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Lipid Extraction from Microalgae Using Ionic Liquid as Solvent and Biodiesel Production †

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Abstract

This work focuses on the production of biodiesel from *Nannochloropsis* sp. microalgae. The study compares the effectiveness of conventional methods, such as Soxhlet extraction and the Bligh and Dyer method with a non-conventional method that uses an ionic liquid as solvent for lipid extraction. The Bligh and Dyer method demonstrated superior efficiency with a lipid yield of 8.19% compared to Soxhlet extraction. Sample parameters were optimized in a maximum lipid yield of 18.29% at a sample volume of 91.9 mL, a duration of 7 min, and a power rate of 0.62 W. We further investigated the use of ionic liquids for lipid extraction from microalgae. We synthesized ionic liquids, and 1-(4 sulphonic acid) butyl-3-Methyl imidazolium hydrogen sulphate and 1-(4 sulphonic acid) butyl pyridinium hydrogen sulphate were characterized using FTIR, NMR and TGA. This method showed lipid extraction efficiency values of 10.7% and 0.402%. Subsequent transesterification of algal oil using 1-(4 sulphonic acid) butyl-3-Methyl imidazolium hydrogen sulphate yielded 19.82% biodiesel. FTIR analysis confirmed the presence of lipids and esters in the biodiesel, and the produced biodiesel met ASTM specifications, indicating its suitability for use as biofuel.

Keywords: ionic liquid; *Nannochloropsis* sp.; microalgae; lipid extraction; biodiesel



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1. Introduction

Renewable energy sources are essential for a sustainable future. Renewable energy enhances energy security by diversifying energy supply and reducing the dependence on imported fuels, thereby increasing resilience to global market fluctuations. The unsustainable and reducing fossil fuel resources, along with their adverse effects on the environment, highlight the importance of using renewable energy sources.

Biofuels, produced from biomass, are the most feasible, renewable, and alternative energy resources to deal with these issues [1]. Biofuels come in different forms, such as vegetable oils. bio-oil, biodiesel, bioethanol, bio-syn, and biohydrogen. Biodiesel, a type of renewable, biodegradable, non-toxic, and ecofriendly fuel, is considered one of the best alternative resources of fossil fuels [2]. Edible and inedible oils, such as jatropha oil, soybean oil, cooking oil, leftover cooking oil, palm oil, and sunflower oil, are commonly utilized as feedstock for the manufacture of biodiesel. However, large-scale production is not feasible due to the high cost and the lack of a consistent and stable supply of raw materials.

These drawbacks could be mitigated by synthesizing biodiesel from microalgae. Microalgae exhibit higher photosynthetic efficiency and faster growth rates than many terrestrial crops and can utilize industrial flue gas as a carbon source. They offer several advantages over traditional crops, such as a high lipid content, greater photosynthetic efficiency, bioremediation potential and versatile growing conditions [3–7]. Algal biodiesel reduce emissions of particular matter, CO, hydrocarbons and SO_x while performing similar to petrodiesel and have no sulfur content. Microalgae produces more oil than macroalgae and grows easier and faster [8].

The lack of cost-effective and efficient extraction and transesterification of lipids limits microalgae from acting as a feedstock for biodiesel production. However, the main barrier for synthesizing biofuel from microalgae is the lack of an economical and effective method for the extraction and transesterification of lipids. Cell disruption is a crucial step in the extraction of lipids from microalgae for biodiesel production. Efficient cell disruption methods help to release intracellular lipids from microalgal cells, making them accessible for further extraction and conversion into biodiesel. Numerous extraction approaches such as chemical, mechanical, and biological extractions, are categorized into two methods: conventional and non-conventional. Conventional methods mostly include chemical extraction procedures, where organic solvents penetrate into the microalgae cell. Non-conventional methods generally disrupt the cell wall of microalgae using mechanical techniques, such as bead milling, expeller presses, and microwave. These methods have been reported to yield higherf lipid recovery; however, they are not economically feasible due to their high energy requirement [9].

Green solvents are a class of solvents primarily recognized for their eco-friendly nature. Ionic liquids (ILs) have lately been identified as promising green solvents in the extraction of microalgal lipids. ILs consist of tiny inorganic or organic anions paired with comparatively big asymmetric organic cations. A ring structure containing nitrogen, such as imidazolium or pyrinidine, typically makes up the cation, to which a variety of functional side groups are connected [10]. ILs have many unique properties, including non-volatility, good thermal, electrical conductivities, large polarities, good thermal and chemical stabilities and potential recoverability [11]. ILs have superior physical properties in chemical reactions, such as the capacity to dissolve polar and non-polar organic, inorganic, and polymeric substances [12]. Ionic liquids can increase the yield of lipids recovered from algae because of their soluble nature in lignocellulosic materials, which are the main constituents of cell walls.

The present research focuses on the integration of ionic liquids into the biodiesel production process from microalgae, which holds promise for overcoming some of the limitations associated with conventional methods. Ionic liquids selectively dissolve lipids, and present an opportunity for improved extraction efficiency and reduced energy consumption, thus contributing to the overall sustainability of the biodiesel production chain. The interdisciplinary approach merges the fields of biochemistry, chemical engineering, and environmental science to develop a more efficient and environmentally friendly pathway for biodiesel synthesis.

2. Experiments

2.1. Chemicals

Microalgae (Nannochloropsis Sp. from Jemmax Nutraceuticals Pvt. Ltd., Tirunelveli, India), hexane, methanol, chloroform, ethanol, KOH, phenolphthalein, carbon tetrachloride, Wiji's solution, sodium thiosulphate, 1,4-butane sultone (99%), 1-methylimidazole, sulfuric acid (98 wt%, RFCL Ltd., Ramagundam, India), diethyl ether (99.5%, AR grade, RFCL), cyclohexane (99.5%, AR grade, RFCL).

