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Production of Biodiesel from Algae

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Biodiesel has gained much attention in recent years due to its eco-friendly nature, non-toxic characteristics, biodegradability and lower net carbon cycle compared to conventional diesel fuels. In the current study, potential algal specie Spirogyra were collected from different districts of Khyber Pakhtunkhwa, Pakistan and employed as a feedstock for biodiesel production. In the first step, oil from algae specie was extracted using n-Hexane and Di-ethyl Ether as solvents, while in the second stage; extracted oil was converted into biodiesel via transestrification reaction. The effects of solvent to oil ratio, size of algal biomass and contact time were studied on the percentage yield of oil extracted. The maximum extracted oil was 0.09 fraction of biomass, by using a blend of both solvents at solvent to biomass ratio of 3.5, algal biomass size of 0.4 mm and contact time of 24 hours. While in transestrification reaction, effects of molar ratio, temperature, reaction time and amount of catalyst (Sodium Hydroxide) were evaluated on the amount of biodiesel produced. Almost 95% conversion of extracted oil into biodiesel was achieved after 25 minutes of contact time at 60 °C with catalyst amount of 0.5% weight of oil and oil to methanol ratio of 8.

Key words: Biodiesel; renewable energy; algae; transestrification; spirogyra; biomass.

Carbon dioxide (CO_2) emissions from the transport sector is contributing a major portion to the environmental pollution and global warming (Balat *et al.*, 2010). The cost of crude oil will continue to rise due to diminishing supply, so production of fuels from alternate sources will be needed in the future decades (Du *et al.*, 2008). In this scenario, biodiesel is the best alternative fuel due to its non-toxic nature (Lapinskiene *et al.*, 2010). Sources of commercial biodiesel include oil from waste cooking, corn, palm, animal fat, canola and jatropha. However, using plant oil for biodiesel production is not only controversial but also requires substantial quantity of land (Lee *et al.*, 2011). Therefore, microalgae is an alternate appropriate raw material for biodiesel production (Chisti *et al.*, 2010; Rawar *et al.*, 2013)

Biodiesel is the monoalkyl esters of long-chain fatty acids, which is derived from transesterification of biological matter (Lapuerta *et al.*, 2008). It is an excellent renewable and safe alternative fuel with environment friendly nature

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(Patil *et al.,* 2011). Biodiesel production from renewable sources can also boost farming and fuel production industries (Xue *et al.,* 2006). Oil of most feed stokes like soybean, sunflower, safflower, cotton seeds, coconut, peanut and rapeseed are investigated to be the potential alternative fuels for diesel engines (Demirbas *et al.,* 2008). Studies showed that tobacco seeds can also be used for biodiesel production (Veljkovic *et al.,* 2006). Biodiesel from oilseeds or animals has higher raw material cost, and it cannot meet the realistic need, and can be used to fulfill only small fraction of existing demand. Therefore microbial oils, produced by various microorganisms like bacillus, fungi, yeast and algae may be considered as the potential feedstock for biodiesel production. They own high lipid content and are cost effective (Meng *et al.,* 2009). Biodiesel obtained from other vegetable oil has the disadvantage of poor performance in cold weather as their polyunsaturated fatty acids tends to decrease the stability, but algal oil has overcome this problem due to the lower melting point of their polyunsaturated fatty acids. The biodiesel produced from these organisms not only have environmental benefits over the fossil fuel, but also economically competitive with the conventional petro diesel.

The accumulated oil in almost all microalgae is mainly triglyceride (>80%), with a fatty acid profile rich in C16 and C18 (Meng *et al.,* 2009). Some challenges associated with the production of biodiesel from algae are exploration of efficient techniques for cultivation and searching for suitable algae strain with fast growth rate and high lipid content (Aliya et al., 2009)..

Spirogyra is the most common green algae, available abundantly in springs, ponds and brackish water. Spirogyra has a simple cellular hair like structure, and has unbranched cylindrical fibers, which is about 1/10 mm in diameter and few centimeter long. The cell wall is composed of two layers, the inner one is made up of cellulose, and the outer is purely pectose by nature. It produces lipids, carbohydrate and proteins that can be utilized for the production of biodiesel, or bioethanol (Meng *et al.,* 2009).

In this study, potential algal strains of spirogyra were used for the production of biodiesel These strains were collected in the Swabi and Mardan disctricts of Khyber Pukhtunkhwa, Pakistan. In the first step, oil from algae specie was extracted using n-Hexane and Di-ethyl ether as solvents, while in the second stage, extracted oil was converted into biodiesel via transestrification reaction. The effect of solvent to oil ratio, size of algal biomass and time was studied on the percentage yield of oil extracted. While in transestrification reaction the effect of molar ratio, temperature, reaction time and amount of catalyst (Sodium Hydroxide) was studied on the amount of biodiesel produced.

