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Soxhlet Extraction of *Spirogyra sp.* as an Energy Source and Physicochemical Characterization of Produced Biodiesel

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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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 biofuel for internal combustion (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.

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1. INTRODUCTION

Due to rapid industrialization, the need for energy increased many folds. Due to exhausting conventional fossil fuels and increasing price of fossil fuels, the demand for alternative fuels has gained much attention [1]. One of the main sources of greenhouse gases (GHGs) is the combustion of petroleum. Another significant source of air pollution is the burning of petroleum. The enormous rise in atmospheric carbon dioxide levels caused by the burning of fossil fuels is one of the primary causes of global warming. Through the process of photosynthesis, biomass is an important renewable energy source that helps to fix CO_2 in the atmosphere. When biomass is grown sustainably, it has no impact on the CO_2 cycle since photosynthesis balances off CO_2 production by fixing carbon. Algae have a higher photosynthetic efficiency than other biomass sources, on average.

One of the key elements to reduce GHGs emissions and replace fossil fuels is bioenergy. Biodiesel is a liquid fuel mostly made of long-chain fatty acid monoalkyl esters (methyl or ethyl) generated from animal fats, plant oils, and algae 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 [3]. Algae can be converted to several types of renewable biofuels depending on the route of conversion employed. These include the anaerobic digestion of algal biomass to produce bio-methane [4], the production of biodiesel from algal oil [5,6] and the production of bio hydrogen from photolysis of biomass [7-9].

Spirogyra is freshwater alga having slimy filamentous green mass. It belongs to a genus of filamentous charophyta in the order Zygnematales and was given this name because the chloroplasts in this genus are arranged in a distinctive helical or spiral pattern. Spirogyra can range in width from 10 to 100 m and can extend to a few centimetres in length. When exposed to enough sunlight, it develops underwater and produces a significant amount of oxygen, which adheres 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 GHGs 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 [10-12]. The fuel property of biodiesel from algal oil is comparable to fuel diesel [13,14].In present research we are trying to investigate the general fuel properties of *Spirogyra* oil based biodiesel.

Algae oil: Algae are unicellular, photosynthesizing organisms that grow quickly and go through their full life cycle in just a few days. Under ideal circumstances, some algae have been found to have high oil content (up to 60% oil by weight) and can produce about 15,000 gallons of oil per acre per year [15].

The first generations of biofuels comprise of maize, wheat, and 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 [16]. 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 [17].

The ability of algae to transform carbon dioxide into potential biofuels, meals, feeds, and value-added products makes them photosynthesis-based cell factories [18–20]. These photosynthetic unicellular organism can also be used as nitrogen-fixing bio fertilizers and in bioremediation applications [21–23]. Algae can produce a variety of sustainable biofuels. These include the anaerobic digestion of biomass to produce methane [19], the transesterification of algal oil to produce biodiesel [6,24], and the photobiological synthesis of biohydrogen [7]. Although the idea of using algae as a source of biodiesel is not new [24,25], it is receiving more attention as a result of the rising cost of petroleum and, more importantly, the growing concern over global warming brought on by the combustion of fossil fuels [19].

2. MATERIALS AND METHODS

Types of feedstocks and its availability production techniques, catalyst and production costs are decide the overall cost of biodiesel production [26]. 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 utilized in IC engines in either pure or blended form, depending on the composition. By blending biodiesel made from algal oil with petroleum, one can increase cetane number, enhance combustion quality, and reduce NOx emissions.

Extraction of oil from algae: Due to the small cell size and low cell densities, the biomass recovery procedure is quite difficult. Traditional techniques for extracting oil include flocculation, filtration, flotation, and centrifugation [27]. Advanced techniques for extracting oil from algae include solvent extraction, super critical fluid extraction, osmotic shock, and ultra sound assisted extraction. Although the expeller press is easy to use, little oil is actually extracted. When compared to traditional fluid extraction, the need for high pressure raises the cost of operation.

Large sample quantities are needed for osmotic shock, and powerful electromagnetic pulses are needed to shatter the cell and trigger the release of the lipids. The techniques like osmotic shocks, ultrasound-assisted extraction, and supercritical fluid extraction produce superior yield, but the method is challenging .It has been reported [28] an improved chemical method to extract hydrocarbons (CH) and fatty acid methyl ester (FAME) from *Spirogyra sp.* using semi critical assisted –solvent (SmCA-Sol). Although, the extraction improvements are associated with an upgraded and improved glassware design. As a result, the present work employed the solvent extraction method.

Procedure to extract the oil: The collected algae from the pond nearby location of Meerut district was dried in an oven at a temperature of 105^0C and pulverized for further use. The Soxhlet extractor's thimble was filled with 25 g of algae powder. Solvent was poured into a round bottom flask in a 1: 10 ratio. Condenser was added to the Soxhlet extractor as part of an experimental arrangement. 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. A group of researchers conducted a comparison study to evaluate the relative efficacy of classic extraction techniques, and the results showed that hydrocarbon/alcohol azeotrophic combinations produced better results than hydrocarbon-only solvents [29]. 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.

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.

