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Enhanced Biodiesel Synthesis from Ultraviolet pretreated (UV) Microalgae Oil through Catalyst Selection and Optimization of Transesterification Parameters

Running Title: Biodiesel Synthesis from Ultraviolet pretreated

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SUMMARY:

In light of the growing concerns about the energy crisis, climate change, and resource depletion, microalgae have attracted a lot of attention as a potential biofuel feedstock. This study focussed on biodiesel synthesis using UV pretreated microalgae oil, selection of potential catalyst and optimization of process conditions to improve biodiesel quality by reducing contaminants and increasing transesterification efficiency. From three various types of catalysts, the best one (KOH) was chosen based on the quantity and quality of biodiesel. By carefully examining the impact of several reaction parameters, including temperature, catalyst wt%, oil to methanol ratio, and reaction time, an ideal biodiesel yield of 79.00% was achieved. The biodiesel that was synthesised was examined using gas chromatography and mass spectrometry (GC-MS), Fourier transform-infrared radiation (FTIR) spectroscopy, and nuclear magnetic resonance (^{13}C NMR). The GC-MS analysis of algae biodiesel showed that it contains saturated fatty acids, which enhance its combustion properties and resistance to oxidation, making it suitable for use in vehicles without modifying the engines too much. The biodiesel fuel's characteristics were evaluated using specific testing protocols that adhered to ASTM and EN standards. By using UV pretreatment, carefully selecting catalysts, and methodically adjusting reaction parameters, a viable approach for sustainable algae based biodiesel generation

Key words: UV pretreatment; Microalgae oil; Transesterification; Biodiesel; GC-MS.

1. INTRODUCTION:

Demand for energy has skyrocketed due to the world's growing population, more cities, and faster industrialisation. The use of oil, natural gas, and all other big fossil fuels has increased at an unprecedented rate.The major drawbacks of fossil fuels are limited sourcehigh cost, and emission of greenhouse gases, which elevate global warming(*1*-*3*). All of these things make it all the more important to look for renewable energy sources that can power transportation (*4*). Syngas, hydrogen, bio-oil, and biodiesel are renewable biofuels that may be produced from biomass feedstocks and they have recently arisen as an alternative to finite fossil fuel reserves (*5*).The blend ability of biodiesel with diesel makes it the most promising of the biofuels described above and engines only require modest adaptations to run on biodiesel(*6*). Biodiesel produced by reacting feedstock triglycerides with a short-chain alcohol in the presence of a catalyst, it is a combination of mono-alkyl esters of long-chain fatty acids generated from microalgae oil, animal fats, and vegetable oils (*7*).

There are many generations of biofuels, each distinguished by the feedstock used to produce them (*8*).Producing edible food crops at a far faster pace than was necessary between 2000 and 2008 to fulfil the world's energy requirement allowed for the development of first-generation biofuels (*9*). To avoid the dilemma that arises when first-generation biofuels are mixed with food, second-generation biofuels are often made from non-food lignocellulosic biomass, such as agricultural wastes, woody crops, and other waste products. Due to ineffective feedstock conversion processes, the yield of second-generation biofuel is currently low(*10*). As an alternative, microalgae oil is considered a third-generation biofuel source due to its higher energy security, social and economic acceptability, and availability. The world's primary source for producing biofuels is presently algae. Among the potential organisms used in biodiesel production, they are deemed the safest, most non-competitive, and fastest-growing. They are superior sources of biodiesel since they grow well on waste nutrients (*11*) and don't include plants that are used for food, which might create food issues (*12*). Microalgae grow at a much quicker pace than plants and need a far smaller quantity of basic nutrients to thrive(*13*) When it comes to producing biodiesel efficiently, the quality of the feedstock is paramount. Despite its potential as a renewable source, microalgae oil isn't always free of contaminants that make transesterification more difficult.

By using pretreatment procedures, contaminants are reduced and reaction efficiency is improved, making it more suitable for biodiesel synthesis. The use of ultraviolet light has recently come to light as a promising non-chemical method for altering oil composition and, by extension, biodiesel output. Biodiesel may be produced from bio-oils via a straightforward chemical process known as transesterification, which reduces the oil's viscosity (*14*). Renewable, non-toxic, and biodegradable biodiesel may be produced from raw micro algal lipids(triacyclglycerols/free fatty acids) by the process of transesterification, a three-step reversible series reaction that begins with the conversion of triglycerides to diglycerides, continues with the conversion of diglycerides to monoglycerides, and finally, converts monoglycerides to esters (biodiesel) and glycerol (by-product)(*15*).Reagents used in the transesterification process typically include oil or fat, a short-chain alcohol (often methanol), and a catalyst (often KOH) (*16*). This biodiesel can then be used directly by unmodified diesel engines.