2.2. Extraction of Lipids from Microalgae

2.2.1. Soxhlet Extraction

The Soxhlet extraction method was used to extract lipids from 15 g of dry microalgae using a hexane and hexane/ethanol (3:1) mixture. The Soxhlet extraction apparatus consisted of three compartments: a round-bottomed (1000 mL) flask, a condenser, and an extraction chamber. The sample was put in a absorbent cotton wool and the flask was heated. During heating, solvent was evaporated and condensation occurred. It was then collected in the extraction chamber, which allowed the lipids to leach into the chamber, and then transferred into the flask. The extraction process was carried out for 10 h. Following the cycles, a mixture comprising solvent and algal oil was obtained. After cooling, the solvent was evaporated using simple distillation. The extracted lipid fraction was dried in a water bath at 60 °C until a constant weight was achieved.

The crude lipid extraction yield was calculated using following Equation (1) [13]:

$$Recovery\ of\ crude\ lipid\ extract = \frac{Weight\ of\ crude\ lipid\ extract}{Initial\ dry\ biomass\ weight} \times 100 \quad (1)$$

2.2.2. Bligh and Dyer Method

Lipid extraction was performed using the procedure described by Bligh and Dyer [14,15]. Two grams of dried microalgae were vigorously shaken with water using an orbital shaker for 2 h at 180 rpm. A total of 10 mL chloroform, 20 mL methanol, and 8 mL water were added, and the mixture was homogenized for 2 min. After that, 10 mL chloroform were added to the mixture, and after blending for 30 s, 10 mL water was added and shaken vigorously for 30 s to form a two-phase system. The homogenate was filtered through Whatman No. 1 filter paper on a vacuum filtration. The residue was then blended with 10 mL chloroform to recover the residual. Centrifugation was used to separate two phases at 3500 rpm for 15 min. Using a Pasteur pipette, the lipid containing the lower phase was carefully removed to avoid contamination of the upper phase. After evaporating the chloroform, the lipid was measured gravimetrically.

2.3. Pretreatment Method—Sonication

A total of 80 mL of wet microalgae was taken in a 100 mL beaker and sonicated using an ultrasonic processor. The horn was inserted 10–20 mm below the sample surface, and sonication was performed at a power setting of 0.6 for 10 min (6 horn). After sonication, lipid extraction was carried out using the Bligh and Dyer method.

2.4. Statistical Design of Experiments

One of the most widely used experimental designs for optimization is response surface methodology (RSM). It is a combination of both mathematical and statistical techniques used for modeling and analyzing problems in which the response is influenced by several variables [16]. Several polynomial regression equations are used by RSM to fit functional connections between values and factors. RSM examines how different factors affect and interact with many response variables; it is frequently utilized in many kinds of optimization [17].

In this optimization study, response surface methodology based on the Box–Behnken method was used to optimize the lipid yield. Three independent parameters (sample volume, sonication time and power rate) were selected to evaluate lipid yield. The parameter ranges were as follows: sample volume (80–100 mL), sonication time (5–15 min) and power rate (0.4–0.8 W). A total of 15 experimental runs were designed using Minitab (Version 22). The experiments were conducted accordingly, and the resulting lipid yields are presented in Table 1.

Table 1. Box-Behnken experimental design matrices.

Sample Volume	Time	Power Rate	Lipid Yield
90	10	0.6	18.16
80	15	0.6	14.49
90	15	0.4	15.06
100	10	0.4	16.41
100	5	0.6	17.67
80	10	0.8	16.45
100	10	0.8	16.84
80	10	0.4	15.77
90	5	0.8	17.41
90	10	0.6	18.26
100	15	0.6	17.81
90	10	0.6	18.01
90	5	0.4	16.68
90	15	0.8	15.21
80	5	0.6	17.52

2.5. Synthesis of Ionic Liquid

1-methylimidazole (15.8 mL, 0.2 mol) and 1,4-butane sultone (22 mL, 0.2 mol) were added to a 250 mL round-bottomed flask. The mixture was stirred magnetically and maintained at 40 °C for 10 h. White solid zwitterions were formed, which were then washed repeatedly with ether to remove non-ionic residues, and dried under vacuum. A stochiometric amount of concentrated sulfuric acid (10.9 mL, 0.2 mol) was added dropwise and the mixture was stirred for 6 h at 80 °C to form 1-(4 sulphonic acid) butyl-3-methyl imidazolium hydrogen sulphate (IL 1). The obtained viscous liquid was washed repeatedly with cyclohexane and diethyl ether four to five times to remove organic residues, and dried in a vacuum. The synthesis of 1-(4 sulphonic acid) butyl pyridinium hydrogen sulphate (IL 2) was carried out according to the procedure described for IL1, from pyridine (16.11 mL, 0.2 mol) and 1,4 butane sultone (22 mL, 0.2 mol) [18].

2.6. Lipid Extraction Using Ionic Liquid Green Solvent

In this extraction method, 1 g of dry microalgae was mixed with ionic liquid in a 1:10 ratio in a 250 mL round-bottomed flask and heated at 95 °C for 2 h. The extracted mixture was cooled to room temperature, and hexane: methanol at a ratio of 2:1 were added. Centrifugation was carried out to separate the tri-phase system. Lipids and hexane were present in the top phase, IL with methanol was present in the intermediate phase, and algal residue was found in the bottom phase. The recovered n-hexane phase was washed twice with 15 mL water to remove polar compounds [11,19]. The lipid fraction was dried in a thermostat oven at 60 °C until a constant weight was achieved, and the residue was weighed to calculate the gravimetric yield using Equation (1).

