Original Research Article

Soxhlet extraction of *Spirogyra sp*. as an energy source and physicochemical characterization of produced biodiesel

Abstract: Due to industrialization demand of energy is increasing and hence to meet the demand without any compromise in growth and development, alternative source of energy needs to be found. The best alternative source is biofuels which are extracted from organic compounds. Algae are aquatic organisms almost available in every type of water bodies and considered as potential candidates for biodiesel productions at large scale. In present study, an experimental set –up was designed to produce algal oil through transesterification process. The Soxhlet extraction method is employed as an oil extraction method. The solvent utilized for oil extraction method is n-hexane, petroleum ether and mixture of both solvents to extract the algal oil. Also the oil extracted from the algae is characterized to identify the suitability of algal biodiesel as a potential biodiesel fuels for IC engine. The characterization revealed that physicochemical properties of obtained biodiesel were in permissible range in comparison with standard diesel fuel.

Keywords: Biodiesel, Soxhlet extraction, *Spirogyra sp*.

1.Introduction:

Due to rapid industrialization, the need for energy increased manifold. Due to exhausting conventional fossil fuels and increasing price of fossil fuels, the demand for alternative fuels has gained much attention [1]. Petroleum combustion is a major contributor of greenhouse gas (GHG). Petroleum combustion is also considered a major source of air contamination. The burning of enormous fossil fuels has increased the carbon dioxide level in the atmosphere manifold and one of the major causes of global warming. Biomass is a major renewable energy source contributing to CO_2 fixation in the atmosphere through photosynthesis process. If biomass is grown in a sustainable way, it has no effect on CO_2 balance as it offset the CO_2 generation by the fixation of carbon through the process of photosynthesis. In comparison to other biomass algae has higher photosynthetic efficiency than other biomasses.

Bioenergy is one of the important components to mitigate GHG emission and substitute for fossil fuels. Biodiesel is a liquid fuel mainly consisting of mono alkyl esters (methyl or ethyl) of long chain fatty acids derived from animal fats, vegetative oils and algal oils. It is a kind of bioenergy having potential to

substitute for conventional diesel [2]. Biodiesel is known as a substitute for conventional diesel fuel due to its renewability and better combustion performance properties. Algae can be converted to several types of renewable biofuels depending on route of conversion employed. These include bio-methane produced by anaerobic digestion of algal biomass [3], biodiesel derived from algal oil[4,5] and bio hydrogen from photolysis of biomass.[6-8]

Spirogyra is freshwater alga having slimy filamentous green mass. It belongs to a genus of filamentous charophyta of the order Zygnematales, named for the helical or spiral arrangement of the chloroplast that is diagnostic of the genus *Spirogyra* measures 10-100µm in width and may stretch few centimeters long.It grows under water and in case of enough sunlight produces large amount of oxygen, which adhering as bubbles between tangled filaments.

Algae owing to ability to fix CO_2, can be utilized for removal of flue gases emitted from power plants, thus reducing the GHG with higher production of biomass and consequently higher biodiesel yield. Few microalgae have convenient fatty acid profile and unsaponification fraction allowing them to produce biodiesel production with high oxidation stability [9-11]. The fuel property of biodiesel from algal oil is comparable to fuel diesel [12, 13].In present research we are trying to investigate the general fuel properties of *Spirogyra* oil based biodiesel.

1.1 Algae oil:

Algae are fast growing photosynthesizing unicellular organisms and complete entire growing cycle in very few days. Some algae reported to have high oil content (up to 60% oil by weight) and can produce almost 15, 000 gallons of oil per acre per year under optimum conditions.

The first generations of biofuels comprise of maize, wheat,& cereal crops having conflict with food supply. The second generation of biofuels mainly comprise of nonedible sources. They include bio hydrogen, bio methanol, mixed alcohol and wood diesel. The third generations of biofuels are most complex and come usually from algal biomass, which produces large amount of energy. The third generation biofuels are consider as carbon neutral and emission less as it absorbs more CO_2 from environment due to high photosynthetic efficiency than that of terrestrial plants.

