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Spirogyra Oil-Based Biodiesel: Response Surface Optimization of Chemical and Enzymatic Transesterification and Exhaust Emission Behavior

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Abstract: Algae are emerging as a major and reliable source of renewable biodiesel that could meet the energy requirements of the world. Like plants, algae produce and store oils in their cells. Algal samples were collected from Gujrat District, Pakistan, their oil content was analyzed, and the best oil producing alga was identified as *Spirogyra crassa*. After collecting sample, oil was extracted using the Soxhlet extraction method. *Spirogyra* oil was characterized physico-chemically for the evaluation of its quality. Acid value, density, saponification value, peroxide value, as well as viscosity and iodine values were determined and their values were 16.67 ± 3.53 mg KOH/g, 0.859 ± 0.050 g/cm^3, 165.33 ± 13.20 mg KOH/g, 4.633 ± 0.252 meq/kg, 5.63 ± 0.833 mm^2/mL, and 117.67 ± 13.01 mg I_2/g, respectively. Chemical as well as enzymatic transesterification protocols were employed for biodiesel production using $NaOCH_3$ and NOVOZYME-435, respectively. Different reactions parameters involved in transesterification were optimized by the response surface methodology. The optimized yield of biodiesel (77.3 ± 1.27%) by the chemical transesterification of algal oil (spirogyra) was observed by carrying out the reaction for 90 minutes at a reaction temperature of 45 °C using 1.13% catalyst ($NaOCH_3$) concentration and 6:1 methanol:oil. Meanwhile, for enzymatic transesterification, the optimized yield (93.2 ± 1.27%) was obtained by conducting the reaction for 42.5 h at the temperature of 35 °C using 1% enzyme concentration and 4.5:1 methanol:oil. Fuel properties, including flash point, pour point, cloud point, fire point, kinematic viscosity, and density, were determined and their values are 125.67 ± 2.11 °C, −19.67 ± 0.8 °C, −13 ± 1 °C, 138.667 ± 2.52 °C, 5.87 ± 2.20 mm^2/mL, and 0.85 6 ± 0.03 g/cm^3, respectively. Fourier transfer infrared spectroscopic (FTIR) and Gas chromatography with flame ionization detector (GC-FID) analysis were performed for the monitoring of the transesterification process and fatty acid methyl acid (FAME) profiling, respectively.

Keywords: Algal oil; biodiesel; process optimization; catalyst and biocatalyst; transesterification

1. Introduction

Energy is a vital necessity for growth and economic development in every sector of life, such as transportation, industrial unit and agriculture [1]. The requirement of energy is increasing day by day owing to the expansion of the population and spread of industries throughout the world to meet the requirements of an expanding human pollution. The world largely depends on fossil fuels which are nonrenewable energy resources, and they are depleting [2]. Among biofuels, biodiesel has emerged as an alternate renewable energy resource which is nontoxic and environment friendly [3]. Among biofuels, biodiesel has emerged as an alternate renewable energy resource which is nontoxic and environmentally friendly. Biofuels are obtained from natural resources. They contain a lesser amount of Sulphur, and as such lead to a very small release of oxides of Sulphur [4,5]. Based upon the feedstock/source and technology used for biofuel production, liquid biofuels can be categorized into three generations, namely first generation, second generation, and third generation biofuels [6]. First generation biofuels correspond to those which are produced from crops and edible oils. However, utilizing food crops as a source of biofuel presents some issues that limit the use of food crops in biofuel production. For instance, as it reduces the land available for agricultural purposes, this will lead to a shortage of food all over the world, in turn raising the prices of food. Deforestation may also arise in this situation, which may cause the destruction of habitats of wildlife and an ecological imbalance [7]. Second generation biofuels are produced from waste vegetable oils, non-edible plant seeds oil, and animal fats [8]. Second generation biofuel has certain advantages over the first-generation biofuels, e.g., non-edible oils are used for the production second generation biofuel which eliminates the competition for land used for food and feedstock because it is not used as a food material. Byproducts obtained are very useful because these can be utilized for heat and power generation [9]. Although second generation biofuels has solved the problems related to first generation biofuels, there are also certain limitations related to second generation biofuels, e.g., second generation biofuels have poor performance at lower temperatures. Furthermore, a large amount of saturated fatty acids is present in animal fats which make the process of transesterification difficult for biodiesel production. This difficulty has led to the search for third generation biofuel [10]. Algae can also serve as a source of bio fuel and algal oil based biodiesel is the third generation biofuel [11]. Microalgae are the organisms capable of photosynthesis and give the best yield of biodiesel [12]. Algae are the best source of biodiesel, because of their biodegradability, non-toxic nature, rapid growth, and such growth being independent of fresh water supply [1]. The algae are economically viable oil sources because they take less amount of water and energy while the oil productivity of algae is higher than other crops [13]. The transesterification of plant seeds or algal oils is the most widely adopted method to produce biodiesel at the micro as well as macro level. The transesterification process brings the viscosity of oil to a lower level. The transesterification process is collectively affected by the molar ratio, type of alcohol, temperature, reaction time, nature and amount of catalysts, reaction mode, and alcohol type. Enzymatic transesterification yields high purity biodiesel and glycerol. This process involves zero soap production and is also environment friendly. Lipases are frequently used for the transesterification of vegetable oils and fats from various sources [14]. Lipase enzyme has emerged as a most important industrial enzyme due to its vast applications. The lipase mediated transesterification process provides an easy approach to remove glycerol from the reaction mixture to bring equilibrium to the alkyl ester regime. Lipase assisted reactions are usually environment friendly and have limited space for side products. Lipase extracted from various sources and even microorganisms are immobilized on a suitable support for enhanced and prolonged usage [15]. Extracellular lipases are more accepted and need downstream extraction followed by immobilization on some feasible support for their repeated use. This approach develops sustainability in the transesterification process and helps to reduce the cost of the process. The current work was designed to optimize the process of lipase catalyzed transesterification of extracted *Spirogyra* oil as a feedstock and to study of the exhaust emission behavior.