Catalyst use is another technical hurdle in biodiesel manufacturing. One may classify the catalysts used as either homogeneous, heterogeneous, nano, or biocatalyst (enzyme) (*17*).Furthermore, basic and acid catalysts are additional ways to categorise homogeneous and heterogeneous catalysts. Due to its shorter reaction time and higher conversion efficiency, the base catalyst is more often used in commercial settings than the acid catalyst(*18*,*19*).Reported homogeneous catalysts for microalgal lipid extraction and transesterification include H_2SO_4, HCl, KOH, NaOH, and CH_3NaO (*20*).A high reaction rate is readily attainable with homogenous catalysts since they are very adaptable and may be utilised in a wide concentration range. Concerning catalysts, potassium hydroxide has achieved industrial-level success in manufacturing biodiesel(*21*). Various catalysts, including NaOH, KOH, and CaO, were tested in this research to find the one with the highest yield. During base-catalysed transesterification, saponification occurs when the free fatty acid level is between 20% and 50%. Biodiesel is a sustainable alternative fuel that may be made from a number of different biological sources, according to research by Kumar Gaurav *et al.,* (2024). The creation of biodiesel from microalgae produces environmentally friendly and renewable energy. It has been determined that this process has great potential as an alternative to traditional fossil fuels(*22*).

Improved biodiesel production by UV pretreating of microalgae oil is a novel approach of this research. Other objectives include finding the best transesterification process factors and determining the efficacy of various catalysts. In order to increase biodiesel output, this study presents a new method that researchers might use as an optimisation strategy: pretreatment of microalgae oil with UV radiation.

2. MATERIALS AND METHODS

2.1Materials

Microalgae oil with 0.095% free fatty acid (% as oleic) used in this study was provided by the company Liable Essential Oil Products Pvt. Ltd. Analytical-grade methanol (CH_3OH, 99.8% purity) and potassium hydroxide (KOH) used for the experimental procedure were purchased from Merck. For FAME content determination by gas chromatography (GC-MS), methyl heptadecanoate (99 %, Sigma-Aldrich) and Tetramethyl silane (TMS) in NMRwere served as the internal standard.

2.2 Methods:

2.2.1Pre-treatment of Microalgae oil:

In cases when the oil is still not completely pure after extraction, ultraviolet light can sterilise it by eliminating any remaining bacteria or spores. Before proceeding for the reaction, UV pre- treatment of microalgae oil was done by taking 50ml of oil in a beaker and subjected to ultraviolet radiation of short wavelength of 256 nm for fifteen minutes in a UV chamber (KUVC- 1; KEMI). Both the processed and untreated feed stocks (microalgae oil) were examined using Fourier transform infrared spectroscopy(*23*,*24*).

2.2.2Transesterification and purification of biodiesel:

Using microalgae oil as a feedstock, transesterification was carried out in accordance with the approach outlined by Celestino *et al.,* 2025 (*25*).The necessary volume of methanol was determined using the oil to methanol molar ratioas 1:30. The KOH pellets, which was previously measured at 1 wt% of oil, were dissolved in this volume. The methanol and catalyst combination were transferred to a 2-necked flask containing the UV pretreated microalgae oil. The flask was then placed on a hot plate and a condenser was attached to it. Using a magnetic stirrer, the reaction conditions were maintained at 60°C for 3h while vigorously swirling. A thick brown layer of glycerol and a yellowish-coloured fatty acid methyl ester (FAME) were present in the mixture, which was properly separated the next day after being placed to a separating funnel (*26*).The separating funnel was used to separate the biodiesel (FAME) from the glycerol (*27*). Without pretreating the feedstock, the same technique was executed. The above said procedure was also repeated with other catalyst like NaOH and CaO to determine the potentiality of the catalyst.