Algae are photosynthesis based cell factories having ability to convert carbon dioxide to potential biofuels, foods, feeds and value added products [14-16]. In addition, these photosynthetic microorganisms are very useful in bioremediation applications [17-19], and as nitrogen fixing bio fertilizers [20]. Algae provide several different types of renewable biofuels. These include production of methane by anaerobic digestion of biomass [15], biodiesel production through transesterification process from algal oil [5,20], and photo biologically produced biohydrogen[6]. The concept of using algae as a biodiesel source is not new, [20,21], but it is gaining the attention due to escalating price of petroleum and more significantly the emerging concern about global warming due to burning of fossil fuels [15].

2.Materials and methods:

Types of feedstocks and its availability production techniques, catalyst and production costs are decide the overall cost of biodiesel production [22]. An attempt to reduce cost of biodiesel algae has been tried by researcher across the globe due to their rapid growth and photosynthetic efficiency as compared to other energy crops. A lots of work going on related to investigation to increase the growth rate of algae as biomass and increase in lipid content by genetic engineering tools for biodiesel production.

Biodiesel can be used either in pure form or blended form at any composition in IC engine. The use of biodiesel from algae oil blended with petroleum improves the cetane number, which in turns improves the quality of combustion and decrease NOx emission.

2.1 Extraction of oil from algae:

The Biomass recovery process is very challenging due to low cell densities and small size of the cell. Flocculation filtration flotation and centrifugation are some traditional methods which are used for oil extraction [23]. Other options like solvent extraction, super critical fluid extraction, osmotic shock and ultra sound assisted extraction are advanced methods for extraction of oil from algae. Expeller press is simple in operation but percentage of oil extracted is low. The requirement of high pressure increases the cost of operation compared to conventional fluid extraction.

Osmotic shock requires large sample volumes and high energy electromagnetic pulses are required to rupture the cell walls and cause the cell to burst to release the lipids. Super critical fluid extraction, ultrasound extraction assisted extraction and osmotic shocks no doubted give better yield but the process is difficult. Therefore the solvent extraction process was adopted for the present work. To avoid degradation biomass are dried in oven (at 105^0C). Although direct sun drying, air drying, spray dryers, freeze dryers, or fluidized beds are commonly used methods.

2.2 Procedure to extract the oil:

The algae collected was dried in oven (at 105^0C) and powdered for further use. 25 gm of algae was taken in thimble of the Soxhlet extractor. A round bottom flask was filled with solvent in the ratio 1: 10. The Soxhlet extractor was fitted with condenser as an experimental setup. The solvent was heated to its boiling point to start the cycle and allowed running for 4 to 6 hours. After such reflux cycles, a portion of oil was dissolved in solvent. The solution was separated and concentrated by use of rotary evaporator. In this study, n-hexane, petroleum ether and mixture of both i.e. n-hexane and petroleum ether was taken for the maximum possible extraction of oil. The insoluble portion of algal biomass remained in the thimble.

2.3 Conversion of oil into biodiesel:

The process employed for conversion of oil to biodiesel is transesterification. In transesterification process, algae oil is reacted with a short chain alcohol in the presence of catalyst to produce fatty acid methyl ester (FAME) and glycerol as a byproduct. The short chain alcohol (usually methanol) used in excess to assist in quick conversion. The catalyst generally basic in nature, like sodium or potassium hydroxide that has already mixed with methanol. In this study freshly prepared sodium methoxide is used as catalyst for better result.

3. Results and discussion:

The manufactured biodiesel from algal oil is taken for physiochemical properties analysis. Physiochemical properties like saponification value, Iodine value, calorific value, acid value and flash point was evaluated for biosynthesized biodiesel.

