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RESEARCH ARTICLE

Biodiesel production from sea green algae using moringa oleifera as heterogeneous catalyst - an approach to definitive screening design optimization technique

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Abstract

Rising concerns over petro-derived fossil fuel depletion and the release of toxic engine exhaust emissions reflect the serious impact of these issues on environmental pollution and their adverse effects on human health. These concerns have stimulated exploration of green, renewable sources of biodiesel feedstocks. Algae biodiesel is regarded as a viable, sustainable, environmentally friendly alternative to fossil fuels. In the current study, oil extraction and biodiesel production were conducted using sea-green algae grown naturally on rocks in marine waters. Primarily, the wet sea green algae were harvested for oil extraction and converted into biodiesel. Sea-green algal oil was extracted using a chemical solvent-extraction method, yielding 24.25%. Further, a novel moringa oleifera dried leaves were used as a heterogeneous catalyst, which were calcinated in an oven with hot air at a temperature of 300 – 600 $^{\circ}$C. The obtained calcined Moringa oleifera was mixed and trans esterified. Thus, the biodiesel yield from sea-green algae oil was 94.99%. Furthermore, a definitive screening design was implemented in this study using MiniTab-21 to maximize biodiesel yield. The design was attempted using a 3-factor, 2-level fractional factorial design from the design of experiments framework. From 18 no. of experimental runs, the sea green algae oil biodiesel yield achieved was 97.14 % at 59 $^{\circ}$C reaction temperature, 9.5 wt. % catalyst concentration, and 12:1 molar ratio. The sea green algae oil biodiesel contained 72.28% saturated and 27.72% unsaturated fatty acids, as determined by gas chromatography. The key fuel properties of biodiesel from sea-green algae oil were tested and confirmed; the values were within standard limits. Correspondingly, the green catalyst reusability, evaluated across 5 experimental trials, resulted in biodiesel yields greater than 50% compared with other reference studies. The definitive screening design analysis reported that the predicted value of R^2 is 97.52 % and Adj. R^2 value is 96.99 % which closely matches the experimental values. Finally, the addition of the green catalyst Moringa oleifera led to a maximum biodiesel yield.Thus, experimental and predicted outputs indicate the potential for utilizing sea green algae oil biodiesel as a renewable fuel in existing diesel engines, serving as an alternative to fossil fuels.

Keywords: Sea green algae, definitive screening design, heterogeneous catalyst, moringa oleifera, catalyst reusability

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1. Introduction

Currently, renewable fuels are drawing extensive attention to operate in the existing diesel engines, as they are eco-friendly fuels, emit low emissions, and mitigate climate change [1]. In earlier days, petro-diesel-derived fossil fuels were considered the primary fuel source, as they are highly efficient, have low cost, and are available [2]. Due to the upsurge in the global population [3], the need for vehicle transportation increased, which resulted in the scarcity of the supply of petro diesel-derived fossil fuels, leading to the exhaustion stage. However, in the present decade, fossil fuels hold 80% of the share in the energy requirement, but due to the concerns that arose, this share will gradually decrease to 50% by 2050 [3],[4]. Furthermore, the toxic exhaust emissions such as CO_2, NOx, SOx, particulate matter, smog, smoke, etc., released from various

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internal combustion engines affected the air quality [5]as a green and carbon-free hydrogen energy carrier, plays an important role in clean energy applications, while the addition of biofuel can also reduce the carbon footprint of engine. Therefore, the ammonia/biodiesel dual-fuel (ABDF, [6], and witnessed global climatic changes [7] [8]as well as the equipment employed. In this research, three liquefaction cycles with different pre-cooling cycle and cryo-cooling cycle have been evaluated. The use of organic Rankine cycle (ORC resulting in adverse effects on human health hazards. Therefore, to mitigate these concerns, there is a need for clean green fuels, known as biodiesels [1].

Biodiesels are biodegradable, eco-friendly, and less toxic, making them promising substitutes for petroleum-derived fossil fuels. These are categorized into three generations, namely, first (1^{st}) generation biodiesel comes under conventional edible oils, second (2^{nd}) generation falls under the category of traditional non-edible oils, and third generation (3^{rd}) represents the algae biodiesel [9]. The 1^{st} generation of edible oils faced the fuel vs. food issue. In contrast, the 2^{nd} generation of non-edible oils faced a few challenges, such as availability, cultivation, low percentage of oil yield, and high free fatty acid composition (FAC). These concerns led to the emergence of clean, green, renewable third-generation algal biodiesel. Compared with the 1^{st} and 2^{nd} generation biodiesel, the 3^{rd} generation algae do not require excess land for their growth, possess high oil yield capacity, can grow in wastewaters, saline waters, sea waters, freshwaters, saline, brackish waters, lakes, ponds, rivers, and reservoirs [10]along with growing environmental concerns, has accelerated the search for renewable energy sources. Marine macroalgae, also known as seaweeds, have gained significant attention for their potential to address these challenges due to their rapid growth, high carbohydrate content, and absence of lignin, which simplifies biofuel production. This review hypothesizes that marine macroalgae offer a more sustainable and economically viable alternative to conventional biofuel sources due to their unique biochemical properties and ecological benefits. The objective of this review is to assess the potential of seaweed-based biofuels, focusing on their role in energy conservation, climate change mitigation, and environmental sustainability. The study highlights macroalgae's carbohydrates and lipids, essential for biodiesel and bioethanol production. A comprehensive analysis of macroalgae cultivation techniques and biorefinery processes is presented, exploring whether optimized processing methods can enhance biofuel yields while minimizing environmental impacts. Additionally, the paper now incorporates life cycle assessment (LCA,[11],[12]this process will require the implementation of inexpensive harvesting methods and productive algal strains that are tolerant to harsh conditions. The use of freshwater in the large-scale cultivation of microalgae is currently unsustainable; hence, the studies which target the use of seawater medium can reduce the demand of freshwater. Chlorella pyrenoidosa has been extensively studied in freshwater. However, information on the cultivation of C. pyrenoidosa for growth productivity in recycled seawater medium, generated after harvesting through autoflocculation is scarce. In this context, the present study investigates the effect of nutrients sufficient seawater medium following reuse of nutrients exhausted seawater medium on the biochemical composition, biomass and lipid productivities of an in-house developed, thermotolerant strain of C. pyrenoidosa under high temperature (45 °C.

Algae are known as one of the oldest living creatures, and they are used in multipurpose applications, such as protein supplements and medicines, as biofuel to operate in automobile engines [13]. The research exploration on the algae species was initiated at the close of the 19th century. It has been estimated that more than 64,000 algae species have been identified worldwide; among these, nearly 35000 species were recognized as having the potential to generate biodiesel [10],[14]. Algae have high lipid content, ranging from 40 to 80% and higher triglyceride content than seed crops. Thus, if the correct species is identified, more than 85% of the oil can be extracted [15]. In India, many coastlines, rivers, ponds, and reservoirs are found in several locations. Based on the natural sunlight, carbon dioxide, and weather patterns, there are seven categories of algae, viz., green, red, brown, red-green, blue-green, phytoplankton, sea-weeds, and marine-algae, which grow naturally in fresh and seawater bodies [6],[16].

It is known that the earth's surface is covered with 72 % of the oceans, in which algae remain the primary producers [10]. Algae grown in marine waters are enumerated as sea-green algae (SGA), red-algae, blue-green algae, green-algae, and seaweeds [17],[18]. These algae were grown under the sea, and the sea-green algae were grown on the marine rocks. These draw a lot of possibilities for the mass production of algae biomass in seawater with complete use, such as seawater temperature management, wave energy for mixing, and culture media preparation [12]this process will require the implementation of inexpensive harvesting methods and productive algal strains that are tolerant to harsh conditions. The use of freshwater in the large-scale cultivation of microalgae is currently unsustainable; hence, the studies which target the use of seawater medium can reduce the demand of freshwater. Chlorella pyrenoidosa has been extensively studied in freshwater. However, information on the cultivation of C. pyrenoidosa for growth productivity in recycled seawater medium, generated after harvesting through autoflocculation is scarce. In this context, the present study investigates the effect of nutrients sufficient seawater medium following reuse of nutrients exhausted seawater medium on the biochemical composition, biomass and lipid productivities of an in-house developed, thermotolerant strain of C. pyrenoidosa under high temperature (45 °C. Marine algae resemble plants and are typically found along the coast, adhering to rocks or other hard surfaces. Marine algae growing on sea rocks are classified as sea green algae (SGA), which resemble green algae and have become an interesting source of third-generation biofuel. These marine algae are freely available and have the potential to meet the energy crisis [11],[18].

