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Oil Extraction from Microalgae *Dunalliela* sp. by Polar and Non-Polar Solvents

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***Abstract*—**Microalgae are tiny photosynthetic plants. Nowadays, microalgae are being used as nutrient-dense foods and sources of fine chemicals. They have significant amounts of lipid, carotenoids, vitamins, protein, minerals, chlorophyll, and pigments. Oil extraction from algae is a hotly debated topic currently because introducing an efficient method could decrease the process cost. This can determine the sustainability of algae-based foods. Scientific research works show that solvent extraction using chloroform/methanol (2:1) mixture is one of the efficient methods for oil extraction from algal cells, but both methanol and chloroform are toxic solvents, and therefore, the extracted oil will not be suitable for food application. In this paper, the effect of two food grade solvents (hexane and hexane/ isopropanol) on oil extraction yield from microalgae *Dunaliella* sp. was investigated and the results were compared with chloroform/methanol (2:1) extraction yield. It was observed that the oil extraction yield using hexane, hexane/isopropanol (3:2) and chloroform/methanol (2:1) mixture were 5.4, 13.93, and 17.5 (% w/w, dry basis), respectively. The fatty acid profile derived from GC illustrated that the palmitic (36.62%), oleic (18.62%), and stearic acids (19.08%) form the main portion of fatty acid composition of microalgae *Dunalliela* sp. oil. It was concluded that, the addition of isopropanol as polar solvent could increase the extraction yield significantly. Isopropanol solves cell wall phospholipids and enhances the release of intercellular lipids, which improves accessing of hexane to fatty acids.

***Keywords*—**Fatty acid profile, Microalgae, Oil extraction, Polar solvent.

I. Introduction

MICROALGAE are unicellular photosynthetic microorganisms, living in saline or fresh water and they make algal biomass using sunlight, water, and carbon dioxide [1]. In addition, they are useful in bioremediation applications and also used as nitrogen fixing biofertilizers. Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil, and photobiologically produced biohydrogen [2].

In the industry, microalgae have been used as source for a wide variety of food supplements, pharmacological substances, lipids, polymers, toxins, pigments, enzymes, biomass, wastewater treatment, and ‘‘green energy’’. They are also important in aquaculture as a source of nutrients, production of oxygen, consumption of carbon dioxide, and nitrogen-based compounds [3], [4].

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Microalgae have been recognized as a promising alternative source for lipid production [5]–[7]. Several species of microalgae can be induced to produce specific lipids and fatty acids through relative simple manipulations of the physical and chemical properties of their culture medium. Microalgae can accumulate substantial amounts of lipids (approximately 5–50% of dry weight).

Lipids can be used as a source for biofuels, as building blocks in the chemical industry and edible oils for the food and health market. One of the bottle-necks in this industry is the lack of a modified and efficient method for extraction of lipid from single cells. Therefore, in this study, the effects of extraction time (20-40 minutes) and type of solvent (Hexane, hexane/isopropanol 3:2 and chloroform/methanol 2:1) on lipid extraction yield from *Dunalliela* sp. strain M_1 were evaluated.

II. Materials and Methods

A. Microalgae Isolate Preparation

The isolate of *Dunalliela* sp. M_1 isolated from Maharloo Lake of Shiraz was used in this research. This isolate was obtained from the microalgae collection of branch for Northwest & West region, Agriculture Biotechnology Research Institute Iran.

B. Microalgae Culturing

The isolate was inoculated in sterile glass pot with modified Johansson culture (Fig. 1). *Dunalliela* sp. has maximum growth rate in temperature ranging from 20 ˚C ~ 30 ˚C and the optimum temperature for this isolate is 26 ˚C [8], [9]. Best light intensity for microalgae growth ranging from 2500 ~ 5000 lux. Light was supplied by fluorescent lamps, and light intensity was set to 4000 lux. For aerating and mixing of culture, a Hailea model 420 air pomp was used. Appropriate pH for microalgae culture is ranging from 7~7.5 [10]. CO_2 cylinder was used to control suitable pH. By injecting CO_2 to the culture, the pH was adjusted on 7.5.

Fig. 1 Microalgae culturing

After eight days in the logarithmic phase, the culture was scaled up to 20 liters in the transparent PET vessel. Ultimately

the culture was scaled up to 300 liters in an open pond photo bioreactor (Fig. 2).

