A Study of Lipid Oil Production from Chlorophyta Species as Biodiesel Potential

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ABSTRACT

The rapid increase of carbon dioxide (CO2) concentration in the atmosphere and high demand for energy security had led to an increased commercial interest in renewable fuels. Green algae from chlorophyta species are favorable feedstock due to their high oil content and fast growth rate. This study investigated the extraction of lipid oil from chlorophyta species collected from Teluk Pelanduk, Port Dickson by using a soxhlet extraction method. Solvent lipid oil extraction from chlorophyta species focus on the efficiency of three different solvents (hexane, ethanol and acetone) and the composition of fatty acids in the lipid oil. Hexane proved to be the most efficient solvent for chlorophyta species extraction with percentage lipid oil of 4.08%, followed by acetone (3.98%) and ethanol

(1.47%).The lipid oil extract from chlorophyta species was analyzed using gas chromatography to identify the fatty acid components in the lipid oil extracts. The results showed that green algae from chlorophyta species have required fatty acid profile for conversion to high quality of biodiesel. From the results obtained, it is interesting to highlight that green algae from chlorophyta species have the required fatty acid profile for conversion to high quality of biodiesel.

Key Words: Green algae, Chlorophyta species, Hexane, Lipid oil, Soxhlet extraction

INTRODUCTION

In recent years, a novel method on studying of green algae as a highest potential for large scale of biodiesel production is increased [1]. The mass production of liquid biofuels such as corn based ethanol and sugarcane has resulted in a series of problem related to food price, land usage and carbon emissions [1].In the literature [2]-[4], the three most common methods for oil extraction are expeller, solvent extraction and supercritical fluid extraction. In expeller process, most of the lipids and the moisture in the biomass is driven out and is later separated [3].Supercritical fluid extraction makes use of medium, commonly CO2 in this supercritical state, for extraction of undesired substance from raw materials [4]. The extraction yield depends on the method and types of solvent used [2].Hexane is extensively used as solvent for oil extraction because of its low vaporization temperature, high stability, low corrosiveness and low greasy residual effects [5]. [6] proposed to use other non-petroleum materials instead of hexane as a solvent. Alcohols are showing great potential for oil recovery from biomass, playing a duel role of extraction of oil as well as dissolution of fatty acid from oil recovered. Algae seem to be very effective feedstock for biodiesel production. Some algae species have been found to have very high oil content, ranging up to 50% dry weight [7]. It is estimated that algae can produce between 58,700 and 136900 L/ha annually and would need to occupy between 2.5-1.1 of the total land area of the US to replace 50% of the total US transportation fuel consumption. These numbers suggest that algae are capable of producing between 10-30 times the amounts of oil per year than high oil producing plants [8].The term lipids will be defined as those substances which are insoluble in water, soluble in organic solvents such as chloroform, ether or benzene, contain long chain hydrocarbon groups in their molecule and are present or derived from living organism[9]

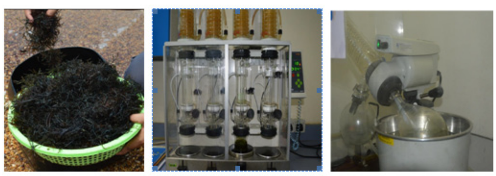
Accordingly, green algae from chlorophyta species were extracted by using solvent extraction method. The present work was conducted to compare the efficiency of various solvents to extract lipid oil. The three solvent used for lipid oil extraction are hexane, ethanol and acetone. Lastly, the compound present in the lipid oil extract is analyzed by using Gas Chromatography technique.

MATERIAL AND METHOD
Materials

The study was carried out in the analytical laboratory MICET, UniKL. Instrument used were soxhlet extractor, rotary evaporator and grinder. Solvent used for this experiment were hexane, acetone and ethanol. Green algae (chlorophyta species) were obtained from Teluk Pelanduk, Port Dickson.

Oil Extraction Method

The algae were immersed in the formalin solution for 48 hours before being processed. Then the sample of algae as shown in Figure 1(a) was dried by using drying oven at temperature of 70°C for 24 hours. The dried algae were grind to form green algae in powder form. Hexane has been used as solvent for lipid extraction from green algae [10] while ethanol and acetone have been found to be effective with marine sample. A comparison of hexane, ethanol and acetone as a solvent was conducted by using soxhlet extraction method, Figure 1(b). The soxhlet extraction set-up consist of three parts; solvent reservoir (round bottom flask), soxhlet apparatus and condenser. In a cellulose extraction thimble, 10 g of the dried algae was placed in the soxhlet apparatus .The reservoir was filled with 125 ml of the extraction solvent. The solvent was allowed to reflux for 4 hours. After cooling, the reservoir containing the solvent and the lipids was detached from the soxhlet apparatus and placed in the rotary evaporator apparatus as shown in Figure 1(c) to recover the solvents. The solvent reservoir was placed in the evaporation apparatus and the evaporated solvent recovered in the collector. The lipid extract was dried completely of the residual solvents and weighed. Lastly, the sample was analyses by using gas chromatography to identify the fatty acids components.



