Production of Biodiesel from *Spirogyra elongata*, a Common Freshwater Green Algae with High Oil Content

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Abstract: The need for exploring nonfood low-cost sustainable sources for biodiesel production is ever increasing. Commercial and industrial algae cultivation has numerous uses in biodiesel production. This study explores *S. elongata* as a new algal feedstock for the production of biodiesel that does not compete with food production. The major fatty acids identified in *S. elongata* oil were oleic (30.5%), lauric (29.9%), myristic (17.0%), and palmitic (14.2%) acids. Transesterification to FAME was conducted using basic (KOH), acidic (HCl), and Zeolitic catalysts for assessment. The yields with acidic (54.6%) and zeolitic (72.7%) catalysts were unremarkable during initial screening. The highest biodiesel yield (99.9%) was achieved using KOH, which was obtained with the optimum reaction conditions of 1.0% catalyst, 60 °C, 4 h, and an oil-to-methanol volume ratio of 1:4. The resulting *S. elongata* oil methyl esters exhibited densities, CNs, and IVs, that were within the ranges specified in the American (ASTM D6751) and European (EN 14214) biodiesel standards, where applicable. In addition, the high SVs and the moderately high CPs and PPs were attributed to the presence of large quantities of short-chain and saturated FAME, respectively. Overall, the composition and properties of FAME prepared from *S. elongata* oil indicate that *S. elongata* is suitable as an alternative algal feedstock for the production of biodiesel.

Keywords: *Spirogyra elongata*; biodiesel; GCMS; oil; cetane number; fatty acid methyl esters

1. Introduction

Fossil fuel resources are being depleted at a high speed because of increasing population and rapid industrialization [1]. The interest in biofuels as a renewable energy alternative is growing fast because of their importance with regard to sustainability and climate change mitigation [2–5]. Biodiesel is derived from vegetables oils, animal fats, and other oleaginous sources, such as algae, and is comprised of long-chain mono alkyl esters of fatty acids [6]. On the international level, the demand for biodiesel is increasing because it can be used as a direct replacement, or blend component, for conventional petroleum diesel (petrodiesel) fuel. The advantages of biodiesel over petrodiesel include renewability, biodegradability, safe handling, lower greenhouse gas emissions, and superior lubricity [7]. The most common method for the production of biodiesel is the transesterification of...
triglycerides to yield fatty acid methyl esters (FAME) [8,9]. Transesterification can be catalyzed by numerous homogeneous and heterogeneous acid, base, and enzymatic methods. However, transesterification via acid catalysis is almost 4000 times slower than the base-catalyzed process [10,11]. One of the disadvantages of biodiesel, relative to petrodiesel, is the increased production costs, which is exacerbated when expensive commodity food crops are utilized [12–14]. Thus, the employment of low-cost nonedible lipid sources is the most effective way to reduce the cost of biodiesel production. Currently, sunflower and rapeseed oils are the most common feedstocks used for the production of biodiesel in Europe. Waste vegetable oils, soybean oil, and animal fats are the most prevalent in Canada and the USA, whereas soybean oil is the most common source of biodiesel in South America. Coconut, palm, and jatropha oils predominate in Asia, soybean and palm oils in Australia, and animal fats and waste vegetable oils are the most common feedstocks for biodiesel production in New Zealand [14].