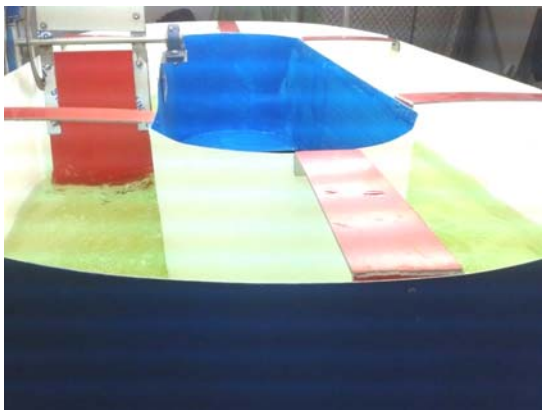

Fig. 2 Open pond photo bioreactor

C. Microalgae Harvesting

The microalgae were harvested by electrocoagulation method [11], [12]. Microalgae culture was poured in the harvester vessel (Fig. 3). A 200 rpm stirrer was used for gently stirring the medium. Two aluminum electrodes were adjusted with 1 cm gap in the medium. Dazheng ps-305D DC power supply was used for electroflucculation of cells. Coagulated microalgae were separated from water and dried in 60 ˚C and then desalinated by fresh water and the samples dried again in 60 ˚C.

Fig. 3 Microalgae harvesting, desalinating and drying

D. Microalgae oil Extraction

Digital Soxhlet Buchi model b-811 was used for lipid extraction. 5 grams of dried biomass was applied in the soxhlet cartouche. Every cycle consists of 15 minutes boiling lipid extraction and 15 minutes for solvent retrieval, that period of second stage was considered 20, 30, and 40 minutes. The hexane, hexane/isopropanol 3:2, and chloroform/methanol 2:1 (HPLC grade) were used as solvent. All of experiments were performed in three replicates. The extracted lipid was analyzed with AJILENT 7890 gas chromatography to determine the fatty acid profile**.** Statistical analysis of the data was performed with SPSS 16.0 software.

III. Results and Discussion

A. Effect of Solvent Type

The selection of the solvent system for oil extraction from microalgae biomass is an important factor. Solvent selection for extraction of oil at the initial step would allow cost-effective oil production without further expense required for the purification of the product.

Extraction of lipids from microalgae is basically a mass transfer operation, which depends on the nature of the solute and solvent, the selectivity of the solvent, and the level of convection in the medium. The Soxhlet method involves washing of solid mass with a solvent that has high solubility and selectivity for the solute. The Soxhlet extraction mechanism is mainly diffusion, and the procedure does not involve application of any shear stress to the biomass [13]. Hexane (non-polar solvent) has been used extensively throughout the world as a solvent for extracting vegetable oils [14]. Hexane is extensively used for oil extraction because of its high stability, low greasy residual effects, boiling point, and low corrosiveness.

The results show that for hexane solvent relying simply on diffusion of lipids through the cell membrane is a slow process and results in low extraction yield. Clearly extraction yield with hexane-isopropanol solvent was better compared to hexane. Total lipid extracted from *Dunaliella* sp. has been shown for all solvents (Fig. 4). Distinctly minimal extraction yield is for extraction by hexane about 5.14%. This is because various lipids have different polarities. Polar lipids are often in the cellular membrane that have strong hydrogenic or electrostatic bond with protein molecules. Adding polar solvent (i.e. ethanol, methanol or isopropanol) causes breaking the link between lipid and protein before extraction. Furthermore, hexane is able to reach neutral lipid. Micelle structures prevent considerable amount of lipid extraction and often adding alcohol helps their rapid destruction. Therefore, solvent or mixture of solvents should be polar enough to solve cellular membrane lipids. The oil extraction yield was increased from 5.14% to 13.93% by adding isopropanol.

B. Effect of Time on Extraction Yield

The extraction time is an important parameter for lipid extraction efficiency. It helps in deciding the optimum residence time required for the extraction process. In this study, the effect of time on lipid extraction yield was investigated with different time intervals varying from 20 to 40 min. The results showed that by increasing the extraction time, oil extraction efficiency was increased. But, by increasing the extraction time for samples that were treated with hexane solvent, no significant effect on extraction yield

was observed (Fig. 5). This illustrates that even with increasing extraction time, hexane is not able to penetrate into cellular tissue and extract their lipids. In the meanwhile, increasing extraction time for samples that were treated by hexane and isopropanol, leads to increasing in the extraction yield significantly.