2. Results and Discussion

2.1. Characterization of Spirogyra Oil

The physico-chemical properties including acid value, density, iodine value, viscosity, saponification value, and peroxide value of *Spirogyra* oil, as calculated by the American Oil Chemists Society (AOCS) methods, are given in Table 1.

Table 1. Physico-chemical properties of *Spirogyra* oil.

Sr. No.	Property	Value
1	Acid value before acid transesterification	16.67 ± 3.53 mg KOH/g
2	Acid value after acid transesterification	1.23 ± 0.53 mg KOH/g
3	Iodine value	117.67 ± 13.01 g I_2/100g
4	Saponification value	165.33 ± 13.20 mg KOH/g
5	Density	0.86 ± 0.05 g/cm^3
6	Peroxide value	4.63 ± 0.25 meq/kg

Acid value describes the presence of free fatty acids in oil. The observed acid value before acid transesterification of the algal oil (*Spirogyra* oil) under study was 16.67 ± 3.53 mg KOH/g. The observed value is comparable to 12.21 mgKOH/g of oil that was reported by Bhraatheraja et al. [16]. For base catalyzed biodiesel production, oil was subjected to acid esterification as a pre-step. After this step, the acid value was reduced from 16.17 ± 3.53 to 1.23 ± 0.53 mg KOH/g.

Iodine value describes the extent of unsaturation in oils [17]. The observed iodine value of the *Spirogyra* oil was 117.67 ± 13.01 mg I_2/g. The saponification value is "the number of grams of NaOH required to completely saponify all the triglyceride esters in one gram of a particular oil" [18]. The saponification value of the spirogyra oil was found to be 165.33 ± 13.20 mg KOH/g, which was slightly lesser than the 173 mg KOH/g reported by Stanelay et al. [18].

"Density is a weight of unit volume of oil" [19]. The density of the *Spirogyra* oil under study was 0.86 ± 0.050 g/cm^3, which is comparable to 0.883 g/cm^3 as reported by Phathak et al. [20].

The extent to which an oil sample undergoes primary oxidation can be defined by its peroxide value. The observed peroxide value of algal oil (*Spirogyra*) in our study was 4.63 ± 0.25 meq/kg that is comparable to 4.26 meq/kg revealed by Ojo-Awo, for the same algal oil [21]. According to International Olive Oil Council (IOOC) standards, a good oil should have less than 10 meq/kg peroxide value.

2.2. Biodiesel Production and Optimization

2.2.1. Summary Statistics of Selected Model

The biodiesel yield data acquired by chemical and enzymatic transesterification of *Spirogyra* oil was examined to select the appropriate statistical models. Selected response surface models were utilized for the optimization of different reaction conditions to get the maximum yield of biodiesel through chemical and enzymatic transesterification of algal oil. Based on the best fitted experimental data, (i.e., model significance, R^2 value, adjusted R^2 value, and lack of fitness test) quadratic models were selected out of linear, cubic, and 2F1 and quadratic models. The summary statistics of selected quadratic models for both chemical and enzymatic transesterification of the oil under study to biodiesel are presented below in Table 2.

Table 2. The Summary statistics of selected model.

Models	Catalyst	Selected Models	Model Significant (*p*-values)	R-Squared	Adjust R-Squared	Lack of Fit
a	$NaOCH_3$	Quadratic	(<0.0001)	0.9933	0.9871	0.0843
b	NOVOZYME-435	Quadratic	(<0.0001)	0.9954	0.99911	0.6693

In model a, for the chemical transesterification of sample oil, *p* value, R^2 value, and adjusted R^2 value were <0.0001, 0.9933, and 0.987. However, in the case of model b for enzyme catalyzed biodiesel production, these values are <0.0001, 0.9954, and 0.9991, respectively. These summary statistics illustrate the validity of quadratic models for the chemical and enzymatic routes of biodiesel production.