2.7. Biodiesel Production from Microalgae

The extracted algal oil was used as a raw material for biodiesel production. The reaction was conducted in a conical flask filled with microalgal oil and methanol in a

1:6 ratio at temperature of 60 °C, and stirred vigorously for 90 min. Heating was provided using an oil bath. No catalyst was used in this procedure.

$$\text{Algal oil} + \text{Methanol} \leftrightarrow \text{Esters (Biodiesel)} + \text{Glycerol}$$

After completing the reaction, the mixture was allowed to settle in a separating funnel. If formed two phases, where the upper phase contained biodiesel and the lower phase comprised glycerol.

3. Results and Discussion

3.1. Characterization of Ionic Liquid

To confirm the identity of the synthesized ionic liquid, the following characterization methods were used.

3.1.1. TGA

Figure 1 shows the thermal stability of the prepared ionic liquids. IL1 shows good thermal stability up to 320.4 °C. It shows a slight weight loss of about 2–3% below 150 °C, which may be due to the loss of residual solvents and water present in the sample. The most significant weight loss occurred between 300 °C and 400 °C, where the loss of sample weight was 70%. This indicates the primary decomposition of the ionic liquid, where the breakdown of organic structure occurred. The maximum decomposition rate was observed around 350 °C, suggesting that this is the temperature where the compound underwent rapid thermal degradation. IL2 showed thermal stability up to 246.16 °C. The significant mass loss occurred between 200 °C and 350 °C. The rate of decomposition slowed down after 350 °C and both curves stabilized with minimal residue by 450 °C. Components that decompose at a higher temperature show better thermal stability.

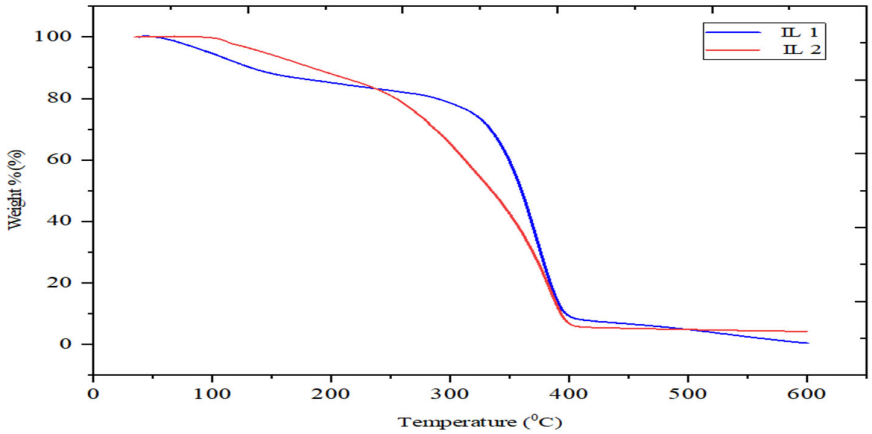


Figure 1. TGA of ionic liquid.

3.1.2. FTIR

The IL1 FTIR spectrum in Figure 2 shows a dip at 1574 cm^{-1} which indicates the presence of C=N imidazole ring stretching. The stretching and bending vibrations of C-H in the imidazole ring appear at 3155 cm^{-1} and 1163 cm^{-1}, respectively, which confirms the presence of the imidazolium ring. At 1022 cm^{-1} there is S=O stretching corresponding to the -SO_3H is observed, and at 745 cm^{-1} tC-S stretching is observed [19]. Peaks in the region around 572 cm^{-1} can be associated with the S-O bending vibrations of sulphonate groups. Peaks around 850–900 cm^{-1} are often attributed to C-H out-of-plane bending vibrations in aromatic or hetero-aromatic compounds. At 2938 cm^{-1}, there is the presence

of methylene asymmetric C-H stretching [20–23]. It confirms the presence of 1-(4 sulphonic acid) butyl-3-methyl imidazolium hydrogen sulphate.

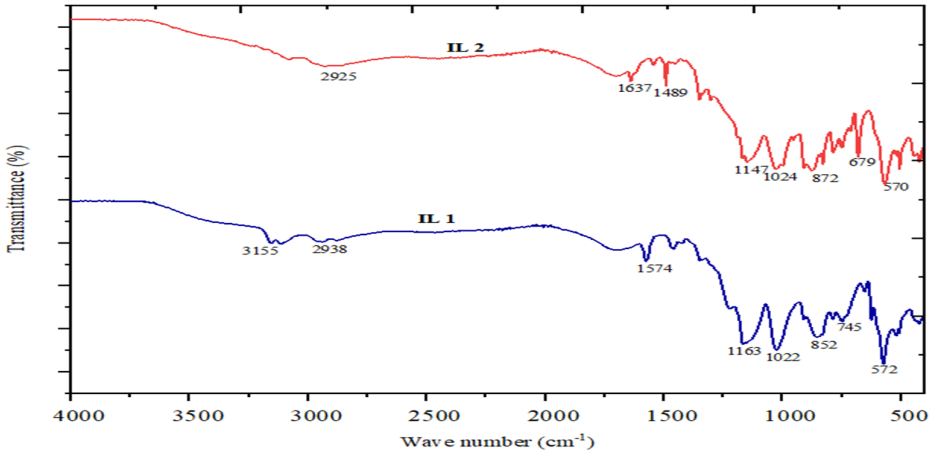


Figure 2. FTIR of ionic liquids.