Therefore, algae grown in marine waters are considered a significant source for producing 3rd generation biodiesel since they possess high oil yields with less input and are also cost-effective. Godvin

Sharmila et. al. [18] reviewed biofuel production using marine algae resources. Their study highlighted that algae grown in marine waters are suitable for biodiesel production and discussed different types of algae, such as green, brown, and red seaweeds, as well as the challenges encountered during cultivation, harvesting, and biofuel production. The study reported that the third-generation algae biodiesel was a sustainable fuel source to replace fossil fuels.

Furthermore, various optimization techniques, such as AI - artificial intelligence, ANN - artificial neural networks, RSM - Response Surface Methodology, DSD - Definitive Screening Design, the Taguchi approach, ANOVA, and others have been identified as prominent tools for analyzing biodiesel yield more effectively and for keeping pace with the latest developments. These tools help to assess the influencing parameters like RT - reaction temperature, MR - molar ratio, T - time, and CC - catalyst concentration [19]. Among these factors, catalyst concentration is vital for obtaining biodiesel.

Apart from the above applications, these methodologies are applied to optimize, the engine combustion, in medical and drug delivery, simulation of heat transfer in the body, where the governing equations of heat exchange are solved using Akbari–Ganji method (AGM), python [20] using semi analytical methods, the heat exchange was analyzed [21], [22] using ANOVA analysis, the dimensionless numbers are investigated [23].

Sunil Kumar et.al. [24] presented the transesterification followed by the performance and emission analysis results using Jatropha–Algae oil biodiesel. Based on their study, a comparative modelling analysis using an ANFIS - adaptive neuro-fuzzy inference system and the RSM - response surface methodology was carried out, varying the process parameters: catalyst concentration, molar ratio, temperature, and reaction time. The output results demonstrated that, under a molar ratio of 6–12, KOH of 0–2% w/w, time of 60–180 min, and temperature of 35–55 ^{o}C, the R^2 value was observed to be 0.2867 using RSM and 0.998 using ANFIS. The study reported that the tested biodiesel blends (B0, B5, B10, and B20 vol%) significantly reduced emissions.

Another researcher, Y. Rajesh et.al. [25] implemented DSD, an optimization technique to achieve maximum biodiesel yield. In their study, fried palm oil was used to produce biodiesel, and the maximum biodiesel yield of 96.23 % was attained at an MR of 6:1, RT of 55 ^{o}C, and the CC of 1 wt.%. Their study concluded that using the DSD optimization technique reduced production costs and improved biodiesel yield.

Thus, evidence from the literature indicates that optimization techniques play a vital role in enhancing the biodiesel yield, and that process variables such as RT, CC, MR, and T are significant parameters for converting the raw oil into biodiesel. Among these process parameters, catalyst selection has an impact on biodiesel production.

Generally, catalysts are classified into two types: homogeneous and heterogeneous. As a general practice, homogeneous catalysts, such as NaOH and KOH, are most frequently used because these are readily available and exhibit high catalytic activity. However, due to the corrosive nature and large water requirement during the washing process, the homogeneous catalyst is expensive to use in transesterification [26]biodiesel has attracted attention worldwide as an eco-friendly alternative to fossil fuel for being renewable, non-toxic, biodegradable, and carbon-neutral. Although the homogeneous catalyst has its own merits, much attention is currently paid toward the chemical synthesis of heterogeneous catalysts for biodiesel production as it can be tuned as per specific requirement and easily recovered, thus enhancing reusability. Recently, biomass-derived heterogeneous catalysts have risen to the forefront of biodiesel productions because of their sustainable, economical and eco-friendly nature. Furthermore, nano and bifunctional catalysts have emerged as a powerful catalyst largely due to their high surface area, and potential to convert free fatty acids and triglycerides to biodiesel, respectively. This review highlights the latest synthesis routes of various types of catalysts (including acidic, basic, bifunctional and nanocatalysts,[27]. Thus, heterogeneous catalysts have attracted increasing interest in the transesterification process to overcome these challenges. The heterogeneous catalyst is suitable for reuse and recycling. Many heterogeneous catalysts are available at low cost, including eggshells [28], moringa leaves [27], orange peel [19], and metal oxides such as αFe_2O_3/CuO nano catalysts [29].

In addition, the 2nd and 3rd generation biodiesel feedstocks were prepared by using homogeneous or heterogeneous catalysts that are mixed with additives such as oxygenated additives, metal oxides, nano fluids, and hybrid nanofluids [2], which enhance the engine performance characteristics and mitigate engine exhaust emissions. Numerous researchers experimented with different second (2nd) and third (3rd) generation biodiesels by mixing heterogeneous catalysts. This garnered significant interest and offered a cost-effective, improved transesterification process. Another researcher, Onome Ejeromedoghene et.al. [7] reviewed various non-vegetable biodiesel feedstocks, for instance, lipids from microalgae, and waste material as a heterogeneous catalyst. Their review studies emphasized biodiesel production, environmental pollution, and economic sustainability. The results showed that 98% of the biodiesel was produced from non-vegetable oils, using waste materials as heterogeneous catalysts, which could be reused and recycled for repeated experiments.

Tarigan JB et. al. [30] used banana peels as a heterogeneous catalyst and mixed them in palm oil to produce biodiesel. The results showed that the potassium oxide (K_2O) was the main constituent of the calcined waste banana peel. The maximum biodiesel yield was 97.7 ± 0.6% and 90.0 ± 2.3% at a 1:15 molar ratio, time of 30 mins, 7 wt.% catalyst concentration. Another researcher, Kalyani et. al. [28], used chicken eggshell as a heterogeneous catalyst, mixed in Spirogyra green algae oil, and achieved 96.18 % of the biodiesel yield at a

10:1 molar ratio (MR), 0.6 wt.% heterogeneous catalyst concentration (CC), 48 °C reaction temperature (RT), and 18 mins. The study concluded that the heterogeneous catalyst was a significant factor.

Ahmad Galadima et. al. [31] discussed the potential of algae oil, and a critical review was conducted to prepare algae biodiesel using a heterogeneous catalyst. A detailed review was conducted on the utilization of homogeneous and heterogeneous catalysts. Homogeneous catalysts such as KOH, KOCH, Ca (OH), and Mg (OH) were used for transesterification. Also, acids, viz., sulphuric acid (H_2SO_4), HCL, etc., are reported with a lower reaction rate, a high oil to alcohol ratio, catalyst concentration, which are reported to be expensive and corrosive in nature. These concerns drove the researchers to explore greener heterogeneous catalysts, as they are low-cost and less corrosive.

In addition, various researchers have shown preference for mixing algae biodiesel feedstock with heterogeneous catalysts during the transesterification process. Therefore, the selection of various second and third-generation biodiesel feedstocks [32], [33]. Especially, the production of algae biodiesel plays a vital role as a bioenergy source for IC engine applications, which results in the mitigation of environmental pollution, reduction in engine exhaust emissions, and better combustion in CI engines [34],[35].

The literature indicates that heterogeneous catalysts greatly mitigate the issues faced by homogeneous catalysts. Initially, the heterogeneous catalyst is in solid form and is mixed in various phases to obtain the liquid form. Few studies have investigated the use of sea green algae (SGA) combined with a calcined Moringa oleifera catalyst to produce biodiesel. Moringa oleifera leaves, also known as the "miracle tree" [36], are identified as rich in minerals, contain high percentages of potassium, calcium, and magnesium, and are used in medical applications [37] and grown abundantly in tropical countries like India and the United States.