The production of biodiesel also involves a purification step.The biodiesel was washed to remove catalyst, glycerol, soap, etc. A saturated brine solution was made by adding NaCl to distilled water (25 vol% biodiesel) until no more salt dissolved. This was done to neutralise the soap that generated while using homogeneous base catalysts, rather than using standard distilled water for washing (*28*).Before pouring the fluid into a separate funnel, it was carefully swirled to prevent foam development. Two distinct phases, one consisting of biodiesel and the other of water impurity, formed when the mixture was allowed to sit overnight. A pH greater than 7 will characterise this layer of water impurities. The process of washing with water is carried out repeatedly until a layer of transparent water with a pH of around 7 forms.

2.2.3. FAME Conversion and Optimization of Process Parameters

The biodiesel yield is the ratio of the biodiesel volume to microalgae oil volume. Equation (1) was used to compute the yield after purification.

$$\text{Yield \%} = \frac{\text{volume of biodiesel (ml)}}{\text{volume of microalgae oil (ml)}} \times 100 \qquad (1)$$

Likewise, the biodiesel yield was computed for various catalysts. The catalyst that produced the highest yield was selected for use in further experiments. The presence of FAME was confirmed using NMR investigation and utilizing conventional methods and in comparison, with ASTM D6751 and EN standards, biodiesel's fuel characteristics, including density, pH, calorific value, flash point, cetane number, kinematic viscosity, and so on, were assessed. The molar ratio of oil to methanol (1:10 to 1:40), catalyst wt% (1wt% to 2.5wt%) , reaction temperature (55^{o}C to 75^{o}C), and the reaction duration (1h to 4h) are the factors that determine the yield and cost of biodiesel synthesis. A number of transesterification reactions were carried out utilizing a catalyst, UV pretreated microalgae oil as input, by adjusting one parameter while holding all other parameters constant. On each occasion, the yield was determined (*29*-*33*).

2.2.4. Characterization of feedstock and product:

The chemical composition of microalgae oil (feedstock) and Biodiesel samples, both with and without UV pretreatment, was analysed using FTIR spectroscopy. A BRUKER spectrometer (alpha - T) operating at 18-20^{O}C was used to acquire the biodiesel's FTIR spectra. The liquid samples of biodiesel are introduced by placing it on the Attenuated Total reflectance (ATR) crystal, allowing direct measurement without the need for extensive sample preparation. Scanners used the mid-infrared range (4000-400 cm^{-1}) to look for functional groups and UV-induced structural changes. Chemical structures of fatty acid methyl esters (FAMEs) in the biodiesel sample were identified and confirmed using Nuclear Magnetic Resonance (NMR) spectroscopy. A 400 MHZ NMR spectrometer was used to conduct the ^{13}C NMR analysis of biodiesel that was made by UVpretreating the feedstock and biodiesel that was made without UV pre-treating the feedstock.Using a 5 mm NMR tube and a volume ratio of 15:0.5, the samples were produced in $CDCl_3$. Tetramethyl silane (TMS) served as the internal standard(*34*).Fatty acid methyl esters (FAMEs) in biodiesel were identified and quantified using GC-MS analysis. Verifying the completion of transesterification and evaluating the chemical make-up and quality of the fuel are both aided by this. Quantification of the peaks obtained in the biodiesel sample on the column

Rtx-5MS (30 m × 0.25 mm × 0.25 μm), using helium as a carrier gas. The injector temperature was maintained at 250 °C. Starting at 50°C, the column oven temperature program increases by 50°C/min to 260°C with a 6-minute pause. Then, it increases by 30°C/min to 270°C with a 15-minute hold. The whole program lasts for 26.53 minutes. An injection temperature of 250°C and a sample duration of 1 minute are specified. It was possible to identify the biodiesel sample's fatty acid methyl ester peaks by comparing them to those in the GC chromatogram of the aforementioned standard(*35*,*36*).