Predicted vs actual graphs and normality plots for both models also helped to validate the quadratic model selection (Figure 1). The linear distribution of data and absence of outliers in the normal plot of residues for both models predict the accuracy of the quadratic model. The predicted values of biodiesel yield were also in agreement with the actual/calculated values of biodiesel yield as illustrated by the normal distribution of data in predicted vs actual plots of both models.

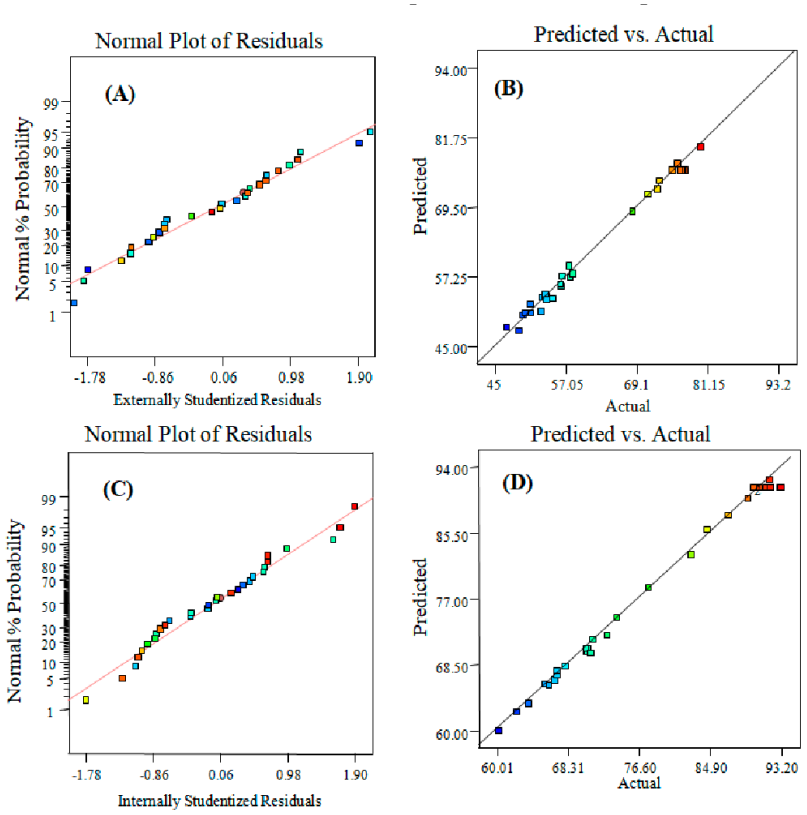

Figure 1. (**A**) and (**C**) showing the normality plots of biodiesel produced by chemical and enzymatic transesterification, Plot (**B**) and (**D**) showing the predicted versus actual plot for biodiesel production.

2.2.2. Optimized Reaction Parameters and Optimum Yield

The optimum yield of biodiesel by the chemical transesterification of algal oil (*Spirogyra*) was achieved by carrying out the reaction for 90 min at reaction temperature of 45 °C using 1.13% catalyst ($NaOCH_3$) concentration and a 6:1 methanol to oil molar ratio (Table 3). Renita et al. [22] also reported the optimization of different reaction parameters involved in algal oil-based biodiesel production using the response surface methodology. They revealed that the maximum yield of biodiesel was obtained when the reaction was performed at 60 °C using 0.3% by wt. catalyst (NaOH) and a 5:1 alcohol to oil molar ratio for 90 min [22].

Table 3. Table showing the optimum conditions for biodiesel production.

Model	Catalysts	Catalyst Conc.%	Methanol:Oil	Reaction Time (Min/Hrs)	Reaction Temp °C	Biodiesel Yield (%)
a	$NaOCH_3$	1.13	6:1	95 min	45	77.3 ± 1.27
b	NOVOZYME-435	1	4.5:1	42.5 h	35	93.2 ± 1.06

In the case of enzymatic biodiesel production, the optimum yield was obtained by conducting the reaction for 42.5 h at the temperature of 35 °C using 1% enzyme (NOVOZYME-435) concentration and 4.5:1 methanol to oil molar ratio.

2.2.3. Analysis of Variance (ANOVA)

The impacts of various reaction parameters on the percentage biodiesel yield in the form of linear terms, first order interactions, and quadratic terms are described by the ANOVA in Table 4. The terms (a) and (b) represent the quadratic model for $NaOCH_3$ and NOVOZYME-435 catalyzed transesterification of *Spirogyra* oil, respectively. In Table 4, the terms having a p value < 0.05 were statistically significant. The linear terms A (catalyst concentration), B (methanol:oil ratio), and C (reaction temperature) were found to be significant for both models, while D (reaction time) was significant for model (a) only.

Table 4. Analysis of variance (ANOVA) for chemical [(a)] and enzymatic [(b)] transesterification of *Spirogyra* oil.