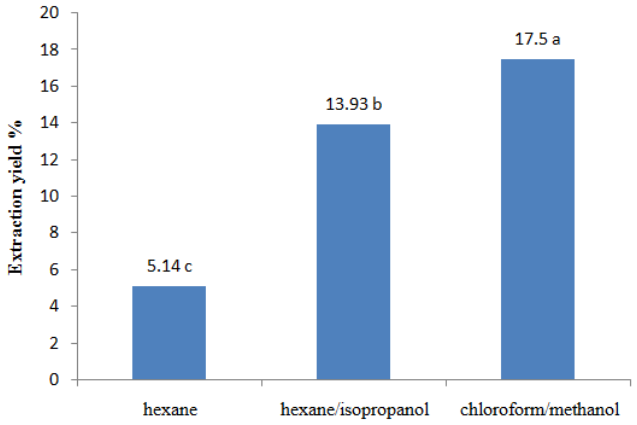

Fig. 4 Effect of solvent type on lipid extraction yield

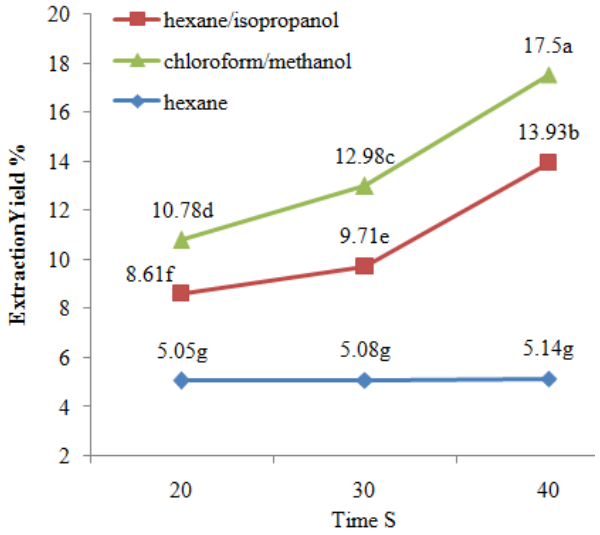

Fig. 5 The effect of extraction time on lipid extraction yield

C. Microalgae Oil Fatty Acid Profile

Fatty acid profile of microalgae oil was analyzed by using AJILENT 7890 gas chromatography mass spectrometry (Table I). From the analysis, the saturated fatty acids (59%) were found to be more compared to unsaturated fatty acids (31%). For the saturated fatty acid, palmitic acid composition with 36.62% was observed to have maximum portion of profile.

D. Microalgae Oil Specifications

Table II shows the physic-chemical properties of *Dunaliella* sp. oil. All of the properties were according to Codex standard for vegetable oils [15].

TABLE I
FATTY ACID PROFILE OF *DUNALIELLA* SP. OIL

Fatty acid name	Carbon number	Fatty acid%
Lauric Acid	C12	-
Myristic Acid	C14	2.64
Palmitic Acid	C16	36.62
Palmitoleic Acid	$C16_1$	5.9
Stearic Acid	C18	19.08
Oleic Acid	$C18_1$	18.62
Linoleic Acid	$C18_2$	3.26
Linolenic Acid	$C18_3$	4.65
Archidic Acid	C20	+

TABLE II
PROPERTIES OF *DUNALIELLA* SP. OIL

Property	Value
Acid value	0.835 mgKOH /g
Peroxide value	7 Meq/kg
Iodine value	48 g/100g
Saponification value	196 mgKOH/g

IV. Conclusion

The effects of solvent type and extraction time on the lipid extraction yield of *Dunaliella* sp. cells were investigated. Test results showed that application of isopropanol as polar solvent in composition with hexane can improve the extraction yield from 5.14% up to 13.93%. Although this yield is less than chloroform/methanol yield (17.5%), the extracted oil with this food grade solvent could be considered for food applications. Also the present results for the fatty acid profile of extracted oil from showed that this method was mostly according to fatty acid profile of edible vegetable oils. The physicochemical properties of the extracted oil with hexane/ isopropanol were according to Codex standard for the edible vegetable oils.

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