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Transesterification of oil extracted from different species of algae for biodiesel production

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In the current study, biodiesel production efficiency of *Chlorella vulgaris, Rhizoclonium hieroglyphicum* and mixed algae culture was measured by transesterification process. Growth rate of algal species was measured on the basis of increase in their dry matter in various media. Protein, carbohydrates and lipids in all selected algae were measured on dry matter basis. Extracted oil was analyzed for water contents, iodine value, saponification value, acid number and fatty acid composition. Transesterification of algal oil was performed by using sodium methoxide as a catalyst. Yield of biodiesel from extracted oil was calculated for *C. vulgaris* (95%), *R. hieroglyphicum* (91%) and mixed algae culture (92%). Produced biodiesel was analyzed for kinematic viscosity (4.9, 5.0 and 4.7 mm^2/s), flash point (160, 156 and 155°C), specific gravity (0.91, 0.914 and 0.912 g/ml), cetain number (51, 49 and 47 min), iodine value (47, 53 and 49 mg/g), acid number (0.49, 0.5 and 0.46 mg.KOH/g), carbon residue (0.01, 0.02 and 0.01 mass%), sulfated ash (0.007, 0.003 and 0.004 mass%), sulphur (0.013, 0.012 and 0.01 wt%) and water contents (15, 23 and 17 mg/kg) for *C. vulgaris, R. hieroglyphicum* and mixed algae culture, respectively. Properties of biodiesel were compared with ASTM standards and it was found with high quality biodiesel.

Key words: Biodiesel, oil extraction, algae, transesterification.

INTRODUCTION

Fuel production from biomass is getting more importance these days due to scarcity, pollutants emission and increase in cost of conventional fossil fuels (Sensoz et al., 2000). Earlier studies revealed the fuel properties of algae and the effect temperature on the yield of algal biofuel (Demirbas, 2006). All algae contain proteins, carbohydrates, lipids and nucleic acids in varying proportions. While the percentages vary with the type of algae and the cultivation conditions, some algae types could accumulate 40% or more of their overall mass by fatty acids (Becker, 1994). Algae have the potential to produce more oil per acre than any other feedstock being used to make biodiesel (Demirbas, 2009).

Biodiesel are monoalkyl esters of long chain fatty acids which are transesterified from vegetable oil or animal fat (Ma and Hanna, 1999). Biodiesel has been commonly used in United States and Europe with annual production of 57 to 67 million L and 500 million to 1 billion L, respectively (Gerpan, 2005). The main obstacle in commercialization of biodiesel is high cost of oil feedstock. Research efforts are made to minimize oil feedstock cost (Zhang et al., 2003; Noureddini et al., 2005; Dhaermadi et al., 2006).

The most significant distinguishing characteristic of algal oil is its yield and hence its biodiesel yield. According to some estimates, the yield (per acre) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils. Many species exhibit rapid growth and high productivity and many microalgal species can be induced to accumulate substantial quantities of lipids,

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often greater than 60% of their dry biomass (Sheehan et al., 1998).

There are several methods used to extract oil from algae that includes mechanical systems, chemical, thermal, plasma and microwave techniques. Most of the traditional methods do not offer the long-term solutions. Algal oil can be extracted by using suitable solvents (Xu et al., 2006). Transesterification is the most common method and leads to monoalkyl esters of algal oil, vegetable oils and fats. The methyl ester produced by transesterification of vegetable oil has low viscosity and improved heating value as compared to those of pure vegetable oil which results in shorter ignition delay (Demirbas, 2010).

The current study was conducted to assess the biodiesel production efficiency of various species of algae. A comparison was made to find out the algal species with high oil contents and biodiesel production efficiency. Growth rate of selected algae was compared by growing in various culture media. Harvested biomass from these algal species was dried, ground and oil was extracted by Soxhlet extractor using hexane as a solvent. The extracted oil was transesterified to biodiesel using sodium methoxide as a catalyst. Biodiesel produced from these oils was analyzed and compared with ASTM standards.