Source	Df	SS(MS) [a]	SS(MS) [b]	*f* value (*p* value) [a]	*f* value (*p* value) [b]
Model	14	3633.03(259.5023)	3648.775(260.63)	160.0411 (<0.0001)	232.81(<0.0001)
A-Enzyme Concentration	1	8.268(8.268)	64.22(64.22)	5.099(0.0393)	57.37(<0.0001
B-methanol/oil	1	10.125(10.125)	20.06(20.056)	6.244(0.0246)	17.91(0.0007)
C-Reaction temp.	1	42.32(42.32)	48.02(48.02)	26.099(0.0001)	42.89(<0.0001)
D-reaction time	1	38.427(38.427)	3.83(3.83)	23.698(0.0002)	3.42(0.0843)
AB	1	3.0625(3.062)	5.18(5.18)	1.888(0.1895)	4.62(0.0843)
AC	1	28.09(28.09)	22.33(22.33)	17.323(0.0008)	19.94(0.0005)
AD	1	0.2025(0.202)	28.36(28.36)	0.124(0.7287)	25.33(0.0001)
BC	1	1.96(1.96)	8.56(8.56)	1.208(0.2889)	7.64(0.0145)
BD	1	0.9025(0.9025)	0.031(0.031)	0.556(0.4672)	0.027(0.8708)
CD	1	0.01(0.01)	0.005(0.005)	0.0061(0.9384)	0.005(0.9444)
A^2	1	795.216(795.216)	565.09(565.09)	490.428(<0.0001)	504.78(<0.0001)
B^2	1	17.103(17.103)	15.79(15.79)	10.548(0.0054)	14.101(0.019)
C^2	1	84.749(84.749)	125.81(125.81)	52.267(<0.0001)	112.383(<0.0001)
D^2	1	17.930(17.93)	0.88(0.88)	11.058(0.0046)	0.782(0.3902)
Residual	15	24.322(1.62)	16.7923(1.119)		
Lack of fit	10	21.368(2.14)	10.1173(1.011)	3.62(0.0843)	0.76(0.6693)
Pure error	5	2.953(0.59)	6.675(1.335)		
core total	29	3657.355	3665.567		

[a] Statistical model for chemical transesterification, [b] Statistical model for enzymatic transesterification.

For model (a), among the first order interaction terms, only AC (catalyst concentration × reaction time) was revealed to be significant with the p value 0.0008, while all other first order terms, i.e., AB, AD, BC, BD, and CD, were found to be insignificant with the p values greater than 0.05, i.e., 0.189, 0.728, 0.288, 0.467, and 0.938, respectively. On the other hand, for model (b), three first order interaction terms, i.e., AC, AD, and BC with p-values 0.005, 0.001, and 0.0145 were depicted to have significant effects on the model response (p-value < 0.05).

All the quadratic terms showed a significant influence on the $NaOCH_3$ catalyzed transesterification (Model (a)) with p values < 0.05. Meanwhile, in the case of enzyme catalyzed transesterification (Model (b)), the terms A^2, B^2, and C^2 have a significant quadratic effect with p values 0.0001, 0.019, and 0.0001, respectively. However, D^2 was insignificant with p value 0.3902.

2.2.4. Response Surface Plots for Chemical Transesterification

The 3D response surface plots are used to determine the process conditions that produce a desirable/maximum response (i.e., biodiesel yield resulted from the chemical transesterification of *Spirogyra* oil). The 3D plot for the only significant first order interaction term (A × C) is presented

as Figure 2. The 3D plot shows that the optimum values for reaction temperature and catalyst concentration are 45 °C and 1.13%, respectively. The steep curves at the edges inferred that varying the reaction temperature or catalyst concentration above and below the optimum point can significantly reduce the biodiesel yield (Figure 2).

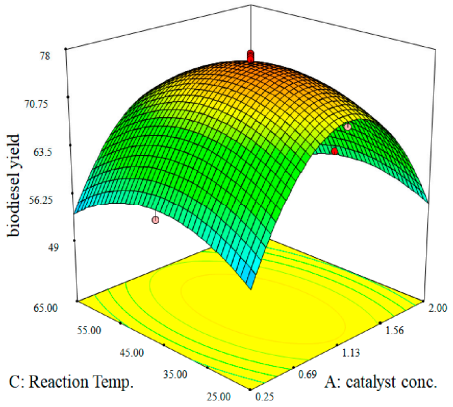

Figure 2. Surface response plot of first order interaction term showing significant impact on yield of biodiesel produced by chemical transesterification.

2.2.5. Response Surface Plots for Enzymatic Transesterification of Algal Oil

The impact of interaction terms on the yield of biodiesel obtained by the NOVOZYME-435 catalyzed transesterification of algal oil is illustrated by 3D response surface plots (Figure 3). It can be observed from plots that the optimal response (biodiesel yield%) was achieved by using 1% enzyme concentration at 35 °C for 42.5 h with a 4.5:1 methanol to oil molar ratio. However, above and below these levels, a decline in the optimal response was observed.

