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Research Article

Biodiesel Production from a Naturally Grown Green Algae *Spirogyra* Using Heterogeneous Catalyst: An Approach to RSM Optimization Technique

Teku Kalyani[a*], Lankapalli Sathya Vara Prasad[a], Aditya Kolakoti[b]

[a]*Department of Mechanical Engineering, Andhra University College of Engineering, Visakhapatnam, 530003, India*

[b]*Department of Mechanical Engineering, Raghu Engineering College(A), Visakhapatnam, 531162, India*

Abstract. The present study focuses on oil extraction and biodiesel production from naturally grown green *Spirogyra* algae. Solvent oil extraction and oil expeller techniques were used to extract the *Spirogyra* algae oil (SALO), and the oil yields were compared to identify the most productive method. Using chicken eggshell waste (CESW) heterogeneous catalyst (HC) was prepared for the production of *Spirogyra* algae oil biodiesel (SALOBD). Furthermore, Box–Behnken (BB) assisted response surface method (RSM), an optimisation technique, was used in this study to achieve maximum algae biodiesel yield. From the 29 experimental trails, 96.18 % SALOBD was achieved at molar ratio (10:1), heterogeneous catalyst (0.6 wt.%), temperature (48 °C), and time (180 minutes). The predicted values of R^2 (97.51%) and Adj. R^2 (95.02 %) are found to be encouraging and fits well with the experimental values. The output results showed that HC was identified as the significant process constraint followed by the time. The fatty acid composition (FAC) analysis by Gas Chromatography (GCMS) reveals the presence of 29.3% unsaturated composition and 68.39 wt. % of the saturated composition. Finally, the important fuel properties of SALOBD were identified in accordance with ASTM D6751. The results obtained using chicken eggshell waste (CESW) for the production of biodiesel were recommended as a diesel fuel replacement to resist energy and environmental calamities.

Keywords: Dried algae powder, dried algae flakes, Heterogeneous catalyst, Response surface method, *Spirogyra*.

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1. Introduction

Globally, the utilisation of non-renewable fuels like petrodiesel-derived fossil fuels stood as one of the primary sources of fuel supply for transportation, agriculture and industries (Höök & Tang, 2013). During the earlier days of its discovery, fossil fuels were identified as the most popularly used fuels because of their high efficiency, accessibility and low cost. However, with the increasing demand and high consumption, the availability of petrodiesel-derived fuels is on the verge of extinction. Also, the exhaust emissions from petro-diesel-fuelled engines contribute to the reduction of air quality and increase environmental air pollution around the globe. Consequently, the search for renewable and clean-burning fuels is attracting a lot of interest. To overcome the challenges, research on various feedstock oils revealed that biodiesels derived from different naturally existing oils might replace the current petrodiesel-derived fuel. Therefore, biodiesels from various edible and non-edible oils have become viable alternatives to traditional hydrocarbon fuels like petro-diesel.

Biodiesels are clean burning renewable fuels, popular for their biodegradable capacity, reliability, less toxic nature and improved engine performance. Biodiesel oils are divided into three (3) generations listed as first (1st) generation, second (2nd) generation and third (3rd) generation. The 1st generation of biodiesel oils is related to edible oils. For the production of biodiesel, these oils raised the debate about food vs fuel issues. This concern led to 2nd generation of biodiesels. These are linked with non-edible oils and are proven to be a better source of producing biodiesel oils compared to the first generation because these biodiesels are readily available and less expensive. However, crop cultivation, production and oil yield are considered significant tasks in the production of second-generation biodiesel oils. These drawbacks made researchers focus on third-generation fuels termed algae biofuels.

The algae biodiesels are emerging worldwide since they are available naturally, easy to grow, have high oxygen profiles, capable of reducing emissions etc. Algae species, known as a clean, renewable fuel, are identified as one of the fast-growing biomasses to convert fuel into biodiesel. Around the globe, more than 60,000 algae species are growing, and nearly 35,000 algae species have been identified (Mata *et al.*, 2010). Algae groups are classified into seven types listed as red, green, blue-green *(Cyanobacteria)*, brown, phytoplankton, seaweeds, and other algae species (Nur *et al* 2015). Green microalgae *Spirogyra* division comes under *Chlorophyta* with lengths ranging from 10 to 100 µm. Irradiation of light and temperature are the two important factors considered in achieving maximum growth of *Spirogyra* algae.

* Corresponding author
Email: kalyanithermal@gmail.com (T. Kalyani)

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Table 1
Presence of oil content in some of the microalgae species

Microalgae species	Oil content (dry wt. %)
Chlorella	27 -32
Schizochytrium	51-77
Dunaliella tertiolecta	35-42
Spirogyra	22-35
Natashia	44-47
Monallanthus salina	22
Nannochloris	20-24
Nannochloropsis	30-68
Neochloris oleoabundans	45-48
Botryococcus braunii	26-75
Tetraselmis sueica	14 - 33
Isochrysis	7- 33

Source: (Bhateria & Dhaka, 2014); (Chisti, 2007); (Konga *et al.*, 2017)

The range of light intensity varies from 32 to 38 μmol $m^{-2}s^{-2,}$ and temperatures from 12 to 29 °C are considered optimum. Altogether, 400 *Spirogyra* free-float algae were identified and commonly found in freshwater near pond surfaces and saline waters (Ananthi *et al.*, 2021).

Species that possess unicellular growth in rivers, open ponds, and freshwater resources are called microalgae. It is evident from research reports that microalgae species produce high oil yields ranging from 20 to 80%, as shown in Table 1 (Bhateria & Dhaka, 2014). The oil yield of microalgae with 70% oil by wt.% in biomass generates 136900 L/ha, and with 30 % oil by wt.% in biomass is 58700 L/ha (Chisti, 2007).

Biodiesel production and characterisation research from various edible and non-edible oils is an ongoing process. Oils that are inexpensive and easily accessible have a minute impact on food vs fuel conflict and are recommended as the better source for biodiesel production. However, due to the inadequate atomisation problem in diesel engines, it is suggested to use low-viscosity biodiesels (less than 40 Cst.) (Kolakoti & Appa Rao, 2020). Hence, the transesterification process is recognised as one of the best processes for reducing kinematic viscosity (Hariyanto *et al.*, 2021); this process also gains high efficiency while converting it into biodiesel. The molar ratio (MR), time, temperature, and catalyst concentration (CC) are four significant process parameters used during transesterification. Among these, CC plays a beneficial role in achieving the maximum amount of biodiesel. Based on the availability and reaction process, two (2) types of catalysts exist, homogenous and heterogeneous catalysts.