MATERIALS AND METHODS

Algal sampling and identification

Samples of *Chlorella vulgaris, Rhizoclonium hieroglyphicum* and mixed algae culture were collected from fish forms and laboratory of Punjab Fisheries Department, Lahore Pakistan. These algal species were identified using standard methods described by Zarina et al. (2005a, b).

Culture media preparation

Aquatic cultures of algae were prepared in artificial ponds having dimensions of 0.3 x 0.3 x 0.15 m with final capacity 13.5 L. The rate of growth of these algal species was measured by the estimation of increase in its biomass for 6 days. Experiments were conducted in the month of November with light intensity of 25.5 K lux and 30°C of average temperature. The following three types of culture media were used for algal culture.

Wastewater

Raw sewage water collected from municipal drain was used for the growth of all selected algae in separate ponds.

Agar solution

This medium was prepared by dissolving 10 g of analytical grade agar in 1 L of a filtered tap water to measure the growth rate of selected algal species in ponds of same dimensions as mentioned earlier.

Synthetic medium

For the preparation of synthetic medium, six stock solutions were prepared in a distilled water as follows: (a) 30 g sodium nitrate per liter, (b) 2 g potassium phosphate per liter, (c) 3 g iron sulfate, 3 g citric acid, 1.5 g boric acid and 1 g manganese chloride per liter, (d) 0.22 g zinc sulphate, 0.79 g copper sulphate, 1.5 g ammonium molybdate, 0.23 g ammonium vanadate, 2.5 g ethylene diamine tatra acetic acid and 0.12 g cobalt chloride per liter, (e) 0.007 g vitamin B_{12} per liter and (f) 3 g ethylene diamine tatra acetic acid, disodium salt per liter. Synthetic medium for algal growth was prepared by mixing 10, 5, 1, 0.1, 20 and 20 ml per liter of each stock solution from serial (a) to (f) respectively (Hur, 2008).

Biodiesel production processes

Oil extraction from algae

In the current study, solvent extraction method was used because solvent used was recycled, reducing processing cost. Three hundred milliliters of n-hexane was used for 40 to 60 g of dried algae for the oil extraction. The extraction was carried out in a Soxhlet extractor (UNE-EN 734-1, 2006) for 4 h in order to determine the algae oil contents. All the experiments were conducted using a 0.5 L round-bottomed glass flask. The resultant solution was separated from solvent by distillation. The solvent was reused in the next batch of extraction.

Transesterification of algal oil

The extracted oil was transesterified to methyl ester (biodiesel) by sodium methoxide. The mixture was heated at 62°C and methanol (10 wt% dry basis) having sodium metal (0.1 wt% dry basis) previously dissolved was added to the reactor (Charoenchaitrakool and Juthagate, 2011). Reaction was conducted at the same temperature for 1 h with constant stirring at 110 rpm. The reaction mixture was cooled to room temperature after this process. Then, the solid phase was separated using a separating funnel under vacuum condition. Finally, the bottom layer of glycerin was separated from the mixture biodiesel and hexane layer (top layer), which was then washed with water to remove the methanol excess and the traces of catalyst (Karaosmanoglu et al., 1996; Lang et al., 2001; Chen et al., 2012). In order to obtain the crude biodiesel, it was necessary to remove the solvent by distillation.

Analysis of algal biomass and extracted oil

Algal biomass was analyzed for protein, carbohydrates and lipids. Protein content was determined by the block digestion method and lipid contents by solvent extraction (Boccard et al., 1981). The fatty acid profile of extracted oil was analyzed by GC-MS in a gas chromatograph (Shimadzu GC-14-A). Acid value, free fatty acids, iodine value and saponification value were determined by methods of Raie (2008).

Biodiesel analysis

The final product of methyl ester identified with the help of thin layer chromatography (TLC) and analyzed for kinematic viscosity, flash point, specific gravity, cetain number, carbon residue, sulfated ash, sulphur and water contents by ASTM standard methods and iodine value, acid number were by methods suggested by Raie (2008). In thin layer chromatography, thin layer chromatogram (0.25 mm of thick) having length of 20 cm and width of 20 cm was prepared by using water and silica gel. The plate was air dried and activated through heating at 105°C in an oven for one hour. Diethyl ether and n-hexane (20:80) were used as solvent system, biodiesel was dissolved in a solvent and 2,7-dichlorofluorescein was used as non-destructive locating agent to see color bands (purple-yellow) under ultra violet light of 366 nm wavelength.