Algae use carbon dioxide and light to grow in aquatic environments to produce biomass. Algae is classified into two types: microalgae and macroalgae. Microalgae are unicellular, small (measured in micrometers), and normally grow as a suspension in water. Macroalgae are large (measured in inches), multicellular, and can often be seen growing in ponds. There are a variety of ways in which macroalgae can grow. Seaweeds are the largest multicellular macroalgae. For example, the giant kelp plant can grow more than 100 feet long. Microalgae are known for rapid biomass production as compared to terrestrial crops, contain relatively high oil contents, and could serve as good sources for biodiesel production. The mass cultivation of algae can be performed using saline or wastewater on nonarable lands. Currently, the interest in algae to produce biofuels is growing. The three main components found in algae biomass are natural oils/lipids, proteins, and carbohydrates. Most of the natural oil is present in the form of triglycerides, which can be converted to biodiesel. Energy can be generated from microalgae in several ways in addition to biodiesel production. There are some algae species that are utilized to produce hydrogen gas under specialized conditions. Algae biomass can also be burned like wood or digested anaerobically for biogas production to generate heat/electricity. Terrestrial crops take a full season to grow and, on average, contain 5% oil, whereas the growth rates for microalgae are much faster [15]. Microalgae grow fast and produce high quantities of oil and generally double their size every 24 h. Some microalgae, during the peak of their growth phase, have doubling times of only 3.5 h [15]. Generally, microalgae contain between 20–50% oil, which can reach as high as 80% in some strains [16,17]. Photoautotrophic microalgae need carbon dioxide and light for growth. Heterotrophic algae can grow in darkness using organic carbon, such as acetate or glucose. The capital, production, and maintenance costs for heterotrophic microalgae are high, which renders them unsuitable for biodiesel production from an economic perspective [18]. The average algae oil yield per hectare is around 50,000 L, although there have been claims of higher yields of up to 100,000 L per hectare per year [19,20]. Algae are, thus, one of the most promising long-term sustainable sources of biomass and oils for fuel, food, feed, and other coproducts. In summary, the advantages of algae include: fast growth rates (they can be harvested daily); high biofuel yields (2000 to 5000 gallons of biofuels per acre per year can be easily produced); they consume CO₂ as they grow and release oxygen; they do not compete with agriculture (algae can be cultivated on wasteland by using sea, brackish, and wastewater); and they have multiple uses (fuel, feed, and food) [21,22].

The Spirogyra genus contains about 400 free-floating green algal species (division Chlorophyta) in freshwater environments around the world. Spirogyra are named for the beautiful helical or spiral arrangement of the chloroplasts that is characteristic of the genus. These filamentous charophyte green algae, from the order Zygnematales, contain chains of thin unbranched cylindrical cells. Spirogyra masses usually float near the surfaces of ponds and streams and, during photosynthesis, are buoyed by the release of oxygen bubbles. The present study reports the use of S. elongata as an easily cultivated and promising candidate for biodiesel production. S. elongata, used in the present study, is a fast-growing alga with
high oil content and is one of the most promising sources for the production of biodiesel because of its favorable fatty acid profile. The transesterification of the extracted oil to produce FAME was performed using acidic, basic, and zeolitic catalysts. The reaction variables, such as temperature, time, catalyst load, and oil-to-methanol volume ratio, were optimized to obtain the maximum biodiesel yield. Lastly, the fuel properties of the resulting FAME were determined and compared with international biodiesel standards, such as EN 14214 and ASTM D6751. Such fuel properties included density, saponification value (SV), iodine value (IV), cloud point (CP), pour point (PP), and the cetane number (CN).

2. Materials and Methods

2.1. Materials

All chemicals and reagents, including methanol, potassium hydroxide (KOH), sodium hydroxide (NaOH), hydrochloric acid (HCl), zeolite, dipotassium hydrogen phosphate (K₂HPO₄), potassium hydrogen phosphate (KH₂PO₄), and n-hexane were purchased from Merck (Darmstadt, Germany) and used as received.

2.2. Collection, Growth, and Oil Extraction of Algae

*S. elongata* was collected from a water pond situated on the campus of the University of Agriculture in Faisalabad, Pakistan. *S. elongata* was cultivated in a Volvox freshwater medium with a pH between 6 and 8. After growth, *S. elongata* was dried, ground into small particles, and soaked in n-hexane for 3 weeks. After soaking, the solution of n-hexane and oil was filtered, followed by the removal of n-hexane by reduced pressure rotary evaporation (Hydrion Scientific, Model RE-302, Vista, CA, USA) to yield *S. elongata* oil, which was filtered again to remove unwanted particles.