The FTIR spectra of IL2 (1-(4 sulphonic acid) butyl pyridinium hydrogen sulphate) shows major peaks at 1637cm^{-1} and 1489 cm^{-1}, which represents the C=N and C=C stretching vibrations in the pyridine ring [21]. The peaks at 1147 cm^{-1}and 1024 cm^{-1} correspond to S=O stretching vibrations [22]. N-C vibrations are seen at 872 cm^{-1} and 679 cm^{-1} [23]. The peak at 2925 cm^{-1} is attributed to CH_2 asymmetric stretching [24].

3.1.3. NMR

Figure 3 represents ^{1}H NMR spectra of 1-(4 sulphonic acid) butyl-3-methyl imidazolium hydrogen sulphate. The multiplet peak at 1.7185 ppm represents CH_2 groups in the butyl chain. N-CH_2 and N-CH_3 in the imidazole ring are seen at 2.532 ppm and 3.191 ppm, respectively. The triplet at 3.772 ppm indicates the CH_2 group adjacent to sulfonic acid. The singlet peak at 6.185 ppm represents protons adjacent to the sulphonic acid group, likely showing downfield. The aromatic C-H protons of the imidazolium are observed at 7.525 ppm and 8.982 ppm.

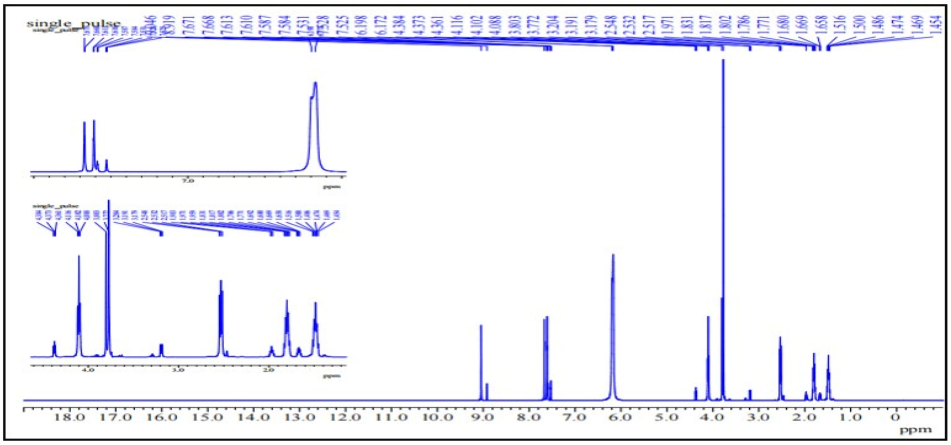


Figure 3. ^{1}H NMR spectrum of IL 1.

Figure 4 represents the ^{13}C NMR spectra of 1-(4 sulphonic acid) butyl-3-methyl imidazolium hydrogen sulphate. The peaks at 23.306 and 28.912 ppm suggest the presence of carbon atoms in the butyl chain. Peaks at 120.148, 122.101, and 136.595 ppm correspond to aromatic carbon in the imidazolium ring. The peaks at 38.943, 50.298, and 75.159 ppm indicate methylene carbons adjacent to an electronegative atom.

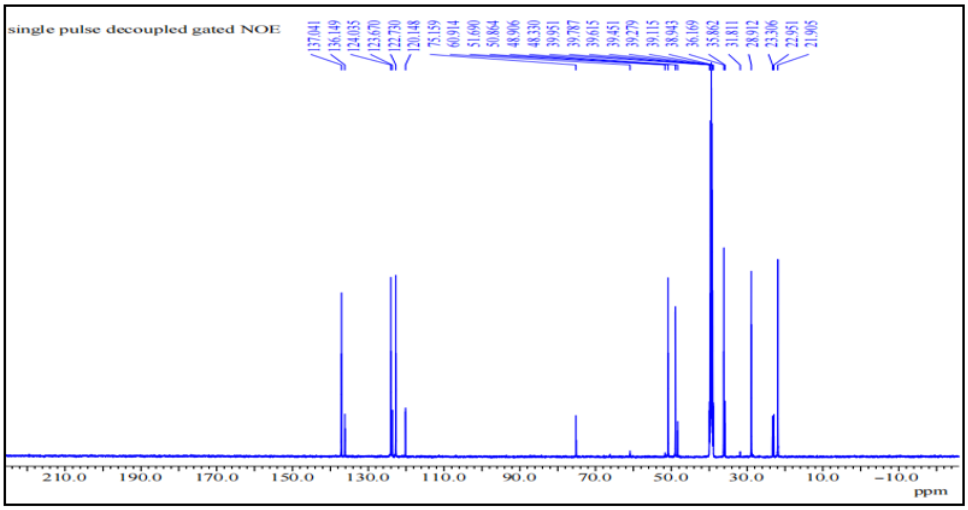


Figure 4. ^{13}C NMR spectra of IL1.

3.2. Lipid Extraction from Microalgae

3.2.1. Soxhlet Extraction and Bligh and Dyer Method

The Soxhlet method was carried out using both hexane and the hexane/ethanol mixture. Using hexane as a solvent, approximately 1.67% of lipids were extracted from the 15 g of microalgae. However, for the hexane and ethanol mixture (1:3 ratio), the lipid extraction efficiency increased to 16.53%.

