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Biodiesel production from Anabaena flos-aquae microalgae

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Abstract

Renewable biofuels are needed to displace petroleum derived transport fuels, which contribute to global warming and are of limited availability. Biodiesel and bioethanol are the two potential renewable fuels that have attracted the most attention. This study demonstrates the production of algal biodiesel from Anabaena flos-aquae. The research showed that the oil content in Anabaena flos-aquae was 40% by weight of dry biomass. Acid-catalyzed transesterification was used to convert fatty acids [FA], monoglycerides [MG], diglycerides [DG] and triglycerides [TG] to fatty acid methyl esters [FAMEs]. Gas chromatography–mass spectrometry [GC–MS] was used to analyze the FAMEs. The major fatty acid composition of the tested microalgae was a mixture of saturated and unsaturated fatty acids.

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Introduction

Currently about 80% of global energy demand is produced from fossil fuels. However, broad utilization of fossil fuels has led to global climate change, environmental pollution, and health problems (Brennan and Owende, 2010). Several countries are, therefore, trying to develop novel sources of energy sources that are clean sustainable. Of the potential alternate sources of renewable energy, biofuels have received the most attention and are expected to play a crucial role in the universal energy infrastructure in the future. Biodiesel, one of the most commonly used biofuels, is accepted as a perfect, recyclable energy carrier as a possible primary energy source (Chisti, 2007).

Commercial biodiesel is presently produced from animal fat, waste frying oil and vegetable oils (Barnwal and Sharma , 2005), whose competition with edible vegetable oil for agricultural land is still a controversial issue (Mata *et al.*, 2010). Therefore, microalgae that can grow rapidly and transform solar energy to chemical energy through CO_2 fixation are being considered as a promising oil source for creating biodiesel (Mata *et al.*, 2010). Under proper culture environments, certain microalgal species are able to accumulate up to 50–70% of oil/lipid per gram of dry weight (Chisti , 2007). The fatty acid profile of microalgal oil is optimal for the production of biodiesel (Gouveia and Oliveira , 2009). An additional advantage is that microalgae can produce up to 58,700 L of oil per hectare, which is one to two magnitudes greater than that of any other energy crop (Chinnasamy *et al.*, 2010).

Nevertheless, mass manufacture of microalgal oil faces a number of technical hurdles that render the current development of the algal industry. Furthermore, it is also necessary, but very problematic, to develop economical technologies that would permit efficient biomass harvesting and oil extraction. However, since microalgae production is considered as a conceivable approach to assuage global warming, it is clear that producing oil from microalgal biomass would provide profound benefits including fuel. The present study was undertaken to determine the ability of green microalgae to produce biodiesel by measuring the amount of biodiesel produced by isolated strain of Anabaena flos-aquae.

Materials and methods

Microorganism

Purified Anabaena flos-aquae microalgae (strain IR, isolated from Anzali international wetland) was purchased from National Inland Water Aquaculture Institute of Iran (Fig. 1A).

Culture medium

Anabaena flos-aquae was cultured in BG11 medium (Rippka , 1988). The pH of the medium was adjusted to 7.2 using 0.1 N NaOH before autoclaving. Cultures were maintained as batch cultures at 26±1°C with a light intensity of 1500–2000 lux, and grown in a light/dark cycle of 12/12 h for 14 days.

Harvesting of Anabaena flos-aquae

Microalgae biomass was harvested using a continuous feed, fixed bowl centrifuge. The plate, consisting of 15–20% dry matter, was directly frozen. The paste was then dried in a vacuum oven at 70 °C and stored at -20 °C.

Extraction of lipid from microalgae

The neutral lipids that were converted to FAME (fatty acid methyl esters) using a fixed bed reactor were extracted from dried algae powder using hexane Soxhlet extraction. Prior to extraction, dried algae flakes were crushed using a blender and then pulverized in a ball grinder. When the cells had been milled properly, the mixture was placed in the Soxhlet extractor until the extracting hexane was colorless. The lipid containing hexane solution was then filtered through a 1.2 lm GFC Whatman filter. The hexane was then removed using a rotary evaporator. The crude oil was then re-dissolved in hexane and filtered through activated carbon to remove pigments (Fig. 1B)(Chisti , 2007).