- This study aims to extract algal oil and produce biodiesel from sea-green algae that naturally grow on marine rocks in marine water resources.
- A novel combination, Moringa oleifera, a heterogeneous catalyst, was mixed with the sea green algae oil (SGAO) to produce sea green algae oil biodiesel (SGAOBD).
- The obtained SGAOBD was tested to fuel properties, fatty acids, and elemental composition.
- A definitive screening design (DSD) was implemented to optimize biodiesel production from sea green algae and to obtain precise results, using the statistical software Minitab-21. Using the DSD optimization technique, the experimental and response biodiesel yields of SGAOBD can be estimated by regression equations.
- A Pareto chart was generated to determine the standardized effects of the contributing process parameters. Further, the optimum values were produced using the response optimizer in DSD, and an optimization plot for maximum yield was generated to identify the optimum contributing factors.
- Additionally, surface and contour plots were generated to analyse the effect of reaction temperature, catalyst concentration, and molar ratio on the yield.
- Further, reusability tests were conducted on the calcined heterogeneous catalyst to assess variation in biodiesel yield.

Thus, using sea green algae oil biodiesel is a positive approach: third-generation algae biodiesel can serve as fuel in existing diesel engines, providing an alternative to fossil fuels and helping to regulate engine exhaust emissions.

2.Materials and methods

The chemical solutions methanol (CH_3OH) and n-Hexane of the analytical grade of 99.8% pure were acquired from Sigma-Aldrich Chemicals in Visakhapatnam, India. The following apparatus were used in the Thermal Fuel Laboratory, Raghu Engineering College (latitude 17°99'34.6"; longitude 83°41'52.8"): hot air oven, conical flasks, measuring jars, Soxhlet apparatus, steam-distillation unit, round-shaped heater, separatory flask, beakers, filter papers, distilled water, thermometers, muffle furnace, hot-plate magnetic stirrer with temperature control, tripod stand, and glassware. The Moringa oleifera leaves, used as heterogeneous catalysts, were obtained from the farmland of Raghu Engineering College, Visakhapatnam, at 17°99'44.8" latitude and 83°41'41.8" longitude.

3. Processing techniques of algae

Algae processing techniques have recently attracted interest, as these are eco-friendly and possess high oxygen and oil content. Therefore, the following processing techniques were applied to produce biodiesel: algae collection, harvesting, oil extraction, and biodiesel production [38]. Therefore, a flow chart representing the methodology used in the present study, from the processing techniques of algae to the application of the optimization technique, Definitive Screening Design (DSD), is presented in Figure 1.

4. Algae collection

The sea green algae naturally grown on the rocks of the marine water resources, Figure 2(a-b), were collected from two (2) coastal areas for 120 days, i.e., from January to April 2025, during the natural sunlight range at 23 °C and 28 °C. The sea-green algae were collected at two locations: the first (Figure 2a), near Bheemili Beach Road, Bheemunipatnam, at 17°88'90.7" latitude and 83°45'49.4" longitude; the second (Figure 2b), at 17°71'42.8" latitude and 83°32'38.2" longitude, near Beach Road, Yoga Village, coastal areas, Pandurang Puram, Visakhapatnam.

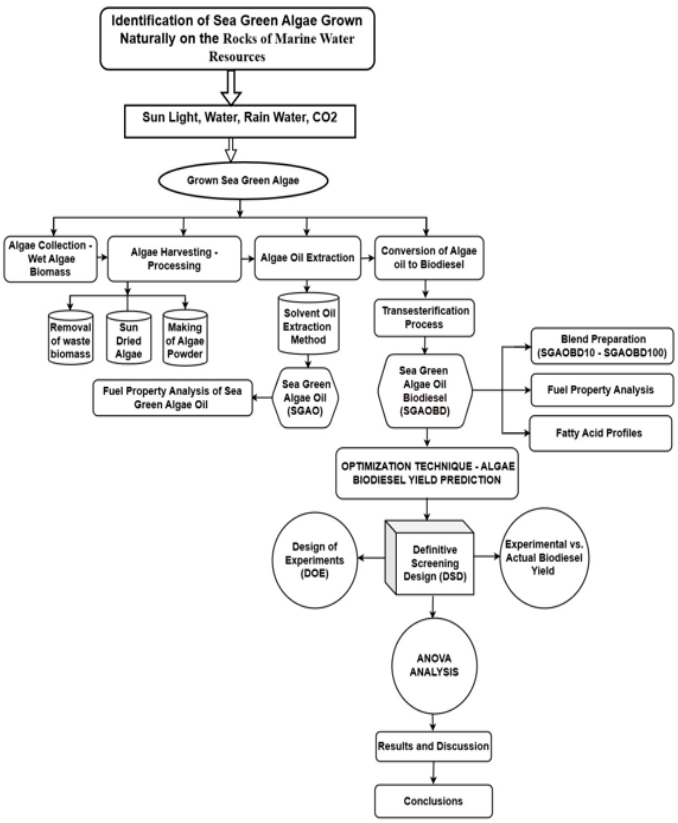


Figure 1. Flow chart representing the methodology of the present study

a. 17°88'90.7" latitude and 83°45'49.4" longitude – near Bheemili beach road, Bheemunipatnam

b. 17°71'42.8" latitude and 83°32'38.2" longitude - near the beach road, yoga village, coastal areas, Pandurang Puram, Visakhapatnam.

Figure 2 (a-b). Locations for collection of sea green algae

5. Algae harvesting

The sea-green algal blooms collected from the two identified locations, as shown in Figure 3 (a-b), were gently harvested and cleaned to remove the mud and stones attached to the algae (Figure 3c). A measured weight of 96 kg of algae blooms was harvested; after washing with distilled water, they weighed 52 kg. The algae blooms were segregated into each layer-wise and were sun-dried in the natural light for 5 days, Figure 3d, with an average time of 7 – 8 hours and temperature of 22 °C to 30 °C. The sun-dried algae were kept in a hot air oven by setting the temperature to 90 °C, to remove excess moisture, as represented in Figure 3e. Further, the dried sea-green algae were ground into a fine powder (Figure 3f–g). Lastly, the net weight obtained after grinding was 7500 grams, i.e., 7.5 kg.

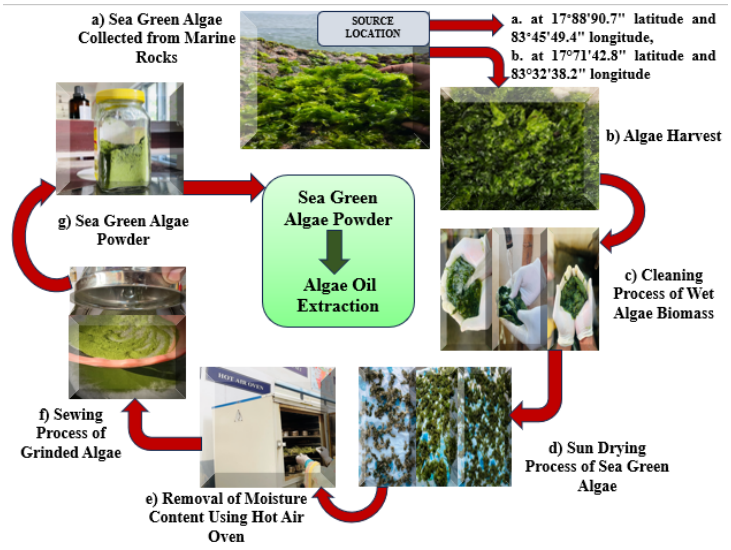
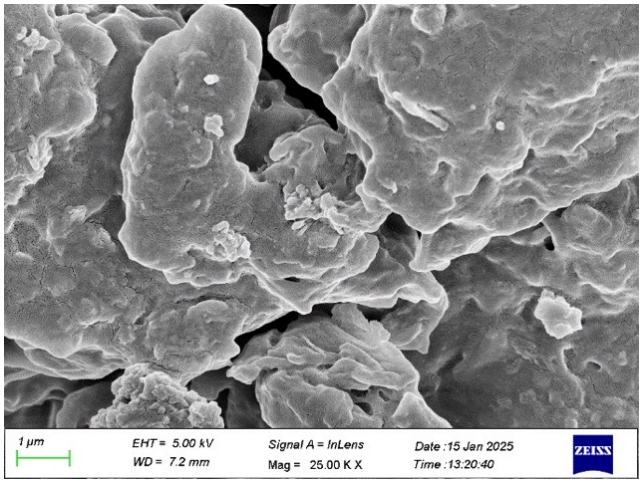


Figure 3 (a-g). Stages of sea green algae - from collection to dried algae powder

6.Sem representation of sea green algae

The SEM images of the sea green algae (SGA), captured at different magnifications to reveal their shape and structure, were obtained using an OXFORD test instrument, as shown in Figure4 (a-b). Results from the test instrument indicate that the morphological structure of the sea green algae exhibits a large surface area.