2.3. Transesterification

*S. elongata* oil was converted into biodiesel by catalytic transesterification in the presence of methanol. Transesterification was performed using acid, base, and zeolite catalysts. Acid-catalyzed transesterification was conducted at five concentrations of hydrochloric acid (HCl; 6.25, 12.5, 25, 50, and 100% by weight compared to oil weight), with a methanol-to-oil volume ratio of 6:1, at 60 °C for 4.5 h, with stirring at 100 rpm. Base-catalyzed transesterification was carried out using potassium hydroxide (KOH; 0.125, 0.25, 0.50, 0.75, and 1% by weight compared to oil weight), with a methanol-to-oil ratio of 3:1, at 60 °C for 1.5 h, with stirring at 100 rpm. Zeolite-catalyzed transesterification was done at five concentrations (0.5, 1, 2, 4, and 8% by weight) using a methanol-to-oil volume ratio of 5:1, a reaction temperature of 40 °C, a reaction time of 10 h, and a stirring speed of 100 rpm. The effect of the different reaction parameters on the biodiesel yield from *S. elongata*, including the reaction time (2 to 10 h), reaction temperature (30 to 70 °C), and the methanol-to-oil volume ratio (1:1 to 5:1), using a fixed KOH concentration, was also optimized. The crude product after transesterification was separated into phases and the top biodiesel-containing phase was washed with excess hot water (80 °C) to remove residual methanol, soap, and impurities. Later, after drying, the purified biodiesel was preserved as a transparent liquid for further study.

2.4. Physiochemical and Fuel Properties

The pH of all of the biodiesel samples was tested using a calibrated pH meter (pHep, HI98107, Hanna Instruments (M) Sdn Bhd, Malaysia). The density (g/mL) of the biodiesel samples was determined by weighing the mass of 1.0 mL of each sample. Specific gravity (SG) was measured using an SG bottle. The following formula was used to calculate SG by taking water as the standard:

\[
SG_{\text{true}} = \frac{\rho_{\text{sample}}}{\rho_{\text{H}_2\text{O}}}
\] (1)

where \(\rho_{\text{sample}}\) is the density of the biodiesel sample, and \(\rho_{\text{H}_2\text{O}}\) is the density of the water.
The iodine values (IVs), saponification values (SVs), and acid values (AVs), were determined using American Oil Chemists’ Society (AOCS) methods, Cd 1-25, Cd 3-25, and F 9a-44, respectively [11]. The cloud points (CPs), pour points (PPs), and cetane numbers (CNs) were measured according to American standards, ASTM D2500, ASTM D97, and ASTM D613, respectively [11]. The biodiesel yields were calculated by measuring the mass of the final product and dividing it by the theoretical maximum mass and is expressed as w/w % (weight/weight percentage).

Gas chromatographic mass spectrometric (GC-MS) analyses were performed using 0.1 µL of sample (with a split ratio of 50:1) on a Perkin Elmer Clarus 600 GC System using an Elite-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) with a maximum temperature range of up to 350 °C. The detection system was Perkin Elmer Clarus 600C MS (ionizing energy 70 eV, scan range 10–600 m/z). Helium used as a carrier gas was of high purity (99.9999%) and was run at a flow rate of 0.2 mL/min. The injection, transfer line, and ion source temperatures were 220, 200, and 200 °C, respectively. The oven temperature program began with a 10 min hold at 35 °C, followed by a ramp rate of 10 °C/min to 200 °C, which was held for 10 min. Unknown compounds were identified by comparison to reference standards, and by comparison of the spectra with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

2.5. Statistical Analysis
The data of the biodiesel yield, fuel quality parameters, and the characterization of the S. elongata oil of each sample were analyzed in triplicates and reported as mean ± SD.

3. Results and Discussion
3.1. Characterization of S. elongata Oil and Fatty Acid Profile of Biodiesel from S. elongata Oil