(a) (b) (c)

Figure 1: (a) Sample of green algae. (b) Soxhlet extractor (c) Rotary evaporator

Fatty Acid Analysis

The lipid extracts had to be converted into more volatile compounds such as methyl esters for analysis by Gas chromatography technique [11]. Up to 0.05g of the lipid sample was dissolved with 0.95 ml hexane and 0.5 ml sodium methoxide into 2 ml vial. The mixtures were shaking in a vortex mixture vigorously. After 5 minutes, the separation of the mixture can be seen. Take out the clear, separate methyl ester layer and dry with anhydrous sodium sulphate. Then, 10 µl of the extract FAME were taken and diluted it with hexane in 1ml volumetric flask. Lastly, 1 µl of sample were injected into the gas chromatography for analysis.

RESULT AND DISCUSSION

Effect of Solvent towards Extraction Process

In this study, the potential of chlorophyta species as alternative sources for biodiesel was identify. The species of green algae sample was confirmed by referring to standard references from previous study by [12] and [13]. The extraction process was carried out for almost 4 hours by using different solvent; hexane, ethanol and acetone. The results obtain from the extraction of green algae by using hexane, acetone and ethanol as solvent shown in Table 1. Based on result (Table 1), the percentage of lipid oil extracted by using hexane, ethanol and acetone as a solvent was compared. Figure 2 shown graph of percentage lipid oil extracted for hexane, ethanol and acetone.

Table 1 Experimental data for extraction of green algae by using hexane, ethanol and acetone

Solvent (average reading from 3 sample collected)	Hexane	Ethanol	Acetone
Weight of thimble (g)	1.601	1.609	1.577
Weight of algae (g)	15.000	15.000	15.000
Weight of flask (g)			
	152.443	152.498	152.497
Solvent used (ml)			
	150.000	150.000	150.000
Weight of flask+ extracted lipid	153.321	152.783	153.183
Weight of extract lipid			
	0.798	0.295	0.686
Actual weight of lipid extract after using rotary evaporator	0.612	0.221	0.597

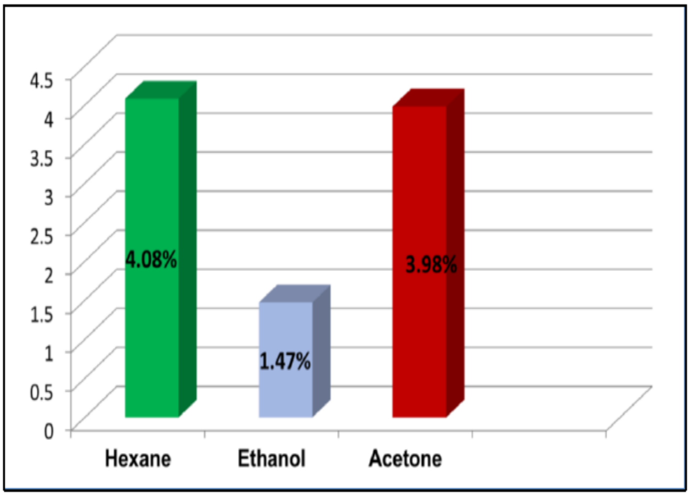


Figure 2: Graph of percentage lipid oil extracted for each solvent

Hexane is symmetrical between the carbons and hydrogen. Thus, hexane can be classified as nonpolar. Meanwhile, ethanol was a very polar molecule due to its hydroxyl group with the high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules. Hence, ethanol and acetone were classified as polar solvent while hexane was classified as non-polar solvent. Based on Figure 3 solvent with lowest relative polarity (hexane), produce highest lipid oil. The percentage of lipid oil for ethanol and acetone were lower because both were polar solvent.