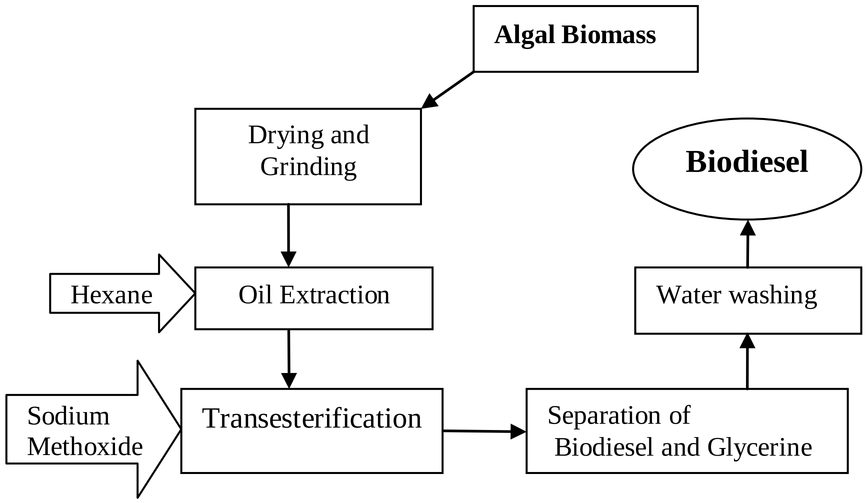

Figure 1. Steps involved in the biodiesel production from algal biomass.

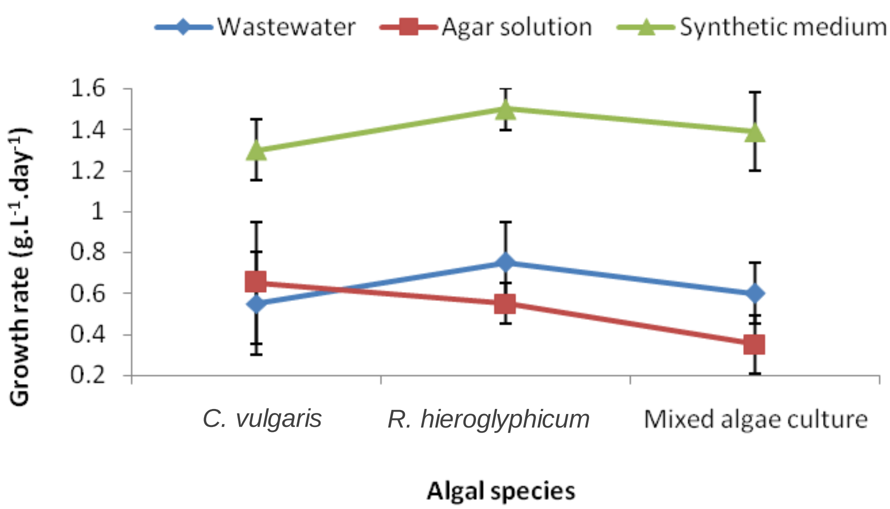

Figure 2. Growth rate of algal species in three different types of nutrient media.

RESULTS AND DISCUSSION

Processing algae for biodiesel

Algae were processed to biodiesel by extracting oil from dried and ground biomass of algae using hexane as solvent. It was transesterified to biodiesel by sodium methoxide catalyst. Biodiesel produced was separated from glycerin by separating funnel and washed with water to get pure biodiesel (Figure 1).