3.RESULTS AND DISCUSSION:

3.1. FTIR analysis of feedstock (microalgae oil) before and after UV pretreatment

Comparison of the FTIR spectra of UV pretreated and untreated algae oil shown in **Fig. 1.**The presence of several functional groups in algae oil is shown by the FTIR spectra. The part of the spectrum linked to stretching C-O bonds which is between 1000 and 1350 cm^{-1}. Near 2920 cm^{-1} and 2850 cm^{-1}, symmetric and asymmetric stretching of the $-CH_2$ and $-CH_3$ groups were noted. The fact that these peaks were unaffected by UV pretreatment suggests that the backbone of the fatty acid remains unbroken. The presence of triglycerides is confirmed by an ester carbonyl (C=O) signal which is located at 1740 cm^{-1}. Moreover, a band at around 1450 cm^{-1} is associated with the bending vibration of the methyl and methylene groups. A very faint signal at around 720 cm^{-1} is attributed to the rocking vibrations of $-(CH_2)n$-long chains, confirming the existence of the long hydrocarbon chains typical of lipids. Triglycerides are partly breaking down into smaller pieces in the UV pretreated sample because the peak at ester carbonyl is not as intense. The decrease in the intensity of the signal at ester carbonyl in the UV pretreated sample indicates that triglycerides are partially hydrolysing into smaller fragments.

3.2. Screening of catalyst and analysis of product:

The most successful option for transesterification was biodiesel made from UVpretreated microalgae oil with KOH as a catalyst, which had the greatest yield of 38% (**Fig.2**). Possible causes include the synergistic impact of the KOH catalyst's high basicity and UV pretreatment, which together eliminate feed contaminants such as free fatty acids. In contrast, contaminants cause a little lower yield of 36% for biodiesel made using the same catalyst but without UV pretreatment

Because of its higher propensity for side reactions, biodiesel made using a NaOH catalyst (with and without pretreatment) has lower yields (32% and 31% respectively) than biodiesel made with a KOH catalyst. It was challenging to purify using the NaOH catalyst due to the excessive soap production that was observed. Additionally, it was noted that the NaOH catalyst-produced transesterification product combination takes more time than the KOH catalyst-produced mixture to settle into transparent layers. Because of its slower kinetics and lesser solubility, CaO, a heterogeneous catalyst, causes partial transesterification and shows the lowest yield (with UV-28% and without UV 30%). Based on the data shown above, it is evident that KOH is the most effective of the three catalysts. The optimal conditions for the transesterification process are achieved by combining UV pretreatment of microalgae oil with KOH catalyst.

3.3.H NMR and ^{13}C NMR analysis of biodiesel:

In order to qualify biodiesel as a viable alternative fuel, the structural criteria have been revealed by the HNMR spectrum. Fatty acid methyl ester (FAME) is confirmed by the H-NMR spectrum of the biodiesel sample, as shown in **Fig. 3(a).** The effective transesterification of triglycerides into biodiesel is confirmed by the distinctive singlet at 3.6ppm, which corresponds to the methoxy protons ($-OCH_3$) of the ester functional group ($-COOCH_3$).

Additional evidence for this conversion may be seen around 2.2-2.3 ppm in the form of methylene protons ($-CH_2$) next to the ester carbonyl. Unsaturated fatty acid methyl esters are confirmed by signals in the 5.0-5.4 ppm range, which show the presence of vinylic ($-CH=CH-$) protons.The peaks seen between 0.8 and 1.6 ppm correspond to the aliphatic methyl ($-CH_3$) and methylene ($-CH_2$) groups found in fatty acid chains.

The ^{13}CNMR spectrum is depicted in **Fig. 3(b).**Fatty acid methyl esters (FAME) are indicated by the ester carbonyl group (C=O) at 174 ppm. Minimal indications in the 1.6-2 ppm range indicate that the moisture content of purified biodiesel is very low. Conversion of feed into biodiesel is further confirmed by the identification of the methoxy($-OCH_3$) carbon from the ester group at a peak range of around 51.22 ppm. It is possible to identify unsaturated fatty acid chains by looking for peaks between 127.72 and 130.08 ppm. There may be trace amounts of glycerol in the signals that fall between 63.02 and 76.91 ppm. The aliphatic chain is clearly shown by peaks at 14.02-31.92 ppm, which correspond to the terminal methyl ($-CH_3$) and methylene ($-CH_2-$) groups. These peaks indicate that the microalgae oil was successfully converted into biodiesel by transesterification.