Homogeneous catalyst is most regularly used in the transesterification process as they are highly catalytic in nature, readily available and available at a low price (Supriyadi *et al.,* 2022). The most commonly used solutions are homogeneous catalysts: Potassium Hydroxide (KOH) and Sodium hydroxide (NaOH). Due to its corrosive nature, the purification process requires more water and remains expensive. Furthermore, the solution mixed in water is tough to collect and reuse. Research was being carried out to mitigate these issues using the heterogeneous catalyst (HC). The key benefits of using a heterogeneous catalyst to prepare biodiesel include its environmental friendliness, ease of accessibility, separability, excellent stability and ability to be reused for up to five trials (Kolakoti & Satish, 2020; Hadiyanto *et al* 2016).

Kolakoti *et al.,* (2022) prepared a green heterogeneous catalyst calcinated at 700°C for 3 hours using *Moringa oleifera* leaves that are available naturally for biodiesel production using palm oil. The maximum biodiesel achieved was 92.82 % at optimum conditions. Fan *et al.* (2019) carried out an investigation using waste banana peel as a heterogeneous catalyst for the production of biodiesel. The results showed the presence of strong alkalinity, recyclable nature, and catalytic performance observed in the unique alkaline catalyst derived from the waste banana peel. Moreover, after the transesterification process, heterogeneous catalyst uses less water for purification, which is inexpensive. As a result, heterogeneous catalyst attracts a lot of interest and suggests this procedure as a practical and cost-effective transesterification method.

With the improvement in the latest technologies, the oil and biodiesel yields were estimated using various tools like Analysis of Variance (ANOVA) and Artificial Neural Network (ANN) (Kolakoti *et al.*, 2020), which show high accuracy in yield predictions (Kolakoti *et al.,* 2020). Another researcher (Kumar *et al.*, 2020) performed an experimental investigation on Algae – Jatropha to produce biodiesel yield using the Response Surface Method (RSM) and ANOVA Analysis. Similarly, (Chozhavendhan *et al.*, 2020) identified significant changes by changing process parameters such as catalyst concentration, oil-to-alcohol ratio, reaction temperature and reaction time parameters and concluded that catalyst plays a beneficial role in processing biodiesel. Kolakoti *et al.,* (2021) used waste chicken egg shells as a heterogeneous catalyst to produce biodiesel from cooking oil. Also, the biodiesel yield was compared using two optimisation techniques RSM and ANN. The results concluded that 91% of biodiesel yield is achieved from the two techniques. The discussion shows that there is more potential for algal biofuel as a sustainable green fuel.

In the current study, naturally grown green *Spirogyra* algae are collected directly from two open ponds from January to May 2022. A four-step technique (collection – harvesting – oil extraction – transesterification) is used to produce the *Spirogyra* algae biodiesel. This study mainly focuses on collecting algae from open ponds without any kind of cultivation process, nutrients and chemicals. Furthermore, the *Spirogyra* algae grow naturally using sunlight, carbon dioxide and rainwater (Reddy & Majumder, 2014). The collected algae were cleaned and processed in two forms to extract oil, and oil yields via two different techniques were compared. One is the solvent extraction method (I) using dried algae powder, and the second is the oil expeller method (II) using dry algae flakes. Furthermore, the obtained *Spirogyra* Algae Oil (SALO) is converted into *Spirogyra* Algae Oil Biodiesel (SALOBD) using a heterogeneous catalyst (HC) prepared from chicken eggshell waste (CESW). Very limited studies in this combination (SALO + CESW) are available to convert raw algae oil to biodiesel with chicken eggshell waste as a heterogeneous catalyst mooted this study. Furthermore, an optimisation technique was used in this study to obtain maximum algae biodiesel yield. For this, Box–Behnken (BB) supported response surface method (RSM) was applied. A number of 29 experimental trials were conducted randomly by varying the significant process constraints such as heterogeneous catalyst, molar ratio, time and temperature.

Hence the main objective of the present study is to extract algae oil and compare oil yields using two oil extraction techniques and to produce biodiesel from a naturally grown green algae *Spirogyra* using chicken egg shell waste as a heterogeneous catalyst. Finally, using RSM optimisation technique, the experimental biodiesel yield prediction was attained by varying the molar ratio, heterogeneous catalyst, time and temperature, conducting 29 experimental runs.

2. Methods and Materials

2.1 Materials

Chemicals such as methanol (CH_3OH) with (99.8 %) purity, sulphuric acid (H_2SO_4) with (99.99 %) purity and n-Hexane with (99 %) purity of different grades were purchased from Sigma Aldrich (Merck) Visakhapatnam, India. The chicken eggshell wastes are collected from a local restaurant in Visakhapatnam.

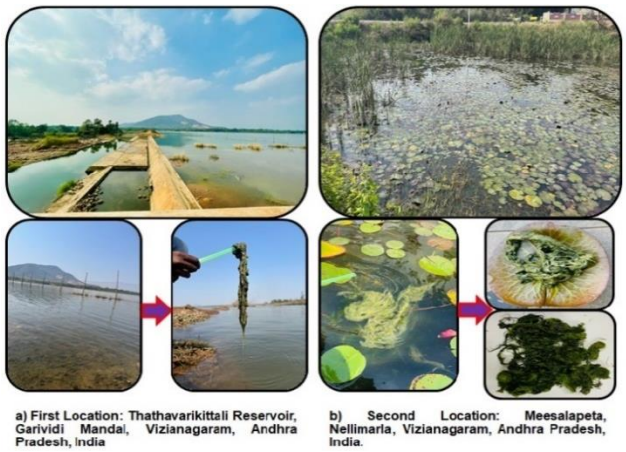

Fig. 1 Locations of *Spirogyra* algae collection

The condenser apparatus required for oil extraction and transesterification processes are soxhlet apparatus, reflux, steam distillation unit, round bottom flask, hot plate magnetic stirrer, thermometers, filter papers, conical flask, separatory flasks, distilled water and glassware of Borosil make. The apparatus required for experimentation are utilised from the research fuel laboratory.

2.2 Four-Step Process for Preparation of Spirogyra Algae Biodiesel

2.2.1. Collection

The naturally grown green *Spirogyra* algae were collected from two open ponds for 120 days (every 15 days) at a recorded temperature ranging from 22 to 29 °C. As shown in figure 1, the algae species were identified in two different locations, the first location is near thathavarikittali reservoirs, Garividi Mandal, at 18°17'03.0"N latitude and 83°28'57.9"E longitude, and the second location are identified at 18°13′40.1″N latitude and 83°28′58.1″E longitude near Meesalapeta, Nellimarla, Vizianagaram, Andhra Pradesh, India.