Growth rate measurement

Growth rate of all three types of algae *(C. vulgaris, R. hieroglyphicum* and mixed algae culture*)* was measured by growing these species in three types of nutrient media (wastewater, agar solution and synthetic medium) and it was observed that all species showed maximum growth in synthetic medium as it contained all the necessary nutrients its required amount. *R. hieroglyphicum* showed growth rate of 1.5 $g.L^{-1}.day^{-1}$ in synthetic medium which was higher than *C. vulgaris* and mixed algae culture. The growth rate of these species in wastewater and agar solution was almost similar with *C. vulgaris* which showed more growth in agar solution while *C. vulgaris* and mixed algae culture showed more growth in wastewater (Figure 2) but it was decreased with time (over 6 days) due to deficiency of nutrients if no fresh medium was added.

Ruiz-Marin et al. (2010) performed many experiment under batch culture condition in which microalgae showed high growth rates in initial days but growth and chlorophyll contents were decreased after four cycles of culture

Table 1. Percentage chemical composition of selected algal species on dry matter basis.

Sample specie	Protein	Carbohydrate	Lipid	other
C. vulgaris	35±3	16±1.5	45±1.9	4±0.4
R. hieroglyphicum	38±2.1	19±1.8	34±0.9	9±0.7
Mixed algae culture	28±1.6	29±3.4	35±2.8	8±1.3

Table 2. Analysis of extracted algal oil for various parameters before converting it to biodiesel.

Analytical parameter	Oil extracted from *C. vulgaris*	Oil extracted from *R. hieroglyphicum*	Oil extracted from mixed algae culture
Water content (%)	0.07±0.01	0.08±0.009	0.05±0.015
Iodine value (mg/g)	63±2	75±1	69±2
Saponification value (mg/g)	173.12±3.4	168.3±4.1	182.6±1.5
Free fatty acid (%)	2.6±0.3	3.6±0.7	3.3±0.1
Acid number (mg KOH/g)	31.46±2.1	34.5±1.8	37.3±2.8

indicating collapse of the culture due to nutrient deficiency.

Algal biomass analysis

Algal biomass was harvested by filtration and gravity sedimentation methods. It was dried and analyzed for protein, carbohydrates and lipid contents. *C. vulgaris* showed higher lipid contents (45%) than *R. hieroglyphicum* (34%) and mixed algae culture (35%) on the basis of their dry mass whereas protein and carbohydrates were observed to be more in *R. hieroglyphicum* (Table 1). An integrated approach of biodiesel production from heterotrophic *C. protothecoides* showed that the lipid content reached 46.1, 48.7 and 44.3% of cell dry weight in samples from 5, 750 and 11,000 L bioreactors, respectively (Li et al., 2007).

Analysis of extracted algal oil

Oil extracted from algal species was analyzed for water contents, iodine value, saponification value, free fatty acids and acid number. It was deduced from the results that there was no much difference in the above mentioned parameters in oil extracted from all three species of algae (Table 2). Properties of oil were comparable with that of the study conducted by Li et al. (2007) in which water content in the extracted oil was found to be less than 1% which means that oil feasible for methyl ester production as transesterification level decreased if the water content increased to 10% of the oil quantity. Saponification value of 189.3 mg KOH g^{-1} and acid value of 8.97 mg KOH g^{-1} were also measured in lipid extracted from heterotrophic chlorella cells (Li et al., 2007).

Fatty acids profile (14:0, 16:0, 16:1, 16:2, 16:3, 16:4, 18:0, 18:1, 18:2, 18:3, 18:4, 20:0, 20:1, 20:2, 20:3 and 20:4) of algal oils was also analyzed in algal oils of selected types of algae and it was observed that unsaturated fatty acids percentage was more in *C. vulgaris* (78.09%) than *R. hieroglyphicum* (77.5%) and mixed algae culture (77.99%), while saturated fatty acids were higher in *R. hieroglyphicum* (Table 3). Feasibility of biodiesel production from algal consortium grown in treated wastewater was checked by Chinnasamy et al. (2010), about 126.7 g (144 mL) of crude algal oil was extracted from 2.3 kg of dry algal biomass and extracted oil was examined for the fatty acid profile using GC. The profile showed 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 20:5, 22:5 and 22:6 fatty acids. These results are also in conformity with the observations made by Gouveia and Oliveira (2009) that microalgal lipids derived from *C. vulgaris, Scenedesmus maxima, Nannochloropsis oleabundans, Scenedesmus obliquus* and *Dunaliella tertiolecta* were mainly composed of unsaturated fatty acids (50 to 65%).