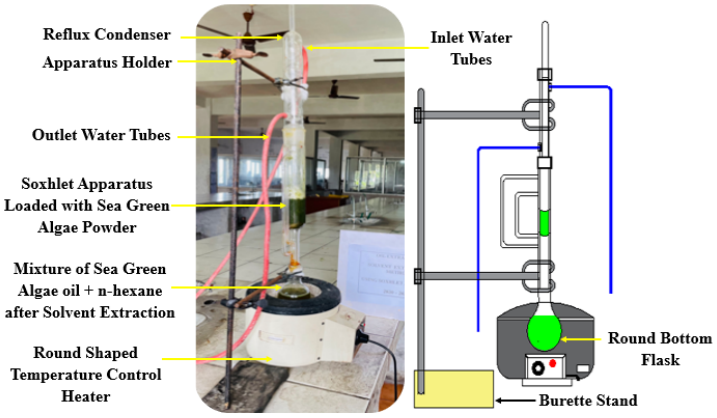


(a) (b)

Figure 4(a-b). Morphological structure of sea green algae

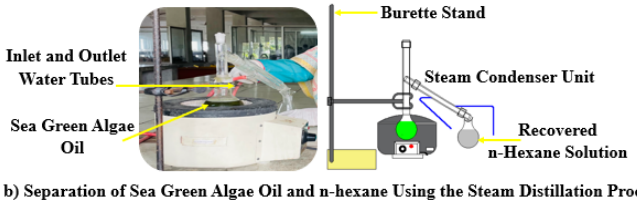
7. Oil extraction

Oil extraction is used to convert the dried algae from a solid into a liquid form. In the present study, the solvent oil extraction process is used to extract the Sea Green Algae Oil (SGAO) from the fine-dried algae powder [39]. The SGAO extraction was conducted at 83°40′09.43′′E (83.400943) longitude and 17°98′86.02′′N (17.988602) latitude. The experimental setup is shown in Figure 5a–d.



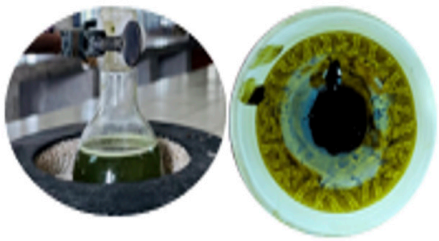
a) Experimental Setup of Solvent Oil Extraction Process Using Soxhlet Apparatus

a) Experimental setup of the solvent oil extraction process



b) Separation of Sea Green Algae Oil and n-hexane Using the Steam Distillation Process

b) Separation of sea green algae oil and n-hexane solvent using the steam distillation process



c) Sea green algae oil

d) Recovered n-hexane solution(reusability)

Figure 5 (a-d). Stages of the sea green algae oil extraction process

Under optimum conditions, a measured quantity of dried sea green algae powder was mixed with n-hexane at a proportion of 1:2 (300 grams algae powder: 600 ml solvent). [39], [40]. The solvent n-hexane was heated in a bottom flask of round shape at 65 °C, and converted into vapors, which were transferred to the reflux condenser. The vapors gradually condensed, and the droplets interacted with the algae powder in the Soxhlet housing chamber forming a warm solvent that extracted algae oil, as shown in Figure 5a. Furthermore, the experimental procedure for oil extraction is repeated several times over approximately 18 hours. Using steam distillation, the SGAO and the solvent n-hexane are separated, as depicted in Figure 5 (b–d). Using this method, 90% of the solvent was recovered and can be reused for another extraction. Furthermore, the procedure is continued until the dried algal powder becomes pale. Finally, the obtained SGAO is stored in an airtight container. Thus, the SGAO yield efficiency was measured using Eq. 1.

$$\text{SGAOYieldEfficiency(wt.\%)} = \frac{\text{Mass of the sea green algae oil extracted(grams)}}{\text{Total mass of the dried sea green algae powder}} \times 100 \quad (1)$$

8. Catalyst preparation

One of the main steps in the preparation of heterogeneous catalysts is calcination. In this work, dried Moringa oleifera leaves were used as a heterogeneous catalyst. The fresh leaves were cleaned with filtered water to remove dust and contaminants, as shown in Figure 6. For five days, the cleaned leaves were exposed to the sun to eliminate any remaining moisture [27]. Further, the dried leaves were placed in the hot air oven for 8 hours to ensure the moisture content was eliminated [28]. Therefore, a fine powder was made from the dried leaves and calcinated using a muffle furnace at temperatures of 300 °C and 600 °C for three (3) continuous hours by maintaining the rate of heating for 5 °C/min [19], [30]. Following meticulous examination, the fine powder of the moringa oleifera at various temperatures was observed to be fully decayed at 600 °C [27]. Figure 6 shows the detailed synthesis of the heterogeneous catalyst from Moringa oleifera.

Figure 6. Step-by-step preparation of the moringa oleifera leaves as a heterogeneous catalyst

Thus, the calcined catalyst was used to produce the sea-green algae oil biodiesel (SGAOBD). Further, EDX analysis was conducted to characterize the heterogeneous catalyst.

9. Biodiesel transesterification

After the steam distillation process, the obtained SGAO product is heated till the temperature reaches 110 °C to remove the H_2O content. One of the most popular methods, known as the transesterification process [41], is used to prepare algae biodiesel [42]. Firstly, the raw SGAO was poured into a conical flask (Figure 7a). The prepared heterogeneous catalyst was mixed in the Methanol (CH_3OH) solution to form a methoxide solution, as illustrated in Figure 7b. Now, the prepared solution was placed on a controlled heater with a hot-plate magnetic stirring device and heated between 55 to 60 °C until it attains the required temperature, Figure 7 (c – d).

Further, the triglycerides in the oil will transform into diglycerides throughout this process, and the diglycerides will turn into monoglycerides, ultimately forming esters and glycerin [43]. The hot

SGAOBD and glycerin were separated in a separatory flask, as shown in Figure 7e. The acquired SGAOBD was rinsed with purified water until the soap solution was removed (Figure 7f). Further, the obtained biodiesel was heated without stirring until it reached 100 °C to eliminate the H_2O content present in the SGAOBD. The recovered heterogeneous catalyst was reused for the subsequent experimental tests, as shown in Figure 7g. Therefore, the experimental SGAOBD yield was assessed [43] from Eq. 2.

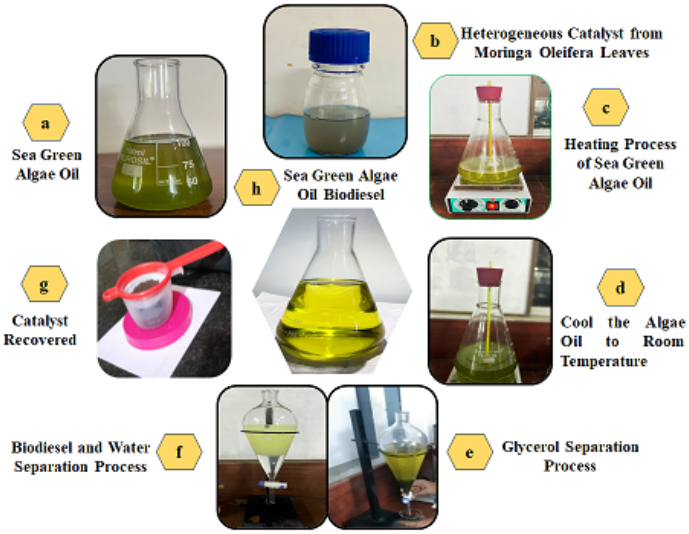


Figure 7(a-h). Stages of sea green algae biodiesel production using a heterogeneous catalyst

$$\text{ExperimentalBiodieselYield(Im)} = \frac{\text{WeightofSEAGREENAlgaeBiodieselYield(SGAOBD)Produced}}{\text{WeightofSEAGREENAlgaeOil(SGAO)}} \times 100 \quad (2)$$

The procedure was repeated by varying three process variables, reaction temperature, catalyst concentration, and molar ratio according to a design of experiments (DOE) implemented in Minitab-21 statistical software, as shown in Tables 1 and 2. Finally, the SGAOBD obtained was characterized for various fuel properties (Table 7) and was blended with diesel.