Transesterification

A two-step protocol was used for the

transesterification of all extracted lipids. The first step used an acid catalyst to methylate free fatty acids (FFA) and to transmethylate acylglycerols (Barnwal *et al.*, 2005). Twenty mL methanol and 10 % H_2SO_4 in 1 mL methanol were added to the lipid in hexane solution stored in each vial. The mixture was transferred into a flask, heated to 50 °C, and moderately agitated for 2 h. Evaporated methanol was frequently replenished. In the second step, 25 wt% KCH_3O in methanol was added dropwise to the gently stirred reaction mixture until a pH 13 was attained. The mixture was then heated again to 55 °C and moderately agitated for 2 h. Evaporated methanol was frequently replenished. The mixture was evaporated in a 60 °C oven to obtain dried post-methylated lipid extract. The lipid was then re-dissolved in 20 mL hexane for FAME analysis.

Gas chromatography–mass spectrometry (GC–MS)

An Agilent 6890 gas chromatograph electron impact mass spectrometer was used to analyze FAMEs in the microalgae biodiesel. One microliter of the sample was injected in splitless mode at a flow rate of 1.0 mL/min with helium as the carrier gas onto a 5% phenyl-methylpolysiloxane. The elution temperature program had an initial temperature of 50 °C and then linearly ramped to 180 °C at 15 °C min-1, then to 230 °C at 2 °C min-1, and finally to 310 °C at 30 °C min-1. The final temperature was held for 13.67 min (total run time = 50 min). Chromatogram was acquired using HP6890 MS software and peaks were identified with NIST MS library. The observed mass range was set from 37 to 800 amu to remove the solvent (Brian *et al.*, 2011).

Results

The oil content in Anabaena flos-aquae was 40% by weight of dry biomass. The major fatty acid composition mainly composed of mixture of saturated and unsaturated fatty acids mixture, such as myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n-7), margaric acid (C17:0), oleic acid (C18:1n-9) and eicosapentaenoic acid (C20:5n-3). Myristic acid was found to be as the major constituent in Anabaena flos-aquae microalgae while eicosapentaenoic acid was the lowest.

Discussion

During recent years a number of investigators have studied seed oils for the construction of biofuels. Production of second generation fuels such as bioethanol and biodiesel from biomass grown on arable lands, especially the use of oil-seeds for biodiesel, have elevated the food prices. Third generation biodiesel from microalgae grown on non-arable land is the obvious answer to the food-fuel competition. The process of microalgal cultivation could be improved for efficient yield of algal lipids through the screening and improvement of microalgal strains (Anoop *et al.*, 2011).

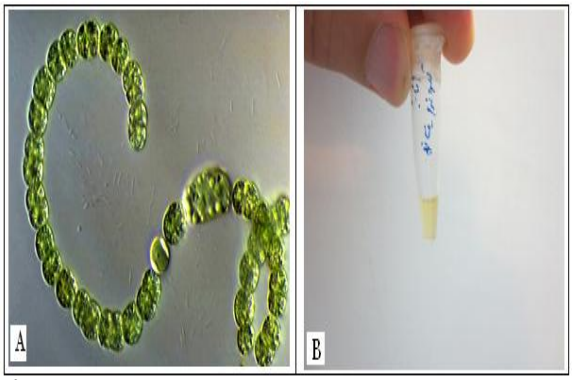

Fig. 1. a) *Anabaena* flos-aquae *isolated from Anzali wetland (left), b)* Biodiesel extracted from *Anabaena* flos-aquae *(right).*

The oil content of *Anabaena* flos-aquae used in this study was 40% by weight of dry biomass. The lipid content in dry biomass of *Anabaena* flos-aquae *is higher than other* microalgae. For example, the lipid content in dry biomass of *Chlamydomonas reinhardtii* is 21%, *Chlorella emersonii* 28–32%, *Chlorella vulgaris* 14-22%, *Crypthecodinium cohnii* 20%, *Dunaliella primolecta* 23%, *Dunaliella salina* 6%, *Dunaliella tertiolecta* 36%, *Euglena gracilis* 14-20%, *Phaeodactylum tricornutum* 20-30%, *Pleurochrysis carterae* 30–50% and *Chlorella protothecoides* 55% (Illman *et al.*, 2000; Becker and Richmond A, 2004).

