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A study on effective lipid extraction methods from certain fresh water microalgae

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ABSTRACT

Chlorella sp., *Scenedesmus sp.*, and *Neochloris sp.* were isolated from fresh water ponds in and around Gandhigram, Dindigul District, Tamilnadu, India and used for lipid extraction. Different methods, including autoclaving, bead-beating, microwaves, sonication and a 10% NaCl solution treatments were tested to identify the most effective cell disruption method. The total lipids from three microalgal species were extracted using a mixture of chloroform and methanol. Fatty acid composition was detected by GC. Finally the sonication method was found to be the most applicable and efficient method of lipid extraction from microalgae. *Neochloris* sp. showed higher oleic acid productivity of 18.09 mg g^{-1} dw but *Chlorella* sp. was linoleic acid productivity of 17.61 mg g^{-1} dw when the cells were disrupted using the sonication method.

Introduction

Lipids including animal fats and plant oils are the main feedstock for biofuels (biodiesel) production. Animals and most microorganisms are heterotrophs. They are able to efficiently synthesize a compact storage of energy-fat while releasing a certain amount of CO_2. Plants, including algae, are autotrophs and they function with bulky storage of energy-starch [1]. Currently, biodiesel is made from a variety of feedstocks, including pure vegetable oils, waste cooking oils and animal fat; however, the limited supply of these feedstocks impede the further expansion of biodiesel production. Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production. In recent years, use of microalgae as an alternative biodiesel feedstock has gained renewed interest from researchers, entrepreneurs, and the general public [2].

In particular, biodiesel has two main advantages, the mitigation of carbon dioxide and as a substitute for petroleum [3]. The processes involved in biodiesel production from microalgae are cultivation, harvest, lipid extraction, and the transesterification of the lipids. Although all these steps are essential, the lipid extraction is particularly important because cell disruption comes in this step [4]. Therefore, the appropriate cell disruption method and device are key to increasing the lipid extraction efficiency. Different methods, such as microwave, sonication and bead beating, have already been used for cell disruption. For example, microwave were recently suggested as an efficient method for vegetable oil extraction [5], while sonication is widely being used to disrupt microbial cells [6]. Bead beating that causes direct mechanical damage to cells based on high speed spinning with fine beads has been used both on a laboratory as well as in an industrial scale [7].

So, the most efficient method for microalgae needs to be studied. The present study envisages developing oil extraction methods from microalgae and compares the different methods.

Materials and methods

Isolation, Purification and Identification of microalgae

Algal samples were collected from different freshwater bodies in and around Dindigul District, Tamilnadu, India. Algal were cultured (modified Bristol medium) and purified by standard plating methods, identified and authenticated (Table 1).

Cultivation and harvest of microalgae

Chlorella sp., Scenedesmus sp., and Neochloris sp., were selected for this study based on their potential for lipid production. The microalgae were incubated in separate 2-L jars using a BG-11 medium [8] with 0.3 v/v/m air and 2500 lux. The biomass of cultured cells was harvested by centrifugation and freeze-dried at -70^{0}C under vacuum.

Cell disruption

An aliquot (0.5 g) of the dry cell biomass was blended with 100 ml of distilled water and the mixture disrupted using five different methods as follows: 1) autoclaving at 121^{0}C with 15lbs for 5 min. 2) bead-beading using a bead beater at a high-speed of 3500 rpm for 5 min, 3) microwaves using a microwave oven at 100^{0}C for 5 min (2450 MHz) [4], 4) sonication using a sonicator at a resonance of 50 Hz for 15 min, 5) osmotic shock using a 10% NaCl solution with vortexing for 1 min and maintained for 48 h.

Lipid extraction

The total lipids were extracted from microalgal biomass using a modified method of Bligh and Dyer [9]. The lipids were extracted with chloroform-methanol (2:1, v/v), and then separated into chloroform and aqueous methanol layers by the addition of methanol and water to give a final solvent ratio of chloroform:methanol:water of 1:1:0.9. The chloroform layer was washed with 20 ml of a 5% NaCl solution, and evaporated by rotary vacuum evaporator (Rotavapor R-210, Buchi). The weight of the crude lipid obtained from each sample was measured using an electronic scale.

Analysis of fatty acid composition

A fatty acid composition analysis was performed using a Shimadzu 2010 gas chromatograph (Shimadzu Scientific

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Instruments, Columbia, MD, USA) equipped with a flame ionization detector and a DEGS capillary column (30 mx0.25x0.25µm). Fifty milligram samples were placed into capped test tubes, saponified with 1 ml of saturated KOH-CH_3OH solution at 75^0C for 10 min, and then subjected to methanolysis with 5% HCl in methanol at 75^0C for another 10 min [10]. Thereafter, the phase containing the fatty acids was separated by adding 2 ml of distilled water and then recovered. The components were identified by comparing their retention times and fragmentation patterns with those for standards [11]. Six fatty acids (C16:1, C17:0, C18:0, C18:1, C18:2, and C18:3) were used as the standard materials.

