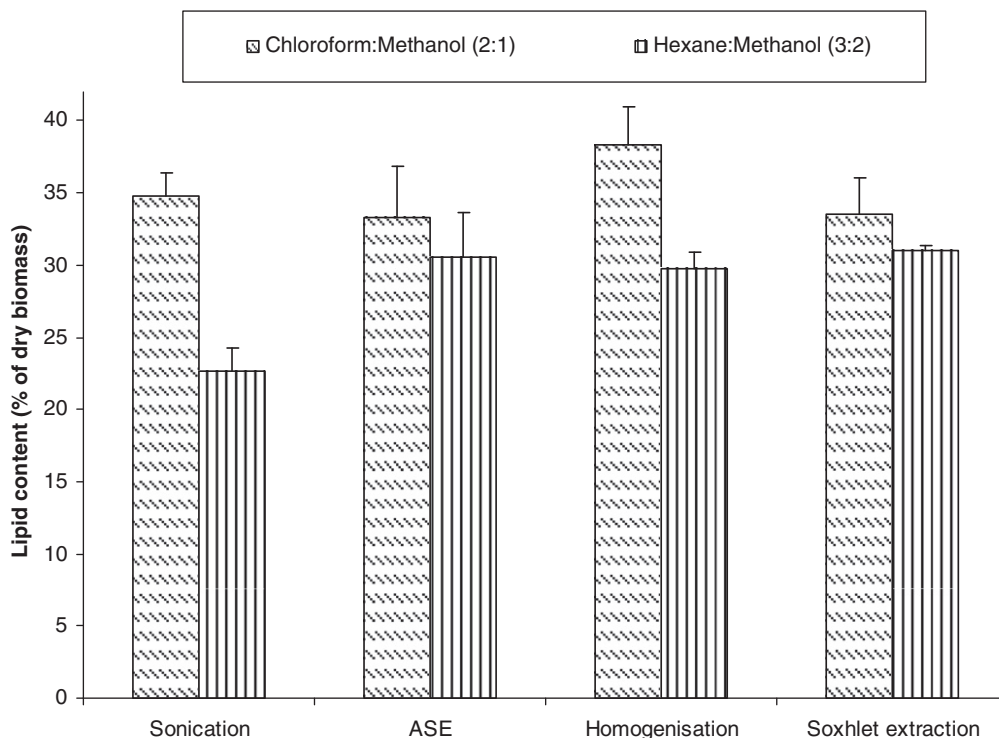


Fig. 2. Comparison of lipid extraction methods using miscible (i.e. chloroform:methanol) and partially miscible solvent system (i.e. hexane:methanol).

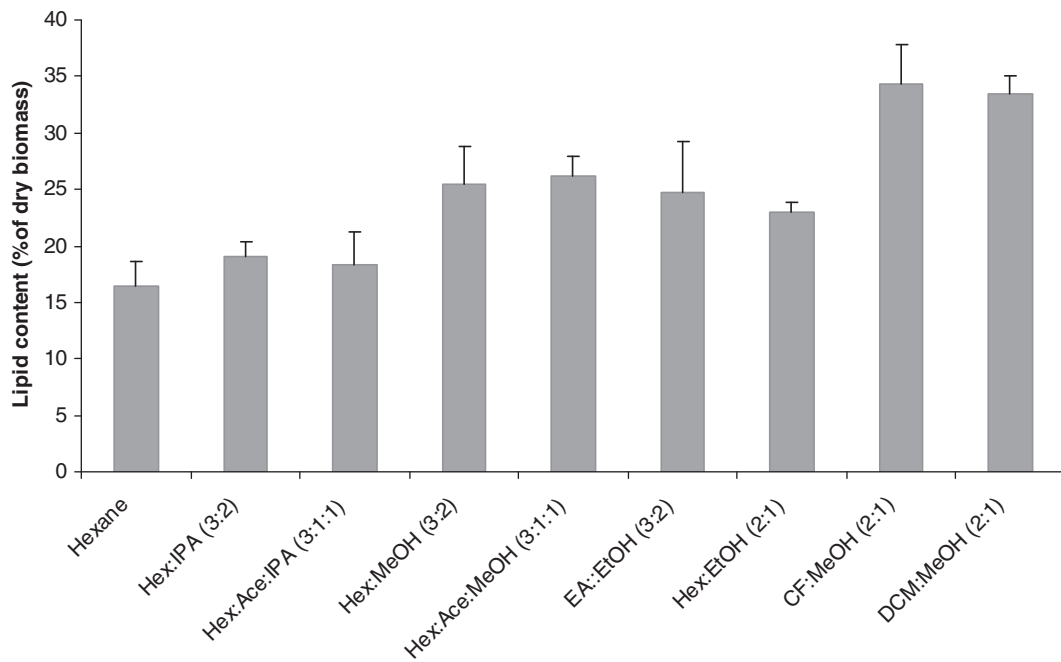


Fig. 3. Effect of solvent system on extraction of lipid from dried microalgae biomass.