10. Blending of sea green algae oil biodiesel with diesel

The SGAOBD, produced through the transesterification process, was blended with diesel in ratios ranging from 10% to 90% in 10% volumetric increments, as shown in Figure 8. For clarity, SGAOBD10 denotes a blend comprising 100 ml of sea green algal oil biodiesel (SGAOBD) and 900 ml of diesel. Similarly, for SGAOBD20, SGAOBD30, SGAOBD40, SGAOBD50, SGAOBD60, SGAOBD70, SGAOBD80, SGAOBD90 and SGAOBD100.

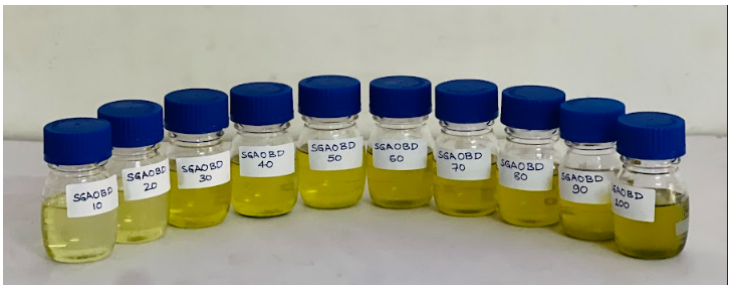


Figure 8. The blending of sea green algae oil biodiesel with diesel

Thus, SGAOBD blends were evaluated for key fuel property values according to ASTM standards.

11.Definitive screening design - design of experiments

The present study implemented a Definitive Screening Design (DSD) using a 3-factor, 2-level fractional factorial design, an optimization technique [25]. The reaction temperature (A), catalyst concentration (B), and molar ratio (C) are the three contributing parameters selected to attain maximum biodiesel yield and to identify the most significant process parameters. The ranges of the 3 process parameters are displayed in Table 1.

A total of 18 experimental trials were conducted in random order by varying the 3 process variables. The maximum and minimum values for experimental and response biodiesel yield (%) are shown in Table 2.

Table 1. Ranges of process variables

S. No.	Variables	Unit	Minimum Value	Maximum Value
A	Reaction temperature (RT)	°C	39	59
B	Catalytic concentration (CC)	Gram (wt.%)	3.5	9.5
C	Molar Ratio (MR)	-------	4:1	12:1

Table 2. Design matrix of process variables for experimental and predicted responses

Experimental Runs	Reaction Temperature (RT)	Catalytic Concentration (CC)	Molar Ratio (MR)	Biodiesel Yield Percentage (%)	
				Experimental Yield	Response Yield
1	39	3.5	4	74.64	75.40
2	59	9.5	8	94.87	95.44
3	39	6.5	4	78.98	78.09
4	49	3.5	4	81.01	81.84
5	49	6.5	8	84.66	86.30
6	59	3.5	4	88.14	88.28
7	39	3.5	8	76.11	77.17
8	49	9.5	12	92.13	90.77
9	39	9.5	12	84.57	84.33
10	39	3.5	12	78.99	78.94
11	39	9.5	4	81.25	80.79
12	59	9.5	4	94.36	93.67
13	59	3.5	8	91	90.05
14	59	6.5	12	94.88	94.52
15	49	6.5	4	85.73	84.53
16	49	3.5	12	87.12	85.38
17	59	9.5	12	94.99	97.21
18	39	6.5	8	79.12	79.86

11.1. Analysis of variance - ANOVA

ANOVA analysis identifies the influencing process parameters [44] from three contributing factors. This analysis examines the significance of contributive parameters by analyzing the response yield at several levels [43], based on the experimental yield matrix. Thus, Table 3 presents the ANOVA for the influencing parameters.

The parameters influencing the biodiesel yield from sea-green algae oil are those with the lowest P-values and the highest F-values. Table 3 reports that P-values are 0.000 for all three contributing parameters, indicating that the design is reliable; the highest F-value was observed for reaction temperature. Lastly, the model's F-value of 183.69 and P-value of 0.000 indicate that it is statistically significant.

Table 4 shows that the T-value specifies the individual role of each parameter and demonstrates that the reaction temperature makes the greatest contribution to the yield, followed by the heterogeneous catalyst concentration. The 'P' value was observed to be 0.000 in all cases, which confirms that the design is safe. A Pareto chart (Figure 9) was generated to represent the standardized effects of contributing factors and their confidence levels.

Table 3. Analysis of variance

No.	Source	DF	Adj-SS	Adj-MS	F-value	P-value
A	Model	3	779.2	259.733	183.69	0.000
B	Linear	3	779.2	259.733	183.69	0.000
C	Reaction Temperature (RT)	1	523.35	523.347	370.12	0.000
D	Catalytic Concentration (CC)	1	90.08	90.078	63.70	0.000
E	Molar Ratio (MR)	1	39.65	39.646	28.04	0.000
F	Error	14	19.8	1.414		
	Total	17	798.99			

Table 4. Definitive screening design analysis

No.	Terms	Coeff.	SE Coeff.	T-value	P-value	VIF
A	Constant	86.303	0.282	306.34	0.00	
B	Reaction Temperature (RT)	6.440	0.335	19.24	0.00	1.03
C	Catalytic Concentration (CC)	2.695	0.338	7.98	0.00	1.04
D	Molar Ratio (MR)	1.773	0.335	5.3	0.00	1.03

11.2. Pareto chart

This chart provides information on the absolute values of the standardized effects from highest to lowest value, and the null-hypothesis is tested using standardized effects [25]. Figure 9 shows a reference line denoted α.

(Where Factor A represents Reaction Temperature, B represents the catalytic concentration, and C represents the molar ratio)

It is evident from Figure 9 that all three contributing factors (RT, CC, and MR) crossed the reference line. The graph confirms that these parameters are statistically significant at a 95 % confidence level, indicating that the null hypothesis can be rejected.

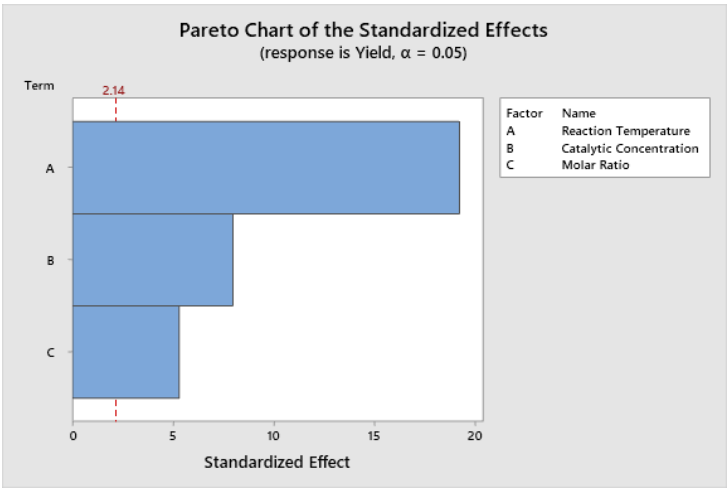


Figure 9. Pareto chart of standardized effects for the process parameters

11.3. Coefficient of determination

The reliability of the selected statistical model fitting can be inferred from the accuracy of the model, which was assessed with the aid of the coefficient of determination, R^2, and Adj R^2 [45]. As tabulated in Table 5, the 'S' denotes the standard deviation between the data and the fitted values; i.e., the smaller the 'S' value, the more accurate the model response. In addition, the 'R-sq.' quantifies how well the model fits the experimental data and indicates the percentage of variation in the responses explained by the model. In this analysis, higher R-sq. values indicate a safer design.

Table 5. Value of R-sq. for the optimized model

No.	S	R-sq.	R-sq.(adj)	R-sq. (pred)
1	1.18911	97.52 %	96.99 %	96.04 %

Thus, DSD analysis showed that the R-sq. value of 97.52 % exceeded the 95% confidence level, indicating that the definitive screening design (DSD)model fits the data. A graph was plotted representing the actual (x-axis) and predicted values (y-axis) of the biodiesel yield [24],[45] as displayed in Figure 10.