2.2.2 Harvesting and processing dried algae powder and dried algae flakes

The wet algae blooms collected from two locations weighed 158 kg and were cleaned with distilled water to remove unwanted plants, snails, mud etc., as shown in Fig. 2. After processing, a net weight of 96 kg of wet algae is obtained and divided into two equal parts (48 kg each) to prepare into two forms: dried algae powder and dried algae flakes.

The dried algae powder is processed as shown in Figure 3. Initially, *Spirogyra* wet algae were separated layer by layer and dried for five (5) consecutive days at 8:1 sun hour during the temperature range from 22 to 29°C and later dried in a hot air oven for one hour by maintaining 90°C to remove excess moisture content. Later, the dried algae layers were converted into dried algae powder (Fig. 3) using a mechanical grinding machine available in the research lab. Finally, the weight of the dried algae powder obtained was 8 kg.

In a similar approach, the processing of dried algae flakes is shown in Figure 4. Firstly, the obtained wet algae were ground into a smooth paste. The smooth paste was transferred into a lab tray and exposed to sunlight for five (5) consecutive days. About 14 kg of dried algae flakes were obtained out of 48 kg of wet algae paste. Furthermore, the *Spirogyra* algae oil extraction was carried out using the solvent extraction method and oil expeller press method. Finally, the algae oil was extracted using chemical and mechanical methods, and the oil yields were compared to identify the most productive method.

Fig. 2 Algae cleaning process

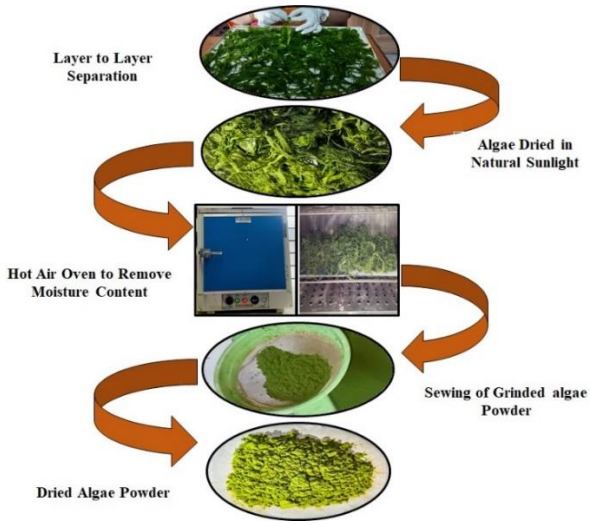

Fig. 3 Processing of dried algae powder

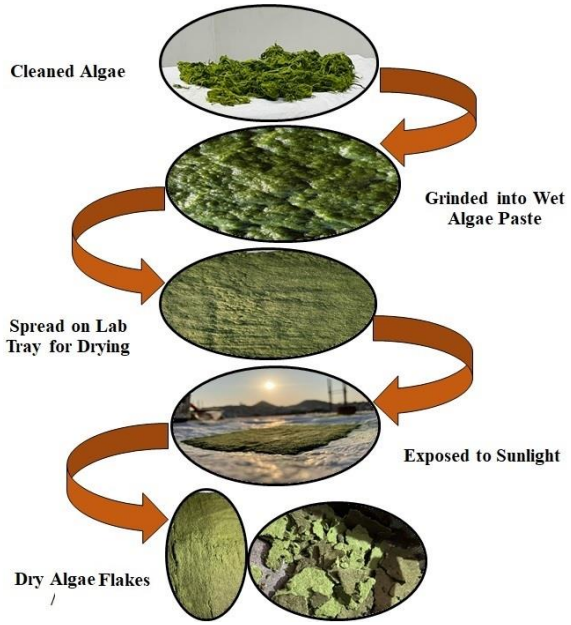

Fig. 4 Processing of dried algae flakes

2.2.3 Oil Extraction

In third-generation algae biodiesels, the oil extraction process plays a beneficial role in which the solid form of dried algae powder/flakes is transferred into a liquid (algae oil) form known as the extraction process (Topare *et al.*, 2011). The oil extraction process uses various chemical techniques like solvent extraction and supercritical (SC) CO_2 methods. Similarly, physical and mechanical techniques like microwave heating, ball milling, ultrasonication and oil expeller methods etc (Mubarak *et al.*, 2015). According to the research that is currently accessible, oil expeller techniques, supercritical CO_2, and solvent extraction procedures are the most efficient ways to recover oil between 50 and 80 percent (Konga *et al.*, 2017). Each oil extraction technique has its own benefits and drawbacks, as mentioned in

the literature that is currently available (Bhargavi *et al.*, 2018). In the present investigation, two techniques were proposed to extract algae oil and to compare the oil yield, viz., solvent extraction process (the chemical method I) and oil expeller press process (mechanical method II).

2.2.3.1 Solvent Extraction Process (Method I)

In the present study, *Spirogyra* algae oil was extracted by a solvent extraction process using the soxhlet apparatus (Konga *et al.*, 2017). The solvent extraction process is the most popular method, renowned for its high extraction efficiency and ease of use. As shown in Fig. 5a, the setup includes a heater control, round bottom flask, soxhlet apparatus, reflux condenser and glass beakers necessary for extraction purposes. In order to obtain the maximum benefit from the solvent oil extraction process, optimum conditions must be maintained throughout the process.

The oil extraction process was initiated by mixing the dried algae powder and n-hexane in the proportions of 1:2. The prepared algae powder with a weight of 300 grams was filled in the soxhlet apparatus, and 600 mL of n-hexane solvent was transferred into the flask (Kalyani *et al.*, 2023) and the mixture is heated until the temperature reaches 65°C. Upon heating, vapours flow up from the side tube through the reflux condenser unit to reach the soxhlet housing chamber, where vapours are condensed and interact with solid dried algae powder.