3.2. Effect of extraction method

The efficiency of lipid extraction could be improved significantly if cell membranes are lysed to facilitate solvent permeation. In this study, sonication, ASE, Soxhlet extraction and homogenization were studied. The lipid extraction efficiency of each technique was compared for the three different drying methods (see Fig. 1a) and two different solvent systems i.e. chloroform:methanol and hexane:methanol (see Fig. 2) used. Two way ANOVA showed no interaction between drying method and lipid extraction efficiency as based on lipid yield ($p > 0.05$), but showed significant interac-

tion between solvent system and extraction method ($p < 0.05$). Similarly, one way ANOVA showed that there is no significant difference between extraction methods using the chloroform:methanol solvent system ($p > 0.05$), but the type of extraction method impacted the lipid yield ($p < 0.05$) for the hexane:methanol solvent system. Further, the NK test showed that only sonication had a significant difference with other extraction methods for the hexane:methanol solvent system which was less efficient with only 22% of cell lipid extracted, but faster than other methods. ASE resulted in maximum lipid extraction at 33%, followed by Soxhlet (30%) and homogenization (28%). The miscibility of the solvent sys-

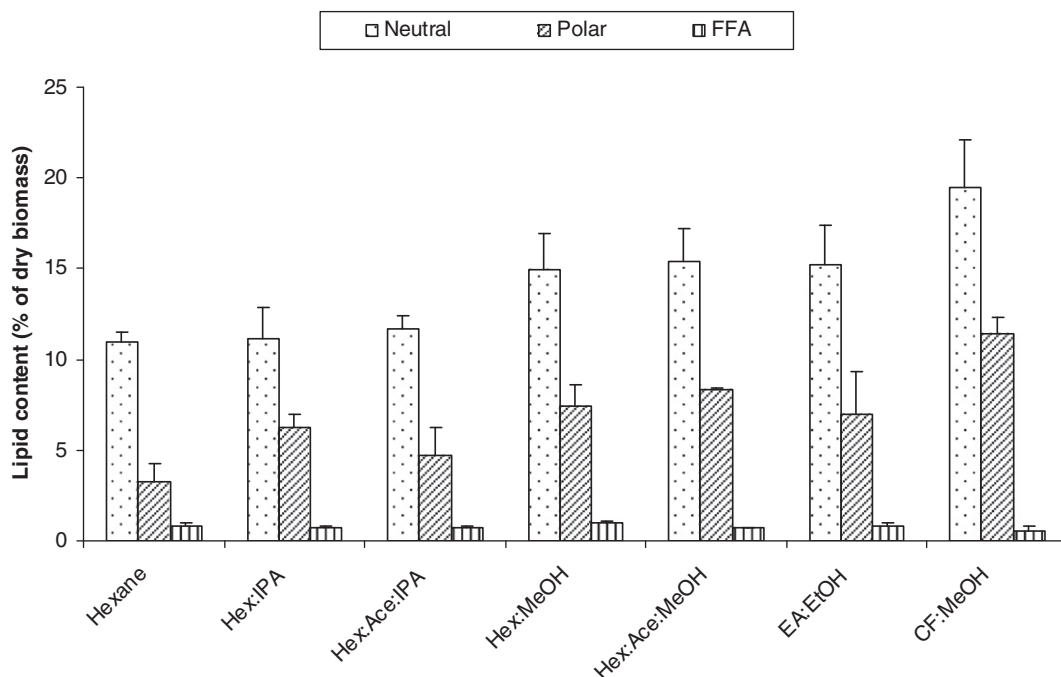


Fig. 4. Classification of lipids extracted from dried microalgae biomass using different solvent systems.

Table 1

Classification of lipids extracted from dried microalgae biomass using different solvent systems. Data were express in mean values \pm SD.