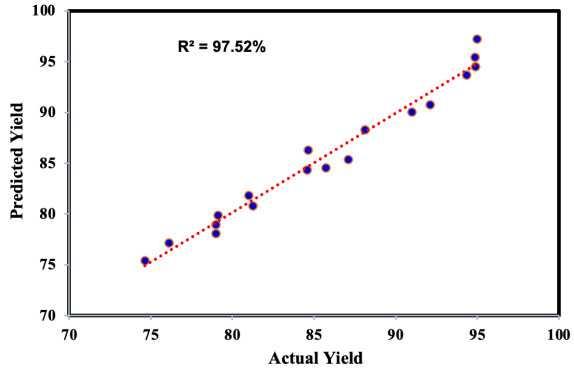


Figure10. Yield interpretation of actual and predicted values

12. Results and discussion

12.1. Sea green algae oil yield estimation

A total of 96 kg of sea green algae (SGA) were collected from January to April 2025, over 120 days, at two different locations. The sea-green algae were cleaned, yielding a net weight of 52 kg. Further, the algae were sun-dried and pulverized into a fine powder, yielding a net weight of 7500 grams, i.e., 7.5 kg. A sample of 300 gms of dried sea green algae powder, mixed with 600 ml of n-hexane solvent, was ready for the lab-scale oil extraction, following 1:2 ratio. After each solvent oil extraction, 69 ml of sea green algae oil (SGAO) was obtained. Finally, for 7500 gms of dried algae powder and 1819 ml of SGAO, a yield efficiency of 24.25% was achieved and was computed using Eqs. 1, 3, and 4.

$$\text{SGAOYieldEfficiency} = \frac{1819}{7500} \times 100 \quad (3)$$

$$\text{SGAOYieldEfficiency} = 24.25\ \% \quad (4)$$

12.2. Sea green algae oil biodiesel yield estimation

The yield of sea green algae oil (SGAO) obtained by solvent extraction was 24.25%. A further 1819 ml of raw SGAO was converted to algae biodiesel using the transesterification method. Hence, from 1728 ml of SGAOBD, the experimental biodiesel yield was 94.99%. The calculations were formulated in the equations below (Eqs. 2, 5, and 6).

$$\text{ExperimentalBiodiselYield(Im)} = \frac{1728}{1819} \times 100 \quad (5)$$

$$\text{ExperimentalBiodiselYield(Im)} = 94.99\ \% \quad (6)$$

12.3. Model fitting – effect of definitive screening design

According to the Definitive Screening Design (DSD) optimization method [25], experiments were conducted using the three process parameters as shown in Table 1. With the intent to determine the optimum combination [24] and to find the impact of parameters on yield, the results are presented in Table 2, where the process parameters are varied for 18 Design of Experiments. The minimum and maximum experimental values of SGAOBD were 74.64% and 94.99%, respectively. Similarly, the minimum and maximum predicted values of SGAOBD were 75.40% and 97.21%, respectively. Therefore, at reaction temperature 59 °C, 9.5 weight percent CC, and 12:1 MR, attains highest experimental biodiesel production of 94.99% is attained % and the highest response biodiesel yield achieved was 97.21%. The mathematical model, derived from the predicted response values expressed as yield, is given in Eq.7. Therefore, three confirmatory assessments conducted under similar conditions indicated that 97.14% of the average SGAOBD yield was attained.

12.4. Maximum yield determination

The biodiesel yield is the weight of the biodiesel produced divided by the weight of the source oil. From Table 2, the optimum values are arrived at using the response optimizer in DSD [25]. From the optimization plot (Figure 11), it is confirmed that at 59 °C reaction temperature (RT), 9.5 wt.% catalytic concentration (CC), and 12:1 molar ratio (MR), the optimum values were identified. In addition, Table 6 shows the contributing factors at the optimum level.

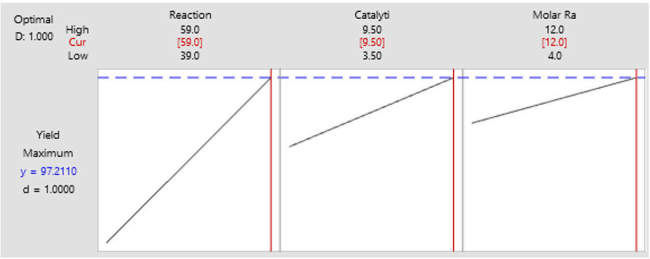


Figure 11. Optimization plot for yield

Table 6. Contributing factors at the optimum level

No.	Parameters	Value
A	Reaction Temperature	59
B	Catalytic Concentration	9.5
C	Molar Ratio	12

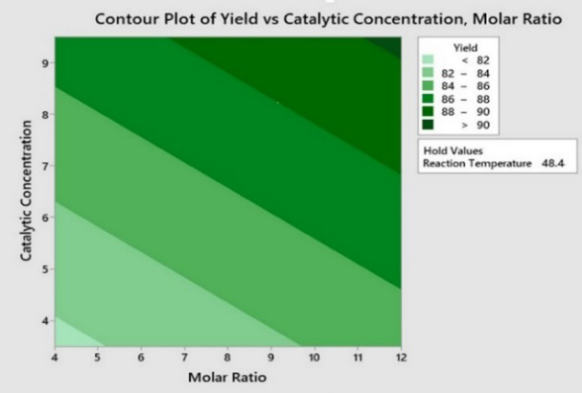
Based on the three contributing factors, the optimum yield of the SGAOBD was determined from the regression equation (Eq.7) [28].

Regression Equation

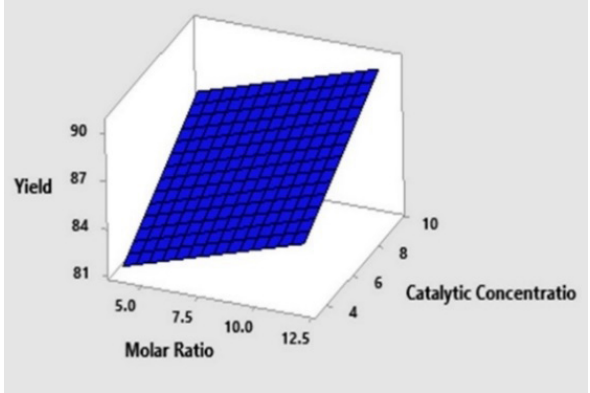
Biodiesel Yield = 45.36 + 0.6440 Reaction Temperature + 0.898 Catalytic Concentration + 0.4431 Molar Ratio = 97.21% (7)

12.5. Effect of molar ratio on yield

During the transesterification process, the molar ratio (MR) plays a significant role because a large mass proportion of reactants promotes greater interaction between oil and methanol molecules. The methyl ester yield can be raised by increasing the MR to an optimum level, provided the percentage of methanol does not increase beyond the desired level for a given mixture, which leads to a reduction in biodiesel [25]. Therefore, it is evident from the contour and surface plots [24], [45] Figure12 (a & b) that more than 90 % of the SGAOBD yield was attained at a molar ratio of 12:1, catalyst concentration of 9.5 wt.%, at a constant temperature of 48.4 °C.



(a)



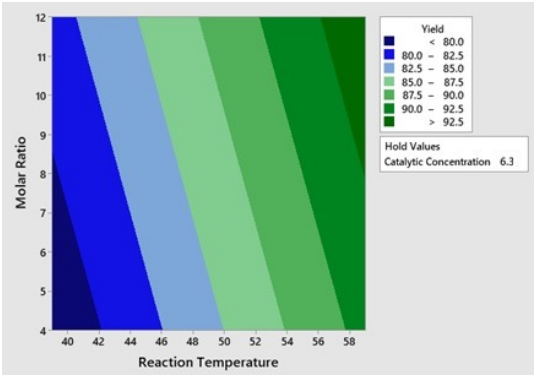
(b)

Figure12(a). Contour plot of yield vs. molar ratio and catalytic concentration

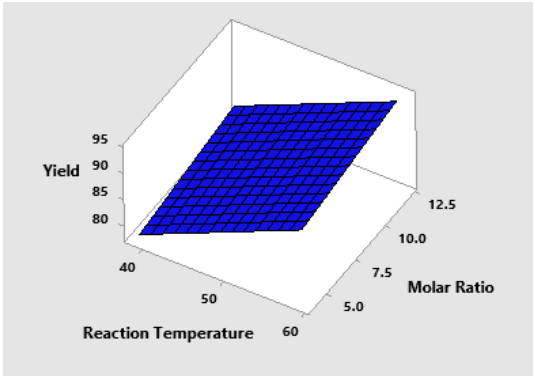
Figure12(b). Surface plot of yield vs. molar ratio and catalytic concentration

12.6. Effect of reaction temperature on yield

Figs.13a and 13b represent the contour and surface plots of SGAOBD at different reaction temperatures and molar ratios. The reaction temperature and the alcohol percentage in the mixture are critical for achieving the maximum biodiesel yield. At huge reaction temperature (<62 °C), the rate of reaction in the given mixture is higher and the contributing yield will rise. However, it is crucial to maintain the reaction temperature (RT) below the boiling point of the methanol (64 °C) to attain maximum biodiesel yield. Thus, it is evident from the above Figure13 (a & b), more than 92.5 % of SGAOBD yield was attained at a reaction temperature of 59 °C, molar ratio 8:1, by maintaining a constant catalytic concentration of 6.3 wt.% (hold value).



