Characterization of Algal Oils and Their Potential as Biofuel in Pakistan

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ABSTRACT

Biodiesel is becoming increasingly attractive fuel because of its ecological advantages. Algae are the fastest-growing plants on the earth and macroalgae are rich in storage lipids that have potential to be converted to biodiesel. In the present study, three high oil yielding, naturally occurring algal species i.e., Cladophora sp. Spirogyra sp. and Oedogonium sp. were collected and their potential was evaluated for oil yields and characterized for lipids composition. Biomass from three different algae was obtained by cultivating in the individual aquarium for about three months. The oil was extracted by solvent extraction method using soxhlet apparatus. These oils were analyzed for lipid contents using thin layer chromatography. Among all the oils extracted from these algae, the lipid contents of Oedogonium sp. were found to be highest. Oil yield of Oedogonium sp. was 26% (based on dry matter) and contained triacylglycerol, free fatty acids and sterol. Algae can be a substitute for oil based fuels and can be grown anywhere i.e., sewage or salt water. Thus, macroalgal oil has potential to be used as biofuel in Pakistan.

Key words: Algae, Biodiesel, Energy, Fossil fuel

INTRODUCTION

Recent energy crisis has shifted the focus to develop eco-friendly and renewable bioenergy resources (Shahir et al., 2014). Owing to the rapid industrialization and ever increasing population, there is an incessant increase in the energy demand (Fazal et al., 2014). The situation has become more alarming due to the exhaustion of worldwide natural fossil fuels. It is expected that the natural resources of oil, natural gas and coal will deplete in year 2044, 2047 and 2117, respectively (Shafiee & Topal, 2009). However, biomass is considered to be the only renewable resource that can replace the carbon based fossil fuels. Biomass based energy includes bioethanol, biodiesel, biomethane and biohydrogen, etc., (Ashnani & Anwar, 2014).

Biodiesel is considered to be eco-friendly and carbon neutral fuel as it does not disturb the carbon dioxide cycle (Ahmad et al., 2011; Sharma & Jain, 2010). Biodiesel is monoalkyl ester of fatty acids that are derived through the transesterification reaction of vegetable oils and fats (Shirazi et al., 2014). As a result, biodiesel has a rising market potential, that can be classified according to its end-use applications in: transportation, non-road applications (mining, forestry, construction, etc.), marine and heating (Rincon et al., 2014).

Though, vegetables oils are the prominent sources for the production of biodiesel (Wen et al., 2010; Ma & Hanna, 1999) but non-edible and algal oils are preferred due to world food crisis (Haq et al., 2014). Algae are rich in oil and grow rapidly (Guschina & Harwood, 2006; Ito et al., 2013), but lipid contents are variable depending upon source. Some species have been reported to contain up to 60% (w/w) fatty acids of dry weight (Baddiley et al., 1994). The harvest of oil from algae (per acre) is 200 times more compared to the best-performing plant/vegetable (Sheehan et al., 1998). Therefore, algal oil can be the promising source for the production of biodiesel. Even the algal waste after oil extraction can be used as feedstock for anaerobic digestion for the production of biogas as well as biofertilizer.

The National Policy of Pakistan encourages the substitution of fuel for transportation and other applications with biofuel to alleviate the energy crisis and meet the objective of environmental protection and climate change. The policy intends to introduce a minimum of 10% biodiesel by the year 2025 (Majeed, 2009). Therefore, there is a need to develop sustainable solution that have no competition with feed directly or indirectly and enhances the economy of the country.

This study is aimed to characterize presence of fatty acid, i.e., triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG) and free fatty acid (FFA) found in the oil yielding macroalgae present in Pakistan for using as a feedstock for biofuel production.

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MATERIALS AND METHODS

Cultivation of Algae and Microscopic Examination

Three different algal species were collected from Botanic Garden, GC University, Lahore Pakistan and were identified by microscopic examination as Cladophora sp. Spirogyra sp. and Oedogonium sp. The starter cultures of these species were cultivated in the individual aquarium with dimensions of 4×10×8 cm³ containing waste water from fish aquarium having KH₂PO₄ (0.2 g/L) and NaHPO₄ (0.6 g/L) as major constituents at 25±3°C under natural sunlight for about 3 months to obtain enough algal biomass.

Oil Extraction from Algal Biomass

Algal biomass was filtered thoroughly using Whatman filter paper (Sigma Aldrich, 2.5 μm) and kept in sunlight for 6-7 h to remove excess water and afterwards was kept in air oven at 100°C for 15 min. Dried biomass was crushed with pestle and mortar. Oils from the algal biomass were extracted using soxhlet equipment by following the procedure of Shahidi (2003).

The solvent vessel was washed and 3-4 boiling chips were added in the vessel, which was afterwards dried in a drying oven at 103±2°C for 1 hr. The weight of dried vessel with chips was recorded and 200 ml of extraction solvent i.e., hexane was added.

