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Comparison of different solvents used in nanochloropsis algae oil extraction

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Abstract

In the past decades, the edible vegetable oil fuels were of no interest as a source of energy due to their higher price compared to fossil fuels. Recent increases in petroleum prices and confusions about the future petroleum availability have increased a renewed interest in vegetable oil fuels for diesel engines. There are large amounts of low-cost oils such as algal oils that could be converted into biodiesel. Microalgae have high potential for production of biodiesel. Thus transition to second generation biofuels, such as microalgae, can also contribute to a decrease in land needs due to their presumed higher energy yields per hectare and non-requirement of agricultural land. This paper presents technological advances made in extraction of microalgae oil. Different solvent extractions were compared using Soxhlet method for maximum oil extraction. Also the most suitable solvent and temperature conditions for the highest oil extraction yield were determined.

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Introduction

The main sources of energy are petroleum, natural gas, coal, hydroelectrical and nuclear energies. The demand for energy is increasing continuously because of the increase in population and industrialization (Afify *et al.*, 2010). Transportation and energy are the major anthropogenic sources, responsible in the European Union, for more than 20% and 60% of greenhouse gas emissions, respectively. With the rapid development of the modern industry, the energy requirements have considerably increased in recent years. To find clean and renewable energy sources ranks as one of the most challenging problems facing civilization in medium to long-term; therefore, alternative energy sources should be explored. Thus "biodiesel" has frequently appeared in many recent reports (Ghasemnejadmaleki, 2014, Fischer *et al.*, 2001).

Biofuel production is expected to offer new opportunities to diversify income and fuel supply sources, promote employment in rural areas, develop long-term replacement of fossil fuels, reduce greenhouse gas emissions, boost the decarburization of transportation fuels, and increase the security of energy supply (Gorji, 2014, Mata *et al.*, 2010).

Conventional biodiesel mainly comes from soybean and vegetable oils (Bunyakiat *et al.*, 2006), palm oil (Al-Widyan *et al.*, 2002), sunflower oil (Antolin *et al.*, 2002), rapeseed oil (Peterson *et al.*, 1996), and restaurants waste oil (Bouaid *et al.*, 2007). The characteristic of biodiesel determines that it is a feasible substitute for conventional energy. Nevertheless, the production cost is generally high for biodiesel (Huang *et al.*, 2010). Since, vegetable oils are also used for human consumption; This can lead to an increase in the price of food-grade oils, causing the cost of biodiesel to increase and preventing its usage, though it has some advantages over diesel fuel. Biodiesel may also be disadvantageous when replacing the crops used for human consumption or if the feedstock is cultivated in the forests and other critical habitats with associated biological diversity (Mata *et al.*, 2010).

In order to avoid competing with edible vegetable oils, low-cost and profitable biodiesel should be produced from low-cost feed stocks such as non-edible oils, used frying oils, animal fats, soap-stocks, and greases. However, the available quantities of waste oils and animal fats are not big enough to satisfy the today demands for biodiesel. Thus transition to second generation biofuels such as microalgae can also contribute to a decrease in land needs due to their presumed higher energy yields per hectare, and also not requiring agricultural land (Reinhardt *et al.*, 2008). To resolve the worldwide energy shortage crisis, seeking for lipid-rich biological materials to produce biodiesel has recently attracted much research attention (Huang *et al.*, 2010). In the past four years, the production of biodiesel from algae has also been an area of considerable interest (Miao *et al.*, 2006).

Algae, especially microalgae, are the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti, 2007). Though the idea of using algae as a source of fuel is not new (Chisti, 1980 – 1981; Nagle and Lemke, 1990), it is just now that this issue is taken seriously mainly because of the increasing price of petroleum and, more significantly, the emerging concern about global warming, which is associated with burning away of fossil fuels. The oil productivity of many microalgae greatly exceeds that of the best oil producing crops (Shay, 1993).

Microalgae production has the potential to grow into a commercial industry because they can be grown at a very fast rate. The prevalence of microalgae makes them easily to be acquired and produced in large quantities (LeBlanc, 2011).

Several methods can be used to acquire microalgae oil. Methods like supercritical fluid extraction (Bunyakiat *et al.*, 2006), mechanical method using

either screw or hydraulic press and solvent extraction such as soxhelt method according to Randall hot extraction procedure have been used. Before now mechanical methods have been widely used in extracting oil at normal atmospheric temperature. However oil produced with this method is usually low grade with poor quality and yield poor quality fuel.