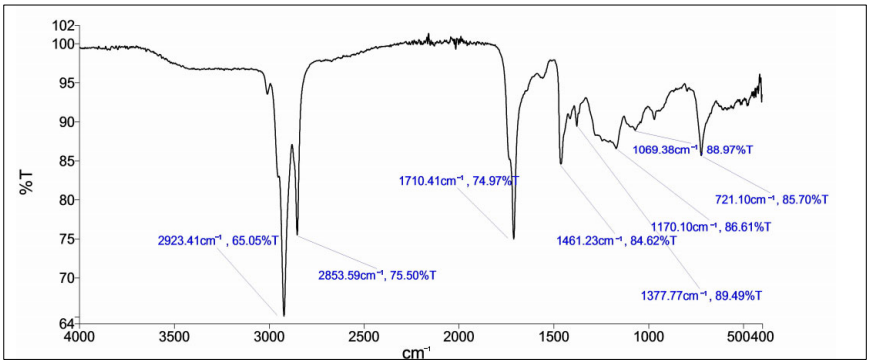
The Bligh and Dyer method was conducted using solvents such as methanol, water, and chloroform, which yielded 8.19% of lipids from 2 g of microalgae. The data shows that the Bligh and Dyer method is more efficient than the Soxhlet method due to the use of both polar and non-polar solvents. In order to increase the efficiency of the Bligh and Dyer method, a sonication pretreatment method is preferred to enhance cell disruption, thereby improving lipid yield.

3.2.2. Characterization of Lipids

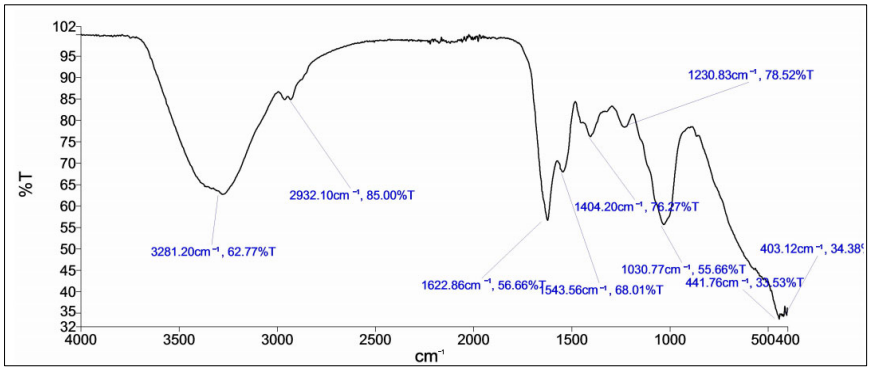
Characterization of extracted lipids carried out using FTIR

Figure 5a shows the FTIR spectrum of Soxhlet extraction using hexane as solvent, showing prominent peaks at 2923 cm^{-1} and 2853 cm^{-1}, corresponding to the asymmetric and symmetric stretching vibrations of CH_2 groups, indicating the presence of long hydrocarbon chains of lipids. A significant peak at 1710 cm^{-1} is attributed to the ester carbonyl stretching vibration, which confirms the presence of ester bonds in triglycerides. Additionally, the spectrum displays peaks at 1461 cm^{-1} for CH_3 asymmetric bending, 1377 cm^{-1} for CH_3 asymmetric bending vibrations, 1170 cm^{-1} for CO-O-C asymmetric stretching, and 1069 cm^{-1} and 721 cm^{-1} for CO-O-C symmetric stretching and CH_2 rocking, respectively, further confirming the presence of ester linkage [22,25,26].

The FTIR spectrum of the Soxhlet extract obtained using an f ethanol-hexane mixture (Figure 5b) shows major peaks at 3281cm^{-1} and 1622 cm^{-1}, corresponding to N-H stretching and the amide I band (mainly C=O stretching),respectively, confirming the presence of proteins. The peak at 1414 cm^{-1} for the =CH stretching associated with methylene and the methyl groups, while the peak at 1045 cm^{-1} corresponds to C-O-P stretching, indicatind the presence of lipids.



(**a**)



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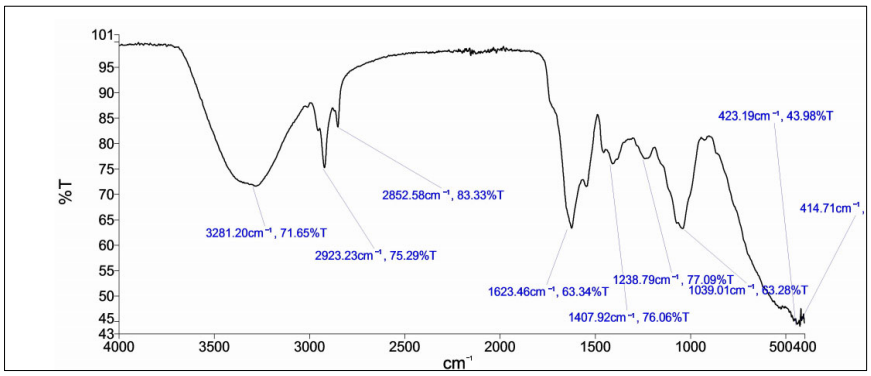

(**c**)

Figure 5. (**a**) FTIR spectrum of lipids extracted from microalgae using Soxhlet (Hexane Solvent). (**b**) FTIR spectrum of lipids extracted from microalgae using Soxhlet (Hexane + Ethanol Solvent). (**c**) FTIR spectrum of lipids extracted from microalgae using Bligh and Dyer after pretreatment.

Similarly, Figure 5c shows the spectrum for the sample obtained using the Bligh and Dyer method after sonication pretreatment. It displays similar key peaks such as 2923 cm^{-1} and 2853 cm^{-1}, confirming the presence of a long hydrocarbon chain. The peak at 1407 cm^{-1} is attributed to symmetric bending of $(CH_3)_3N+$, indicating the presence of lipids. The peaks at 1238 and 1039 cm^{-1} correspond to phosphoryl group vibrations in lipids, while the bands at 3281 and 1623 cm^{-1} indicate the presence of proteins [27–29].