Solvent systems	Neutral	FFA	Polar
Hexane	70.9 \pm 5.0	7.4 \pm 1.0	21.7 \pm 4.8
Hexane:IPA (3:2)	62.7 \pm 8.0	3.6 \pm 0.5	33.7 \pm 2.8
Hexane:acetone: IPA (3:1:1)	68.8 \pm 5.3	3.7 \pm 0.9	27.4 \pm 7.6
Hexane:methanol (3:2)	64.1 \pm 4.4	4.4 \pm 0.1	31.5 \pm 2.0
Hexane:acetone:methanol (3:1:1)	63.0 \pm 3.4	2.8 \pm 0.3	34.2 \pm 1.5
Ethylacetate:ethanol (3:2)	66.2 \pm 4.9	3.6 \pm 0.4	30.2 \pm 5.3
Chloroform:methanol (2:1)	61.9 \pm 1.5	1.6 \pm 0.3	36.5 \pm 0.5

tem used for the extraction explains the lower extraction efficiency achieved for biomass sonication. Completely miscible solvent systems i.e. chloroform:methanol (2:1) gave similar levels of extraction efficiency to other extraction methods, but partially miscible solvent systems i.e. hexane:methanol (3:2) yielded a lower extraction efficiency for sonication than other extraction methods. It was observed hexane and methanol formed two distinct layers, where algae biomass was in contact with polar solvent methanol at the bottom layer during sonication. Even though small droplets of hexane and methanol dispersed in each layer, microalgae had more contact with the methanol at the base of the solvent system. When the completely miscible solvent system i.e. chloroform:methanol was used, the lipid extraction efficiency of sonication improved from $22.7 \pm 1.5\%$ to $34.7 \pm 1.6\%$. In the case of Soxhlet extraction, methanol with a lesser boiling point than hexane (i.e. 65°C versus 68°C), resulted in more methanol evaporated initially until the hexane:methanol ratio reached 75:25 (v/v). Hexane and methanol forms a negative heteroazeotrope which boils at 50°C with a molar distribution of hexane:methanol (49:51) and a volume ratio of hexane:methanol (75:25). Hence, the algae were exposed to intermittent polar and non-polar solvent in the Soxhlet method and this resulted in comparable lipid extraction efficiency for the ASE and homogenization methods.

3.3. Effect of solvent extraction system

In addition to the extraction method used, the strength of solvent systems also affects extraction efficiency. Based on the results,

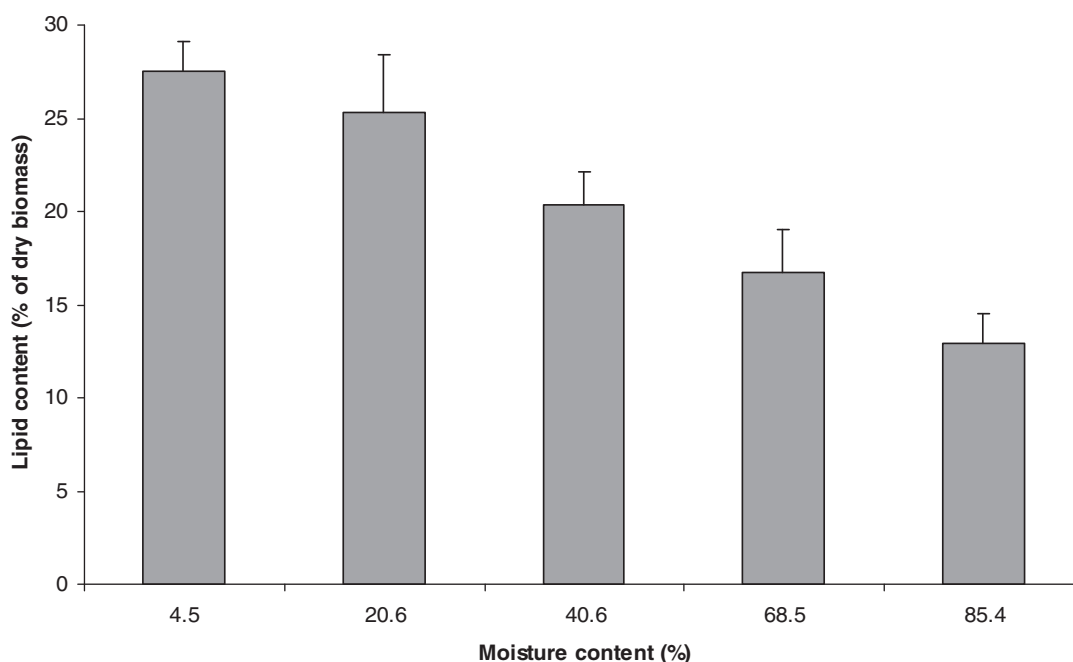


Fig. 5a. Effect of biomass moisture content on lipid recovery.