The aim of this work is to investigate, estimate and compare the potentiality and sustainability of the use of different extraction solvents from microalgae. It further compares the properties of biodiesels obtained from oil microalgae and vegetable oils.

Materials and methods

Microalgae cultures

In this experiment, we used nanochloropsis algae that can grow rapidly and live in the particular temperature of about 22°C. Nanochloropsis algae have high percentage of lipids (>50%) (Rodolfi, 2009). Algae nutrient solutions are made up of a mixture of chemical salts and water (Table I).

Table I. Compositions of algae nutrient solutions (Laing, 1991).

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric Chloride ($FeCl_3$)	0.8g
Manganous Chloride ($MnCl_2.4H_2O$)	0.4g
Boric acid (H_3BO_3)	33.6g
EDTA, di-sodium salt	45g
Sodium di-hydrogen Orthophosphate ($NaH_2PO_4.2H_2O$)	20g
Sodium Nitrate ($NaNO_3$)	100g
Solution B	1ml
Make up to 1 liter with fresh water	Heat to dissolve
Solution B	
Zinc Chloride ($ZnCl_2$)	2.1g
Cobaltous Chloride ($COCl_2.6H_2O$)	2g
Ammonium Molybdate ($(NH_4)_6MO_7O_{24},4H_2O$)	0.9g
Cupric Sulphate ($CUO_4,5H_2O$)	2g
Concentrated HCl	10ml
Make up to 100 ml with fresh water	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B1	0.2g
Solution E	25ml
Make up to 200 ml with fresh water	
Solution D (for culture of diatoms- used in addition to the solutions A and C, at 2ml per liter of culture)	
Sodium Metasilicate ($Na_2SiO_3,5H_2O$)	40g
Make up to 1 liter with fresh water	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1g
Make up to 250 ml with fresh water	

After providing the growth media solution in 1 liter basis, nanochloropsis algae were cultured in 10 bottles using fresh water and growth media, and reached the stationary stage of growth phase after 3 weeks (Fig. 1 & 2).

The Randall hot extraction procedure

The nanochloropsis algae were extracted using different solvents in Soxhlet according to Randall (Model: Behr E4).The Randall hot extraction procedure consists of three steps, Decocting, rinsing and evaporation (Fig. 3). During the first step, the extraction thimble containing the sample is immersed in the boiling solvent in the beaker - just like a tea bag in a cup of hot water. Much of the algae that are to be extracted will already dissolve during this step, and disperse in the solvent.

The upper part of the apparatus just works as a reflux condenser; the reflux will drip into the thimble, thus helping to dissolve the algae.

In the second step, the extraction thimble is lifted out of the beaker. It still contains some adhering extract;

and it might still contain some algae that have not yet been dissolved. Now the reflux from the condenser will rinse the adhering extract and also, given time, dissolve the parts of the algae that are yet undissolved.

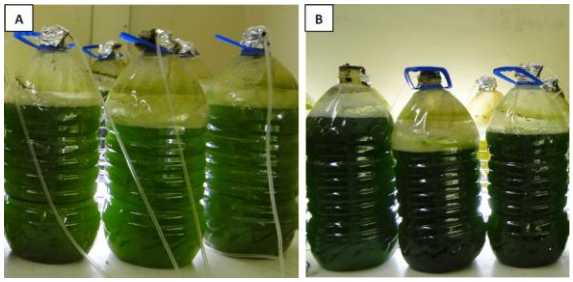

Fig. 1. Nanochloropsis algae: A) after 2 weeks, B) after 3 weeks.

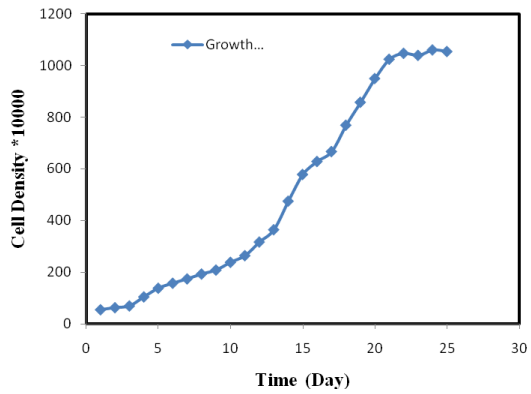

Fig. 2. Growth curves of Nanochloropsis algae.