3.4. Fuel properties of biodiesel

Biodiesel is a potential alternative fuel for diesel engines; however, it must first satisfy international standards for its physical and chemical qualities before it can be utilised effectively. According to the findings, the majority of the fuel's characteristics met the requirements set by the United States. The acid number, which indicates the degree of biodiesel breakdown, was within the acceptable range (0.2 mg KOH/g) according to biodiesel regulations. The biodiesel's kinetic viscosity was determined to be 4.54 mm^2/s, which is likewise within the acceptable range. The fuel's flammability is indicated by its flash point. The flash point was recorded as 149°C. Having a higher flash point makes handling and storage safer. Using biodiesel with a high cetane number (52 observed) produced the best ignition qualities. Density: 870 kg/m^3, calorific value: 45.26 MJ/kg, pH: 9, refractive index: 1.446, ash content: 0.016, etc., were within the limits of ASTM/ EN standard (**Table 1**).

3.5 Optimization of Parameters:

3.5.1. Effect of Reaction Time:

An essential transesterification process parameter is reaction time. From**Fig.4(a)**, it can be observed that yield was increased as long as reaction duration was increasing and reached its maximum value at 3h. This is becausesufficient time is readily available to complete the task. Extending the reaction period beyond 3 hours reduced biodiesel yield, which makes sense given that prolonged contact with catalysts and reactants might cause biodiesel hydrolysis or soap production. Thus, 3 h was the optimum response time to maximize biodiesel yield.Similarly, de Luna et al., 2017 (*37*)) achieved a 33% yield in 3h of reaction time from *Chlorella sp*. Microalgae at 80 ° C reaction temperature.

3.5.2. Effect of Reaction Temperature:

The reaction temperature has a significant impact on the pace of the reaction and the yield of biodiesel. A larger conversion rate is attained as the temperature rises, as seen in **Fig. 4(b).**When oil and methanol are mixed at high temperatures, the mixture becomes more homogeneous. In this case, 65ºC produced the highest output. The boiling point of methanol is 64.7°C, so it's rather near. Exceeding 65ºC resulted in a loss in yield for two reasons: first, because methanol evaporates at that temperature, and second, because too much heat might stimulate unfavourable reactions that lead to undesirable byproducts. Therefore, the transesterification process is best carried out at a temperature of 65ºC.Singh *et al.,*2019(*38*) studied the impact of different temperature conditions (40ºC to 70ºC) on the biodiesel yield and found that 65ºC was the optimum temperature with maximum FAME yield.

3.5.3. Effect of oil to methanol molar ratio:

One of the most influential factors on conversion efficiency, biodiesel output, and biodiesel production cost is the oil-to-methanol molar ratio.**Fig. 4(c)**shows that increasing the oil-to-methanol ratio from 1:10 to 1:20 initially increased the yield by providing an adequate amount of methanol to drive the reaction.The yield decreased when the oil-to-methanol ratio was increased, especially between 1:30 and 1:40. It is likely that the catalyst becomes inactive due to an overabundance of methanol, which prevents it from interacting with the oil molecules and causes a poor transesterification process, which is the likely cause of this decrease.Additionally, biodiesel may be lost during purification if there is an excess of methanol, which makes product separation more difficult. Therefore, a ratio of 1:20 between oil and methanol is optimal for increasing biodiesel output.

These results were agreed upon by Moradi et al. (2022(*39*)in which sufficient yield was achieved at theoptimum oil-to-methanol ratio (1:20)

3.5.4Effect of catalyst wt%:

To speed up the transesterification process, catalyst wt% is critical as shown in **Fig. 4(d)**The biodiesel yield was affected by the catalyst wt%. Increasing the catalyst wt% from 1 wt % to 1.5 wt % raised the ester yield from 40% to 79% because the catalyst was more readily available, which led to a faster reaction rate.The biodiesel yield decreased as the catalyst wt% increased. This is because an emulsion was formed due to an overabundance of catalyst, which made purification difficult. This research determined that 1.5 wt% of microalgae oil was the ideal catalyst wt%. Similar results were obtained by Sharma et al. (2016(40)observed maximum biodiesel yield from *Microalgae Chlorella vulgaris*using 1.5% KOH as base catalyst.

