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Biodiesel production using marine microalgae *Dunalialla salina*

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Abstract

Alternative demand is unquestionable in order to reduce gaseous emissions (fossil CO_2 ,nitrogen and sulfur oxides) and their purported greenhouse, climatic changes and global warming effects, to manage the frequent oil supply crises, as a way to help non-fossil fuel producer countries to decrease energy dependence. Biodiesel from oil crops, waste cooking oil and animal fat cannot practically satisfy even a small fraction of the existing demand for transport fuels. Microalgae are a promising alternative supply of lipid for biodiesel production. This study discusses the perspectives concerning the green alga Dunaliella salina Toed for biodiesel manufacturing purposes. The alga was cultivated under controlled lab conditions. Biomass concentration at early stationary grown microalga was 1.9 mg/L dry weight, while the algal oil was about 24.8% of the biomass. Algal oil was directly transesterified and analyzed using GC/MS technique. Six fatty acid methyl esters were identified. The amount of saturated with sixteen and eighteen carbon ester fractions were38.57% .this result showed that Dunaliella salina oils were suitable for production of biodiesel.

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Introduction

Since Fossil fuels are starting to reveal their limitations as an energy source, there is a demand to find out an alternative fuel to find out the energy need of the world. Biodiesel is one of the finest available sources to fulfill the energy demand of the earth (Basha *et al.*, 2009). The petroleum fuels play an essential role in the development of industrial growth, transportation, agricultural sector and to meet other basic human needs. In addition, continuous use of coal base fuel is alarming the environmental issues including NO_2 SO_2 and CO_2. Hence, the scientists are seeking alternative fuels (Shenbaga *et al.*, 2012).

Biodiesel is a non-toxic and biodegradable with low pollutant and currently receiving much attention because of its potential as a sustainable and environmentally friendly alternative to petrodiesel. Normally, biodiesel is produced through transesterification reaction, in which short chain alcohol (e.g. methanol) in the presence of catalyst (e.g. sodium hydroxide, NaOH) reacts with oil .Seeds' oils such as soybean, sunflower, rapeseed and palm oil are very common feedstock to produce biodiesel (Vasudevan *et al.*, 2008 and Lam *et al.*, 2012).

Important benefit of microalgae is that, unlike other oil crops, they are able to double their biomass within 24 hr. actually; the biomass doubling time for microalgae during exponential growth could be as short as 3 to 4 hrs. Which is significantly quicker compare to the doubling time for oil crops (Banerjee *et al.* 2002)? Recently, microalgae have been identified as a superior feedstock for biodiesel production, mainly because of their rapid growth rate (100 times faster than terrestrial plant) and their ability to double their biomass in less than 24 hours under certain culture conditions (Lam *et al.*, 2012; Tredici *et al.*, 2010). It is for this reason microalgae are potential for synthesizing more oil per acre than the terrestrial plants which are currently used for the production of biodiesels (Huang *et al.* 2010) and using microalgae to make biofuel will not compromise fabrication of food, fodder and other products derived from crops. Furthermore, microalgae could be cultivated in brackish water on non-arable lands, and therefore might not incur land-use change, minimizing associated environmental impacts (Huang *et al.* 2010 and Searchinger *et al.*, 2008). Several of microalgae, e.g., Chlorella, Dunaliella and Spirullina could accumulate considerable amount of oil (up to 60% of dry weight) when cultured under certain environmental conditions, such as high in temperature, light intensity and hyper salinity (El-Baky *et al.*, 2004).

Dunaliella salina is a unicellular species of green algae without cell wall (Hounslow, 2010). It lives in areas of fluctuating salinity and can tolerate extreme salinities. This microalga is quite easy to cultivate and has a relatively high growth rate and lipid content (Tang *et al.*, 2011).

In the same context, the current study attempts to examine the capability of biodiesel production from microalgae. The main goal of this paper is to evaluate and measure the amount of biodiesel and fatty acid composition produced by isolated strains of Dunaliella salina.