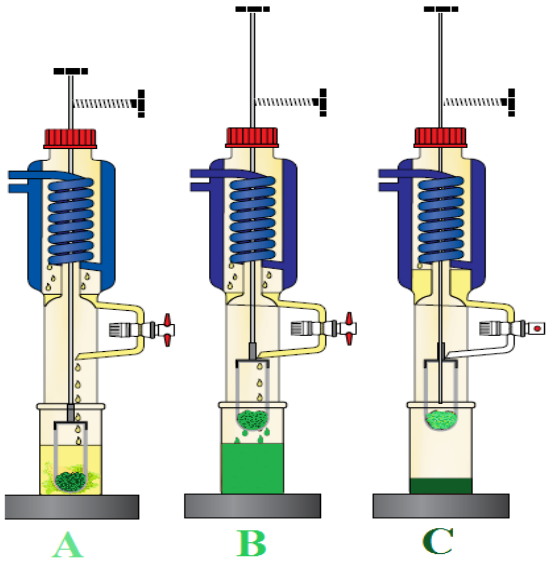

Fig.3. The Randall hot extraction procedure. A) Decocting, B) Rinsing C) Evaporation.

In order to evaporate the solvent, the reflux stopcock closes below the condenser. Now the condensate will gather in the lower part of the condenser; we can use it again for the next extraction. Due to the short distances in the apparatus, it is possible to evaporate the sample almost to dryness.

For this extraction, the used solvents were Chloroform, Methanol, Ether, normal Hexane and 2-Propanol. First, a 100 ml sample of the culture was obtained and centrifuged at 2000 rpm for 20 min; then the algae and water were separated, and the excess water was thrown away. The above steps were repeated until enough nanochloropsis algae were separated. Next the separated algae were filtered through a Millipore membrane (0.2 µm) and put aside for about 8 hours at 60°C to dry. After drying, the filtered material was taken to measure its dry biomass weight. After preparation of 7 samples placed in extraction thimbles, 60 ml of the solvents was poured into the Soxhlets. Extraction time was about 7 hours at 70°C (Fig. 4).

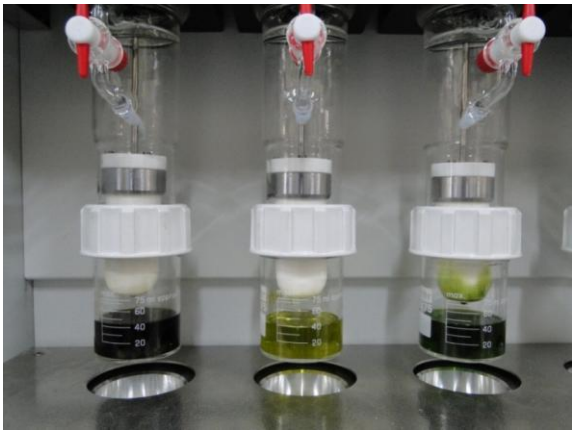

Fig. 4. Extraction of lipids from algae by Behr E4.

Results and discussion

Calculation of the lipid content

The yield of the microalgae lipids was calculated by the equation:

$$Y(\%) = \frac{Wi - Wf}{Wi}$$

Where Wi and Wf are the weights of the Initial dry algae (mg) and the Final dry algae (mg), respectively.

Comparison of Different Solvents

The lipid extraction of nanochloropsis algae by Soxhlet method according to Randall hot extraction procedure showed that using different solvents in the same conditions leads to different results. Table II shows the results obtained through using different solvents in terms of the samples' behavior, volume percentage of the solvents, extraction temperatures, and the initial and final weights of dried nanochloropsis algae.

Table II. Extraction of lipids by different solvents.

