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Evaluation of factors affecting on lipid extraction for recovery of fatty acids from *Nannochloropsis oculata* micro-algae to biodiesel production

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Abstract

Background: This study aimed at determining the appropriate method for dewatering and drying biomass and selecting a suitable organic solvent for lipid extraction.

Methods: *Nannochloropsis Oculata* was cultured in Gillard F/2 medium and after reaching the end of the stationary growth phase, algal biomass was separated from aqueous by centrifuge and dried through three methods: Oven, Air-dried and Lyophilized. Soxhlet apparatus achieved lipid extraction of all samples: diethyl ether, n-hexane and n-pentane using three solvents. At each stage, the quantity and quality of the extracted lipids were determined by Gas Chromatography.

Results: In all three drying methods, palmitic acid and palmitoleic acid, and most significantly fatty acid composition of microalgae were extracted. The fatty acid composition of palmitic acid extracted by Diethyl ether was significantly more than the other two solvents. Maximum production of triglyceride was observed in Lyophilized and air-dried microalgae where lipid extraction was performed with diethyl ether solvents and are 75.03% and 76.72% of fatty acid.

Conclusion: The use of Lyophilized method for dewatering and drying of biomass and Diethyl ether as solvent for the extraction of lipids from biomass, studied in this paper, as compared to other methods, had higher yields and researches proved that the production of biodiesel from microalgae's lipid was more efficient.

Keywords: Nannochloropsis oculata, Lipid, Solvent, Biomass, Biodiesel

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Introduction

Rapid depletion of fossil fuels with increasing energy consumption and global warming have resulted in a move towards alternative energy sources with less emissions (1,2). In recent years, biodiesel as a renewable, biodegradable, and nontoxic energy has received considerable attention (3-5). The raw materials for biodiesel production now mainly include vegetable seed oil, soybean oil, some recovered animal fats and microalgae (3). Due to high growth rate, short growth time, high biomass production and low land use, microalgae have certain advantages compared to other biodiesel sources (1,4). The application of microalgae to economical production of biodiesel requires that a high percentage of microalgal biomass be composed of fatty acid containing compounds that can be converted to Fatty Acid Methyl Esters (FAME) (6). Microalgae are theoretically capable of producing much more lipids than any conventional crop and depending on the species; microalgae produce many different kinds of lipids (4,7).

The key processes involved in biodiesel production using microalgae are cultivation, biomass harvesting, dewatering and drying of biomass, lipid extraction from biomass, and conversion of lipid to biodiesel (1). Each one of these steps is important in the economic production of biodiesel from microalgae. Meanwhile, researches continue in this field to achieve the optimal condition for each step. For example, different studies are being conducted on the quality and quantity of microalgae cellular lipid as the result of variety of growth condition (temperature and light

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intensity) or type of the medium (nitrogen, phosphate and iron concentration) (8-10). Furthermore, some studies are being done on the methods of microalgae extraction from medium by centrifuge, chemical coagulation and electrical coagulation or converting lipid to biodiesel using various catalysts (11-14).

Drying and lipid extraction from oleaginous microalgae are important steps in biodiesel production. The diversities of composition and structure of lipids from various kinds of microalgae call for different methods of extraction, thereby affecting the cost of operations (2). Lipid extraction from microalgae biomass is done through various means: mechanical, organic solvent, enzymatic extraction, osmotic shock, supercritical fluid extraction, and microwave/ultrasound assisted extraction (5,7,15). In spite of recently developed extraction methods including supercritical fluid extraction or microwave/ultrasound assisted extraction, lipid extraction from biomass by organic solvents was still found superior in comparison to other because of simplicity, relatively low cost and the non-requirement for special equipment. This method is nowadays used extensively in researches related to biodiesel (5). In studies conducted so far, only the effect of solvent on the quantity of lipid has been surveyed while quality of extracted lipid has an important role in biodiesel production and its correspondence with standards. The purpose of this study was to investigate the effect of solvent type on fatty acids composition extracted from microalgae. Taking into consideration the temperature's effect on fatty acids composition, three common methods of drying microalgae biomass were studied, and the quality of fatty acid attained from each method was determined in biodiesel production.

Methods

The study was conducted in the first half of 2012 at the Environmental Health Engineering Research Center of Kerman University of Medical Science using pilot in laboratory scale.