it was apparent that ASE was the most effective method for lipid extraction. Therefore in this experiment, lipid was extracted from microalgae biomass using ASE in conjunction with seven various solvent systems shown in Fig. 3. One way ANOVA showed that the type of solvent system used had a significant impact on lipid yield ($p < 0.05$). Chloroform:methanol (2:1) was the best solvent system for lipid extraction from microalgae for any given condition. A similar result was obtained from Lee's study [7] when a micro-bead beater was used for cell disruption. In our study, the extraction efficiency of dichloromethane:methanol (2:1, v/v) was as effective as chloroform:methanol (i.e. $33.1 \pm 1.6\%$ and $34.3 \pm 3.5\%$ respectively). Since dichloromethane (DCM) is less toxic than chloroform, it has replaced chloroform as extraction solvent in several studies [18,19]. The usage of chlorinated solvents is also hazardous and not eco-friendly; hence other non-polar solvents were investigated in this study. Hexane is good candidate solvent as it is used commercially for a wide range of extraction applications [20]. However, the use of hexane alone gave only a 16% lipid yield, but addition of a polar solvent with hexane improved yield. A hexane:isopropanol (3:2) solvent system gave a 19% total lipid yield; where addition of acetone with this system did not change the lipid yield considerably. Methanol improved extraction efficiency relative to iso-propanol when mixed with hexane increasing lipid yield to 26%. A similar lipid yield was obtained using an ethyl acetate:ethanol (3:2) solvent system, but usage of ethyl acetate is not recommended as it absorbs moisture quickly and is potentially explosive.

Fig. 4 shows the classification of lipid obtained using different solvent extraction systems from dried microalgae biomass. One way ANOVA showed significant differences ($p < 0.05$) for all lipid fractions (neutral, FFA and polar). Addition of polar solvents to hexane not only increased the polar lipid fraction, but also increased the neutral lipid fraction. For example, when isopropanol was included with hexane, the polar lipid fraction yield was increased by 85% and neutral lipid by 5%. Similarly, when methanol was included with hexane, the polar lipid fraction was doubled and the neutral lipid fraction was increased by 60%. The hexane:methanol solvent system extracted higher levels of neutral and polar lipids than the hexane:iso-propanol solvent system. The chloroform:methanol solvent system extracted 68% more neutral lipid,