Materials and methods

Microalgae culture

Dunaliella salina was obtained from the Biotechnology laboratory, Botany Department, Faculty of Science, University of Urmia. This organism was originally isolated from the brine water at the salty Lake of Urmia. The indoor stock cultures of microalgae, Dunaliella salina was maintained in air conditioned room. Johnson's medium was prepared using autoclaved Deionized water in 2 L flasks. All glass and plastic ware were washed with 10% HNO3and rinsed with distilled water (Sathasivam *et al.*, 2013). 10 ml of culture inoculums from the exponential phase was transferred to the culture flasks and incubated at

3000 lux using two fluorescent bulbs for 16:8 hrs. light and dark cycle. NaCl concentration during the growth phase was at 2M per ml. The maximum exponential phase was obtained after 8-10 days. The continuous aeration was provided for culture. For large scale production of microalgae, 100 liters FRP tanks were used.

Biomass Estimation

Algal growth was characterized based on cell counts. 1 ml of algal culture was removed from the replicate flasks at regular intervals throughout the experiment. It preserved with iodine solution prior to counting using a haemocytometer slide under bright-field contrast microscopy (Venugopalan *et al.*, 1989). The cultures were harvested by centrifugation at 5000 rpm for 15 min. Pellets were then washed twice with distilled water and dehydrated at 30˚C until a constant weight was achieved. The dry weight of the algal biomass was estimated from the average of at least three representative samples.

Lipid Extraction

At early stationary phase after 9 days of experimentation, lipids were extracted by using chloroform: methanol (2:1) solvent mixture according to the methods of Bligh and Dyer (1959). The extracted lipid was separated into two layers, the upper layer methanol together with water was removed and the chloroform layer including lipid was collected. The residues were subjected to repeated extraction twice. The entire extracts were mixed together forming crude oil extract and the chloroform was evaporated.

Biodiesel Production

The biodiesel from microalgae was produced by direct transesterification as described by Johnson and Wen (2009). The dried algal biomass (1 g) was placed in a glass test tube and mixed with 3.4ml of methanol, 0.6ml of sulfuric acid and 4.0 ml of chloroform. The reaction mixture was heated in a water bath at 90ºC for 40 minutes. After the reaction was completed, the tubes were allowed to cool at room temperature. 2 ml of distilled water was added to the tubes and mixed for 45 seconds. Then the samples were centrifuged at 3000 rpm for 10 min. to accelerate phase separation. The organic layer that contained biodiesel (FAME) was collected and transferred to a pre-weighed glass vial. The solvent was evaporated using nitrogen gas and the biodiesel was determined gravimetrically.

Fatty Acid Methyl Esters (FAMEs) Analysis Using GC/MS

The yield of biodiesel produced from direct transesterification methods was estimated gas chromatograph (GC). Agilent Technologies 7890A Gas Chromatograph and Agilent Technologies 5975C Mass Spectrometer. One mL was diluted to 2 mL hexane in a screw cap tube (Lee *et al.*, 2007). Helium was a carrier gas at flow rate of 54 ml/min. Chromatographic data was recorded and integrated using Agilent data analysis software. The components were identified by comparing their retention times and fragmentation patterns with those for standards from library.

Results

Algal Biomass and Oil Content

The maximum number of cells after 9 days of experimentation achieved a value of 5.3×10^6 cells/ml (Table 1). This value corresponding to 1.9 mg/L dry weight and a relatively high oil content of %24.8. For the purpose of biodiesel production, high oil content and rapid growth rates are major factors in selecting an algal strain (Chisti, 2007).

Fatty Acid Compositional Profiles

The fatty acids composition of *D.* salina was evaluated at early stationary growth phase. Table 1 revealed that the profile composition and percentage of FAMEs. The amount of lipid content wasbetween 25% the fatty acid compositional profiles of algal strains are influenced by specific growth conditions such as nutrient levels, temperatures, and light intensities (Hu *et al.,* 2008). This makes it more difficult to define a single compositional profile for algal-based biodiesel (Hoekman *et al.,* 2012). As well, clear differences in carbon chain length and degree of unsaturation are important algal oil characteristics for biodiesel production and may influence its properties and performance (Griffiths *et al.*, 2009).

Table 1. profile composition and percentage of FAMEs.