Culture conditions of Nannochloropsis oculata

Stock culture of *Nannochloropsis oculata* was obtained from the Research Institute for Aquaculture in the south of the Iran. This microalga is a eukaryotic photosynthetic microorganism and given its simple structure, it had a fast growth rate (8). Stock culture was cultivated on liquid of F/2 Gillard medium in bubble column photobioreactors. Column photobioreactors were made of Pyrex glass tubes (650 mm in height and 35 mm of internal diameter). Fluorescent lamps (FL20D, OSRAM, Korea) were used as the light source for their growth. According to the study of Banerjee *et al* (16) and Sen *et al* (17) continuously illuminated 70 µE m^{-2} s^{-1} and the photoreactors were operated at 20 °C on the basis of data reported in the literature (8). Sterile-air containing 2% (v/v) CO2 by filtering using glassfiber was aerated into the column through an air sparger at the bottom of the column at 0.5 v/v flow rate.

Dewatering and drying of biomass

After the microalgae reached the end of the stationary growth phase, the algal biomass was extracted from medium by centrifuge (8000 rpm, 20 minutes) (1,5) and was dried by the three different methods. Drying in oven (105 °C for 4 hours), drying in ambient temperature (20 to 25 °C for one week) and Lyophilization (by freeze dryer machine, model of EYELA and temperature of -69 °C) were some of the methods of study (7).

Extracting lipid from biomass by the method of soxhlet

20 g of every sample of dried biomass, unified gently by mortar and lipid extraction from it was done by soxhlet model of Soxtee 2050 in this way: Each cycle entailed boiling for 25 minutes, lipid extraction for 40 minutes, and solvent recovery for 15 minutes. The extraction of all the samples was done in 5 cycles. For the extraction process, 3 common solvents, Diethyl ether, N-hexane and N-pentane with different boiling temperatures having purity levels were used. The extracted lipid by 0.45 µm filter, was smoothed to remove microalgae leftovers (8). The drying and extracting lipid steps were repeated three times, and the results were reported on averages. After determining the amount of lipid, the dried lipid was solved in 0.4 ml Isopropyl alcohol, and the amount of triglycerides in lipid was measured according to Xin *et al* (18).

Analyzing and determining the fatty acid characteristics

To determine the compositions of fatty acid, 150 mg of each sample of extracted lipid was derived by BF3 according to the EN Iso5509 standard (6). The organic phase, attained by Gas chromatography machine model Technologies (7890 A-5975c Agilent) equipped with DB-wax pole (30 m length, 0.25 mm internal diameter, 0.25 µm thickness) and Flame Ionization Detector (FID), was analyzed. The temperature of injector and detector was fixed at 250 °C. The temperature attained 180 °C within 5 minutes and 220 °C at the speed of 4 °C/min and was maintained at this degree for 25 minutes. Helium was also used as carrier gas in the amount of 1.5 ml/min. The compositions of fatty acid were calculated on the basis of the percentage of total fatty acid and according to the peak attained from analyzing (9,19).

Statistical analysis

Data were analyzed using one-way ANOVA, and Tukey test was used to determine the statistical difference between media. A value of $P<0.05$ was considered statistically significant (20,21).

Results

The results of important fatty acid compositions extracted using different organic solvents in *Nannochloropsis oculata* microalgae that dried in oven, in the vicinity of open air, and through the lyophilization method are presented in Figures 1 to 3. The results showed that in all of these methods, the highest percentage of compositions was related to palmitic and palmitoleic acids. The results related to the quantity of extracted compositions also showed that in lyophilization and open-air methods, the Diethyl ether was more suitable than the two other solvents. The results of triglyceride extracted using different organic solvents in *Nannochloropsis oculata* microalgae dried by different methods are presented in Figure 4.