The FTIR spectra revealed several key peaks, which were interpreted to confirm the presence of lipid specific functional groups. Hexane extraction using the Soxhlet method appears to be most effective method for extracting lipids; it showed strong peaks of lipids. Notably, the absence of significant protein peaks suggests minimal protein extraction with this method. The Soxhlet extraction using the ethanol–hexane mixture appears to be best for isolating proteins. Ethanol increases the extraction of protein content from microalgae. The Bligh and Dyer method seems to be a good choice for extracting both lipids and proteins.

3.2.3. Pretreatment Method—Sonication

Sonication was carried out at 0.6 W using 80 mL of wet microalgae sample for 10 min. Further application of the Bligh and Dyer method yielded 13.65% of lipid extraction. This indicates that sonication plays a crucial role in lipid yield, possibly due to its ability to enhance cell disruption.

3.2.4. Optimization of Sonication Parameters

ANOVA Analysis

Analysis of variance (ANOVA) is a crucial statistical method used in RSM to determine the significance of model terms and to identify the factors that influence response variable and the fitness of the quadratic model. As per this model, an F-value of 12.14 and a *p*-value of 0.007 indicated that the model terms are significant. If the *p*-value (probability of error value) is less than 0.05, it indicates that the model terms are significant, while *p*-values greater than 0.1 indicate that the model terms are not significant. In this model, sample volume, time, quadratic effects of time and power rate, and interaction effects of volume and time are significant terms because the *p*-value is less than 0.05. This suggests that these factors have an important effect on the response variable.

In this experimental model, the coefficient of determination (R^2) was used to evaluate the fit of the regression model. If R^2 value is closer to 1, the model will be stronger and provide good prediction of the response. The model demonstrates a strong fit to the data, with a high R^2 value of 95.62%, which indicates that it explains the majority of the variability in lipid yield. The adjusted R^2 value of 87.75% accounts for the number of predictors and still shows a high degree of fit.

Parameter Study

Contour plots are two-dimensional representations of the surface plot. Figure 6 represents the contour and surface plot of interaction effects of time and sample volume on lipid yield by assuming power rate as constant. The highest lipid yield (>18%) was obtained between 7.5 and 12.5 min and between 85 and 95 mL. Both sample volume and time have a positive effect on lipid yield.

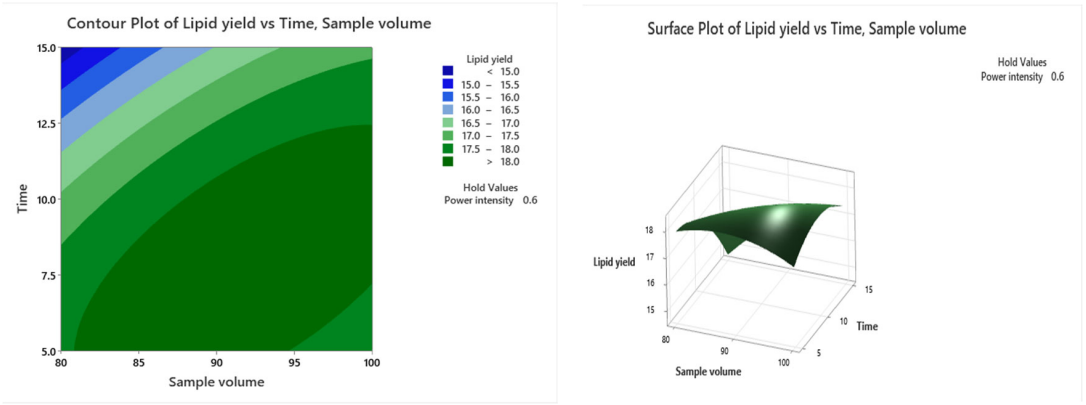

Figure 6. Contour and surface plots of lipid yield vs. time, sample volume.

Figure 7 represents the contour and surface plot of interaction effect of time and power rate on lipid yield. It shows a high lipid yield at 0.54–0.72 W and between 5 and 10 min. The lipid yield initially increased with time, reached a maximum, and subsequently decreased. The contour and surface plots illustrating the interaction effect of power rate and sample volume on lipid yield are shown in Figure 8. A higher power rate yields higher lipid output

across all sample volumes. It shows maximum lipid yield at 0.54–0.66 W and having a sample volume between 90 and 100 mL.

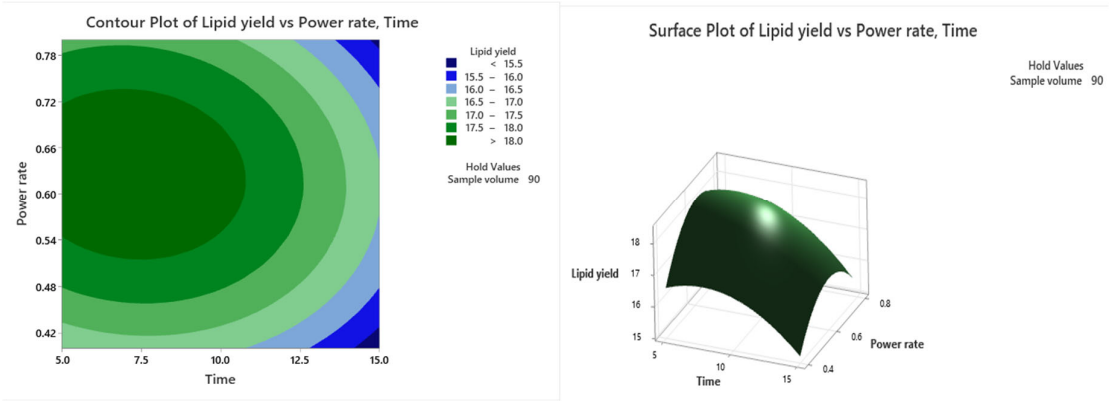

Figure 7. Contour and surface plots of lipid yield vs. power rate, time.