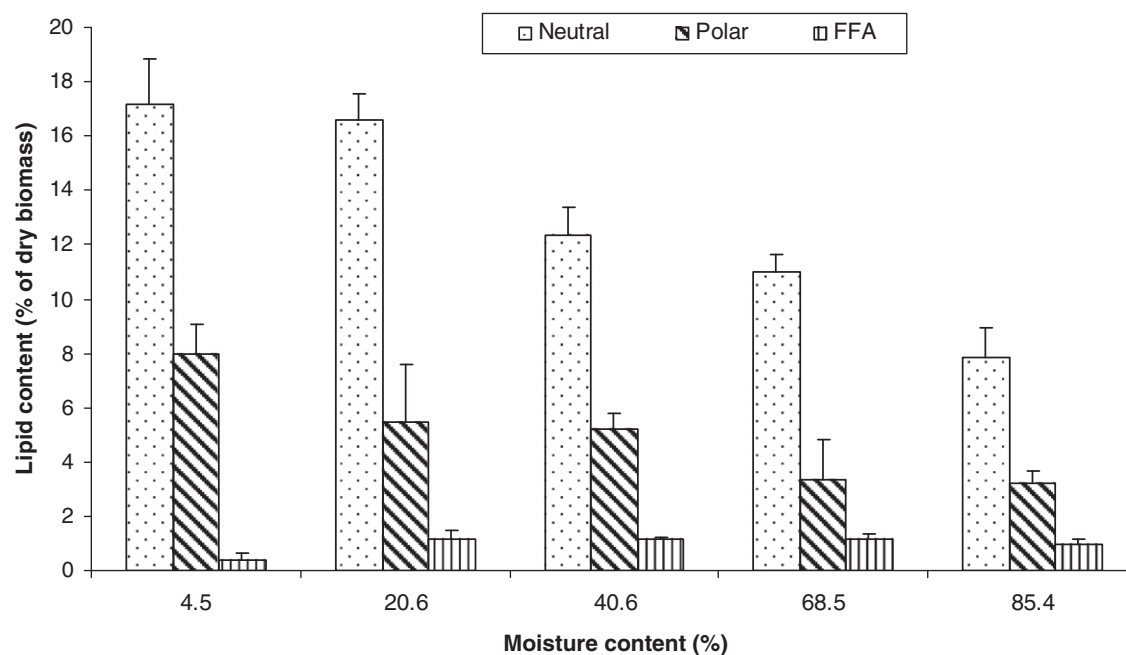


Fig. 5b. Effect of biomass moisture content on relative lipid fraction recovery.

Table 2

Relative lipid fractions extracted from microalgae biomass with different moisture content. Data were express in mean values \pm SD.

Moisture content (%)	Neutral	FFA	Polar
4.5	67.8 \pm 3.5	1.5 \pm 0.5	30.7 \pm 2.5
20.6	71.6 \pm 2.5	5.0 \pm 0.8	23.3 \pm 5.2
40.6	65.3 \pm 3.4	6.2 \pm 0.4	28.5 \pm 1.9
68.5	70.9 \pm 2.4	7.3 \pm 0.7	21.8 \pm 2.0
85.4	65.7 \pm 5.3	7.8 \pm 1.0	26.5 \pm 2.2

Table 3

Fatty acid distribution in different fractions of microalgae lipid extracted from oven dried biomass using a hexane:methanol solvent system via ASE.

FAME	Neutral	FFA	Polar
C14:0	7.14	7.72	6.77
C15:0	0.52	0.84	0.33
C16:1	28.03	15.22	18.99
C16:0	34.83	48.15	36.50
C18:3n3	0.06	-	-
C18:2n6c	0.95	3.98	0.21
C18:1n9c	12.28	3.57	17.21
C18:2n6t	0.63	2.27	4.47
C18:1n9t	2.29	5.45	12.11
C18:0	1.14	8.58	3.08
C20:4n6	1.72	-	-
C20:5n3	9.50	-	-
C20:3	0.06	-	-
C20:0	0.02	0.65	-
C21:0	-	0.12	-
C22:0	-	1.43	0.02
C23:0	-	0.19	-
C24:0	0.02	0.38	0.01
Others	0.87	1.47	0.33

3 times more polar lipid than for hexane and 26% more neutral lipid, 50% more polar lipid than the hexane:methanol solvent system. Table 1 shows the percentage of lipid fractions obtained using different solvent systems. When hexane was used as the solvent, there was no decrease in the FFA concentration. When the lipid

is used as feedstock for biodiesel production, the FFA content is a critical factor as it interferes with the alkali catalysts used for the transesterification reaction. Based on the results obtained, we hypothesize that a drop in lipid extraction efficiency will increase the FFA content in the extracted lipid.

3.4. Effect of moisture content

Lipid was extracted by ASE using a hexane:methanol solvent system to study the effect of biomass moisture content on lipid extraction. After centrifugation at 1790g for 5 min, the wet algae biomass contained 85.4% moisture by weight. Microalgae with moisture contents of 68.5%, 40.6%, 20.5% and 4.5% were then prepared by drying algae in oven at 55 °C for 10, 20, 30 and 60 min respectively. Fig. 5a shows that higher biomass moisture content reduces the lipid extraction efficiency. One way ANOVA showed that the moisture content had a significant effect on the lipid yield ($p < 0.05$). It was hypothesized that water molecules surrounding the hydrophilic outer layer of the cell wall resist the non-polar solvent penetration inside the cell which then hinders lipid extraction. Further, the NK test showed that the difference in the mean of lipid yield of microalgae biomass with 4.5% and 20.6% moisture content was not statistically significant ($p > 0.05$). Using microalgae biomass with a 20% moisture content for extraction will significantly reduce the energy demand for drying. However, this may simultaneously increase the moisture content of the extracted lipid which will then need further treatment prior to transesterification.