EXPERIMENTAL

Collection and pretreatment of algae specie

The algae samples were collected from Swabi and Mardan districts of Khyber Pukhtunkhwa, Pakistan. The samples were spread under sun in an open area for 48 hours to evaporate the amount of water associated with biomass. The dried samples were grinded, and the fine powder was passed through a 500 micron sieve, to remove the oversize particles. This step is necessary, to get smaller size particles which will have a physical contact with the solvent used for extraction.

Treatment with Solvent

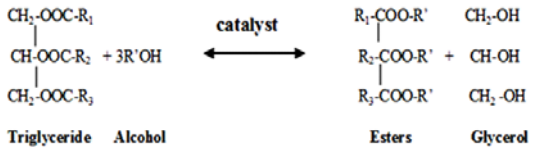
20 gram of grinded algal biomass was treated with 20 ml of solvent. Solvents n-Hexane and Di-ethyl Ether were used for the extraction of oil from algal biomass. Both solvents were used alone as well as a blend of n-Hexane and Di-ethyl Ether was employed for oil extraction. The results are shown in Table 1. The mixture was kept at room temperature for 24 hours. A layer of oil on the solvent surface was formed, which was separated from the residue. We obtained a higher fraction of oil extracted by suing the combination of both the solvents.

The extracted oil was separated by evaporating the solvent in a rotary evaporator. Initially, the evaporator was kept at 34 ^{o}C for 15 minutes to evaporate Di-ethyl Ether. Temperature was then raised to 69 ^{o}C to remove n-hexane. This process left behind solvent free oil in the evaporation flask. The different steps involved in extraction of oil from algal biomass are shown in the flow chart in Figures 1 & 2.

Transesterification of oil with methanol

About 400 ml oil was leached and

separated through the extraction process from 20 kg of algal biomass sample. The extracted oil was converted to biodiesel through transesterification reaction in the presence of methanol. In this process triglycerides react with the alcohol to form the fatty acid ester (biodiesel) and the glycerol, as shown in the equation (1) below (Georgogianni *et al.*, 2007).



During this reaction the algal oil was allowed to react with the methanol in the presence of alkali.

RESULTS AND DISCUSSION

Extraction of oil from algal biomass

Effect of solvent to algae ratio

The effect of solvent to algae ratio on percent yield of extracted oil is shown in Figure 3. It was observed that the percent yield of oil increases as the solvent to algae ratio increases. The higher yield at solvent to algae ratio is attributed to the excess solvent available to extract oil from the algal bio mass. For the same amount of solvent and biomass, the oil extracted is only 4 %. This suggested the use of excess amount of solvent to extract more oil from the algal biomass.

Effect of the algal biomass size

The size of algal biomass also effect the amount of oil extracted. Figure 4 shows that when the size of biomass decreases, the oil yield increases. This can be justified by the improved interaction between the algae specie and solvent, due to larger surface area of smaller algal specie. The smaller sized particles have a good interaction with solvent as compared to large particles and thus enhance the yield.

Effect of contact time

The effect of contact time on the amount of extracted oil is shown in figure 5. It can be seen that percentage yield increases as the contact time increases. The higher yield at larger contact time can be explained by the fact that enhanced interaction between the solvent and biomass occurred after long contact time, which lead to homogenous mixing. Therefore, oil is extracted from all portions of the algae specie. The contact time was varied from 5 to 24 hours, while the other parameters i.e solvent to algae ratio and size of algae remains constant.

Transesterification reaction

Effect of molar ratio of oil to methanol

Yield of biodiesel depends on the amount of extracted oil as well as the methanol used in the reaction. As per stoichiometry, 3 mole of alcohol is required to convert 1 mole of extracted oil into 3 mol of fatty esters (biodiesel) and 1 mole of glycerol. It was observed that biodiesel yield increases almost linearly by increasing oil to methanol ratio. At higher molar ratio, the excess amount of oil promotes the forward reaction. The

Table 1. Amount of oil extracted using different solvent combination

S. No	n-Hexane (ml)	Di-ethyl Ether(ml)	Fraction of oil extracted
1	40	0	0.0862
2	0	40	0.082
3	20	20	0.09

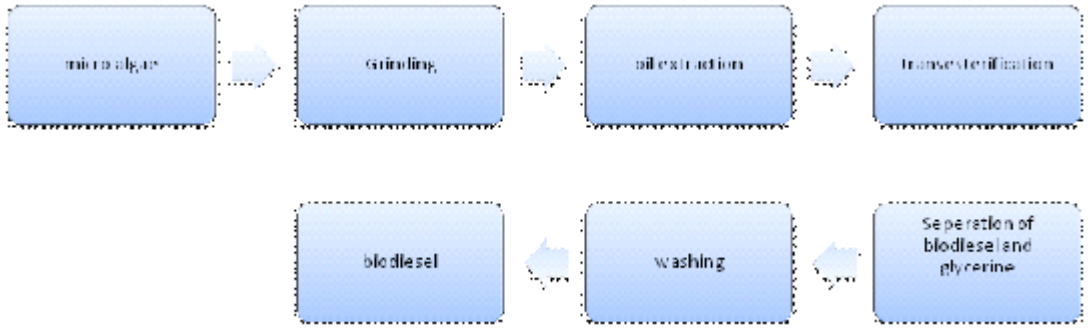


Fig. 1. Schematic of biodiesel production process

range of oil to methanol ratio was 2 to 8, with a maximum yield of 95% of biodiesel (Figure 6).