D. salina FAME	Percentage
Dodecanoic acid	9.21
Nonanedionic acid	7.45
Octadecanoic acid	16.42
Propionic acid	17.17
Hexadecanoic acid	18.40
Palmitic acid	3.93

Discussion

Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon-neutral transport fuels are necessary for environmental and economic sustainability (Chisti, 2007).

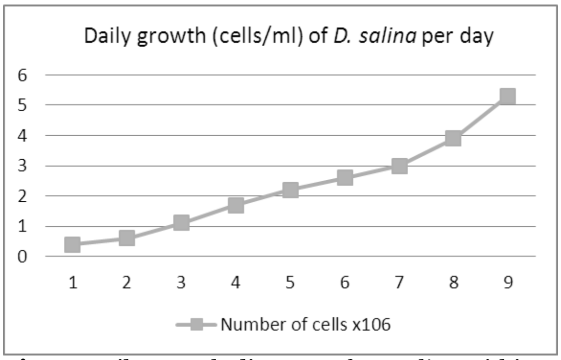

Fig. 1. Daily growth diagram of *D. salina* within 9 days (cells x 10^6/ml).

Producing biodiesel from algae has been touted as the most efficient way to make biodiesel fuel. Algal oil processes into biodiesel as easily as oil derived from land-based crops. The difficulties in efficient biodiesel production from algae lie not in the extraction of the oil but in finding an algal strain with a high lipid content and fast growth rate that is not too difficult to harvest and a cost-effective cultivation system (i.e., type of photo bioreactor) that is best suited to that strain.

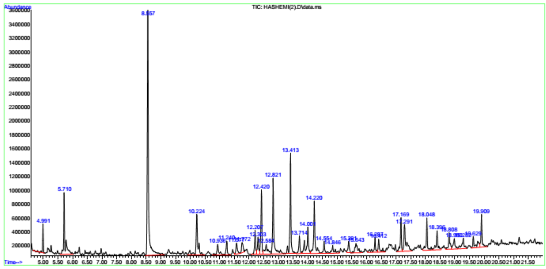

Fig. 2. Gas chromatogram of fatty acid methyl esters from *D. salina*.

Algae are the fastest growing plants in the world. Microalgae have much faster growth rates than terrestrial crops. The per-unit area yield of oil from algae is estimated to be between 18,927 and 75,708 L/acre/year; this is 7 to 31 times greater than the next best crop, palm oil, at 2,404 L/acre/year. Algae are very important as a biomass source. Different species of algae may be better suited for different types of fuel. Algae can be grown almost anywhere, even on sewage or salt water, and does not require fertile land or food crops, and processing requires less energy than the algae provides. Algae can be a replacement for oil-based fuels, one that is more effective. Algae consume CO2 as they grow, so they could be used to capture CO2 from power stations and other industrial plant that would otherwise go into the atmosphere. Tables 6.7 and 6.8 show the advantages and disadvantages of biodiesel from algal oil (Chaumont, 2005).

Based on the profile of fatty acid composition, D. salina was evaluated as oil producer. The alga was cultivated batch-wise under optimum laboratory conditions. Cell density of 5.3×10^6 cells/ml culture, equivalent to a dry weight of 1.9 mg/L produces 24.8% oil. Fatty acid profiles were identified as Dodecanoic acid, Nonanedionic acid, Octadecanoic acid, Propionic acid, Hexadecanoic acid and Palmitic acid. The properties of Dunaliella salina biodiesel indicates that it has a high ratio of >C:12

fatty acids and assumed that D. salina is suitable for biodiesel production which is proved by Knote.

It is very clear that the marine microalgae are able to grow extremely rapidly, generally doubling their biomass within a day. Moreover, they can grow on saltwater found in the coastal belt which is not suitable for domestic and agriculture purpose. The commercial production of biodiesel from microalgae practically successful in developed countries. Moreover, the microalgae are attractive is that they can assimilate carbon dioxide as the carbon source for growth which contributes to atmospheric CO_2 reduction. In addition, microalgal biofuel is similar to those produced by fossil and crops and it can be used directly to run existing diesel engines or as a mixture with crude oil diesel (Johnson *et al.*, 2009). So, due to the immeasurable advantages behind marine microalgae, it is understood that presently studied marine microalgae D. salina can be considered as potential feed stock for biodiesel production to compete the future energy crisis.

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