Dried and ground algal mass (40 g) was placed in porous cellulose thimble which was then, loaded into the main chamber of the soxhlet extractor. The Soxhlet extractor was placed onto the flask extraction vessel containing the extraction solvent. The Soxhlet was then equipped with the condenser. Lipids were extracted at 70°C. After completion of extraction of (5-6) h, the solvent was removed from the extracted oil in the rotary evaporator.

Total mass of lipids extracted from algae were estimated using following formula.

\[
\text{Mass of lipids extracted} = (\text{wt. of flask} + 3 - 4 \text{ boiling chips} + \text{extracted oil}) - (\text{wt. of flask} + 3 - 4 \text{ boiling chips})
\]

Lipid contents in algae were calculated as below:

\[
\text{Oil (lipids) contents (g) per mass of algae} = \frac{\text{mass of lipids extracted (g)}}{\text{mass of algae (g)}} \times 100
\]

TLC Analysis

In order to determine the lipid composition of the algae, a thin layer chromatography was carried out. Oil samples of (30 μl) were carefully spotted onto silica gel plate (Merck 8×6.5 cm). The mobile phase consisted of solvents having chloroform, hexane and methanol. The ratio (based on volume) of chloroform: hexane: methanol were 8:6:1, respectively. The plate was placed in the mobile phase for about 45 min for the movement of mobile phase and samples moved by capillary action. The spots were pictured by spraying the plate with 10% (v/v) H₂SO₄ in ethanol and heating on a hot plate until charring occurred (Patil et al., 2011).

Measurement of Rₖ

The distance covered by substance and mobile phase was measured and Rₖ value was calculated as follows:

\[
Rₖ = \frac{\text{Distance travelled by visualized spot}}{\text{Distance travelled by solvent front}}
\]

RESULTS AND DISCUSSION

In the present study, three macroalgae species that are abundantly distributed in the Pakistan were used for the evaluation of oil yield and lipids composition by using thin layer chromatography.

Microscopic Identification

The algae were identified microscopic examination. Cladophora is reticulated filamentous green algae, grows as microscopic thin, hair-like threads. It has multinucleated cells containing oval shaped reticulated, pyrenoid-packed chloroplasts.

Genus spirogyra is a multicellular green algae and contains cellulosic cell wall. Long unbranched filaments are surrounded by slime. In each cell a large, coiled shape d chloroplast, containing numerous pyrenoids is also present along with large vacuole.

Oedogonium is also a multicellular filamentous algae containing convulated Chloroplast (one or more pyrenoids located in the chloroplast). The oil extraction was carried out with n-hexane by soxhlet extraction method using solvent. Among all three algae, maximum oil yield (26% which corresponded to 31.70 kg/acre/anum of dry matter) was obtained from Oedogonium sp. However, the oils extracted from Spirogyra sp. and Cladophora sp. on dry matter basis was 16.20 % and 20.64 % which corresponded to 26.0 and 29.16 kg / acre / annum, respectively (Table I).
There are many microorganisms which have the ability to accumulate oils under some special growth conditions (Li et al., 2008; Doan et al., 2011). Berglund et al. (2001) reported that both the quantity and quality of lipids produced will vary with the identity of the algal species. The fatty acid proportion in algal cell varied and depended on growth conditions (Cohen, 1988; Thompson et al., 1990) e.g. environmental or culturing parameters such as light intensity, growth phase, photoperiod, temperature, salinity, CO₂ concentration, nitrogen and phosphorous concentration (Wu et al., 2011).

These oils were analyzed for lipid contents using TLC (Fig. 1). The results of fatty acid composition of algal oil with respect to their Retardation factor (Rf) values are given in Table II. The Rf of the bands indicated the presence of triacylglycerols, free fatty acids, diacylglycerol when compared with the standard Rf values of neutral lipids. Oedogonium sp. contained triacylglycerol, free fatty acids and sterol which proved to be the best algal oil for the biodiesel production.

Presence of the free fatty acids (FFA) was not observed in the oil extracted from three algae studied. During transesterification presence of the FFA (>1% w/w) caused the side-saponification initiation and as such could not be used for biodiesel production (Hingu et al., 2010; Atadashi et al., 2011). FFA scavenge catalyst and reduce catalyst effectiveness ultimately lowered the yield of biodiesel (Kusdiana & Saka, 2004). However, the oil could be pretreated by number of ways to reduce the FFA to less than 1% w/w, and afterwards could be converted to biodiesel by transesterification.

In addition to, the production of biodiesel as chief product from the extracted algal oils, the waste of algal biomass could be anaerobically digested for the production of biogas, thus, making biodiesel production more economical. This excess amount of energy (biogas) could be utilized for domestic purpose or could be sold to improve the economics of integrated system (Chisti, 2007).

The growth of algae was not optimized in the present work. The optimization of the growth of algae would certainly enhance the oil yield.

**Conclusion**

This research supports the concept that macroalgae present in Pakistan can be a potential...
source of biofuel as lipid contents ranged from 15-26% (MAG, DAG and TAG). The oil from these algae can be successfully converted into biodiesel by transesterification. Overall regional cultivation of macroalgae processing into biofuels may provide economic benefits to Pakistan rural communities.

REFERENCES


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