3.6. Characterization of product:

3.6.1^{13}CNMR :

Biodiesel produced from microalgae oil that has been treated with UV radiation and microalgae oil that has not been treated **(Fig. 5(a) and 5(b))**exhibit distinct structural variations according to the ^{13}C NMR investigation. At around 174 ppm, the ester carbonyl region (-COO-) was noticed.Increasing increments of δ 174.472 ppm and δ 174.424 ppm were observed for the UV pretreated sample, with peaks exhibiting steeper slopes.However, it seems that the transesterification process was more efficient in the instance where the sample was treated with UV radiation since a single carbon peak was seen in the untreated sample, just above However, it seems that the transesterification process was more efficient in the instance where the sample was treated with UV radiation since a single carbon peak was seen in the untreated sample, just above δ 174.322 ppm, but with less intensity.Unsaturated carbon signals also show variations in the spectrum. The biodiesel that has not been treated with ultraviolet radiation has weaker peaks at 130.203 ppm and 128.038 ppm. In contrast, the peaks at δ 130.315 ppm, δ 130.262 ppm, and δ 128.336 ppm seen in the UVpretreatedbiodiesel suggest a variation in the unsaturation level. The existence of FAME was confirmed by the observation of the methyl ester-associated peak (-OMe) at δ 51.490 ppm for UV pretreatedsamples and δ 51.424 ppm for non-UV pretreated samples.The terminal methyl group signals at δ 14.316 ppm for UV pretreated and δ 14.051

ppm for non-UV pretreated chains, which show slight variations in chain termination. However, there are structural differences in the aliphatic chain region (δ 34.162-δ 29.155 ppm for UV pretreated and δ 34.098-δ 29.105 ppm for non-UV-treated). According to the altered unsaturation patterns and sharp carbonyl peak, biodiesel's composition and efficiency are enhanced by exposure to UV light, which might result in improved fuel attributes(*40*).

3.6.2 Fourier Transform- Infrared spectroscopy (FTIR) analysis of product:

The Fourier Transform Infrared (FTIR) spectra of biodiesel derived from UV pretreated and untreated microalgae oil feedstocks are presented in **Fig. 6.**The stretching vibrations of the ester carbonyl (C=O) were detected at 1740 cm^{-1}, indicating successful transesterification. This proves that FAME is present; the fact that it is more pronounced in the UV Pretreated sample suggests that its ester concentration is larger. Although the -OH stretching absorption band is less intense in biodiesel that has been treated with UV, it nevertheless indicates the presence of residual alcohols, moisture, or free fatty acids in the fuel. This indicates better feedstock purity. The presence of long-chain hydrocarbons is indicated by the strong peaks seen at 3000 and 2800 cm^{-1}, which correspond to the symmetrical and asymmetrical $-CH_2-$ and $-CH_3-$ stretching, respectively. In the 1460–1375 cm^{-1} region, both samples have peaks associated with the C–H bending of alkanes. There is further proof of improved transesterification when the C-O stretching vibrations of the ester functional groups appear between 1350 and 1000 cm^{-1}, are somewhat amplified in UV pretreated biodiesel.

.3.6.3 Gas chromatography – Mass spectrometry (GC-MS) analysis of product:

The biodiesel produced from microalgae oil, with and without UV pretreatment, was analysed using Gas Chromatography-Mass Spectrometry (GC-MS)**(Fig. 7(a) & Fig 7(b)).**Validating the effective transesterification of microalgae oil into biodiesel, the GC-MS analysis highlights the presence of different fatty acid methyl esters (FAMEs). The estimation of the major compounds in both samples is shown in **Table 2**, together with the area % attributed to each compound. The presence of dominant peaks corresponding to 7,10-

octadecadienoic acid methyl ester (18.10% in UV pretreated biodiesel and 18.86% in non-UV biodiesel, respectively) and 7-hexadecenoic acid methyl ester (68.80% in UV pretreated biodiesel and 68.39% in non-UV biodiesel) confirmed that the main biodiesel components conform to standard FAME profiles. So, it was established that microalgae oil could be successfully converted into biodiesel.

The findings of this study showed that UV pretreatment of microalgae oil resulted in compositional changes in the produced biodiesel. One sign that UV pretreatment aids in the breakdown of long-chain fatty acids was the reduction in heavier methyl esters, such as hexacosanoic acid methyl ester, which is present in 0.16 percent of UV pretreated biodiesel compared to 3.1 percent in non-UV pretreated biodiesel. This further improves the biodiesel composition. This agrees with earlier result that found that greater molecular weight chemicals are reduced by UV pretreatment, leading to increased molecular fragmentation and better fuel quality. Also, the fact that the UV pretreated sample was the only one to contain z-7-tetradecenal (0.11%), lends support to the idea that an intermediate must be formed in order to improve oxidative stability. It may be inferred that fatty acid structures are altered by isomerisation or oxidative cleavage in samples that were pretreated with ultraviolet radiation, as no 13-docosenoic acid methyl ester was detected in the untreated samples. This suggests that processes may be converting some 13-docosenoic acid fatty acid precursors into unsaturated fatty acids. The observation of the lack of 8,11,14-docosatrienoic acid methyl ester in biodiesel lends evidence to the argument that prolonged exposure to UV radiation accelerates the degradation of these unstable long-chain fatty acid esters, resulting in superior biodiesel.