Another positive note about *Anabaena* flos-aquae microalgae *is that it can grow on waste water(Jia et al., 2011). This aspect is very important for an*

economic production of fuel in comparison to fossil fuels. Fossil fuels are the major energy sources but their overconsumption leads to disastrous effects such as air pollution. Burning of fossil fuels releases carbon dioxide, nitrogen monoxide, nitrogen dioxide, sulphur dioxide, and carbon monoxide etc. These gasses have severe consequences for habitats and also affect human health. *In addition, t*hey are non-renewable sources of energy which derive from pre-historic fossils and are no longer available after usage. Their sources are limited and they are depleting at a faster rate. When extracted, this fossil fuel poses a severe damage to the landscape as they are to be dug out from the underground wells. In comparison to fossil fuels, microalgae fuels do not have such problems and above all, they are renewable. Nevertheless, further research and development are necessary to establish an economical industrial scale production of *Anabaena* flos-aquae biodiesel.

Conclusions

This study has demonstrated that *Anabaena flos-aquae* biodiesel is technically feasible. It is a renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum.

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References

Anoop S, Poonam SN, Jerry DM. 2011. Renewable fuels from algae: An answer to debatable land based fuels. Bioresource Technology **102,** 10-16. http://dx.doi.org/10.1016/j.biortech.2010.06.032

Barnwal BK, Sharma MP. 2005. Prospects of biodiesel production from vegetables oils in India. Renewable & Sustainable Energy Reviews **9,** 363-378. http://dx.doi.org/ 10.1016/j.rser.2004.05.007

Becker EW, Richmond A. 2004. Microalgae in human and animal nutrition *Handbook of Microalgal Culture*. Published by Blackwell Publishing: England, 312-351 P.

Brennan L, Owende P. 2010. Biofuels from microalgae - a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable & Sustainable Energy Reviews **14**, 557-577. http://dx.doi.org/10.1016/j.rser.2009.10.009

Brian JK, Clayton VM, Bingwen Y, Daniel N. 2011. Production of algae-based biodiesel using the continuous catalytic Mcgyan_ process. Bioresource Technology **102,** 94-100. http://dx.doi.org/ 10.1016/j.biortech.2010.05.035

Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technology **101,** 3097-3105. http://dx.doi.org/ 10.1016/j.biortech.2009.12.026

Chisti Y. 2007. Biodiesel from microalgae. Biotechnology Advances **25,** 294-306. http://dx.doi.org/ 10.1016/j.biotechadv.2007.02.001

Gouveia L, Oliveira AC. 2009. Microalgae as a raw material for biofuels production. The Journal *of* Industrial Microbiology *and* Biotechnology **36,** 269-274. http://dx.doi.org/ 10.1007/s10295-008-0495-6

Illman A, Scragg A, Shales S. 2000. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme and Microbial Technology **27**, 631-635. http://dx.doi.org/ 10.1016/s0141-0229(00)00266-0

Jia Y, Ming X, Xuezhi Z, Qiang H. 2011. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. Bioresource Technology **102,** 159-165. http://dx.doi.org/ 10.1016/j.biortech.2011.03.029

Mata TM, Martins AA, Caetano NS. 2010. Microalgae for biodiesel production and other

applications: a review. Renewable & Sustainable Energy Reviews **14**, 217-232. http://dx.doi.org/ 10.1016/j.rser.2009.07.020Rippka **R.** 1988. Isolation and purification of cyanobacteria. Methods in Enzymology **167**, 3-27. http://dx.doi.org/ 10.1016/0076-6879(88)67004-2