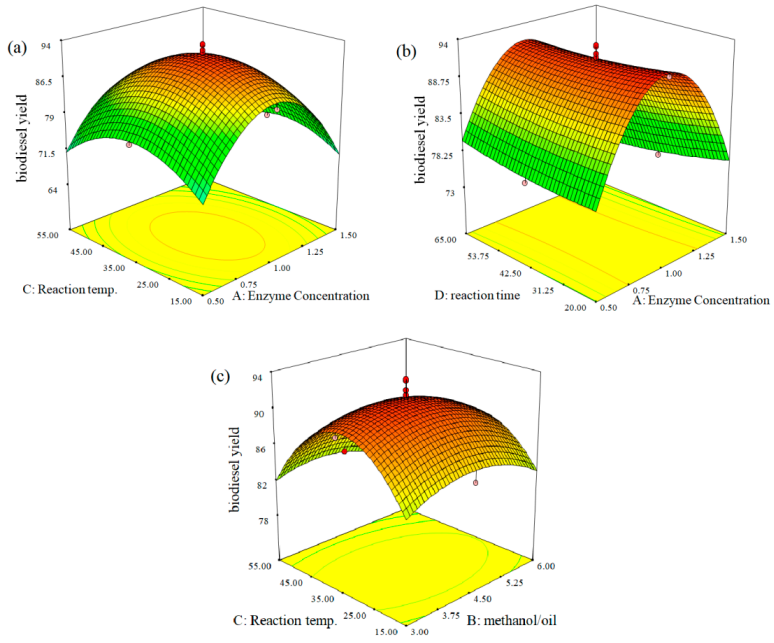

Figure 3. Response surface plots of first order interaction terms showing significant impact on the yield of biodiesel produced by enzymatic transesterification. Figure 3(**a**) = (A × C), Figure 3(**b**) = (A × D), Figure 3(**c**) = (B × C).

2.3. Fuel Properties of Resultant Biodiesel

The fuel properties of synthesized biodiesel; which were calculated by the American Society for Testing and Materials (ASTM) standard methods, are given in Table 5. The kinematics viscosity of the *Spirogyra* oil-based biodiesel was depicted to be 5.87 ± 2.20 mm^2/mL, which was slightly higher than 5.52 mm^2/mL reported by Ashokkumar et al. [23]. Biodiesel exhibits a higher kinematic viscosity than that of conventional diesel [23]. The kinematic viscosity of biodiesel increases due to high polarity in biodiesel as compared to petro-diesel [24]. According to international standard ASTM 6751, the value of kinematic viscosity ranges from 4 to 6 mm^2/mL. Our reported value also lies in this range. The density of synthesized biodiesel was found to be 0.856 ± 0.03 g/cm^3. The observed value was closer to 0.853 g/cm^3 that was described by Ashokkumar et al. [23]. In another previous study, the density for the algal oil is reported to be 0.866 g/cm^3, which is also comparable with the value observed in current study. Biodiesel also possesses a higher density than conventional diesel, and that is why it produces a flame with less smoke. The density reported in this study is comparable with the value of 0.864 g/cm^3 according to ASTM D 6751 standard. The temperature at which the vapors over the fuel become flammable is known as flash point [25]. The observed value of flash point of *Spirogyra* oil-based biodiesel was 125.67 ± 2.11 °C, that was comparable to 140 °C as reported by Ashokkumar et al. [23], whereas it is slightly lower than 145 °C as revealed by Ahmad et al. [26]. The flash point is related to the safety of an engine. If the value of the flash point is higher than is considered safe to use, it becomes easier to store the biodiesel [24]. The flash point in our study falls within the range of 100–170 °C as described in ASTM D 6751. Pour point is the temperature at which the quantity of wax out of solution is adequate to gel the fuel [27]. In this study, the pour point of *Spirogyra* oil-based biodiesel was found to be −19.67 ± 0.8 °C, which was comparable to −19 °C as reported by Ashokkumar et al. [23]. However, the pour point value reported in another study was −10 °C, which is different than our observed value [28]. Fire point is the lowest fuel temperature at which the diffusion flame is sustained above the fuel pool without any external heat supply. The fire point of the sample oil-based biodiesel was depicted to be 138.66 ± 2.52 °C. According to international standards, fire point should range from 120 to 180 °C and our observed value falls in this range, owning to the good fuel properties biodiesel is getting attention as a renewable fuel [29]. The cloud point (CP) is referred to as the temperature at which crystals starts to form in the fuel [30]. In our study, the value of cloud point is observed to be −13.00 ± 1 °C. Cloud point affects the starting of a passenger car and heavy-duty engine. An engine shows good performance when the cloud point is around −20 °C. The above mentioned fuel properties have a large impact on the combustion of biodiesel and efficiency of the engine. Viscosity affects the starting of the engine. If the fuel is viscose, then there will be an incomplete combustion of fuel, as black smoke and incomplete combustion cause the lowering of engine power. Pour point and cloud point determine the lowest operating temperature, below these temperatures fuel become useless. Flash point is related to safety of fuel, and if the value of flash point is higher, then it is easy to use and easy to store at elevated temperatures.