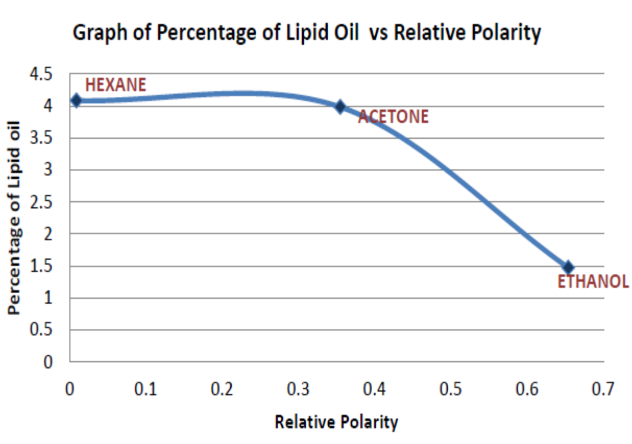


Figure 3: Graph of percentage of lipid oil versus relative polarity

Thus, the total lipid content determine by solvent extraction depends on the nature of the solvent. Other than polarity, the boiling point of solvent was another parameter should be considered to determine the most suitable solvent for extraction process. Generally, a solvent was chosen based on boiling point where the solvent boiling point is well above the extraction. In soxhlet extraction process the solvent was evaporates, the boiling point should not be too high. Boiling point of ethanol was higher compared to hexane and acetone. Boiling point for hexane is 68 ºC, ethanol 78 ºC and acetone 56 ºC .Higher boiling point will reduce the solvent volume and affecting the accuracy of measurement. In addition to the above considerations, a solvent should also be inexpensive, non -toxic and nonflammable for safety reasons.

Determination of Lipid Oil compound by using Gas Chromatography

Based on the chromatogram of lipid oil extract (Figure 4), the data was tabulated as Table 2. The data of chromatogram for the lipid oil extracts showed the three compounds elute between 1.482 minutes and 6.164 minutes. Only three compounds are being identified. These compounds were identifying to be Methyl Palmitate at peak 3, Methyl Stearate at peak 5 and Methyl Linoleate at peak 6.

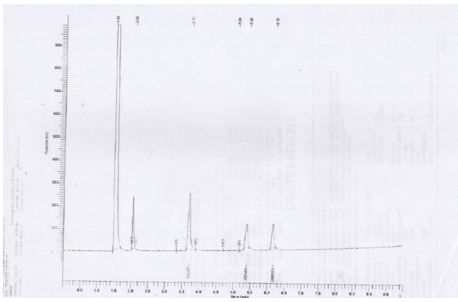


Figure 4: Chromatogram of lipid oil extract

Table 2: Data of lipid oil extraction

Peak	Retention Time (min)	Peak Area (µ V.s)	Area (%)	Fatty Acid
1	1.482	6094566.92	70.44	-
2	2.049	571764.21	6.61	-
3	3.705	1065960.47	12.32	Methyl Palmitate (16.0)
4	5.044	863.92	0.01	-
5	5.385	464872.16	5.37	Methyl Stearate (18.0)
6	6.164	454392.47	5.25	Methyl Linoleate (18.2)

Important fatty acids for biodiesel production have been proposed to be C16:0 (palmitate), C18:1(stearate) and C18.2 (linoleate) [14]. According to the result obtained, (Figure 5) the highest composition of fatty acids in algae oil was palmitic acid(12.32%) , followed by linoleic(5.25%) and stearic (5.37%) . For this analysis, palmitic and steric acid were identify as saturated fatty acid while linoleic acid as unsaturated fatty acids. Lower percentages of unsaturated fatty acids are better since highly unsaturated fatty acid are known to be responsible for the poor volatility, the low oxidation stability, and the tendency for gum formation observed in some oil-derived biodiesel [15]. In term of lipid classes, lower degree of unsaturation fatty acid is more suited for biodiesel conversion.

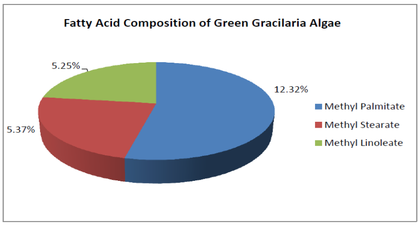


Figure 5: Composition of fatty acid in green gracilaria algae

CONCLUSION

The lipid oil from chlorophyta species have a potential to be used as biodiesel product. The objective of this study was to study the extraction of chlorophyta species by using soxhlet extraction method. This study also aims to compare the efficiency of various solvents for their ability to extract lipid oil. The three solvent used for lipid extraction were hexane, ethanol and acetone. Of the three solvents, hexane proves to be the most effective solvents for the green gracilaria species with the percentage lipid extracted of 4.08% followed by acetone and ethanol with 3.98% and 1.47% respectively. By using gas chromatography technique, the compound present in the lipid extract was identified. The fatty acid compounds were identified to be methyl palmitate, methyl stearate and methyl linoleate. Based on the content of fatty acids for biodiesel production that has been proposed by [14], it can be conclude that green gracilaria species appear to have the required fatty acid profile for conversion to high quality of biodiesel

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