As a result of the interaction, some of the desirable chemicals in algae dissolve in the hot solvent. After the reaction, the soxhlet apparatus was filled with the hot algae oil mixture. Furthermore, the solvent and the algae oil mixture were transferred into the round bottom flask through a siphon tube. The process was repeated until the dried algae powder turned into pale colour for an extraction time of 18 hours. The hot algae oil and the n-hexane solvent were separated using a steam distillation process. Finally, 64 mL of SALO was obtained, and 90% of the n-hexane was recovered. Finally, the percentage of SALO yield was calculated using Equation 1.

$$\% \text{ Yield of algae oil (wt\%)} = \frac{\text{Mass of oil extracted (grams)}}{\text{The total mass of dried algae powder or flakes}} \times 100 \quad (1)$$

2.2.3.2 Oil Expeller Process (Method II)

The second method used to extract SALO is a mechanical screw-type oil expeller press technique. In this process, the algae oil was extracted using dried algae flakes (see Fig. 4) without using solvents. To extract algae oil from the oil expeller press method, initially, 48 kg of wet algae paste was processed and finally, 14 kg of dried algae flakes were obtained. These flakes were positioned directly into the oil expeller and crushed in the local oil crushing machine unit, as shown in Fig. 5b. Finally, from the oil expeller process, 1680 mL of SALO was extracted and 12 % of the oil yield was obtained. Though this method does not require any chemicals to extract oil, it requires a huge quantity of algae biomass and it is tough to recover the traces of oil stored under the expeller. Finally, the oil extraction rate of 70 to 75% is noted (Mubarak *et al.*, 2015), which is less than the solvent extraction process (Topare *et al.*, 2011). Hence, it is evident that the solvent extraction method is more effective than the oil expeller press.

The *Spirogyra* algae oil extracted using methods I and II signify that the solvent extraction method is more effective than the oil expeller method. Therefore, the solvent extraction process was used to extract *Spirogyra* algae oil (SALO), and the *Spirogyra* algae oil biodiesel (SALOBD) was prepared using the transesterification process.

2.2.4 Catalyst Preparation

The consumption of eggs has increased vigorously in recent years after the outbreak of the alarming national situation. Worldwide, 76.7 to 85.2 MMT (Million Metric Tons) of eggs are consumed, with India, the US, the EU, and China ranking as the top four producers (Kolakoti & Satish, 2020). These chicken eggshell wastes (CESW) were relinquished to surroundings causing a rapid increase in environmental pollution. Proper usage of the best out of this waste helps to reduce ecological defilement. In this context, the HC prepared from the CESW (Piker *et al.*, 2016) is one of the significant steps for preparing neat SALOBD. A sample of 11 nos. of the whole chicken egg shell waste was collected near the neighbourhood restaurant. Further, cleaned with deionised water and gently wiped with filter paper. Further, the egg shells covered inside with shrill layers were removed and dried in a hot air oven for 24 hours. The dried CESW are crushed into tiny parts and calcinated in a muffle furnace by maintaining 600°C for 8 hours, as shown in Fig 6. Pandit & Fulekar, (2017) prepared catalyst by maintaining it at 900°C for about 8 to 9 hours. Similarly, Kolakoti & Satish, (2020) performed an experimental investigation to prepare low-grade cooking oil using heterogeneous catalysts from waste egg shells. After several experimental trials, the HC was found desirable at 600°C. Finally, the product CaO was stored for its expedited usage.

Fig. 5 (a) Stages of the solvent extraction process (method I), **(b)** Stages of oil expeller press process (method II)

Fig. 6 Preparation of chicken eggshell waste as heterogeneous catalyst.

2.2.5 Design of Experiments by Response Surface Method (RSM)

The response surface method is one of the most accepted optimisation techniques for experimental conditions. It is one of the beneficial approaches to show the actual and predicted values by varying the significant parameters. This method fits well in various applications such as biodiesel yield prediction, composites, nanocomposite designs, etc. Sohail *et al*., (2020) performed experimental investigations for the production of *Spirogyra* biodiesel and determined their physicochemical properties. The biodiesel yield was optimised using response surface methodology. Chemical and enzymatic behaviour for *Spirogyra* biodiesel was determined by considering four significant parameters, viz., molar ratio, catalyst concentration, time, and temperature. The optimised yield was reported as (77.3 ± 1.27%) and in enzymatic transesterification, the yield attained was (93.2 ± 1.27%).

In another area of study, Tran *et al*., (2020) worked on a central composite design based on response surface methodology to reach the optimal dyes removal conditions, using graphene oxide@$CoFe_2O_4$ nanocomposite for Congo red (CR), methyl red (MR), and crystal violet (CV). The results obtained from confirmation tests exhibited very low errors and outstanding removal efficiencies attained between 93.0% and 99.7%. A similar approach is carried out by (Tran *et al*., 2019) using an efficient optimisation technique and response surface methodology to optimise the experimental conditions for removing chloramphenicol pharmaceutical from wastewater using $Cu_3(BTC)_2$-derived porous carbon as an efficient adsorbent. Also, the quadratic model was used to assess compatibility and suitability using ANOVA analysis. The results reported that a low magnitude p-value (<0.0001) was obtained with a coefficient of determination R^2 = 0.9457, and adequate precision (AP) ratio was observed to be close to 0.1. Nguyen *et al*., (2022) examined the construction of zeolitic-imidazolate framework (ZIF-8) and ZIF-8-derived porous carbon using response surface methods. The findings showed that the quadratic regression models are statistically significant. Hence based on the literature studies, an RSM optimisation technique is used to estimate the actual and predicted value of the biodiesel yield by varying the significant parameters such as HC, MR, Temperature and Time with 29 design of experiments using Minitab -19 software. The four significant process constraints are shown in Table 2.

Table 2
Four process constraints with ranges

Constraints	Units	Low	Medium	High
Heterogeneous catalyst (HC)	(wt.%)	0.6	1.2	1.8
Molar Ratio (MR)	----	8:1	10:1	12:1
Temperature	^{O}C.	38	48	58
Time	mins	120	150	180

