

Biodiesel Properties of Native Strain of *Dunaliella Salina*

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Abstract- Cyanobacteria and microalgae can be suitable microorganisms for the production of biofuels to meet our needs of safe and clean sources of energy. Among them, *Dunaliella* strains are considered good feedstocks for biofuel production. In this regards, a naturally isolated strain of *Dunaliella salina* was investigated to be used as potential biodiesel feedstock. The microalgal strain was isolated from water samples collected from Maharlu Salt Lake, 30 km southeast of Shiraz, Iran. At the end of exponential phase of growth, total content of the lipids was extracted, esterified and then identified using GC/MS analysis. Several types of fatty acid methyl esters (FAMES) were identified in the isolated microalgae. The composition of fatty acids in the studied species of microalgae was mainly hexadecanoic acid methyl ester and tetradecanoic acid methyl ester.

Keywords: Biodiesel, *Dunaliella salina*, Microalgae, Fatty acid methyl esters

1. Introduction

In recent years, as the world's population increased, environmental pollutions due to use of fossil fuels, energy crisis and the demands for generating renewable energy sources had grown dramatically [1]. Due to the mentioned problems and fossil fuel limitations, there is increased concern for finding sufficient supplies of clean energy. Cyanobacteria and microalgae can be suitable microorganisms for the production of biofuels to meet our needs of safe and clean sources of energy [2]. High efficiency, high lipid production (in some cases up to 80% of weight), fast growth, biofixation of waste CO₂ [2], contribution to prevent greenhouse effect, and possibility of culture on inappropriate farm lands make microalgae a suitable tool for this purpose [4].

Dunaliella salina is a halophile green microalga which can be found in sea salt fields, salty ponds and marine waters. Because of its high content of carotenoids, it is a good source of foods with antioxidant activity [5].

Furthermore it can be used for biodiesel production. Tang et al. [6] showed the potency of producing biofuels by methylation of different fatty acids such as linolenic and palmitic acids and introduced *Dunaliella* as a good feedstock for biofuel production. Manufacturing renewable fuels by hydrothermal liquefaction of *Dunaliella* biomass residues have also been investigated [7]. Due to undefined features of native strains, in this study a naturally isolated strain of *D. salina* was examined for the presence of suitable fatty acids to be used as biodiesel supply.

2. Material and Methods

2.1. Isolation and Cultivation

D. salina was isolated from water samples collected from Maharlu Salt Lake, 30 km southeast of Shiraz, Iran. The identification was done using physiological, morphological and molecular approaches.

The inoculation culture of *D. salina* was cultivated in the modified Johnson liquid medium in 250 mL Erlenmeyer flasks containing 100 mL culture medium under steady conditions. Afterwards, *D. salina* was grown under continuous illumination ($60 \mu\text{Em}^{-2} \text{s}^{-1}$) and unlimited aerated condition for three weeks. Temperature was adjusted at $25 \pm 2^\circ\text{C}$.

2.2 Sampling and Analysis

For cell growth determination, optical density and cell number were measured during the cultivation period in the batch culture. Optical density was measured at 660 nm using a UV/Visible spectrophotometer (PG instrument Ltd.) For cell counting, 1 mL of algal suspension was harvested in a sampling tube each time and then direct counting was performed using Neubauer hemocytometer and light microscope. In addition, the dry weight of the algal cells was also measured.

2.3 Total Lipid Extraction

For this purpose, algal cells at the end of exponential phase of growth were centrifuged at 5000 rpm, 4°C for 5 min. Afterwards, the obtained pellets were freeze dried using freeze dryer (Christ, Germany). Dried algal cells were used for lipid extraction. Lipid extraction was done as described before [2].

2.4 Fatty Acid Esterification and GC/MS Analysis

Crude extract of microalgal body (0.5 g) was dissolved in 3 mL methanol in the presence of catalytic amount of sulfuric acid. The mixture was heated to reflux using Dien-Stark apparatus. After cooling, the reaction mixture was washed twice with saturated sodium hydrogen carbonate aqueous solution and dried over anhydrous sodium sulfate. The solvent was removed by distillation under reduced pressure to give an oily substance. The obtained product was injected in GC/MS for analysis. The GC/MS analysis was carried out using a Hewlett–Packard 6890. The gas chromatograph was equipped with a HP-5M capillary column (phenyl methyl siloxan, $25 \text{ m} \times 0.25 \text{ mm}$ i.d., Hewlett–Packard Part No. 190915.433, USA). The oven temperature was programmed from 85°C (5 min) to 265°C at the rate of $7^\circ\text{C}/\text{min}$ and finally held at 265°C for 10 min. The carrier gas was helium with the flow rate of 1.2 mL/min. The mass spectrometer (Hewlett–Packard 5973, USA) was operated in EI (Electron Ionization) mode at 70 eV. The interface temperature was 265°C and the mass range was 15–650 m/z. The identification of fatty acids was performed, comparing the obtained mass spectra with Wiley (275) libraries [8].