(a)



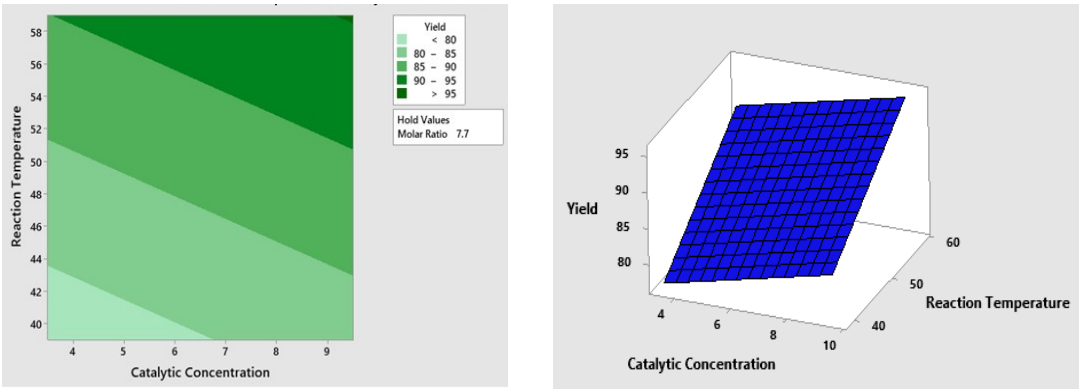
(b)

Figure13(a). Contour plot of yield vs. reaction temperature and molar ratio

Figure13(b). Surface plot of yield vs. reaction temperature and molar ratio

12.7. Effect of catalytic concentration on yield

The catalyst concentration is an important process parameters used in the production of SGAOBD yield. The contour and surface plots presented in Figure 14 (a and b) indicate the SGAOBD biodiesel yield (%) as a function of catalyst concentration and reaction temperature, with the molar ratio held constant. From Figure14 (a & b), it is observed that more than 95% of the SGAOBD was achieved at CC (9.5 wt.%), RT (52 - 59 °C) at the constant molar ratio of 7.7 (hold value). However, the maximum biodiesel yield of 95% was attained at a constant molar ratio of 7:7, which indicates that the design is safe.



(a) (b)

Figure14(a). Contour plot of yield vs. catalytic concentration and reaction temperature

Figure14(b). Surface plot of yield vs. catalytic concentration and reaction temperature

12.8. Model validation

Equation (Eq.2), derived from regression analysis, indicates the theoretical yield. This was developed by performing experiments that varied three process parameters, viz. reaction temperature, catalyst concentration, and molar ratio. To obtain an accurate response yield, experiments were repeated 3 times, and an average yield of 97.14% was attained. The experimental yield was within a reasonable range of the expected yield, with a 2.15 percent error that may be attributed to human error and can be disregarded. Hence, experimental results indicate that the DSD technique performed well in forecasting the most vital response parameters for biodiesel production.

13.Catalyst characterization

The calcined moringa oleifera catalyst [27] was characterized to determine the essential elemental composition using an Energy Dispersive X-ray (EDX) analysis test, as represented in Figure 15. The test reported the presence of five key elements: Ca (calcium), K (potassium), O (oxygen), C (carbon), and Mg (magnesium), with respective abundances of 28.88 wt.%, 27.59 wt.%, 24.52 wt.%, 15.59 wt.%, and 3.42 wt.%.

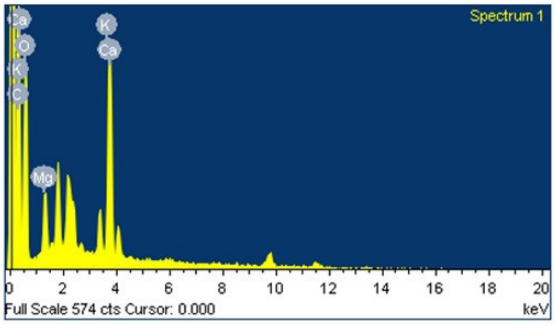


Figure 15. Elemental composition using an energy-dispersive X-ray

However, the current analysis reports that the calcination process improves key components such as potassium, calcium, and other elements that are significant for the transesterification process. The enhancement of certain elements is due to crystalline and lattice rearrangement. Thus, the output obtained from the EDX analysis is found to be in similar agreement with the studies conducted by [27], [46], [47].

14. Fuel property analysis

The growing demand for renewable fuels, especially third-generation biodiesels, is drawing significant attention because of their clean burning and reduced emissions. Biodiesel can compete with diesel and is substantial in storing, handling, transporting fuel, and commercialization [44]. The fuel properties of sea green algae oil biodiesel (SGAOBD) studied here were measured according to ASTM standards and compared with diesel and selected algae biodiesel studies from the literature, as shown in Table 7.

Table 7. Significant fuel properties of the present study and different algae strains

Fuel Properties	Limits	Diesel [28]	Present Study	Spirogyra Green Algae		Microalgae Biodiesel [48]
				[28]	[44]biodiesel was produced from a naturally grown green algae (Spirogyra)	
Kinematic viscosity (KV) (mm^2/sec)	D1298	2.75	4.2	4.24	4.23	2-5.2
Density (kg/m^3)	D1298	831	883	888	887	850-870
Cetane number (CN)	D613	48	54.3	53.86	53.49	37-72
Calorific value (CV) (kJ/kg)	D4868	44000	41000	42100	41243	31700 -41000
Flash point (FP) (^oC)	D93	86	143	145	146	115
Fire point (FP) (^oC)	D93	96	150	153	153	------
Pour point (PP) (^oC)	D613	-13	8.0	5.8	6	-6
Cloud point (CP) (^oC)	D613	------	14.0	12.4	13	------

Table 7 shows that the test values of fuel properties kinematic viscosity, density, cetane number, flash point, and fire point are higher than those of diesel fuel. This indicates a favorable sign for fuel storage facilities, considering biodiesels have a shorter retard time during combustion [49]. Additionally, biodiesel fuels with low calorific value, high density, and high kinematic viscosity have been shown to affect engine performance characteristics. The fatty acid composition (FAC) appears to be a contributing factor to these changes.

Furthermore, Table 7 shows that the density and kinematic viscosity of SGAOBD are 5.88% and 52.72% greater than those of diesel; however, these increases could lead to a drop in brake thermal (Br. Th.) efficiency and an increase in fuel consumption. In addition, the flash point and fire point values measured in the present study (SGAOBD) were higher than those of diesel due to the increased carbon number of saturated fatty acids. This is a positive indication of safe handling and storage. Furthermore, the cetane number (CN) of SGAOBD was higher than that of diesel and fell within the limits owing to its saturated fatty acid composition. This confirms a beneficial sign as the increase always indicates better engine performance by limiting the delay period during engine combustion.

The calorific value (CV) of SGAOBD, reported to be less compared to diesel, as there are fewer hydrocarbons and zero Sulphur [27]. Particularly at low temperatures, biodiesels are more susceptible to atmospheric conditions. The pour point and cloud point occur at low temperatures; they are measured according to ASTM standards. The cloud point indicates the lowest temperature at which wax crystallizes when the fuel cools [44]. Similarly, at the minimum temperature, the fuel becomes gel-like at the pour point and loses its flowability. However, the pour and cloud points fall within the ASTM standards. Thus, Table 7 confirms that the test values of the present study are in close agreement with the reference studies [28], [44], [48], making biodiesel feedstocks appropriate for efficient functioning of the existing compression ignition engine.