Effect of temperature

Reaction temperature also affects the amount of biodiesel production as shown in Figure 7. The temperature was varied from 45 to 65 ^{o}C. Reaction is carried out near the boiling point of methanol under atmospheric conditions. It was observed that higher temperature favors the biodiesel production. In this study, maximum biodiesel produced at 60^{o}C, which is in agreement with the available literature (Khan *et al.,* 2009; Ma *et al.,* 2009; Pramanik *et al.,* 2003; Srivastava *et al.,* 2000).

Effect of reaction time

As shown in Figure 8, the higher the reaction time, the higher the yield of biodiesel. The reaction time for this study was varied between 10 to 25 minutes and it was observed that the yield increases as the reactants spend more time in the reacting vessel. The higher yield (>95%) was obtained at 25 minutes of reaction time**.**

Effect of catalyst amount

The role of catalyst in transesterification reaction is very important. Transesterification reaction can be carried out with both acidic or alkali catalyst. However, using acidic catalyst has the disadvantage due to its corrosive nature as

Fig. 2. Stpewise process of extraction (a) grinding of algae (b) grounded algae (c) mixing of solvents and biomass (d) evapoation of solvent in rotary evapator (d) extracted oil

compared to the alkali catalyst. Sodium hydroxide was used as a catalyst, with amount ranging from 0.3 to 0.5 % of weight of oil. Presence of catalyst has increased the rate of reaction and hence the yield was increased. Maximum yield was obtained with the higher catalyst amount i.e 0.5 % of weight of oil (Figure 9).

Biodiesel analysis

The quality of biodiesel was assessed by measuring its properties such as flash point, viscosity, density, fire point and cloud point, as shown in Table 2. The higher flash point of biodiesel as compared to petro diesel makes it safer. The viscosity of produced biodiesel is higher than the petro diesel; however, further modification can be done to reduce it to an acceptable range. However, it is still in the range of biodiesel standards (Srivastava *et al.*, 2000). Furthermore the higher cetane number of biodiesel make at an excellent alternative fuel. The other properties of biodiesel are also very close to petro diesel.

The Fourier transform infrared spectroscopy (FTIR) results for produced biodiesel and petro diesel are shown in Figure 10. The peak at 1200 and 1750 cm^{-1} corresponds to stretching vibrations of CC(=O)–O bonds of the ester and C=O of methyl ester respectively (Xue *et al.*, 2006).