3. Results and Discussion

At the end of exponential phase the cells were harvested and biomass concentration was measured. Biomass dry weight was about 1.2 g/L. Doubling time of *D. salina* was

approximately 5 h. Growth curve was derived from optical density and cell numbers.

The profile of fatty acid methyl esters (FAMES) was identified through comparison of their mass spectra with those in Wiley libraries. The FAMES profile is displayed in Table 1. Several types of FAMES were detected through GC/MS analysis. The results show that tetradecanoic acid and hexadecanoic acids are the most abundant fatty acids in this strain. Other types of fatty acid methyl esters such as docosanoic acid, eicosanoic acid, pentacosanoic acid, octadecanoic acid, undecanoic acid, dodecanoic acid, nonadecanoic acid are also present in *D. salina*. Selecting high-oil content strains and finding cost effective methods of harvesting, oil extraction and conversion of oil to biodiesel are requirements for producing biodiesel from algae [9].

Table 1. The profile of FAMES of studied *D. salina*

Fatty acid methyl ester (FAMES)	FAME content (% total)
Docosanoic acid methyl ester	7.1
Eicosanoic acid methyl ester	4.6
Pentacosanoic acid methyl ester	5.5
Hexadecanoic acid methyl ester	23.7
Octadecanoic acid methyl ester	7.3
Undecanoic acid methyl ester	5.9
Tetradecanoic acid methyl ester	20.3
Dodecanoic acid methyl ester	4.1
Nonadecanoic acid methyl ester	6.1
Hexadecanoic acid ethyl ester	7.9

Measurement of FAMES in algae biomass is a desirable procedure to indicate amount of suitable lipids which can be converted to biodiesel [10]. The results indicate that different types of fatty acids are present in *D. salina*. All of these fatty acids are saturated. Comparing to our previous study on *Chlorella vulgaris*, both strains have saturated fatty acids but there is a difference in fatty acids compositions present in the microorganisms. Culturing conditions, growth phase, and environmental factors are the criteria that can affect both lipid content and fatty acid profile [10]. Furthermore, biodiesel qualities are dependent on fatty acid composition [11]. Among the fatty acids, hexadecanoic acid and tetradecanoic acid are principal components. Hexadecanoic acid, also known as palmitic acid, which is the most common fatty acid contained in biodiesel [2] constitutes up to 23.7% of total fatty acids in *D. salina*. Octadecanoic acid (stearic acid), another common fatty acid regarded as biodiesel is also present in value of 7.3% in investigated strain. In compare to our previous studies on *Chlamydomonas* and *Chlorella* [2,8], this strain has a different fatty acid methyl ester (hexadecanoic acid ethyl ester) which is not present in FAMES profile of those other species. As same as *Chlorella* [2], all of the fatty acids in *D. salina* are saturated. Highly saturated fatty acids give an excellent cetane number and oxidative stability to biodiesel [2]. Octadecanoic acid (stearic acid) content of *D. salina* is higher than *Botryococcus braunii*, *Chlorella vulgaris*, *Scenedesmus* sp. [11,12], *Chlamydomonas* [8], *Chlorella* sp. and *Navicula* sp. [2,13],

Himantialia elongate, *Synechocystis* sp.[14], *Scenedesmus obliquus* and *Neochloris oleoabundans* [15].

D. salina is a fast growing strain (doubling time ~5.1 h) compared to *Chlamydomonas* (~5.5 h) and *Chlorella* sp. (~9 h). According to the results, it is considerable that this strain is suitable to be used as biodiesel feedstock due to its fatty acids content and fast growth rate.

4. Conclusion

The results of this study show that *D. salina* is a fast growing strain with ideal fatty acid composition. Because of these features, it can be a potential feedstock for biodiesel production. Furthermore this is a naturally isolated strain and has unknown features, so that further investigation should be done in order to establish this strain as a commercial biodiesel producer.

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