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Original Research Article

Biodiesel Production from *Azolla filiculoides* (Water Fern)

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Abstract

Purpose: *To assess the potential of Azolla filiculoides, total body collected from a rice farm in northern Iran as source for biodiesel production.*
Methods: *Solvent extraction using Soxhlet apparatus with chloroform-methanol (2:1 v/v) solvent blend was used to obtain crude oil from freeze-dried the Azolla plant. Acid-catalyzed transesterification was used to convert fatty acids (FA), monoglycerides (MG), diglycerides (DG) and triglycerides (TG) in the extracts to fatty acid methyl esters (FAMEs) by acid-catalyzed methylation. Gas chromatography–mass spectrometry (GC–MS) was employed to analyze the FAMEs in the macroalgae biodiesel.*
Results: *The presence of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), myristic acid (C14:0), stearic acid (C18:3), oleic acid (C18:1) and linoleic acid 9C18:2), eicosenoic acid (C20:1), eicosapentaenoic acid (C20:5), erucic acid (C22:1) and docosahexaenoic acid (C22:6) in the macroalgae biodiesel was confirmed.*
Conclusion: *The results indicate that biodiesel can be produced from macroalgae and that water fern is potentially an economical source of biodiesel due its ready availability and probable low cost.*

Keywords: *Biodiesel, Azolla filiculoides, Water fern, Fatty acid methyl esters*

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INTRODUCTION

In the last two decades, growing international concern about the negative environmental impact of fossil energy has drawn significant attention to renewable liquid biodiesel as a way to replace petroleum-based fuels [1]. Biodiesel, a common term for long chain alkyl esters, is a renewable, biodegradable, and non-toxic biodiesel that shows great promise. Biodiesel is derived from the transesterification of mono-, di- and tri-acylglycerides [TAGs] and the esterification of free fatty acids [FFAs] that occur naturally in biological lipids, such as animal fats and plant oils. As a result, biodiesel has the potential to be a carbon neutral fuel [2]. Furthermore, in comparison to petroleum diesel, biodiesel emits lower levels of environmental pollutants including volatile organic compounds, particulate matter, and sulfur-compounds during combustion [3].

Commercial biodiesel is currently produced from animal fat, waste frying oil and vegetable oils, whose competition with edible vegetable oil for agricultural land is still a controversial issue. Consequently, microalgae that can grow rapidly and convert solar energy to chemical energy via CO_2 fixation are now being considered as a promising oil source for making biodiesel [4].

The fatty acid profile of macroalgal oil is suitable for the synthesis of biodiesel [5]. The major attraction of using macroalgal oil for biodiesel is the tremendous oil production capacity of macroalgae, as they could yield up to 58,700 L of oil per hectare [6], which is one or two magnitudes higher than that of any other energy crop. However, mass production of macroalgal oil faces a number of technical hurdles, for example, high energy demand in harvesting steps and none feedstock specific conversion process, that render the current development of the algal industry economically not feasible. In addition, it is also necessary, but very difficult, to develop cost-effective technologies that would permit efficient biomass harvesting and oil extraction [7].

Azolla has been shown to effectively adsorb Pb, Cd, Cu and Zn from the wastewater [8] and removal of gold (III) from aqueous solution [9]. *Azolla* can grow in any depth of water but is not tolerant of waves or turbulence and can be flushed away in fast-flowing waters. *Azolla* fern has an adverse effect on the Northern Iran ecosystem by increasing the nutrient load and thus contributing to eutrophication of rivers, lagoons, lakes, wetlands and ponds. Dense *Azolla* mats prevent light penetration in open-water areas, which causes oxygen deficiency in the waters of the already poor life conditions for fish there [10].

The main purpose of this paper is to measure the amount of oil produced by *A. filiculoides* macroalgae.

EXPERIMENTAL

Chemicals and reagents

FAME standards and methylation catalysts (10 wt % H_2SO_4 in methanol and 25 wt % KCH_3O in methanol) were obtained from Sigma–Aldrich. All organic solvents (n-hexane, isopropanol, and methanol) were of analytical grade.

Sampling of macroalgae

Fresh *Azolla filiculoides* (as raw living biomass) was collected from the surface of rice fields of Guilan province in the northern part of Iran. Sampling of *Azolla* was carried out in October 2011. The *Azolla* cultures were collected in polythene bags and transferred to the laboratory immediately. Prior to experimentation, the cultures were washed twice with water to remove epiphytes and sand particles.

Extraction of lipids with chloroform-methanol

Crude oil was extracted from the freeze dried *Azolla* (1 g) in a Soxhlet apparatus using chloroform-methanol (2:1 v/v). The dried macroalgae was mixed and homogenized with IKA T18 homogenizer in chloroform-methanol solvent.