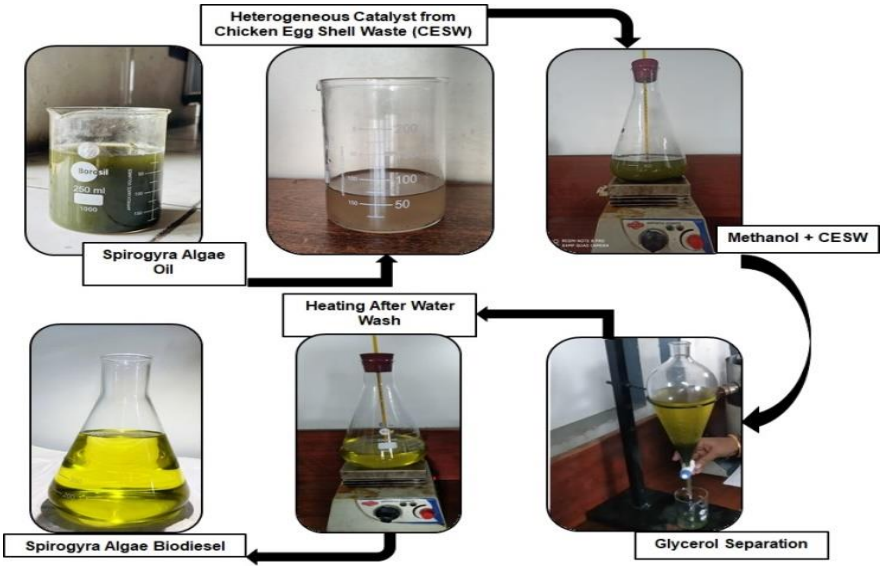

Fig. 7 Stages of *Spirogyra* algae biodiesel oil production with heterogeneous catalyst

Table 3
Design experimental runs with experimental and RSM biodiesel yield percentages

Experimental Runs	HC	Temperature	Time	MR	Exp. Yield %	RSM Yield %
1	1.8	48	150	12	53.65	54.82
2	1.2	58	180	10	93.54	89.84
3	1.2	58	150	12	92.21	89.80
4	0.6	48	150	8	91.04	88.23
5	1.2	38	150	12	77.69	77.98
6	1.2	58	120	10	76.44	76.35
7	1.2	48	150	10	75.8	75.71
8	0.6	48	120	10	86.98	86.63
9	1.2	38	120	10	79.21	82.21
10	1.2	58	150	8	74.85	73.97
11	1.2	48	180	12	92.63	94.87
12	1.2	48	150	10	75.47	75.71
13	1.2	48	150	10	74.58	75.71
14	1.8	48	180	10	62.01	62.71
15	1.2	38	150	8	89.84	91.65
16	1.8	38	150	10	71.24	66.98
17	1.8	48	150	8	55.89	57.18
18	1.2	48	120	12	64.69	66.34
19	1.8	58	150	10	52.47	54.00
20	1.2	48	150	10	75.54	75.71
21	0.6	48	150	12	95.69	92.75
22	0.6	38	150	10	91.65	91.42
23	0.6	58	150	10	92.99	95.54
24	1.2	48	150	10	75.25	75.71
25	1.2	48	120	10	76.99	73.21
26	1.2	38	180	10	90.45	89.84
27	1.2	48	180	8	75.24	75.83
28	1.8	48	120	10	52.14	51.71
29	0.6	48	180	10	95.97	96.76

2.2.6 Transesterification Process

The *Spirogyra* algae oil (SALO) obtained from the steam distillation process was heated up to 110°C, to remove the presence of water (H_2O) content in the SALO. Furthermore, the SALO fuel parameters were tested and it was reported that the kinematic viscosity was 19.20 mm^2/s, density of 898.21 kg/m^3, and FFA of 1.68 % (Yadav *et al*., 2019). It is evident that the algae oil is reported with high kinematic viscosity, which is not recommended to use in the existing diesel engine. However, to reduce the kinematic viscosity of SALO, a widely used transesterification process was performed to convert it into low-viscous SALOBD. Initially, the raw SALO oil was collected in a conical flask and positioned on a magnetic heater with a hot plate. Further, heated until it reached the desired temperature and then a heterogeneous catalyst (CaO) with methanol (CH_3OH) was added. The mixture was stirred continuously using a magnetic stirrer, as shown in Fig. 7. The catalyst (CaO) was built up with an oxygen anion, which initiated to form a strong methoxide anion, thereby assisting the presence of triglycerides in the oil to transform diglycerides to monoglycerides. Finally, esters and glycerine were formed.

As represented in Fig. 7, the hot mixture of biodiesel and heavy glycerine was transferred into a separatory flask. Later, the glycerine was collected into a beaker, and the desired *Spirogyra* algae oil biodiesel SALOBD was obtained. Further, the SALOBD was washed with distilled water and shaken to remove the soap solution. Finally, neat biodiesel was produced by heating the washed biodiesel to a temperature of 100 °C using a magnetic heater. The procedure was repeated by diversifying the four process constraints, as shown in Table 3.

The experimental approach of biodiesel yield (%) was calculated using Equation 2. As shown in Table 3, the RSM optimisation technique was repeated randomly with 29 experimental runs to obtain the predicted RSM biodiesel yield (%), which is presented in Fig. 9

$$SALOBD\ Yield(\%) = \frac{Weight\ of\ spirogyra.algae\ biodiesel}{Weight\ of\ spirogyra\ algae\ oil} \times 100 \quad (2)$$

3. Result and Discussions

3.1 Comparison of SALO yield using solvent extraction and oil expeller methods

The *Spirogyra* algae bloom was collected from two open ponds for 120 days (every 15 days) from January to May 2022, with a total weight of 158 kg. A net weight of 96 kg of fresh wet algae was obtained after cleaning and divided into two equal shares (48 kg each) to prepare dried algae powder and dried algae flakes. The oil is extracted using solvent extraction (method I) and an oil expeller press (method II), and the oil yield percentages were estimated and compared. In solvent extraction, 48 kg of wet algae is separated layer by layer, from which 8 kg of dried algae powder was obtained. A sample of 300 grams of dried algae powder and 600 mL of n-hexane solvent was prepared in the ratio of 1:2 (dried algae powder: solvent), and 64 mL of SALO was obtained after extraction. Finally, from 8 kg of dried algae powder, 1707 mL of SALO with an oil yield of 21.33% was obtained. Whereas, in the oil expeller process, 48 kg of wet algae blooms were ground into a fine paste, transferred into a lab tray, and exposed to sunlight. 14 kg of dried algae flakes were obtained and pressed in the oil expeller. Finally, 1680 mL of SALO with 12 % of oil yield was obtained, as represented in Table 4. Therefore, based on the oil yield percentage, the solvent extraction method is more effective than the oil expeller method. It is evident from Table 4 that the solvent extraction method I, with 21.33 % of algae oil yield, is effective compared with the oil expeller method II, with 12 % of algae oil yield.