Yield of biodiesel

Hundred grams algal biomass of each species was used to measure the production of oil and biodiesel. Maximum quantity of oil was obtained from *C. vulgaris* biomass (45 ml/100 g), it was higher than *R. hieroglyphicum* (35 ml/100 g) and mixed algae culture (33 ml/100 g). Similarly yield of biodiesel was 94, 92 and 91% by *C. vulgaris, R. hieroglyphicum* and mixed algae culture, respectively (Table 4). Li et al. (2007) showed that 98.15% of the oil was converted to monoalkyl esters of fatty acids in 12 h by transesterification of the microalgal oil, catalyzed by immobilized lipase from *Candidia* sp.

Identification of biodiesel by thin layer chromatography

The oil extracted from algal species, biodiesel produced from *C. vulgaris* (Sample 1), *R. hieroglyphicum* (Sample 2) and mixed algae culture (Sample 3) were analyzed and compared with standards of fatty acids and methyl

Table 3. Percentage composition of fatty acids of oil extracted from *C. vulgaris, R. hieroglyphicum* and mixed algae culture algae.

Fatty acid profile	Oil extracted from *C. vulgaris*	Oil extracted from *R. hieroglyphicum*	Oil extracted from mixed algae culture
C14:0	2.26	0.35	1.35
C16:0	18.9	20.89	19.33
C16:1	9.53	11.39	15.01
C16:2	0.04	0.02	n.d.
C16:3	6.29	5.34	4.36
C16:4	7.62	10.03	8.94
C18:0	0.65	1.26	1.15
C18:1	19.58	15.65	14.75
C18:2	11.15	10.16	15.6
C18:3	22.17	23.73	18.07
C18:4	n.d.	0.07	0.02
C20:0	0.1	n.d.	0.18
C20:1	0.91	0.65	0.57
C20:2	0.79	0.43	0.67
C20:3	n.d.	0.03	n.d.
C20:4	0.01	n.d.	n.d.
Saturated	21.91	22.5	22.01
Unsaturated	78.09	77.5	77.99

*n.d. = not detected.

Table 4. Quantification of yield of biodiesel and by product synthesized from algal biomass.

Products and by products	*C. vulgaris*	*R. hieroglyphicum*	Mixed algae culture
Total biomass used (g)	100	100	100
Residual biomass (g)	57±2	69±3.5	71±2.6
Quantity of oil extracted (ml)	45±0.9	35±0.6	33±1.1
Yield of biodiesel (%)	94	92	91
Glycerin and other by products (%)	6	8	9

ester by thin layer chromatography. Figure 3 show that biodiesel produced from all three species was pure methyl ester as there were no traces of glycerides found in all three samples.

Biodiesel analysis

Biodiesel produced by transesterification of algal oil was analyzed for various parameters and results showed that quality of biodiesel obtained from all species was comparable to ASTM D-6751~02 standards. It was also observed that there was no significant difference in the properties of biodiesel produced from *C. vulgaris, R. hieroglyphicum* and mixed algae culture (Table 5). Li et al. (2007) obtained similar results in which properties of biodiesel produced from *Chlorella* sp. were comparable to conventional diesel fuel and comply with the US Standard (ASTM 6751) for Biodiesel.

Conclusions

In the current study, three algal species were used to extract oil and its conversion to biodiesel. The study revealed that algae are fast growing and effective organism for biodiesel production as these can be grown in wastewater as well as in artificial media. Raw municipal wastewater can be used as a medium of growth for algae as growth rate of *C. vulgaris, R. hieroglyphicum* and mixed algae culture were comparable with artificial nutrient media. Oil extracted from harvested biomass of these algae was transesterified to biodiesel using sodium methoxide as a catalyst. Resultant biodiesel was analyzed and compared with ASTM standards. It was found that properties of biodiesel were in accordance with standards limits so it can be blended with fossil fuels or can be used individually.