Extract was concentrated in a rotavapor followed by vacuum-drying with a temperature controlled oil bath and then it was cooled to room temperature. The oil yield of *Azolla* was 15%.

Transesterification of extracted oil

The extracted oil was transesterified by acid-catalyzed methylation to convert fatty acids (FA), monoglycerides (MG), diglycerides (DG) and triglycerides (TG) to fatty acid methyl esters (FAMEs). In the one-step transesterification procedure, the 5 mL of extracted lipid fraction along with 75 mL of 2 % H_2SO_4 in methanol was refluxed for 4 h. After complete conversion, the solvent was partially removed and extracted with ethyl acetate (EA, 2 × 100 mL). The two EA layers were washed with distilled water to neutralize the pH, dried over anhydrous sodium sulphate and evaporated under reduced pressure in a rotary evaporator.

Gas chromatography–mass spectrometry (GC–MS)

An Agilent 6890 gas chromatograph electron impact mass spectrometer was used to analyze the FAMEs in the macroalgae biodiesel. One microliter of 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). Mass spectra were acquired using HP6890 MS software and peaks identification was aided with the NIST MS library. The observed mass range was set from 37 to 800 amu to remove the solvent [11].

RESULTS

Oil yield in Azolla filiculoides was 15 % by weight of dry biomass. The presence of myristic acid (C14:0), palmitic acid [C16:0), palmitoleic acid (C16:1), stearic acid (C18:3), oleic acid (C18:1) and linoleic acid (C18:2), eicosenoic acid (C20:1], eicosapentaenoic acid (C20:5), erucic acid (C22:1), docosahexaenoic acid (C22:6) was confirmed (Table 1). Some of these acids have a good biodiesel property.

Table 1: Oil content of *Azolla filiculoides*

Fatty acid	Structure (C : DB)
Myristic acid	C14:0
Palmitic acid	C16:0
Palmitoleic ***acid***	C16:1
Stearic acid	C18:3
Oleic ***acid***	C18:1
Llinoleic acid	C18:2
Eicosenoic acid	C20:1
Eicosapentaenoic acid	C20:5
Erucic acid	C22:1
Docosahexaenoic acid	C22:6

C: DB = ratio of no. of carbon atoms: no of double bonds

DISCUSSION

Azolla filiculoides is one of the floating fern found in Guilan province of Iran. *Azolla filiculoides* was first imported from Philippines to Iran in 1986 for studies on its nitrogen fixation capacity, making it an ideal fertilizer for paddy fields and an additive to cattle feed. The invasive plant colonized rapidly and formed into dense mats covering vast expanses of surface water.

Biodiesel production generally will be influenced by prices, costs and potential returns compared to using the land over recourses in other ways. First the decision to grow oilseed rather than other crops will be influenced by the opportunity cost of land, thus taking account of market prices for seed, oilseed oil for the biodiesel markets, edible oil and meal [12]. Second, the market price for biodiesel, compared to on-farm production and processing costs, will influence farmer decisions regarding investments in on-farm crushing and processing. These decisions also may be affected by changes in seed and meal prices relative to cost of crushing and price of oil. In comparison, *Azolla* is not an oil seed and is not affected by these factors. Furthermore, *Azolla* can grow in isolated pools at low cost.

Although the oil content of *Azolla filiculoides* is not high in comparison of some microalgae but due to the environmental problems posed by the microalgae, using this macroalgae for producing biodiesel has some benefits [7]. The oil content of *Azolla filiculoides* in comparison of oils used for biodiesel shows it is suitable for using as biodiesel. Also producing *Azolla filiculoides* biomass is generally not more expensive than growing the crop [13]. Biodiesel has immense potential but its high cost and limited supply of organic oils prevent it from becoming a serious contestant for petroleum fuels. As petroleum fuel costs rise and supplies decrease, alternative fuels will become more attractive to both investors and consumers. For biodiesel to become the substitute fuel of choice, it requires a huge amount of cheap biomass. Using new and innovative techniques for cultivation, algae may allow biodiesel production to achieve the price and scale of production needed to compete with, or even replace petroleum [14].

Although different microalgae have previously been investigated for the production of of biodiesel, this is the first report on the use of *Azolla* macroalgae for biodiesel producing.

Biodiesel fuel is a renewable energy source unlike petroleum-based diesel. One of the main biodiesel fuel advantages is lower pollution than petroleum diesel. The lubricating property of the biodiesel may lengthen the lifetime of engines. The lack of sulfur in 100 % biodiesel extends the life of catalytic converters [15].

CONCLUSION

The findings of this work suggest that biodiesel can be produced from macroalgae, and that the algae is potentially an economical raw material source for biodiesel production due to its ready availability and probable low-cost.

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