Fig. 5b shows the variation in the extracted lipid fraction as a function of moisture content in microalgae biomass. One way ANOVA of mean values of neutral, polar and FFA showed that moisture content had a significant impact on lipid fractions. The absolute value of FFA was increased from 3.9 ± 1 mg/g to 11.7 ± 2 mg/g when moisture content increased from 4.5% to 20.6%. There was no significant change in the absolute value of FFA content when the moisture content of algae was increased above 20.6% ($p > 0.05$); however the relative percentage of FFA increased (Table 2) as other lipid fractions decreased.

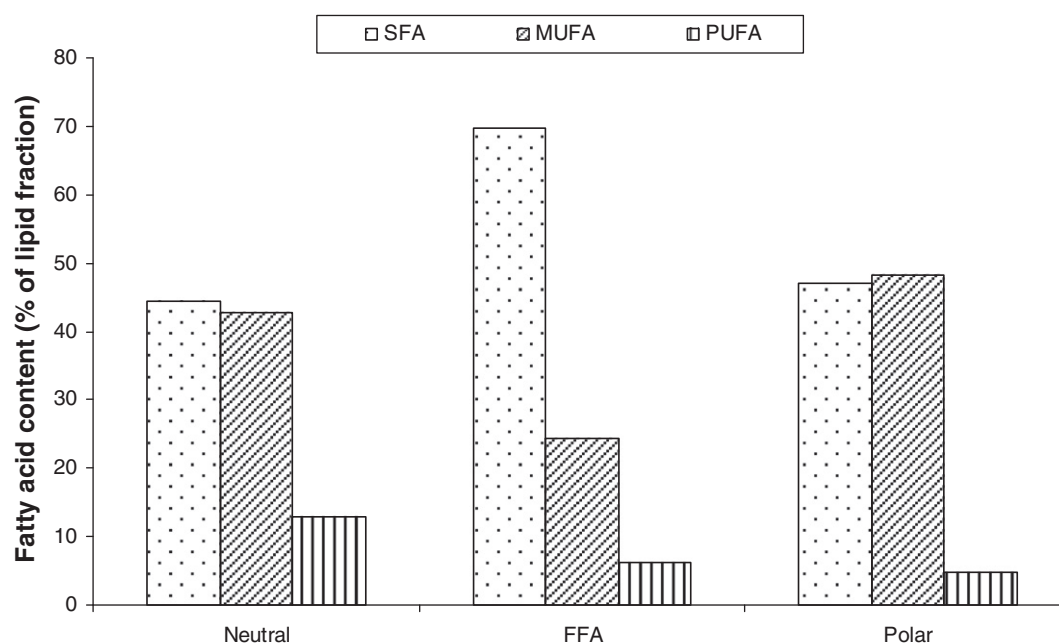


Fig. 6. Distribution of SFA, MUFA and PUFA in the different lipid fractions of microalgae.

3.5. Fatty acid distribution of lipid fractions

Fatty acid profile is an indicator of the suitability of algal lipid for use as a biodiesel feedstock. To understand characterize the fatty acid profile in each lipid class, fatty acid quantification was performed. The fatty acid profile of the extracted lipid fractions from oven dried biomass using a hexane:methanol solvent system via ASE was shown in Table 3. It can be noted that long fatty acids (>C18) did not accumulate in the polar lipid fraction, and that eicosapentaenoic acid (EPA) was present only in the neutral lipid fraction. Fig. 6 shows the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) content of the neutral, FFA and polar lipid fractions. Normally oxidation and enzyme degradation of triglycerides increases FFA content in lipids. In the FFA fraction, 70% of the total fatty acids were present as saturated fatty acids. This is supported by an earlier study that reported saturated fatty acids were more easily hydrolyzed than unsaturated fatty acids from the glycerol backbone of the triglyceride molecule by lipase enzyme [21].

4. Conclusion

Factors affecting extraction efficiency and lipid quality have been studied in detail. Drying methods had no effect on lipid extraction efficiency, but the lipid profile was affected where FFA content increased three times in extracted lipid for microalgae dried in natural sunlight. Complete extraction of neutral lipid from the microalgae biomass was not possible without polar lipid extraction. Overall, the extraction of microalgae biomass with a moisture content of less than 5%, using a polar and non-polar solvent system is effective for achieving high lipid yields with a low FFA content which is suited for conversion into biodiesel.

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