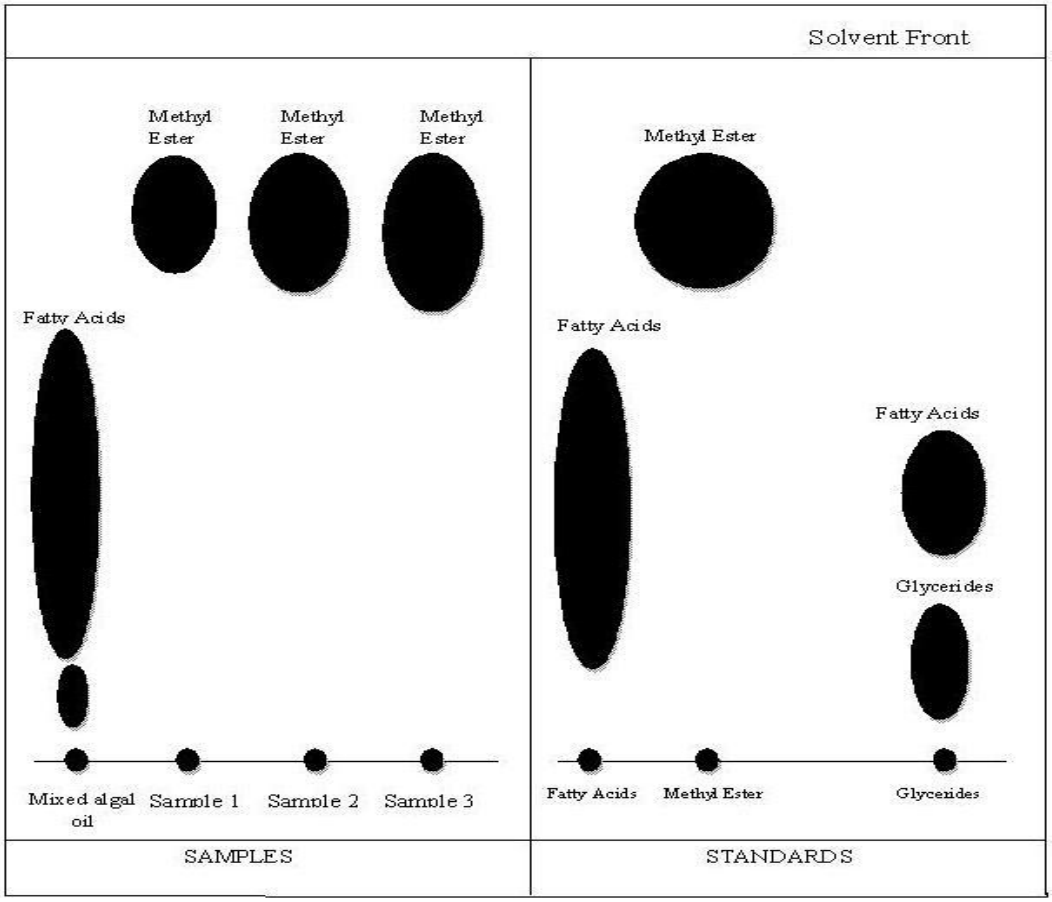

Figure 3. Identification and comparison of biodiesel (methyl ester) with standards using solvent system of n-hexane to diethylether ratio of 80:20.

Table 5. Analysis of biodiesel produced from selected algae and its comparison with international standards.

Properties	Unit	*C. vulgaris*	*R. hieroglyphicum*	Mixed algae culture	ASTM D-6751~02 Standard
Kinematic viscosity at 40°C	mm^2/s	5.2	5.0	4.8	1.9–6.0
Flash point	°C	145	146	140	>130
Specific gravity at 28°C	g/ml	0.916	0.914	0.912	0.88
Cetain number	min	53	51	49	>47
Acid number	mg.KOH/g	0.49	0.5	0.46	0.8 max.
Water contents	%vol	0.04	0.05	0.07	0.05 max.
Calorific value (gross)	MJ/Kg	41.2	35.3	37.2	-----

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