The S. elongata oil had an AV of 5.80 mg KOH/g, a density of 886.3 kg/L, an SV of 240 mg KOH/g, and an IV of 70 g I₂/100 g prior to transesterification. The GC-MS analysis indicated that the major fatty acids present in S. elongata oil were lauric (29.9%), myristic (17.0%), palmitic (14.2%), and oleic (30.5%) acids (Table 1). The fatty acids identified in minor quantities were caprylic, capric, palmitoleic, margaric, linoleic, stearic, gondoic, arachidic, erucic, behenic, tricosanoic, and lignoceric acids. Thus, the majority of the fatty acids identified in S. elongata oil were saturated (66.2%), with only 31.8% monounsaturated (palmitoleic, oleic, gondoic, and erucic), and 2.5% polyunsaturated (linoleic), fatty acids detected. In addition, a majority of the fatty acids (61.8%) contained 16 or fewer carbons in the fatty chain, with lauric, myristic, and palmitic representing the most prominent examples. The high SV and low IV observed for biodiesel prepared from S. elongata oil is directly attributed to the presence of relatively short-chain and saturated FAME, respectively. The relatively high (>0 °C) CP and CN of S. elongata oil biodiesel was due to the low level of polyunsaturated FAME, combined with the high amount of saturated FAME, such as methyl laurate (29.9%), methyl myristate (17.0%), and methyl palmitate (14.2%). The major fatty acids identified in S. elongate oil noted in a previous study [23] were as follows: oleic acid (33.3%), palmitic acid (25.2%), and linoleic acid (10.8%). Oleic acid (30.5%) and palmitic acid (14.2%) were present in major quantities in S. elongata oil used in the present study as well. The other major fatty acids found in the present study, including lauric acid (29.9%) and myristic acid (17.0%), were present in minor amounts (lauric acid, 1.1% and myristic acid, 6.4%) in the previous study [23]. The amount of the linoleic acid in the present study was 2.5% and in the previous study [23] it was 10.8%. The differences in fatty acid composition not only depends upon the environmental conditions but also on the method used for the extraction of oil from spirogyra.
Table 1. Fatty acid composition of S. elongata oil.

| No. | Fatty Acid       | % Amount |
|-----|------------------|----------|
| 1   | Caprylic acid    | 0.3      |
| 2   | Capric acid     | 0.3      |
| 3   | Lauric acid     | 29.9     |
| 5   | Myristic acid   | 17.0     |
| 7   | Palmitoleic acid| 0.1      |
| 8   | Palmitic acid   | 14.2     |
| 9   | Margaric acid   | 0.1      |
| 10  | Linoleic acid   | 2.5      |
| 11  | Oleic acid      | 30.5     |
| 13  | Stearic acid    | 3.0      |
| 14  | Gondoic acid    | 0.4      |
| 15  | Arachidic acid  | 0.3      |
| 16  | Erucic acid     | 0.8      |
| 17  | Behenic acid    | 0.2      |
| 18  | Tricosanoic acid| 0.1      |
| 19  | Lignoceric acid | 0.3      |

3.2. Screening of Different Catalysts for Biodiesel Production

Three catalysts of different natures were selected to evaluate the biodiesel yield using the previously optimized parameters, except for the concentration of catalysts. Table 2 depicts the screening of the different catalyst types (KOH, HCl, and Zeolite) for biodiesel production from S. elongata oil. The most effective catalyst was KOH, using a molar ratio of 3:1 at 60 °C for 1.5 h, with stirring at 100 rpm. Biodiesel yields using the other two catalysts, HCl utilizing a 6:1 molar ratio at 60 °C for 4.5 h, with stirring at 100 rpm, and Zeolite applying a 5:1 molar ratio at 40 °C for 10 h, with a stirring speed of 100 rpm, were significantly lower. The maximum yield of biodiesel using nonoptimized reaction conditions was 85.9% using 1% KOH. As a result, further optimization studies proceeded with KOH as the best catalyst biodiesel yield produced from S. elongata oil.

Table 2. Screening of different catalysts for biodiesel production from S. elongata oil.

| Catalyst Types | Concentration of Catalyst (wt.%) | Biodiesel Yield (%) |
|----------------|----------------------------------|---------------------|
| HCl            | 6.25 6.25 12.5 12.5 25 25 50 50 100 100 | 28.4 ± 0.1 38.0 ± 0.2 44.0 ± 0.4 52.4 ± 0.3 54.6 ± 0.8 |
| KOH            | 0.125 0.125 0.25 0.25 0.5 0.5 0.75 0.75 1.0 1.0 | 70.5 ± 0.9 72.0 ± 0.6 75.5 ± 0.4 82.5 ± 0.7 85.9 ± 0.3 |
| Zeolite        | 0.5 0.5 1 1 2 2 4 4 8 8 | 65.0 ± 0.8 69.5 ± 0.5 72.7 ± 0.7 67.6 ± 0.4 54.4 ± 0.3 |