3.2 Comparison of oil yield with different percentages of dried algae powder samples – Method I

Three varied percentages (100 %, 75 %, and 50 %) of dried algae powder samples were used to estimate and compare the extracted oil yield with the effective solvent extraction process with the same extraction time and temperature. The SALO yield obtained at various percentages of dried algae raw samples was presented in Table 5. It is evident from Table 5 that 21.33 % of algae oil yield was obtained from 100 % dried algae powder. Similarly, 19.33 % of the algae oil yield was obtained from 75 % dried algae powder, and 18 % of the algae oil yield was obtained from 50 % dried algae powder. Finally, the results confirmed that with 100% dried algae powder, the oil yield attained was higher and contained low moisture content compared with 75 % and 50 % dried algae powder.

Furthermore, when compared with the present study and reference study (Konga *et al*., 2017), an increase of 4.55 % in algae oil yield was observed at 100% dried algae powder. Similarly, 15.05 % improvement was observed near 75 % dried algae powder and 18.42 % improvement was observed near 50 % dry dried algae powder. The improvement in algae oil yield for three dryness samples, compared with the present and reference study, were indicated in Fig. 8

Table 4
Algae oil extraction using chemical and mechanical methods

Description	Method I	Method II
Method of Extraction	Solvent	Oil expeller press
Apparatus	Soxhlet	Screw type
Treatment - form	*Spirogyra* - dried algae powder	*Spirogyra* - dried algae flakes
Solvent mixing	n- Hexane	----
Weight of the dried algae (kg)	8	14
Algae oil obtained (litres)	1.707	1.680
% Yield of algae oil (%)	21.33	12

Table 5
Effect of percentage dried algae sample and comparison of *Spirogyra* algae oil yield obtained using a solvent extraction process

No.	Description	Method I		
1	Dryness (%) of algae samples	100	75	50
2	Extraction method	solvent	solvent	solvent
3	Form	dried algae powder	dried algae powder	dried algae powder
4	Time (hrs.)	18	18	18
5	Temperature (°C)	65	65	65
6	Sample wt. (kg)	0.3	0.3	0.3
7	Total wt. of dried algae powder (kg)	8	8	8
8	Total algae oil yield (litres)	1.707	1.624	1.512
9	% Yield of algae oil **(present study)**	21.33	19.33	18
10	% Yield of algae oil (Konga et al., 2017)	20.4	16.80	15.20
11	Improvement (%)	4.55	15.05	18.42

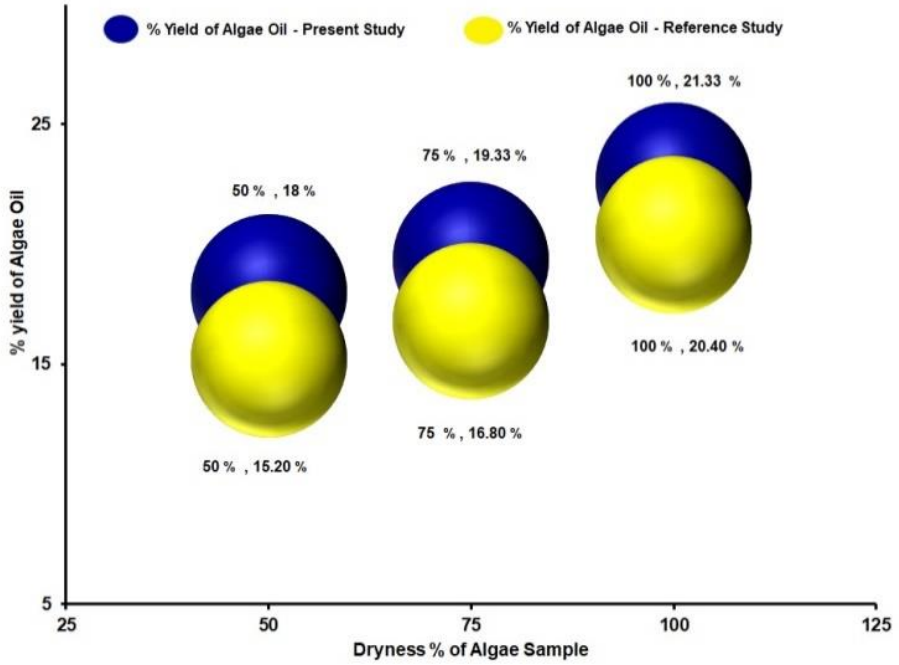

Fig. 8 Improvement in algae oil yield for three dryness samples compared with present and reference study

3.3 Model Fitting

Using the RSM optimisation technique, the four process constraints indicated in Table 2 were varied for 29 design of experiments (DOE), as shown in Table 3. The maximum amount of experimental biodiesel yield of 95.97 % was achieved at MR (10:1), HC (0.6 %-wt), Temperature (48°C), and Time (180 minutes). However, for the same process constraints, the RSM biodiesel yield attained was 96.76 %, as shown in Fig. 9. Finally, three confirmation tests were conducted under the same conditions and revealed that 96.18% of the average SALOBD yield was achieved when compared with the experimental biodiesel yield. Equation 3 represents a quadratic equation that was generated to calculate the relation between the SALO biodiesel yield and the four process constraints for the 29 DOE. The model summary of accuracy was estimated by the coefficient of determination. From Fig. 10, the R^2 value representing actual and predicted biodiesel yield for 29 DOE attained was 97.51%, confirming ≥ 97% of the data was consistent with the achieved values.

$$\begin{aligned}\text{SALO BIODIESEL Yield \%} = {} & 694 + 50.9\,HC - 9.39\,Temperature - 2.498\,Time \\ & - 46.06\,MR - 11.22\,HC*HC \\ & + 0.0607\,Temperature*Temperature \\ & + 0.00309\,Time*Time + 0.394\,MR*MR \\ & - 0.838\,HC*Temperature + 0.0122\,HC*Time - 1.44\,HC*MR \\ & + 0.00488\,Temperature*Time + 0.3689\,Temperature*MR + 0.1497\,Time*MR \end{aligned} \quad (3)$$

Furthermore, the adjusted R^2 attained was 95.02 %, which affirms that the quality and fitness of the model picked were quite encouraging and concluded that the values predicted fit well with experimental values. Hence the optimisation technique proved that the model is satisfactory in estimating the biodiesel yield from SALO.

3.4 The Analysis of Variance (ANOVA)

The influence of four process constraints (HC, MR, Temperature, and time) is beneficial in attaining maximum

biodiesel yield. The significant changes in process parameters during the transesterification process until biodiesel preparation were analysed using ANOVA analysis. It was apparent in Table 6 that the HC was observed as an influencing parameter, followed by the time.