Sample	Solvents	v/v %	Extraction temperature °C/(boiling point °C)	Initial dry algae mg	Final dry algae mg	Percent lipid %
1	n-Hexane	1	70/(68)	479.35	413.87	13.66
2	n-Hexane	1	75/(68)	451.21	384.32	14.82
3	Chloroform	1	70/(61.2)	300.74	239.42	20.39
4	Chloroform	1	75/(61.2)	342.34	262.12	23.43
5	Methanol	1	70/(64.7)	325.54	198.1	39.07
6	Methanol	1	75/(64.7)	355.44	212.12	40.32
7	Ether	1	40/(34.6)	227.78	217.97	4.31
8	2-Propanol	1	90/(82.5)	446.58	202.67	54.61
9	Chloroform /Methanol	1/1	70/(#)	424.33	312.98	26.18
10	Chloroform /Methanol	1/2	70/(#)	295.96	117.27	60.37
11	Chloroform /Methanol	2	70/(#)	410.18	232.39	43.34

Pure and mixed solvents were used in different temperatures. The third experiment was conducted from the soxhelt method according to Randall hot extraction procedure using 2-Propanol. The 2-Propanol solvent was more efficient in extracting the lipids from the nanochloropsis algae. The results showed that 2-Propanol with high boiling point has a lipid extraction of 54.61%, and that of Ether with low boiling point is 4.3%. Ethers have relatively low boiling points due to their inability to form hydrogen bonds with each other. Because of the electro negativity difference between the oxygen and carbon atoms of ether, the molecule is slightly polar. Although they have a low reactivity overall, the two lone pairs of electrons on the oxygen atom do afford the ether molecule some reactivity; the ether molecule is subject to reacting with strong acids and serves as a Lewis base.

Effect of volume percentage of the solvents

Neutral lipids or generally storage lipids are extracted with relatively non-polar solvents such as diethyl ether or chloroform but membrane-associated lipids are more polar and require polar solvents such as ethanol or methanol to disrupt hydrogen bondings or electrostatic forces. Extraction of oil by Chloroform/Methanol (1/2 v/v%) solvent was higher than by Chloroform/Methanol (2 v/v%) because Chloroform/Methanol (1/2 v/v%) makes azeotrope in this composition (Fig. 5). However, the azeotrope of Methanol/Chloroform boils at 53.5 °C, Which is significantly less than either that of the components. The mixed polarity solvent system with Methanol gave better overall yield than the hydrocarbon–only-extractions. The azeotropic mixture of Chloroform/Methanol (1/2 v/v%) gave the highest yield.

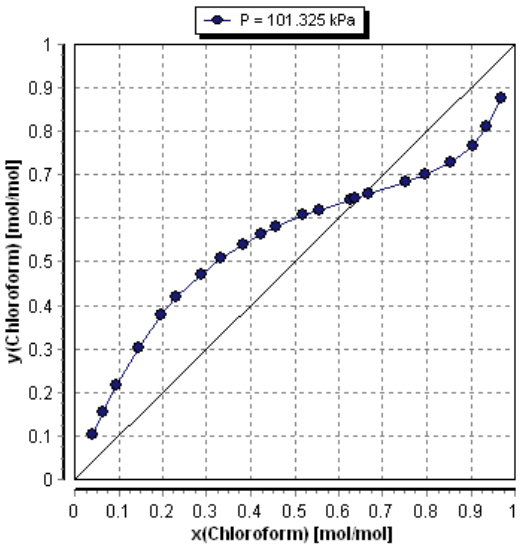

Fig. 5. Vapor-Liquid Equilibrium Chloroform/ Methanol.

Effect of temperature on the rate of extraction

The temperature has effect on the rate of extraction of crude oils from microalgae with solvents. Quantitative data have been presented relating extraction rate and temperature for nanochloropsis algae with n-Hexane, Chloroform and Methanol solvents in Table II. The results show a decrease in the growth temperature from 70 to 75 °C led to an increase in the lipid extraction from 13.66 to 14.8%, 20.39 to 23.43%, and 39.07 to 40.32% for n-Hexane, Chloroform and Methanol solvents, respectively.

Conclusion

Comparison of the laboratory Soxhlet extractions showed that by using different solvents in constant conditions, lipids extraction gets simpler and the extraction percentage increases. The results showed that higher percentage of lipid was extracted by using the Soxhlet extraction method. The percentage extraction of oil was 60.37 for Chloroform/Methanol (1/2 v/v %) solvent. Also the mixed polarity solvent system with Methanol gave better overall yield than the hydrocarbon-only-extractions, though the extraction of oil via Ether had the lowest percentage (about 4%). Finally, 2-Propanol solution had higher boiling point, which is desirable for oil extraction.

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