3. 1 Saponification value:

Additives of petroleum products react with alkali to form metal soaps. Fats are example of such additives. Used engine oil obtained from turbine or internal combustion engine contain chemicals that will similarly reacts with alkali. Saponification value expressed as the number of milligram of potassium hydroxide required to saponify one gram of fat under the specified condition [24]. It is measure of all fatty acids present. Oil possessing high saponification values is good for making soap. If saponification is above the prescribed limit the oil would precipitate and form soap. The saponification of synthesized biodiesel is 98.175 mg KOH/g and it is in accordance with standard limit(ASTM D221).

3.2 Iodine value:

It is the mass of iodine in grams that is consumed by 100gm of chemical substance. Iodine value measures the unsaturation in fatty acids. It is in the form of double bonds in fatty acid. The higher the iodine number the more C=C bonds are present in the fat. The iodine value of a good lubricant should be low. The iodine value determined for biodiesel is 110g I_2/100g oil [24,25] and it is close to standard permissible range(ASTM D96) as shown in table 1.

Table.1: The physicochemical value of algae biodiesel and its comparison with standard value of biodiesel [26,27]

S. No.	Physicochemical properties	Unit	Biodiesel from algal sps. Spirogyra	Standard value of biodiesel	Protocol
1.	Saponification value	mg KOH/g	98.175	------	ASTM D 221
2.	Iodine value	g I_2/100g oil	110	120	ASTM D 96
3.	Calorific value	Kcal/Kg	8871	8901	ASTM D 240
4.	Flash point	^{O}C	167	130	ASTM D 664
5.	Acid value	mg KOH/g oil	0.57	0.50	ASTM D 6751
6.	Density	Kg/m^3	.889	0.88	ASTM D 56

3.3 Calorific value:

The calorific value is the amount of energy released when fuel undergoes complete combustion in presence of oxygen under standard conditions. [28] In the chemical reaction hydrocarbon reacts with oxygen to form carbon dioxide, water and heat. The obtained calorific value is 8871 Kcal/Kg close with standard protocol of algae biodiesel (ASTM D240).

3.4 Flash point:

Flash point is the minimum temperature at which the fuel undergoes a flash. It is desirable to have low flash point temperature so that it can be burned at lower temperature [29].The flash point is investigated as 167^{0}C which is falls little bit more than that of standard value of biodiesel. Although it is considered as an added advantage, because of high non-flammability of the biodiesel relative to petro-diesel. The standard range of algae biodiesel is 130^{0}C as shown in table 1.

3.5 Acid value:

The acid value is measure of the amount of carboxylic acid groups present in chemical compound, such as fatty acid or mixture of the compounds. The observed acid value of algal biodiesel is 0.57mg/KOH/g and it is within permissible range (ASTM D 6751). It is desirable to have low acid value of biofuels due to affecting the material in which it can be stored or transported [24,30,31]

3.6 Density:

It has been observed that density of biodiesel is .889 kg/m^3 which is comparable with normal diesel (ASTM D56) [32]. Results show that biodiesel obtained from transesterification of algae oil can be used as source of alternative fuels**.**

3.7 Infrared spectroscopy (FTIR) analysis:

Fourier transform infrared spectrometry analysis used to identify and evaluate the possible unknown compounds and functional groups present in oil and biodiesel produced. The presence and nature of functional groups gives depth insight about reactivity and stability of biodiesel as well as conversion efficiency of oil to biodiesel. The generated spectrum provides information about the functional groups in the fuel samples for quality analysis and associated type of vibrations. The samples of oil and biodiesel were scanned in mid-infrared region 4000-400cm^{-1} with Agilent technologies Carry 630 FTIR. The FTIR spectrum for oil and biodiesel were interpreted [33] and it indicated that the functional groups in oil and biodiesel are present with characteristics bands of esters (C=O), alkenes (C=C) and alkanes(C-H). The absorption bands in oil from *Spirogyra sp.* indicate that the oil contains characteristics functional groups of esters like C=O esters carbonyl group and C-O-C esters. The functional group of C-H (alkanes) and C=C (alkenes) that must be present in good oil was noticed in FTIR spectrum of *Spirogyra sp.* oil. The two peaks at wave number 1437cm^{-1} and 1200-1173cm^{-1}, in case of biodiesel of *Spirogyra sp.* is observed due to conversion of oil into biodiesel by transesterification process. As, these peaks corresponds to methyl and methoxy stretching mode of vibrations respectively. The details of functional groups and type of vibrations of oil and biodiesel are given in table 2 & 3.