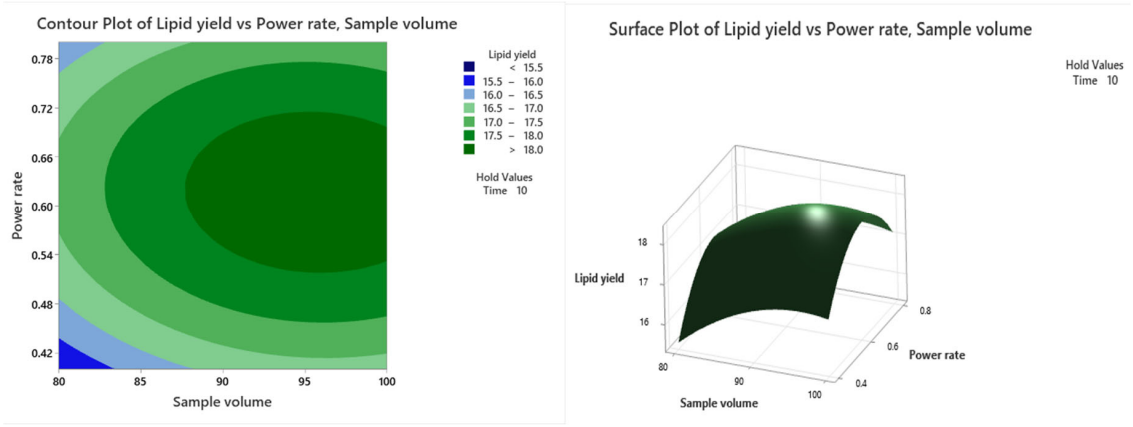

Figure 8. Contour and surface plots of lipid yield vs. power rate, sample volume.

Optimization Plot

Optimization plot in Minitab is a graphical tool used to identify the optimal settings for input variables that will maximize or minimize a response variable in a designed experiment. From the Minitab data (Figure 9), the maximum yield is obtained as 18.403%.

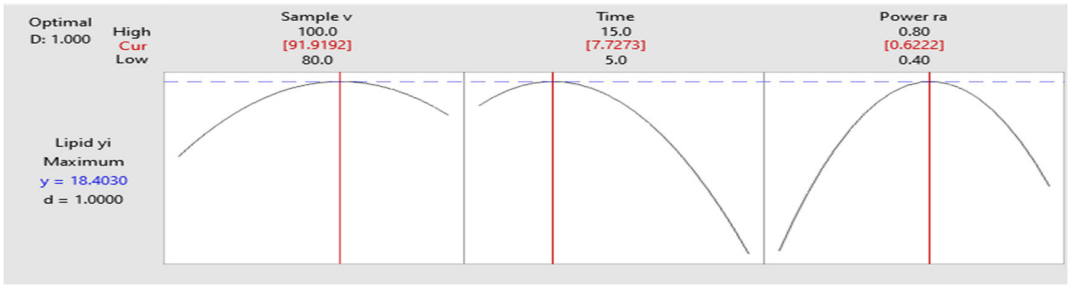

Figure 9. Optimized plot of lipid yield.

Experimentally, a maximum lipid yield of 18.29% was obtained, which is very close to the optimized conditions, indicating that the experimental setup performed well compared to the theoretically optimal settings.

3.3. Lipid Extraction Using Ionic Liquid

By adding the ionic liquids IL1 and IL2 to dry microalgae in a 10:1 ratio, lipid extraction efficiencies of 10.7% and 0.402% were achieved.

The ionic liquid method shows similarity with hexane extraction, which highlights its capability to extract esters and lipids efficiently. In the Figure 10, the significant peaks at 2923 cm^{-1}, 2853 cm^{-1}, 1742 cm^{-1}, 1460 cm^{-1}, 166 cm^{-1} and 721 cm^{-1} confirm the presence of lipids. Ionic liquids effectively solubilize the lipids due to their unique chemical properties. Moreover, they have the potential to reduce the environmental impact compared to traditional organic solvents.

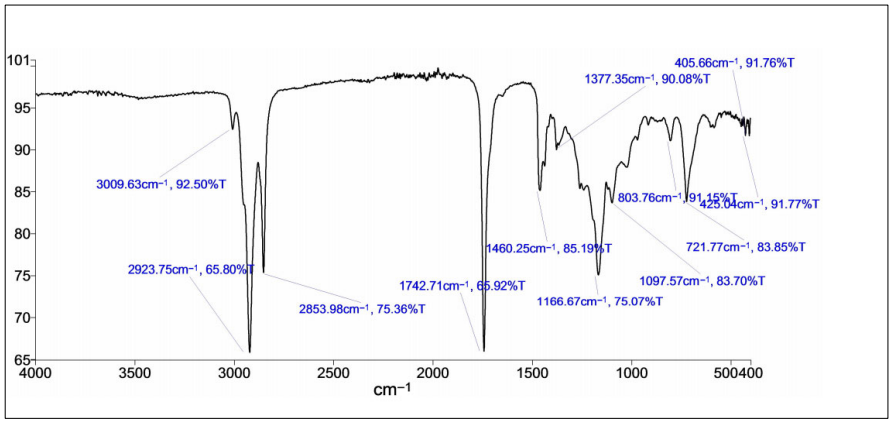

Figure 10. FTIR spectrum of lipid extracted using ionic liquid as solvent.