Table 5. The fuel properties of *Spirogyra* oil-based biodiesel.

Sr. No.	Property	Value
1	Kinematic viscosity	5.87 ± 2.20 mm^2/mL
2	Density	0.85 6 ± 0.03 g/cm^3
3	Flash Point	125.67 ± 2.11 °C
4	Pour Point	−19.67 ± 0.8 °C
5	Fire Point	138.66 ± 2.52 °C
6	Cloud Point	−13.00 ± 1 °C

2.4. Characterization of Spirogyra Oil-based Biodiesel

For monitoring the progress of the transesterification reaction of algal oils, Fourier transform infra-red (FTIR) spectroscopic analysis was employed. The presence of IR bands in the region of

1425–1447 cm^{-1} for (methyl) CH_3 asymmetric bending and 1170–1200 cm^{-1} for (methoxy) O-CH_3 stretching in the FTIR spectrum of biodiesel clearly revealed the conversion of *Spirogyra oil* in to biodiesel by the transesterification process, as these bands were absent in the IR spectrum of *Spirogyra oil* (Figure 4).

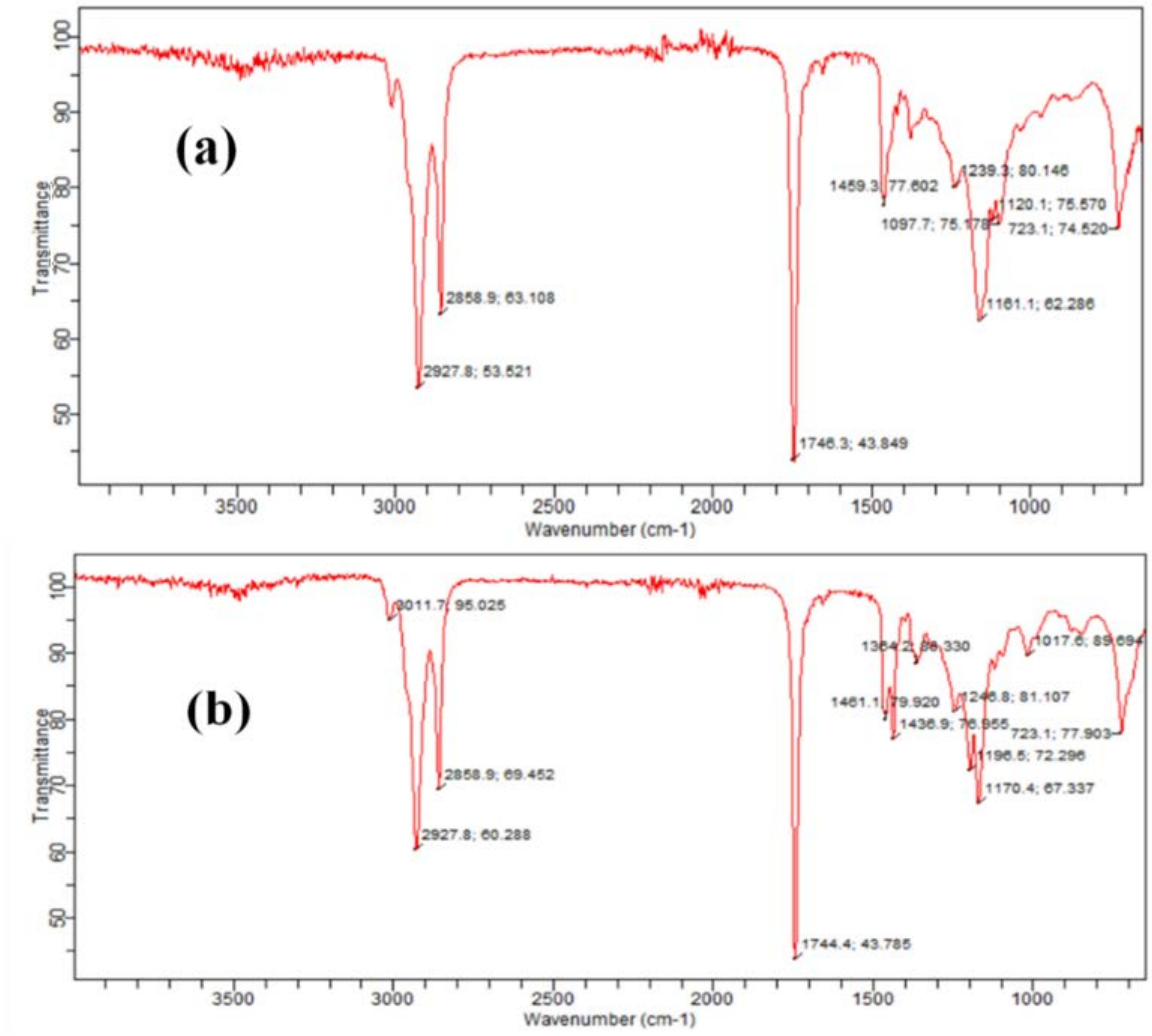

Figure 4. FTIR Spectrum (**a**) *Spirogyra* oil (**b**) *Spirogyra* oil-based biodiesel.