15. Fatty acid composition

Fatty acid composition (FAC) is one of the significant characteristics of biodiesel, and it provides information about the tendency of combustion and engine exhaust emissions [44]. The FAC test was performed using GC-MS. The total saturated FAC composition in the SGAOBD was 72.28%, and the unsaturated FAC composition was 27.72%. The FACs of Palmitic acid (28.96 %), Lauric acid (20.77 %), and Myristic acid (14.22 %) were high in composition, and the unsaturated FAC of Oleic acid was 26.52 %. Therefore, FAC profile showed that SGAOBD contained the highest amount of saturated fatty acids, which supports its use as a fuel in CI engines.

16. Reusability test analysis

One of the advantages of employing heterogeneous catalysts is thought to be catalyst reusability [7]. In the current investigation, the catalyst was recovered after each experimental use. The catalyst was cleaned using a methanol solution and vacuum-dried for 2 hours using a hot air oven, setting the temperature to 50 ^oC for the subsequent experimental trial [27] [49]. As illustrated in Figure 16, a comparative bar graph was plotted for the catalyst reutilization capability for five (5) successive experiments [49].

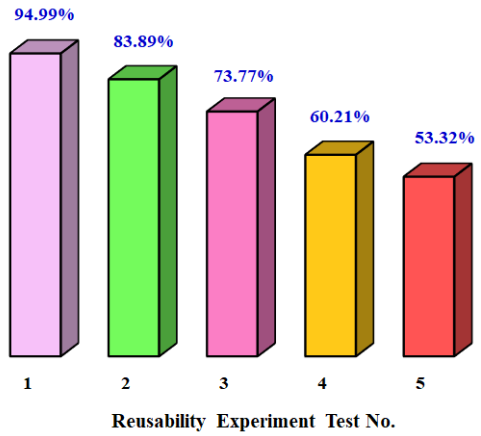


Figure 16. Reusability test for the catalyst

For the first application, the biodiesel yield was 94.99%, and after consecutive uses of the catalyst, the biodiesel yield was above 50%. Furthermore, Table 8 presents a comparison of the present study with various heterogeneous catalysts reported in the literature.

Table 8. Comparison of various heterogeneous catalysts with the current study

	Present Study	Reference Studies		
No. of Experiments	**Biodiesel Yield (%)**	**Orange Peel Oil Biodiesel Yield (%) [19]**	**Waste Chicken Eggshell Catalyst [49]**	**Moringa Oleifera Catalyst [27]**
1st	94.99	86.76	92	92.82
2nd	83.89	76.23	86	85.62
3rd	73.77	67.39	75	76.38
4th	60.21	59.78	68	63.45
5th	53.32	49.23	48	51.31

Therefore, five reusability trials were conducted in the current study, and the results were compared with those of other heterogeneous catalysts. From Table 8, it is observed that [19] used waste orange peel as a heterogeneous catalyst in orange peel oil and obtained 86.76% of the biodiesel yield. [49] used a waste chicken eggshell as a heterogeneous catalyst in waste cooking oil, a second-generation biodiesel, and obtained 92% of the biodiesel yield. In a similar approach, [27] used moringa oleifera as a heterogeneous catalyst in palm oil, a second-generation biodiesel, and obtained 92.82% of the biodiesel yield. Thus, in the present study, a Moringa oleifera heterogeneous catalyst was mixed with third-generation sea green algae oil, producing a biodiesel yield of 94.99%.

Finally, it is observed that the recyclability and the reusability ratio are higher (94.99%) in the present investigation compared with the other low-cost heterogeneous catalysts [19], [27], [28], and [49].

Thus, catalyst reusability testing, and reference studies have shown that sea-green algae mixed with calcinated Moringa oleifera attained a maximum biodiesel yield of 94.99%.

17. Conclusion

The preparation of biodiesel from renewable sea green algae oil holds considerable potential because of its high conversion rate, i.e., from raw oil to biodiesel. This study aims to harvest sea green algae that grow naturally on rocks in marine environments, extract oil from them, and produce biodiesel.

The following significant experimental findings from the current research are summarized below:

- The amount of sea green algae oil obtained by oil extraction was 1819 ml, with an oil yield of 24.25 %. Therefore, 90% of the solvent was collected and reused as n-hexane.
- Energy-dispersive X-ray analysis of Moringa oleifera indicated that calcium and potassium were present at 28.88 wt.% and 27.59 wt.% respectively, followed by oxygen, carbon, and magnesium.
- The experimental yield of SGAOBD obtained was 94.99%, and the response yield obtained using definitive screening design was 97.21% at 59 $^{\circ}$C reaction temperature, 9.5 wt.% catalyst concentration, and 12:1 molar ratio.
- The biodiesel yield from sea green algae oil (response) obtained using the Definitive Screening Design was 97.21%. The response biodiesel yield R^2 (97.52%) and Adj. R^2 (96.99%) affirms that the quality and the fitness of the model chosen were encouraging, and concluded that the predicted biodiesel yields are in good agreement with the experimental biodiesel yield. The regression model was developed and validated using confirmatory experiments conducted under optimal conditions.
- To achieve an accurate response yield, experiments were repeated three times, and the average yield was calculated to be 97.14%. The experimental yield was in reasonable agreement with the predicted yield, with an error of 2.15%, which may be attributed to human error and can be regarded as negligible.
- Contour and surface plots showed biodiesel yield from sea green algae oil reached 90–95% at the selected reaction temperature, catalyst concentration, and molar ratio, which indicates that the design is safe.
- From the catalyst reusability test, it was observed that in 5 experimental trials the biodiesel yield exceeded 50%, confirming that biodiesel yield decreases with increasing catalyst usage.
- The vital fuel properties of the current study, such as kinematic viscosity, density, cetane number, calorific value, flash point, and fire point, fall within the standards. The tested fuel-property values of sea-green algae oil biodiesel are in close agreement with those of diesel and with reference studies.
- The fatty acid composition analysis revealed that 72.28% of

the fatty acids in the sea green algae oil biodiesel were saturated.
- Thus, the experimental and predicted outputs support the use of the sea green algae oil biodiesel as a green, renewable fuel.
- The calcined Moringa oleifera, as a heterogeneous catalyst mixed with the sea green algae oil, facilitates a novel combination of green methods for biodiesel production.

Therefore, the results show a significant production of algae-derived biodiesel and efficient catalyst reusability, thereby enhancing the viability of biofuels derived from algae. Although second-generation biodiesel feedstocks produce higher yields, their adoption is constrained by limited land availability, which has led to the use of algal biodiesel. Interest in algae biodiesel has increased because it has high oil content, high oxygen content, requires less land, has rapid growth rate, is cost-effective, emits less pollution, and is environmentally friendly. However, processing and separation of the algae require more time. This study primarily focused on algae cultivation, harvesting, oil extraction, and biodiesel production. Thus, to attain the maximum biodiesel yield, experimental and predicted response yields were assessed using a definitive screening design, an optimization technique.

The future scope of work was proposed to include engine experiments using several combinations, viz., sea green algae oil biodiesel, algae biodiesel blended with additives, and algae biodiesel without additives. These combinations are tested under various operating conditions, such as varying loads and compression ratios, to evaluate the performance characteristics and exhaust emissions of the existing diesel engine. Subsequently, a cost estimate was proposed to carry out tests on sea-green algae, catalyst, and engine experiments. In addition, a detailed experimental investigation using different algal species was proposed to analyze the biodiesel yield and the feasibility of using biodiesel in existing diesel engines. Finally, combining algae, a green renewable energy source, with the green heterogeneous catalyst Moringa oleifera could achieve improved algae biodiesel yield and high recyclability and reusability, making it suitable for use in existing diesel engines and for replacing fossil fuels.

Authorship contributions

Kalyani Teku: Conceptualization, Data curation, Investigation, Methodology, Writing original draft, Review and Editing, NSC Chaitanya: Formal Analysis and Validation, K P V Krishna Varma: Methodology, Validation, VS Subrahmanyam: Methodology, V Sai Srikanth: Data curation.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Nomenclature

DSD	Definitive Screening Design
EDX	Energy Dispersive X-ray
FAC	Fatty Acid Composition
SGA	Sea Green Algae
SGAO	Sea Green Algae Oil
SGAOBD	Sea Green Algae Oil Biodiesel

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