Table .2: FTIR absorbance spectra of *Spirogyra sp.* oil

S. No.	Wave number (cm^{-1})	Functional groups & type of vibration
1	3012	Vinyl –C-H stretch
2	2912-2945	Mehthylene -C-H asymmetric/symmetric stretch
3	2852-2860	.-C-H stretching, symmetric vibration: (-CH_2) mainly from lipids
4	1739-1754	Ester group vibration of triglycerides, -C=O, -C=C-, lipids, fatty acid
5	1463	Asymmetric bending -C-H(alkanes)
6	1378	Bending vibrational of -C-H(alkanes)
7	1166	-CH_2 Wagging
8	726	Long chain methyl rock of –C-C-bonds

Table.3 :FTIR absorbance spectra of *Spirogyra sp.* biodiesel.

S. No.	Wave number (cm^{-1})	Functional groups & type of vibration
1	3012	Vinyl –C-H stretch
2	2927	Methylene -C-H asymmetric/symmetric stretch
3	2856	-C-H stretching, symmetric vibration: (-CH_2)mainly from lipids
4	1746	Ester group vibration of triglycerides, -C=O, -C=C-, lipids, fatty acid
5	1460	Carbonate Ion , Aliphatic (– CH_3)bend
6	1437	Methyl(-CH_3) asymmetric bending
7	1247	-O-H & -C-H bending vibration, mainly from carbohydrates
8	1200-1173	Methoxy (-O-CH_3) stretching
9	726	Long chain methyl rock of –C-C-bonds

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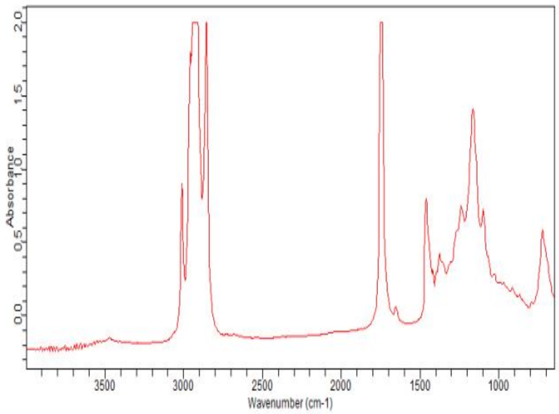

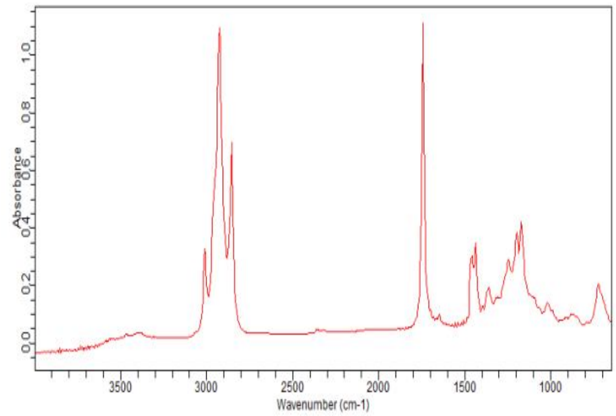

Figure 1: FTIR Image of algae (*Spirogyra*) oil.

Figure 2: FTIR image of algae (*Spirogyra*) biodiesel.

4.Conclusion:

Based on the above study it can be concluded that spirogyra oil based biodiesel is perfect option as a biofuels. The physicochemical parameters like saponification value, iodine value, calorific value, acid value, flash point and density obtained in this study has reasonable accuracy as per standard. It must be noted that solvent extraction method can be generalized for commercial applications as promising biodiesel generation method.

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