Gas chromatography with flame ionization detector (GC-FID) analysis was conducted to establish the compositional profile of synthesized biodiesel. Based on GC-FID results, it was found that the *Spirogyra* oil based biodiesel consisted of palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1), linoliec acid (C18:2), and arachidic acid (C20:0). In a previous study, Prakash et al. revealed the presence of stearic acid (C18:0), myristic acid (C14:0), linoleic acid (C18:2), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), oleic acid (C18:1), linolenic acid (C18:3) and docosadienoic acid (C22:2) as major fatty acids [31]. Our results are comparable with those of reported by Ojo-awo also analyzed the *Spirogyra* oil-based biodiesel. They identified palmitic acid (C16:0), stearic acid (C18:0), α linolenic acid (C18:3), linoleic acid (C18:2), oleic acid (C18:1), and palmitoliec acid (C16:1) [21], and these results are comparable with our findings.

2.5. Emission Profiling

To evaluate the impact of *Spirogyra* biodiesel blending on the emission characteristics of conventional diesel engine, NO*x*, CO, and HC were monitored upon combustion in an IC engine at full load (Table 6). The comparison of pure biodiesel (S100) and neat diesel (D100) was also made on

the basis of exhaust gas emissions. The findings reflected significant reductions in CO and HC for biodiesel blend (S5, S20, S40, and S100) when compared with conventional diesel. The reduction CO for S5 blend relative to D100 was 4.16% at full engine load. A reduction of 33.47% was calculated for S100 when compared with D100. The S20 blend exhibited a 16.36% reduction of CO emission as emitted by D100. Similar trends were observed for HC emissions and reductions in HC concentration were observed for all biodiesel blends in comparison with D100. The HC emissions were reduced by 7.48% for S5, 38.19% for S100, and 22.86% for S20 as compared with D100. However, NO*x* emissions were expectedly higher than D100. It was found that, by increasing the biodiesel fraction in blend, NO*x* emissions started to increase and the S100 blend exhibited 23.88% higher emissions than D100. The S5 blend showed a negligible rise of 2.55% in NO*x* when compared with D100, which can be tolerated. The high NO*x* values for *Spirogyra* biodiesel blends were probably due to the high oxygen contents of algal oil used for biodiesel synthesis [32]. The emission rate of CO and HC for biodiesel blends was substantially lower than the conventional diesel, which indicated the environment friendly domain of biodiesel from algal biomass.

Table 6. Exhaust emission profile of various oxides of *Spirogyra* oil-based biodiesel.

Blends	NO*x* (ppm)	CO (ppm)	HC (ppm)
D100	146.53 ± 5.95	605.33 ± 6.92	68.36 ± 1.99
S5	150.37 ± 3.63	580.11 ± 4.47	63.24 ± 1.72
S20	165.58 ± 5.10	506.27 ± 8.95	52.73 ± 2.65
S40	177.56 ± 2.69	445.26 ± 5.07	49.95 ± 1.50
S100	192.50 ± 4.59	402.70 ± 5.55	42.25 ± 1.55

3. Materials and Methods

3.1. Extraction of Spirogyra Oil

Algal samples were collected from the ponds situated in different areas of Gujart and were identified as *Spirogyra crassa* by one of the co-authors from the Institute of Industrial Biotechnology, GC University Lahore, Pakistan. Algal oil was extracted by Soxhlet apparatus using n-hexane and diethyl ether (1:1) as solvents. Extra solvent was removed on rotary evaporator under vacuum.

3.2. Physico-Chemical Characterization of Spirogyra crassa Oil

The oil extracted from *Spirogyra crassa* was characterized by measuring density, acid value (AV), saponification value (SV), peroxide value (PV), and iodine value (IV) using standard analytical protocols.