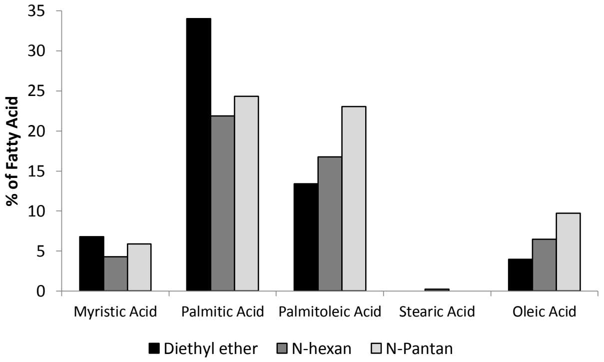

Figure 1. Fatty acid compositions in dried microalgae by oven and using different organic solvents (% of total fatty acids)

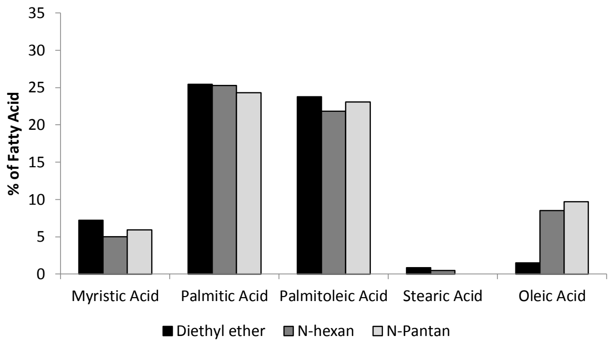

Figure 2. Fatty acid compositions in dried microalgae by open air and using different organic solvents (% of total fatty acids)

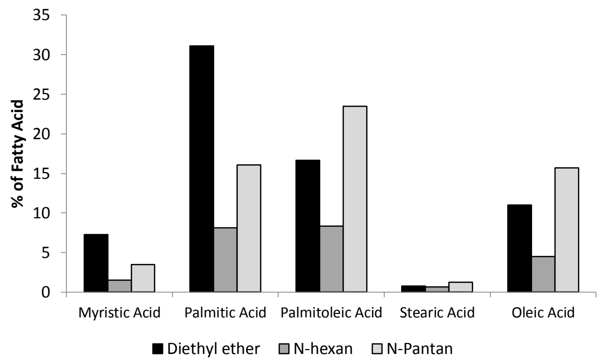

Figure 3. Fatty acid compositions in dried microalgae by lyophilization and using different organic solvents (% of total fatty acids)

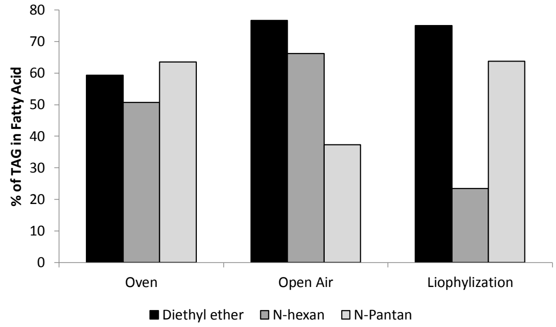

Figure 4. Triglyceride extracted by using different organic solvents in *Nannochloropsis oculata* microalgae that dried by different methods (% of TAG in total fatty acids)

Discussion

Effect of biomass drying on fatty acid extracted from microalgae

Vegetable oils currently used for biodiesel production are mainly C16 and C18. Olofsson *et al* proposed myristic acid, palmitic acid, palmitoleic acid, stearic acid, and oleic acid as important fatty acids for biodiesel production (22). In this study, the one-way ANOVA results and Tukey test showed that in all three methods of biomass drying, the highest composition of microalgae fatty acid are palmitic and palmitoleic acids ($P<0.05$). Comparison of these two compositions among the three methods of drying microalgae also showed that the amount of palmitic acid in lyophilization and oven methods is more than its amount in the other method. But the amount of palmitoleic acid in dried microalgae in open air is more than its amount in the two other methods. The comparison between Figures 1 to 3 showed that the proportion of palmitoleic acid to palmitic acid is decreased by increasing temperature as in the biomass dried in open air than biomass dried in oven. This proportion is decreased because of increasing temperature and reached 0.66% from 1.398% that is according to Kleinschmidt *et al* results (23). Also Widjaja *et al* (5) in a study on the effect of drying temperature on the lipid extracted from Chlorella vulgaris concluded that the temperature of 60 °C caused the quantity in the amount of extracted lipid to decrease. But by increasing temperature to 80 °C or more, the amount of lipid decreased substantially. The temperature can affect the pattern, route and metabolism activity of microalgae cell and, as a result, affect the cellular composition (24). The result further showed that the highest percentage of fatty acid in dried microalgae was attained in open air and that the prolixity of drying time was one weakness in this method that made it uneconomical. Whereas the preparation of microalgae by lyophilization method is done in a shorter time, does not require special storage conditions, and dewatering is done easily (9).