3.4. Biodiesel

Extracted lipids from ionic liquids underwent transesterification with methanol, resulting in a yield of 19.82% of biodiesel.

3.5. Characterization of Biodiesel

FTIR detects assemblages of crucial compositions in biodiesel. In Figure 11, the peak at 1744 cm^{-1} indicates the presence of C=O (aldehyde/ketone) stretching. The peak at 1451.28 cm^{-1} represents C-H bending in alkanes. A significant peak of 1023 cm^{-1}, for C-O stretching vibrations found in esters, confirms the presence of biodiesel [30–32]. In the biodiesel spectrum, the peak higher than 3000 cm^{-1} is similar to -OH of carboxylic acid, and indicates absolute transesterification [32].

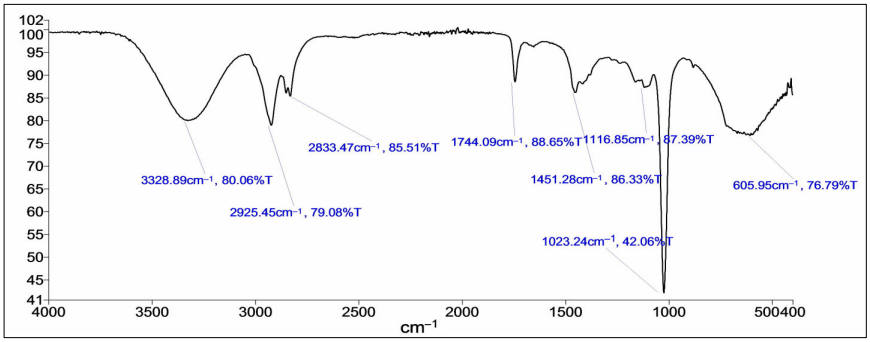

Figure 11. FTIR of biodiesel.

Table 2 represents properties of biodiesel derived from microalgae. The pH of 7 is within the specified range, indicating that the algal biodiesel meets standards for pH, ensuring it is neither too acidic nor too basic, which helps in preventing corrosion in fuel systems. The density of algal biodiesel produced in the study is 860 kg/m^3; according to

ASTMD6751, the limit of density is 862 kg/m^3. Density plays a major role in its engine performance and its ability to blend with the diesel fuel made from petroleum. It ensures that the fuel has the right energy content and flow parameters, which is important for efficient combustion and engine performance. The acid value of the biodiesel is 0.5047 mg KOH/g, which falls very close to the specified limit of 0.50 mg KOH/g. A higher acid value can indicate the presence of free fatty acids, which can cause corrosion in engines.

Table 2. Properties of biodiesel.

Properties	Algal Biodiesel	Limits ASTM D6751
pH	7	7–9
Density (kg/m^3)	862	860–900
Acid value (mg KOH/g)	0.5047	0.50
Free Fatty Acid (%)	0.252	0.8
Iodine value (gI_2/100 g)	48.86	120
Calorific value (kJ/kg)	31,397	46,560

The iodine value of the biodiesel is 48.86 gI_2/100 g, which is considerably lower than the specified limit of 120 gI_2/100 g. The iodine value reflects the degree of unsaturation in the fatty acid chains. A higher value indicates a higher content of unsaturated fatty acids, which can be less stable and more prone to oxidization. The low iodine value suggests a higher proportion of saturated fatty acids, which is beneficial as it implies better oxidative stability and lower tendency for deposits and gums in the ends. The calorific value of biodiesel is 31,397 kJ/kg, indicating that it provides higher engine performance and better fuel construction.

4. Conclusions

Biodiesel production from microalgae Nannochloropsis Sp. was conducted through a two-step process of lipid extraction and transesterification. Lipid extraction using the Soxhlet and Bligh and Dyer methods yielded 1.67% and 8.19% lipid, respectively. The Bligh and Dyer method was found to be most efficient conventional method for lipid extraction from microalgae. In order to improve the efficiency of the Bligh and Dyer method, sonication was employed as a pretreatment method to disrupt microalgal cells. Lipid yield was optimized by sonication parameters such as time, sample volume and power rate using response surface methodology, which showed a maximum lipid yield of 18.403% at sample volume = 91.9 mL, time = 7 min and power rate = 0.62 W, which was confirmed with the experimental results as 18.29%.

As an environmental friendly method, ionic liquids can be utilized as extraction solvents. Synthesized ionic liquid, 1-(4 sulphonic acid) butyl-3- methyl imidazolium hydrogen sulphate, and 1-(4 sulphonic acid) butyl pyridinium hydrogen sulphate were characterized using TGA, FTIR and NMR. Lipid extraction using ionic liquid resulted in yields of 10.7% and 0.402%, respectively. As compared to conventional methods, extraction using ionic liquid 1-(4 sulphonic acid) butyl-3-methyl imidazolium hydrogen sulphate showed higher lipid efficiency.

Production of biodiesel through transesterification was carried out at an algal oil: methanol ratio of 1:6, and the efficiency of the process was 19.82%. FTIR was used to analyze the presence of esters in biodiesel. The produced biodiesel possessed characteristics such as pH 7, density 862 kg/m^3, acid value 0.5047 mg KOH/g, free fatty acid 0.252%, iodine value 48.86 gI_2/100 g, and calorific value 31,397 kJ/kg. The produced biodiesel with significant properties meets the terms of ASTM specifications.

5. Future Scope

The future scope of this study includes evaluating the reusability of ionic liquids after lipid extraction. By integrating microalgae cultivation, the process can be scaled up for industrial production. Additionally the -SO_3H functionalized ionic liquids may have the potential to function as both solvent and Brønsted acidic catalyst, which represents a promising direction for future research.

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