Results and discussion

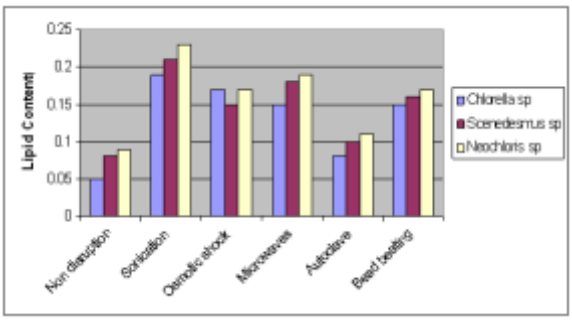
Identification and mass multiplication of microalgae

In the initial phase of the work, from the nine different water bodies samples were collected and 20 genera (Table. 1) of fresh water microalgae were identified authenticated. Out of 20 number of fresh water microalgae three isolates viz., *Chlorella* sp., *Scenedesmus* sp., *and Neochloris* sp., selected for this study based on their potential for lipid production. The three microalgal species were grown for 14 days in 27^0C after which the productivity and lipid content of the algae were analysed. *Chlorella* sp., showed the highest biomass productivity at 52.7 mg $L^{-1}d^{-1}$ on day 14, followed by *Scenedesmus sp*. 42.5 mg $L^{-1}d^{-1}$ and *Neochloris* sp. 39.2 mg $L^{-1}d^{-1}$.

Comparison of lipid extraction methods

The lipid content from *Scenedesmus* sp. was about 132.3 mg L^{-1} which was higher than *Chlorella sp*. and *Neochloris* sp. The lipid productivity of *Scenedesmus* sp. was highest at 9.5 mg $L^{-1}d^{-1}$ (Table 2). Higher lipid content was deserved in all isolates when using the sonication method when compared to other methods (Fig. 1).

Fig. 1. Lipid extraction efficiency according to microalgae species and method

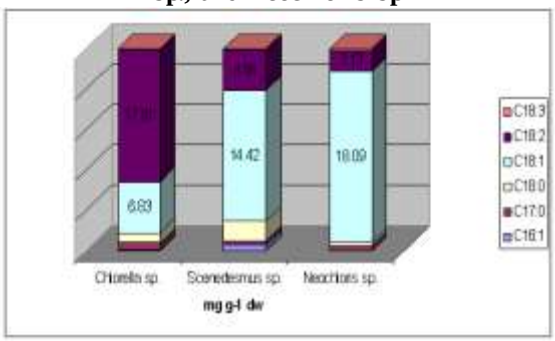


The microwaves and osmotic shock methods were also efficient among the methods tried recording 0.19 g and 0.17 g, respectively, for *Neochloris* sp. while the autoclaving method recorded the lowest efficiency at 0.11 g. Previous study, by the bead-beating method was also shown to extract higher lipid content from *Botryococcus braunii* than sonication, homogenization, french press, and lyophilization [6]. Further studies by the same group or researchers showed the highest efficiency of lipid extracted from *Botryococcus* sp., *Chlorella vulgaris* and *Scenedesmus* sp. using microwave oven method [4]. For *Chlorella* sp., the sonication methods showed the highest efficiency, whereas the autoclave method showed the lower efficiency. For *Scenedesmus* sp., sonication method showed highest efficiency followed by microwaves, bead-beating, osmotic shock and autoclave. A similar lipid extraction method using microwaves has already been reported for vegetable oils and animal fats [12]. This study concludes that the sonication method was found to be the most applicable for large scale lipid extraction from microalgae. Furthermore, this lipid extraction method can be easily scaled-up.

Composition of fatty acid analysis

The fatty acid composition of the three microalgae was determined using GC analysis (Fig. 2). In a previous report, palmitic, stearic, oleic and linolenic acid were recognized as the most common fatty acids contained in biodiesel [13]. Oleic acid (C18:1) and linoleic acid (C18:2) were commonly dominant in three microalgae. Oleic acid was higher in *Neochloris* sp. and lower in *Chlorella* sp. at 18.09 mg g^{-1} dw and 6.83 mg g^{-1} dw, respectively, while linoleic acid was higher in *Chlorella* sp. at 17.61 mg g^{-1} dw. The properties of a biodiesel fuel, including its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity, and lubricity, are determined by the structure of its component fatty esters and also as the oleic acid content increases the oxidative stability for longer storage is enhanced [14]. So among the tested microalgal species, *Neochloris* sp. and *Scenedesmus* sp. showed the highest oleic acid content, making it the most suitable for the production of good quality biodiesel.