Saponification value: Metal soaps are produced when alkali and petroleum product additives react. Examples of these additions include fats. Chemicals included in used engine oil from internal combustion or turbine engines react similarly with alkali. The amount of potassium hydroxide in milligram needed to saponify one gram of fat under the given conditions is known as the saponification value [30]. It measures each fatty acid that is present in a sample. Oil with a high saponification value is suitable for soap making. If the prescribed limit of saponification is exceeded, the oil will precipitate and turn into soap. The saponification of synthesized biodiesel is 98 mg KOH/g and it is in accordance with standard limit (ASTM D221).

Iodine value: The amount of iodine consumed by 100 grams of chemical material, measured in grams. Iodine value gauges a fatty acid's degree of unsaturation. In fatty acids, it takes into accounts the number of double bonds. The more C=C bonds there are in the fat, the higher the iodine number. A good lubricant should have a low iodine value. The iodine value determined for biodiesel is 110g I_2/100g oil [30,31] and it is close to standard permissible range(ASTM D96) as shown in Table 1.

Calorific value: The quantity of energy generated when fuel undergoes complete combustion in the presence of oxygen under typical conditions is known as the calorific value [34]. In a chemical reaction, oxygen and hydrocarbon react to produce carbon dioxide, water, and heat. The obtained calorific value is 8871 Kcal./Kg close with standard protocol of algae biodiesel (ASTM D240).

Flash point: The fuel's flash point is the lowest temperature at which it ignites. Low flash points are preferred because they allow for lower burning temperatures [35]. The flash point is investigated as 167^0C which is falls little bit more than that of standard value of biodiesel. The significant non-flammability of biodiesel in comparison to petroleum-based diesel is regarded as an additional benefit. Table 1 demonstrates that the typical range for algal biodiesel is 130^0C.

Acid value: The number of carboxylic acid groups contained in a chemical compound, such as a fatty acid or combination of compounds, is measured by the acid value. The observed acid value of algal biodiesel is 0.57mg KOH/g and it is within permissible range (ASTM D664). It is desirable to have low acid value of biofuels due to affecting the material in which it can be stored or transported [30,36,37].

Density: It has been observed that density of biodiesel is 0.88 kg/m^3 which is comparable with normal diesel (ASTM D56) [38]. Results show that biodiesel obtained from transesterification of algae oil can be used as source of alternative fuels.

Infrared spectroscopy (FTIR) analysis: In order to find and assess any potential unknown compounds and functional groups contained in the oil and biodiesel produced, Fourier transform infrared spectrometry analysis was performed. Functional groups existence and nature provide detailed information about the reactivity, stability, and effectiveness of biodiesel conversion of oil into the fuel. The resultant spectrum offers details on the functional groups in the fuel samples for quality examination and the kinds of vibrations that go along with them. With Agilent Technologies Carry 630 FTIR, the samples of oil and biodiesel were scanned in the mid-infrared range 4000-400cm^{-1}.The FTIR spectrum for oil and biodiesel were interpreted [39] 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 Tables 2 & 3.

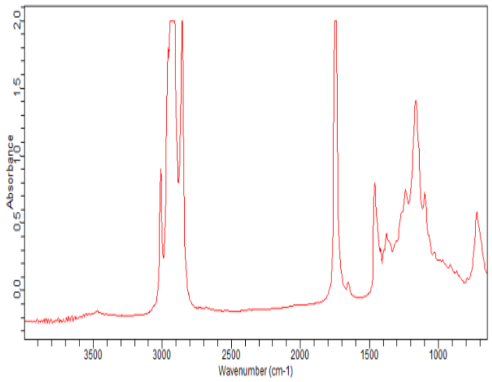

Fig. 1. FTIR Image of algae (*Spirogyra*) oil

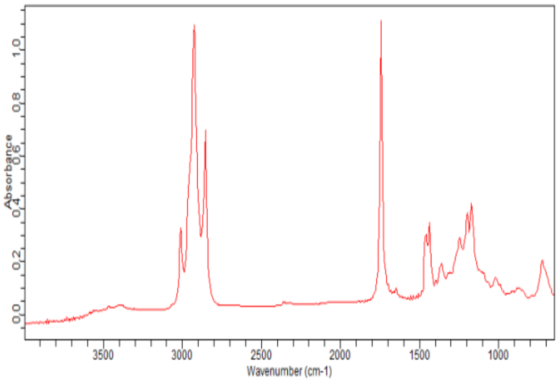

Fig. 2. FTIR image of algae (*Spirogyra*) biodiesel

Table 1. The physicochemical value (average) of algae biodiesel and its comparison with standard value of biodiesel [32,33]

S. No.	Physicochemical properties	Unit	Biodiesel from algal sps. Spirogyra (avg.)	Standard value of biodiesel	Protocol
1.	Saponification value	mg KOH/g	98	------	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	°C	167	130	ASTM D 664
5.	Acid value	mg KOH/g oil	0.57	0.50	ASTM D 664
6.	Density	Kg/m^3	0.88	0.88	ASTM D 56

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	2945-2912	Mehthylene -C-H asymmetric/symmetric stretch
3	2860-2852	.-C-H stretching, symmetric vibration: (-CH_2) mainly from lipids
4	1754-1739	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

4. CONCLUSION

According to the aforementioned study, spirogyra oil-based biodiesel is the ideal choice for a biofuel. The physicochemical characteristics, such as the density, flash point, iodine value, calorific value, acid value, and saponification value, determined in this study have a good degree of accuracy by industry standards. It should be highlighted that the solvent extraction process is a potential biodiesel production technology that can be generalized for commercial purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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