The lyophilization method leads to better extraction of lipid by increasing the sample's contact area (25). Microalgae further consist of special cellular environment that is thick. It needs the suitable method of defeat cell before lipid extraction. Hence the lyophilization method is considered as one of the important methods in this field (2).

The effect of solvent's type on fatty acid extracted from microalgae

There are two major steps in lipid extraction from microalgae using solvent: one is the full extraction and solving lipid in organic solvent and the other is the omission of non-lipid components in extracted solvent. The retrieval of used solvent and the type of solvent have crucial roles in determining the efficiency of lipid extraction and economic performance of the process (2).

Different solvents solve different kinds of lipid in themselves that regarding the relationship between microorganism lipid and other cellular components the relationship between lipid environment and solvent coexistence and also specifications of solvent, such as polarity and molecular chain length is different (2). The one-way ANOVA test results and Tukey test results between case study solvents showed that the amount of palmitic acid and myristic acid in extracted fatty acid composition by Diethyl ether was more than the two other solvents ($P<0.05$). Also, the amount of palmitoleic acid, stearic acid and oleic acid in fatty acid composition extracted by N-Pentane was more than the two other solvents ($P<0.05$).

These results showed that the used solvent polarity had an important effect on yield and composition of lipid extracted from microalgae. In this study, three different organic solvents were used; Diethyl ether is being a solvent with high polarity and N-hexane and N-pentane being non-polar solvents. The results showed, like the study of Widjaja *et al* (5) and the study of Ryckebosch *et al* (7) on Nannochloropsis salina, that N-hexane is not a suitable solvent to extract lipid from it, whereas in the study on the types of Botryccous, Chlorella vulgaris, and Scenedesmos the solvent of hexane mixture/iso propanol showed better results as compared to other solvents in different temperatures and pressures (26). Also in the study of McNichol *et al* (6), the result of the survey relating to extraction by the method of soxhlet and solvent polarity's effect on lipid yields showed that polar solvents like Chloroform/Methanol, Ethanol, and Acetone had higher lipid yield than non-polar solvents like N-hexane. The spot welding of solvent is another factor that influences the choice of a solvent in biomass lipid extraction. Among the case study of solvents, Diethyl ether has the lowest spot welding and has advantage over the two other solvents.

Effect of biomass drying method and solvent's kind on biodiesel production from microalgae

According to the interpretation of different results attained from fatty acid compositions as the result of different methods of drying biomass and the type of solvent, biodiesel production in the amount of attained triglyceride in each method is meaningful because the oil extracted from microalgae in the form of triglyceride is convertible to biodiesel (21). The triglyceride in Nannochloropsis oculata includes saturated fatty acids and monounsaturated fatty acids stored in cell vacuoles (22)and in some of the microalgae kinds consist of 40 to 70% of biomass dry weight (21).

According to Figure 4 and the results of one-way ANOVA test and Tukey test, the amount of triglyceride in the dried microalgae fatty acid composition in open air and lyophilization method in which lipid extraction is done by Diethyl ether solvent, are more and equal to 76.72% and 75.03% of fatty acid. In this study, three temperatures of high (oven method), average (open air) and low (liphilization) were used. The temperature was one important factor in the amount of triglyceride in the composition of microalgae fatty acid and in the production of biodiesel (27,28). By increasing extraction temperature in oven method, the amount of triglyceride decreased. It was because of triglyceride oxidation in high temperature, and as the result of formation of Hydroproxid (-OOH) by Aldehydes, Ketones and fatty acids, showed more reactions (5).

Conclusion

Because of the temperature effects on microalgae cellular structure and solvent type effects on fatty acid composition, method of biomass drying is one of the determining factors in the extraction of microalgae biomass lipid. The results of this study showed that using lyophilization method in order to dewater and dry biomass and also Diethyl ether as solvent in order to extract lipid from Nannochloropsis oculata biomass had higher yield as compared to other study methods of this research and in researches related to biodiesel production from microalgae lipid.

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Ethical issues

we certify that all data collected during the study is presented in this manuscript and no data from the study has been or will be published separately.

Competing interests

Authors declare that they have no competing interests.

Authors' contributions

SD and BH conceived and designed the study. MM and AR performed the literature search and wrote the manuscript. All authors participated in the data acquisition, analysis and interpretation. All authors critically reviewed, refined and approved the manuscript.

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