Fig. 2. Fatty acid composition of Chlorella sp., Scenedesmus sp., and Neochloris sp



Conclusions

The efficient lipid extraction from microalgae depends upon algal species and extraction method. The highest lipid content was extracted from *Scenedesmus* sp. and the sonication method showed the highest efficiency for its recovery. Fatty acid composition detected by GC, showed the highest C18:1 in *Neochloris* sp. and *Scenedesmus* sp. but *Chlorella sp* showed highest C18:2, when the cells were disrupted using the sonication method. Thus, it was concluded that the sonication method would be the most easy and efficient method for lipid extraction from microalgae.

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References

1. Xiong, W., Gao, C., Yan, D., Wu, C., Wu, Q., (2009) Double CO_2 fixation in photosynthesis-fermentation model enhances algal lipid synthesis for biodiesel production. Bioresour. Technol. 101, 2287-2293.

2. Wen, Z., and Michael B. Johnson. (2009) Microalgae as a feedstock for biofuels production Virginia Cooperative Extension publication 442-886.

3. Chisti, Y., (2008) Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 26, 126-131.

4. Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y., and Oh, H. M., (2009) Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 101, 75-77.

5. Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., Cintas, P., (2008) Improved extraction of vegetable oils under high-inensity ultrasound and /or microwaves. Ultrason. Sonochem.15, 898-902.

6. Lee, S.J., Yoon, B.D., Oh, H.M., (1998) Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. Biotechnol. Tech. 12, 553-556.

7. Geciova, J., Bury D., Jelen, P., (2002) Methods for disruption of microbial cells for potential use in the dairy industry- a review. Int. Dairy J. 12, 541-553.

8. Rippka, R., Deruelles, J., Waterbury, J. B. E et al. (1979) Generic assignments, strain histories and properties of pure culture of cyanobacteria. J. Gen. Microbiol.,111, 1-61.

9. Bligh, E.C., Dyer, W.M., (1959) A rapid method of lipid extraction and purification. Can.J. Biochem. Physiol. 37, 911-917.

10. Schreiner M. (2006) Optimization of solvent extraction and direct transmethylation methods for the analysis of egg yolk lipids. Int. J. Food. Prop. 9:573-581.

11. Xu, N., Zhang, X., Fan, X., Han, L., Zeng, C., (2001) Effects of nitrogen source and concentration on growth rate and fatty acid composition of *Elipsoidion sp*. (Eustigmatophyta). J.Appl. Phycol.13, 463-469

12. Mahesar, S.A., Sherazi, S.T.H., Abro,K., Kandhro, A., Bhanger, M.I., Van de Voort, F.R., Sedman, J., (2008) Application of microwave heating for the fast extraction of fat content from the poultry feeds. Talanta 75, 1240

13. Knothe, G., (2008) "Designer" biodiesel: optimizing fatty esters composition to improve fuel properties. Energy Fuel 22, 1358-1364.

14. Knothe, G., (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel process. Technol. 86, 1059-1070.

Table 1. Isolation of microalgae from different location in Dindigul District

S. No	Location	Name of the microalgae
1	Dharmathupatti Dam	*Euglena, Haematococcus, Oscillatoria*
2	Kamarajar Dam	*Chlorococcus, Oocystis, Ulva*
3	Manjalar Dam	*Cylindrospermum, Oedogonium*
4	Marudhanadhi	*Zygnema, Spirogyra*
5	Pannapatti	*Chlorella, Rivularia*
6	Parapalar Dam	*Nostoc, Tolypothrix*
7	Palar- Porandalar Dam	*Scenedesmus, Dunellia*
8	Varadamanadhi Dam	*Ulothrix, Scytonema*
9	Kuthiraiyar Dam	*Neochloris, Spirulina*

Table 2. Lipid and growth productivity of *Chlorella* sp., *Scenedesmus* sp., *and Neochloris* sp

Character	Algal Species		
	Chlorella sp.	*Scenedesmus* sp.	*Neochloris* sp.
Dry weight ($g\,L^{-1}$)	0.5	0.5	0.5
Biomass production($mgL^{-1}d^{1}$)	52.7	42.5	39.2
Average Lipid content(mgL^{-1})	111.6	132.3	128.3
Lipid production ($mgL^{-1}d^{-1}$)	8.0	9.5	9.2