CONCLUSION:

This study's findings highlight the importance of UV pretreatment of microalgae oil and catalyst selection in enhancing the yield of environmentally friendly biodiesel. When comparing the three catalysts (NaOH, KOH, and CaO), KOH produced the highest quality and quantity of biodiesel. Purification of biodiesel was done using brine solution, which acts as a demulsifying agent. Its higher ionic strength compared to normal water helps breaking emulsions, making the purification process more efficient. Under optimised transesterification conditions, the maximum yield of fatty acid methyl ester (FAME) was 79% using a KOH catalyst and UV pretreated algae oil. Biodiesel may be a viable alternative fuel option as its physiochemical properties were within the range of ASTM standards. The creation of esters with distinct peaks, like the C=O stretching of methyl esters, was verified by structural investigation using FTIR. Gas chromatography-mass spectrometry (GC-MS) testing further backs up the biodiesel structure perception; moreover, the long range revealed a high methyl ester ratio, which is often associated with high-quality biodiesel. Further evidence of the transesterification process was provided by nuclear magnetic resonance spectroscopy, which revealed methoxy protons and reduced glyceride peaks, proving that triglycerides had been converted into methyl esters. Finding a suitable catalyst, doing thorough product analysis, and maximising UV exposure are all necessary steps towards improving the yield and quality of microalgae-based biodiesel. This study utilises the development of permanent biofuel technologies to provide the groundwork for expanding the biodiesel production process on an industrial scale.

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DECLARATION OF COMPETING INTEREST:

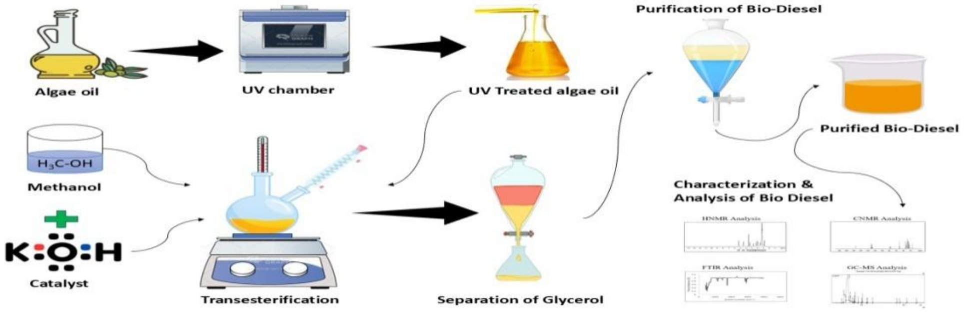
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

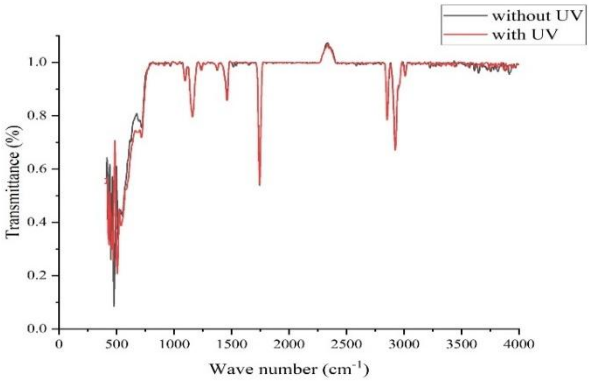
DATA AVAILABILITY:

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

FUNDING:

No funding received.