3.3. Effect of Reaction Time, Temperature, and Oil-to-Methanol Ratio Using KOH Catalyst on Biodiesel Yield

The effects of the different reaction parameters, produced by varying the reaction times (2 to 10 h), reaction temperatures (30 to 70 °C), and oil-to-methanol volume ratios (1:1 to 1:5) for biodiesel production from S. elongata using 1% KOH catalyst at 100 rpm stirring, are given in Figure 1. The determination of an optimum time to produce biodiesel is essential, as it contributes to production costs on the pilot and commercial scales. The effect of
temperature on the biodiesel yield was investigated for reaction times (2–10 h). An oil-tomethanol molar ratio of 6:1, and 1% KOH by weight of oil, were used. Figure 1a shows the variation in biodiesel yields versus reaction times. The biodiesel yield increased with the increasing reaction time, from 2 h to 4 h. There was not any significant increase in the yield of biodiesel after 4 h (Figure 1a). The maximum biodiesel yield was obtained at 4 h. A further increase in the reaction time did not result in an improvement in the biodiesel yield, presumably caused by unwanted hydrolysis and other side reactions at extended reaction times [24]. The methyl ester yields produced at different reaction temperatures (30–70 °C) during transesterification, at a fixed oil-to-methanol molar ratio of 3:1, a reaction time of 4 h, and 1% KOH catalyst at 100 rpm, are presented in Figure 1b. The transesterification rate is strongly dependent on the reaction temperature and, hence, it greatly affects the biodiesel yield. The maximum biodiesel yield was attained at 60 °C (Figure 1b). At higher temperatures, the biodiesel yield decreased. Although higher temperatures increase the reaction rate, temperatures above the boiling point of methanol (>65 °C) resulted in a reduction in the biodiesel yield due to the loss of methanol caused by evaporation [25]. The effect of the oil-to-methanol proportion on the methyl ester yield was studied by varying the oil-to-methanol molar ratio from 1:1 to 1.5, while maintaining the temperature (60 °C), potassium hydroxide concentration (1%), and 4 h time, at 100 rpm. Five molar ratios for oil-to-methanol were examined (1:1, 1.2, 1.3, 1.4, and 1:5). The optimized oil-to-methanol volume ratio was 1.4 (Figure 1c). When the oil-to-methanol molar ratio was increased above 1.4, the methyl ester concentration decreased. A further increase of methanol in the reaction mixture did not increase the biodiesel yield significantly. Moreover, it was found that, when the molar ratio of oil-to-methanol was increased above, or decreased below, the optimum level, there was no significant increase in the biodiesel production. Rather, it contributed to the increased formation of glycerol and emulsion [24].

![Figure 1](image_url)

**Figure 1.** Effect of different reaction parameters on biodiesel production from *S. elongata* oil using 1% KOH catalyst: (a) varying reaction time at a fixed molar ratio of 3:1 with a reaction temperature of 60 °C; (b) varying reaction temperature at a fixed molar ratio of 3:1 with a reaction time of 4 h; (c) varying oil-to-methanol molar ratio at a fixed reaction time of 4 h at a temperature of 60 °C.

### 3.4. Evaluation of Fuel Quality Parameters

Biodiesel with a high density may produce incomplete combustion, while low-density fuel could be highly volatile [10,26,27]. The densities of *S. elongata* biodiesel samples were from 0.81–0.89 kg/L (Table 3). The minimum density was 0.81 kg/L for biodiesel...
produced with 0.25% KOH. Most samples had densities within the range specified in EN 14214 (860–900 kg/m³ at 15 °C). The American biodiesel standard, ASTM D6751, does not contain a density specification. Biodiesel produced using the optimized reaction conditions had a density (0.89 kg/L) that was within the limit prescribed in EN 14214.