3.3. Experimental Design for Transesterification

Two central composite response surface designs consisting of 30 experimental runs were constructed separately for the chemical and enzymatic biodiesel production utilizing Design Expert 7. The reaction parameters selected for the optimization of the chemical transesterification of algal oil were arranged as catalyst concentration (0.25–2%), methanol:oil (3:1–9:1), reaction temperature (35–65 °C), and reaction time (40–150 min.), while for the enzyme catalyzed reaction, the reaction parameters were enzyme concentration (0.5–1.5%), methanol:oil (3:1–9:1), reaction temperature (15–55 °C), and reaction time (20–65 h). To perform the acid catalyzed esterification, 100 grams of oil was taken in a 250 mL three neck round bottom flask. Then, 0.05 g concentrated sulphuric acid (H_2SO_4) and 2.25 g of methanol for each gram of free fatty acid (FFA) present in the oil were added to the flask. The reaction mixture was stirred for 120–180 min at 65 °C. The acid value was determined periodically after 15 min. Base catalyzed transesterification reactions were carried out in a 250 mL three necked flask. For this purpose, 50 g of oil was taken in a three neck flask. Then, the designated amount of catalyst $NaOCH_3$ was added in alcohol according to the central composite rotatable design (CCRD) and this was transferred into the flask containing *Spirogyra* oil (algal oil) that was attached to a thermometer,

condenser, and magnetic stirrer. The reaction mixture was stirred according to the CCRD design at a specific temperature for a designated time. *Spirogyra* oil and methanol were mixed together and then NOVOZYME-435 was added to this mixture. After that, the reaction mixture was placed in a shaker incubator. The reaction conditions were selected as per CCRD. At the end of reaction, enzyme was separated from the reaction mixture through filtration process. Biodiesel was separated from glycerol and was purified.

3.4. Statistical Optimization

Analysis of variance (ANOVA), diagnostic checks, 3D response surface plots, and descriptive statistics were built to study optimization process. Based upon experimental results, the best fitted model was selected by considering the following parameters: lack of fit values, statistical significance of selected model, and higher values of R^2 and adjusted R^2.

3.5. Biodiesel Characterization

Chemical characterization of the synthesized biodiesel was performed by FTIR spectroscopy (Carry-660-Agilent Technologies, Santa Clara, CA, USA) and GC-FID (Shimadzu, Tokyo, Japan) techniques. FTIR analysis was performed to confirm the conversion of fatty acid into fatty acid methyl esters using an FTIR spectrophotometer. Spectra were taken for a scanning range of 400–4000 cm^{-1}. Major fatty acid methyl esters (FAME) were detected by gas chromatography equipped with a flame ionization detector (FID). The capillary column used was made of fused silica (with 30 m × 0.25 mm with 0.25 μm film thickness). The controlled column temperature program was used and the initial temperature was maintained at 100 °C for 4 min. Then, it was increased to 220 °C at the rate of 3 °C per minute and maintained for 4 min, followed by an increase in temperature up to 260 °C with a hold-up time of 10 min. Temperatures of the injector and detector were set at 250 °C and 260 °C, respectively. With a flow rate of 1.0 ml/min, Helium was used as a carrier gas. Varian WS software was used to record the chromatogram. Fatty acids were identified by a comparison of the relative retention times of FAME peaks from samples with those of reference standards procured from Sigma-Aldrich (St. Louis, MO, USA).

3.6. Fuel Properties of Produced Biodiesel

The fuel properties of *Spirogyra* oil-based biodiesel were estimated by performing standard ASTM tests for the following properties: density (ASTM D 5002), Fire point, flash point (ASTM D93), kinematic viscosity (ASTM D445), cloud point (ASTM D2500), and pour point (ASTM D97).

3.7. Exhaust Emission Profiling

Biodiesel synthesized from *Spirogyra* oil was mixed with fossil diesel to evaluate the emission profile of blends at full engine load conditions. The B100 (Diesel 100%), S5 (*Spirogyra* biodiesel 5% and diesel 95%), S20 (*Spirogyra* biodiesel 20% and diesel 80%), S40 (*Spirogyra* biodiesel 40% and diesel 60%), and S100 (*Spirogyra* biodiesel 100%) blends were prepared and combusted at 100% load. The internal combustion engine (IC) used to quantify exhaust gas emissions was a diesel engine having 11.75 KW power with bore stroke 110 × 125 mm at 2200 rpm. The minimum fuel consumption was 2.5 L/hr. The engine was operated at 100% load to measure gaseous emissions. Testo 350 portable emission analyzer was employed to measure CO, NO*x*, and HC emissions upon burning the fuel fractions.

3.8. Statistical Analysis

Statistical analysis of all the experimental results was performed using SPPS statistical software (Chicago, IL, USA) and Design Expert-7, Stat Ease (Minneapolis, MN, USA).

4. Conclusions

A locally isolated *Spirogyra crassa* was found to be a good source of oil which was used for the synthesis of biodiesel. Based on the acid value, saponification value, peroxide value, density, viscosity, and iodine values, spirogyra oil was found to be suitable for biodiesel production. Fuel properties, such as flash point, fire point, cloud point, density, and kinematic viscosity of the *spirogyra*-based biodiesel were found to be within the limits of ASTM standards. The biodiesel production process was monitored by FTIR spectroscopy and the fatty acid profiling of resultant biodiesel was done by GC-FID analysis. Based on biodiesel characterization, it was ascertained that the synthesized biodiesel exhibited technically compatible fuel characteristics and hence may be used as a potential green fuel.

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