3.5 Free Fatty Acid Analysis

The free fatty acid (FFA) composition significantly impacted the fuel properties, specifically density, viscosity, cetane number, and calorific value. The fatty acid composition (FAC) profile was tested using gas chromatography (GCMS), and the results of the present study with the saturated and unsaturated composition were reported in Table 7.

It is evident from Table 7 that the total saturated composition was reported as 68.39 wt.% and the unsaturated composition was 29.3 wt.%. As shown in Fig.11 and Fig. 12, the significant contribution of saturated FAC was palmitic acid with 24.84 wt.%, and unsaturated FAC was oleic acid with 26.12 wt.%. Therefore, from Table 7, it was apparent that the composition of the FFA profile in the present study was observed with very close compositions compared with the available literature studies. Finally, the FAC of the *Spirogyra* biodiesel falls in good agreement compared with the literature studies.

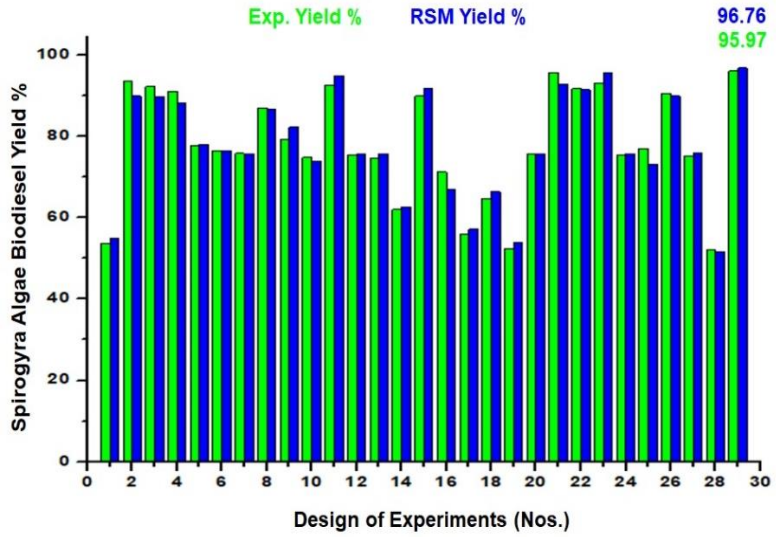

Fig. 9 Experimental and RSM biodiesel yield

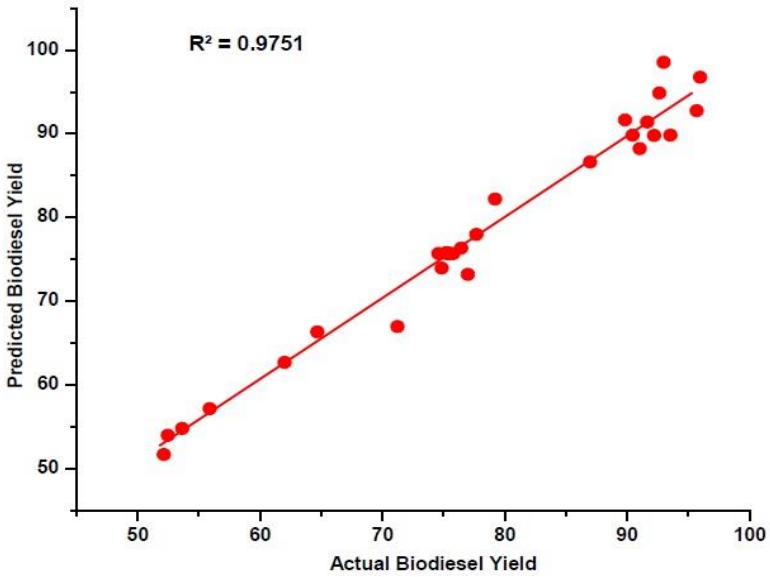

Fig. 10 Actual and predicted biodiesel yield interpretation

Table 6
The analysis of variance (ANOVA)

Source	DOF	Sum of Squares	Mean Square	F-Value	P-Value
Model	14	5060.84	361.49	39.14	<0.0001
Linear	4	3905.85	976.46	105.73	<0.0001
HC	1	3567.99	3567.99	386.33	<0.0001
Temperature	1	25.75	25.75	2.79	0.117
Time	1	311.36	311.36	33.71	<0.0001
MR	1	2.93	2.93	0.32	0.582
Square	4	462.46	115.62	12.52	<0.0001
HC*HC	1	107.01	107.01	11.59	0.004
Temperature*Temperature	1	241.52	241.52	26.15	<0.0001
Time*Time	1	47.00	47.00	5.09	0.041
MR*MR	1	14.65	14.65	1.59	0.228
2-Way Interaction	6	545.12	90.85	9.84	<0.0001
HC*Temperature	1	101.10	101.10	10.95	0.005
HC*Time	1	0.19	0.19	0.02	0.887
HC*MR	1	11.87	11.87	1.29	0.276
Temperature*Time	1	8.58	8.58	0.93	0.351
Temperature*MR	1	217.71	217.71	23.57	<0.0001
Time*MR	1	205.66	205.66	22.27	<0.0001
Error	14	129.30	9.24		
Lack-of-Fit	10	128.45	12.84	60.20	0.001
Pure Error	4	0.85	0.21		
Total	**28**	**5190.14**			

Table 7
Comparison of fatty acid composition profiles

Composition	Type	Wt.%			
		Spirogyra			Algae
		Present study	Reference (Chozhavendhan et al., 2020)	Reference (Richmond, 2004)	(Kumar et al., 2020)
Arachidic Acid	Saturated	2.1	0.3	1.28	2.24
Behenic Acid	Saturated	1.55	0.2	1.39	0.33
Capric Acid	Saturated	0.41	0.3	0.3	-
Caprylic Acid	Saturated	0.55	0.3	0.3	-
Lauric Acid	Saturated	22.68	29.9	21.90	-
Linoleic Acid	Unsaturated	3.18	2.5	2.5	4.88
Myristic Acid	Saturated	13.05	17.0	15.29	-
Oleic Acid	Unsaturated	26.12	30.5	21.62	54.89
Palmitic Acid	Saturated	24.84	14.2	28.63	15.64
Stearic Acid	Saturated	3.21	3.0	4.86	2.10
Other		2.31	1.8	1.28	

3.6 Fuel Property Analysis

Biodiesel fuel plays a crucial role in commercialisation and have the ability to compete with diesel fuels. The SALOBD produced from the transesterification process is characterised by vital fuel properties: density, kinematic viscosity, cetane number, calorific value, pour point, cloud point, flash point, and fire point, as shown in Table 8. The earlier studies on biodiesel fuels stated that the vital properties of the fuel, like flash point, fire point, and cetane number, are higher than diesel fuel. This signifies a positive indicator to depot fuel and the retard time during combustion is lower for biodiesels (Kalyani *et al.,* 2023). Also, biodiesel fuels with high viscosity, density, and low calorific value, report an adverse effect on the engine operating characteristics. One reason for these variations is the appearance of unsaturated and saturated FAC.