### Table 3. Fuel properties of *S. elongata* oil biodiesel produced using different catalysts.

| Type of Catalysts | Concentration of Catalyst (%) | Density (kg/L) | SV (mg KOH/g) | IV (g I₂/100 g) | CP (°C) | PP (°C) | CN |
|-------------------|-------------------------------|----------------|---------------|-----------------|--------|--------|-----|
| **HCl**           |                               |                |               |                 |        |        |     |
| 6.25              | 0.89 ± 0.007                  | 237 ± 5.0      | 59 ± 0.54     | 4.1 ± 0.082     | -4.6 ± 0.023 | 57.8  |
| 12.5              | 0.86 ± 0.001                  | 232 ± 2.9      | 58 ± 0.55     | 5.0 ± 0.093     | -4.1 ± 0.036 | 58.6  |
| 25                | 0.86 ± 0.006                  | 241 ± 3.9      | 59 ± 0.87     | 4.3 ± 0.084     | -3.9 ± 0.016 | 57.6  |
| 50                | 0.86 ± 0.003                  | 250 ± 3.6      | 57 ± 0.40     | 4.1 ± 0.082     | -4.6 ± 0.023 | 58.8  |
| 100               | 0.89 ± 0.007                  | 237 ± 5.0      | 56 ± 0.74     | 5.0 ± 0.093     | -4.1 ± 0.036 | 59.0  |
| **KOH**           |                               |                |               |                 |        |        |     |
| 0.125             | 0.86 ± 0.006                  | 239 ± 3.3      | 59 ± 0.66     | 4.9 ± 0.089     | -3.8 ± 0.049 | 58.6  |
| 0.25              | 0.81 ± 0.009                  | 239 ± 3.3      | 57 ± 0.58     | 4.6 ± 0.090     | -4.4 ± 0.039 | 57.9  |
| 0.5               | 0.89 ± 0.008                  | 241 ± 3.9      | 60 ± 0.43     | 3.7 ± 0.060     | -4.8 ± 0.042 | 58.6  |
| 0.75              | 0.89 ± 0.007                  | 243 ± 3.9      | 58 ± 0.29     | 3.9 ± 0.063     | -4.8 ± 0.039 | 57.2  |
| 1.00              | 0.89 ± 0.008                  | 251 ± 3.1      | 58 ± 0.14     | 4.2 ± 0.082     | -4.9 ± 0.023 | 56.9  |
| **Zeolite**       |                               |                |               |                 |        |        |     |
| 0.5               | 0.86 ± 0.001                  | 237 ± 5.0      | 59 ± 0.79     | 5.0 ± 0.093     | -4.1 ± 0.036 | 57.9  |
| 1                 | 0.86 ± 0.006                  | 232 ± 2.9      | 57 ± 0.55     | 4.3 ± 0.084     | -3.9 ± 0.016 | 58.6  |
| 2                 | 0.86 ± 0.003                  | 241 ± 3.9      | 56 ± 0.88     | 4.1 ± 0.082     | -4.6 ± 0.023 | 59.7  |
| 4                 | 0.86 ± 0.001                  | 250 ± 3.6      | 60 ± 0.37     | 5.0 ± 0.093     | -4.1 ± 0.036 | 60.7  |
| 8                 | 0.86 ± 0.006                  | 239 ± 3.3      | 59 ± 0.54     | 3.7 ± 0.060     | -4.8 ± 0.042 | 57.0  |

The saponification value (SV) provides information about the chain length and, thus, the average molecular mass of the biodiesel. Higher SVs indicate a lower average chain length and molecular weight, and vice versa. The SVs of *S. elongata* biodiesel were from 232–258 mg KOH/g (Table 3). The high SV of *S. elongata* FAME was caused by the presence of high percentages of methyl laurate (29.9%) and methyl myristate (17.0%, Section 3.1). For comparison, the average SVs of biodiesel produced from palm and soybean oils are typically 230–254 and 187–195 mg KOH/g, respectively. Neither EN 14214 nor ASTM D6751 contain specifications for the SV.

The iodine value (IV) is an indication of the degree of unsaturation. Fuels with higher IVs have poor oxidative stability and low CNs. The IVs (Table 3) ranged from 56 to 60 g I₂/100 g for *S. elongata* oil biodiesel. There is no standard value set for the IV in ASTM D6751. However, EN 14214 specifies that the IV should be at, or below, 120 g I₂/100 g. As seen in Table 3, all samples were within the limit specified in EN 14214. The low IV of *S. elongata* oil FAME was due to the presence of low amounts of unsaturated (methyl oleate, 30.5%; methyl erucate, 0.8%), and especially polyunsaturated (methyl linoleate, 2.5%), FAME.