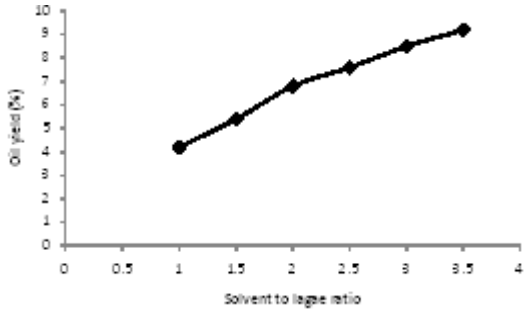


Fig. 3. Effect of solvent to algae ratio on the extracted oil yield

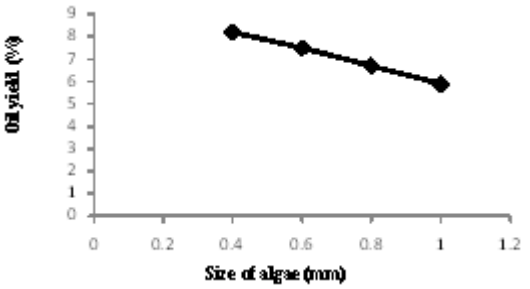


Fig. 4. Effect of size of algae on the extracted oil yield

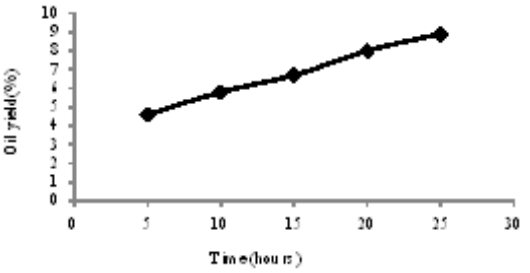


Fig. 5. Effect of contact time on the extracted oil yield

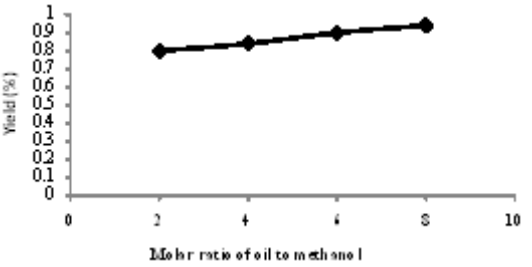


Fig. 6. Effect of oil to methanol ratio on the amount of biodiesel produced

Table 2. Comparison of the physical properties of produced biodiesel with different types of diesels

Properties	Petro Diesel (Lapuerta*et al.* 2008)	Standard Biodiesel (Lapuerta*et al.* 2008)	Produced Biodiesel
Density(kg/m^3)	861	870.3	876.2
Kinematic viscosity(mm^2/s)	1.3-4.1	4-6	4.9
Flash point (°C)	60-80	100-170	140
Fire point (°C)	68-82	120-180	160
Cloud point (°C)	-15-5	-3-12	5

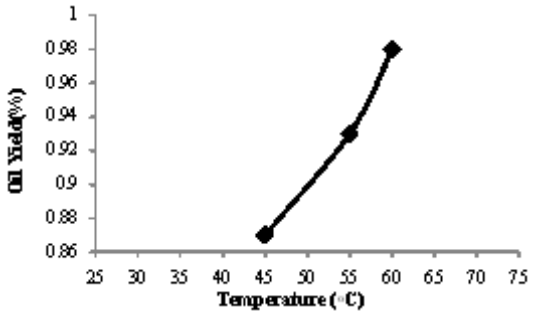


Fig. 7. Effect of temperature on the amount of biodiesel produced

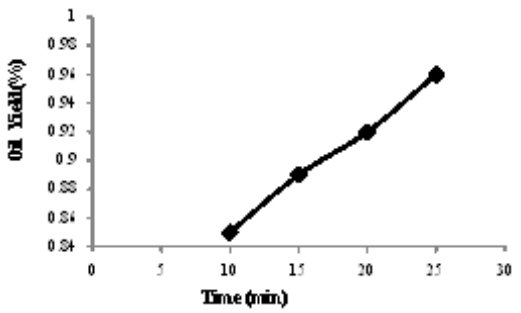


Fig. 8. Effect of reaction time on the amount of biodiesel produced

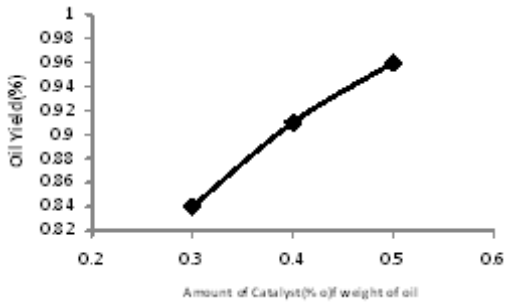


Fig. 9. Effect of amount of catalyst on the amount of biodiesel produced

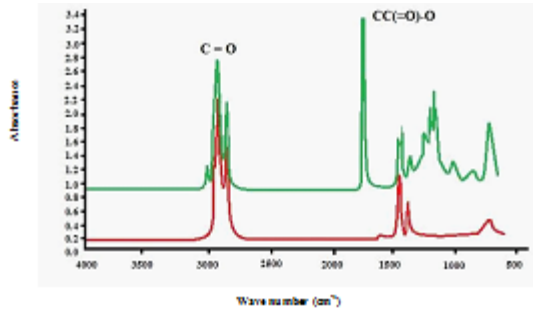


Fig. 10. FTIR plot for produced biodiesel (green) and petro diesel (red)

CONCLUSION

Algae specie (Spirogyra) was successfully used as a raw material for biodiesel production. The process involved two steps e.g. oil extraction and transesterification. It was noted that the maximum amount of oil was extracted from algal biomass using combination of n-hexane and Di-ethyl Ether. Also higher algal to solvent ratio, smaller biomass size and longer contact time enhanced the yield of extracted oil. The maximum extracted oil was 0.09 fraction of biomass, by using a blend of both solvents at solvent to biomass ratio of 3.5, algal biomass size of 0.4 mm and contact time of 24 hours. For transesterification, the variables affecting the process were oil to methanol ratio, amount of catalyst, reaction time and temperature. It was noted that the maximum yield >95% was achieved at a temperature of 60°C, oil to methanol ratio of 8, reaction time of 25 minutes and catalyst amount 0.5 % of weight of oil. The quality of biodiesel was assessed by FTIR and some physical tests, which was in close agreement with the available biodiesel standards and petro diesel.

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