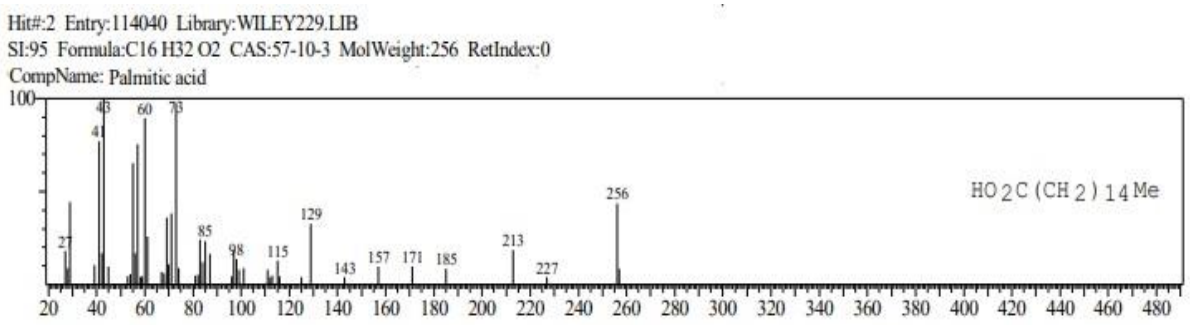

Fig. 11 GCMS spectrum for palmitic acid

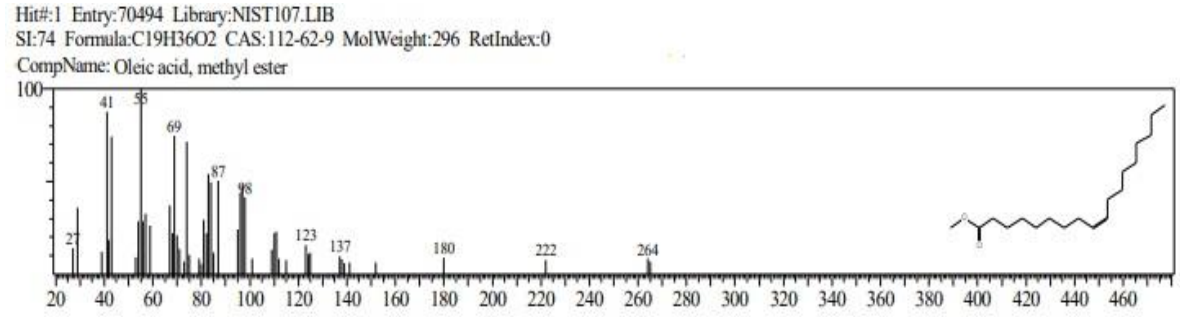

Fig. 12 GCMS spectrum for oleic acid

Table 8
Key fuel properties of *Spirogyra* algae oil biodiesel

Properties	Units	ASTM D6751 (Kalyani *et al.*, 2023)	Diesel (Kalyani *et al.*, 2023)(Saeed *et al.*, 2021)	SALOBD (Present Study)	Microalgae (Kalyani *et al.*, 2023); (Kalyani et al., 2023)(Piloto-Rodríguez *et al.*, 2017)
Kinematic viscosity	mm^2/sec	3.5 to 5.0	2.75	4.24	2 to 5.2
Density	kg/m^3	860 to 890	831	888	850 to 870
Cetane number	-	Min 51	48	53.86	37 to 72
Calorific value	MJ/kg	-	44	42.1	37 to 41
Pour point	°C	-15 to -16	-13	5.8	- 6
Cloud point	°C	-3 to -12	-	12.4	-
Flash point	°C	Min 120	86	145	115
Fire point	°C	Min 132	96	153	-

It is evident from Table 8 that the kinematic viscosity and density of SALOBD are 54.18 % and 6.85 % higher compared to mineral diesel fuel. This change may result in an increase in fuel consumption and a decrease in brake thermal efficiency. The increase may be due to the presence of long-chain fatty acids, i.e. Palmitic acid (Fig. 11). The flash point and fire point temperatures were observed to be high in SALOBD. This is due to the rise in carbon number from saturated fatty acids like palmitic acids, a beneficial sign for storage and safe handling**.** Furthermore, the cetane number (CN) of SALOBD was reported with an increment of 12.20 % than the diesel fuel due to the high composition of saturated FAC. For better engine performance, the increment in cetane number is always recommended since it restricts the delay period during combustion. A low calorific value was identified in SALOBD compared to mineral diesel fuel due to the existence of zero sulphur and low hydrocarbons (Kolakoti *et al.,* 2022)**.** Lastly, the vital fuel properties of SALOBD, associated with the literature (Piloto-Rodríguez *et al.*, 2017) observed to be in a close relationship, make biodiesel fuels suitable for the smooth operation of the existing diesel engine.

4. Conclusions

Chicken eggshell waste (CESW) prepared as a heterogeneous catalyst (HC) was effectively tested on the *Spirogyra* algae oil (SALO) for the production of biodiesel. With the emergence of the RSM optimisation technique, a better yield was attained. Based on the experimental investigations, the following conclusions were drawn. The *Spirogyra* used in this study is naturally grown in an open pond, and it was collected every 15 days a month for 120 days without using any cultivation process, nutrients, or chemicals. The maximum SALO yield obtained was 21.33% for the solvent extraction process and 12% SALO yield for the oil expeller press process, which confirms that the solvent extraction process was more effective than the oil expeller method. It was observed that the oil yield percentage of SALO achieved an increment of 4.55 % over the reference study. About 96.18 % of the SALO biodiesel yield was attained during the transesterification process. The analysis of variance (ANOVA) confirmed that the heterogeneous catalyst concentration followed by time is the most influencing constraint. The key fuel properties of the present study compared with the literature studies are observed within the

range of ASTM D6751. Therefore, using chicken eggshell waste (CESW) for biodiesel production, especially for *Spirogyra* algae oil, was recommended as a diesel fuel replacement to resist the energy and environmental calamities.

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