The CPs and PPs of *S. elongata* biodiesel are given in Table 3. Although the yields were different using the various catalysts, their CPs and PPs were approximately similar. Biodiesel has higher CPs and PPs than petrodiesel because of the higher melting points of FAME compared to diesel-range hydrocarbons. The CPs and PPs of biodiesel depend on the feedstock chemical composition from which they were obtained, as longer chain lengths and lower degrees of unsaturation increase the melting point and, thus, increase the CPs and PPs [28,29]. Neither ASTM D6751 nor EN 14214 contain limits on the CP and the PP, although guidance is provided.

The cetane number (CN) is a dimensionless indicator of the ignition quality, with a higher CN indicating a shorter ignition delay within the combustion chamber [30]. Increasing the degree of saturation and/or the chain length of FAME will increase the CN. The optimal CN range is between 41 and 56, but generally must not be higher than around 65 [31]. The CNs of *S. elongata* biodiesel are given in Table 3. Biodiesel is, of course, composed of FAME, unlike petrodiesel, which contains hundreds of hydrocarbons that boil within a specific range [32]. The minimum specified CNs of biodiesels are 51 in EN 14214,
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and 47 in ASTM D6751. The minimum CNs for petrodiesel are 51 in EN 590, and 40 in ASTM D975 [32,33]. Biodiesels have higher CNs than the parent vegetable oils [28,34–36]. Biodiesel prepared from S. elongata oil provided CNs that were within the ranges specified in ASTM D6751 and EN 14214, which can be explained by the presence of low amounts of polyunsaturated FAME, and the high amount of saturated FAME (Section 3.1), both of which increase the CN.

In a previous study, the optimum biodiesel production from Spirogyra yielded a density of 0.86 kg/L, which was within the range specified in EN 14214. This value was comparable to a previous study and was within the range specified in EN 14214. However, the CP and PP noted in a previous study of S. elongata biodiesel were 4 °C and 9 °C, respectively. A biodiesel with lower CPs and PPs was produced in the present study (Table 3), which exhibited better performance at low temperatures [37].

3.5. The Pros and Cons of Biodiesel Production from Spirogyra elongata

The production of biodiesel from algae is easy and economical. Algae-based biofuel is an essentially carbon-neutral energy source that has been getting a lot of attention lately. The biodiesel produced from S. elongata oil in the present study demonstrated much better cold flow properties, as represented by its CPs and PPs, as compared to a previous study [37]. Biodiesel produced from S. elongata produced under optimized reaction conditions had a density of 0.89 kg/L, which was within the range prescribed in EN 14214. The relatively high SV indicates that S. elongata biodiesel is of comparatively high volatility and low density compared to biodiesel fuels prepared from other oil sources [11,27]. The presence of low amounts of polyunsaturated FAME, and high amounts of saturated FAME, as indicated by an IV of 59 g I$_2$/100 g, not only increased the CN, but is reportedly also helpful in reducing engine deposits. The presence of high amounts of saturated fatty acids, such as lauric acid in S. elongata oil, renders it a better feedstock than has been previously reported [23]. However, there remain challenges associated with the production of biodiesel from algae oil, such as the availability of nutrients, temperature conditions, and other relevant production costs.

4. Conclusions

This study demonstrates that S. elongata is a promising new nonfood algal feedstock for the production of biodiesel that does not compete with food production. The major fatty acids identified in S. elongata oil were oleic (30.5%), lauric (29.9%), myristic (17.0%), and palmitic (14.2%) acids. Transesterification to FAME was conducted using basic (KOH), acidic (HCl), and zeolitic catalysis. The highest biodiesel yield (99.9%) was achieved using KOH, which was obtained with the optimum reaction conditions of 1.0% catalyst, 60 °C, 4 h, and an oil-to-methanol ratio of 1:4. Yields with acidic (54.6%) and Zeolitic (72.7%) catalysts were unremarkable. The resulting S. elongata oil methyl esters exhibited densities, CNs, and IVs that were within the ranges specified in the American (ASTM D6751) and European (EN 14214) biodiesel standards, where applicable. In addition, the high SVs, and moderately high CPs and PPs were attributed to the presence of large quantities of short-chain and saturated FAME, respectively. Overall, the composition and properties of FAME prepared from S. elongata oil indicate that S. elongata is suitable as an alternative algal feedstock for the production of biodiesel.

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