(Canvas was utilized as the primary design tool)

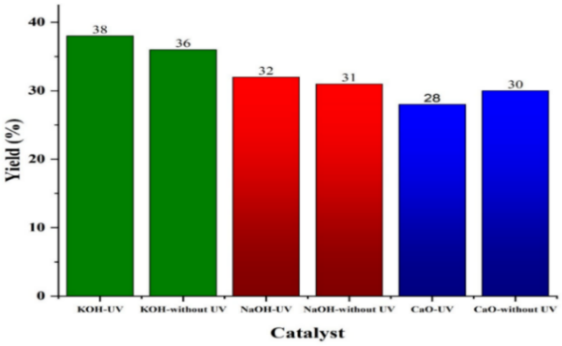
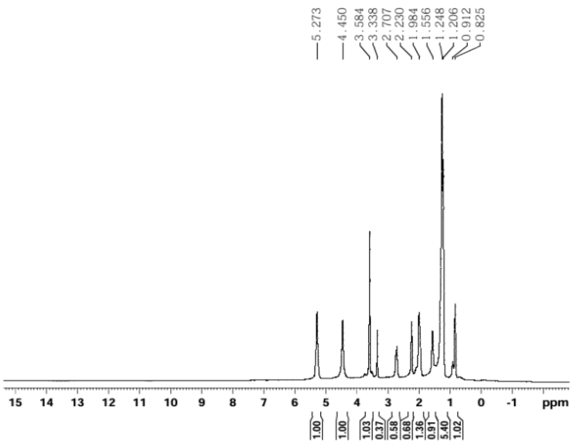


Fig. 1FTIR analysis of feedstock (microalgae oil) before and after UV pretreatment

(Fig. 1.Origin was utilized as the primary design tool)



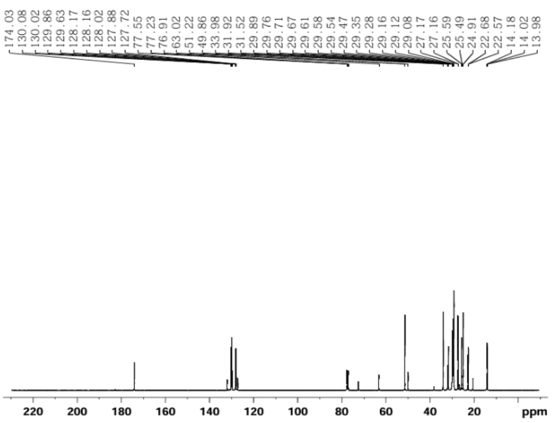
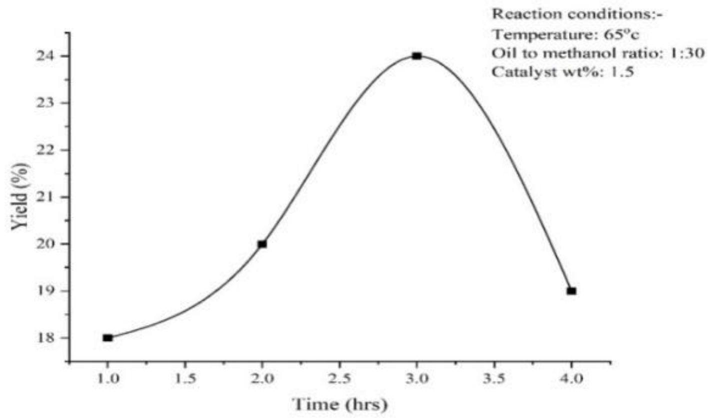


Fig. 2:Comparison of biodiesel yields from various catalysts (before and after feedstock

(Fig. 2. Canvas was utilized as the primary design tool)



Figs. 3 (a) & 3 (b) H-NMR and ^{13}C NMR of biodiesel

(Fig. 3 (a)&3(b).Origin was utilized as the primary design tool)

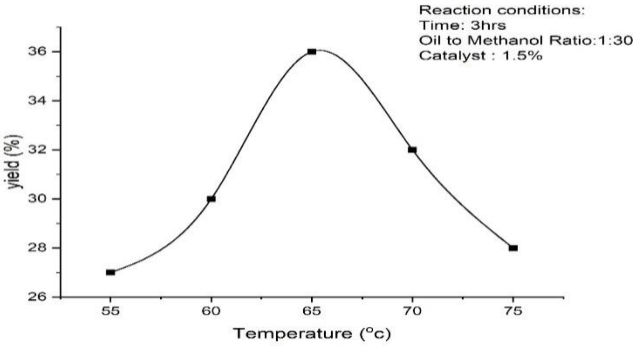


Fig. 4(a) Effect of reaction time on biodiesel yield

(Fig. 4(a).Origin was utilized as the primary design tool)

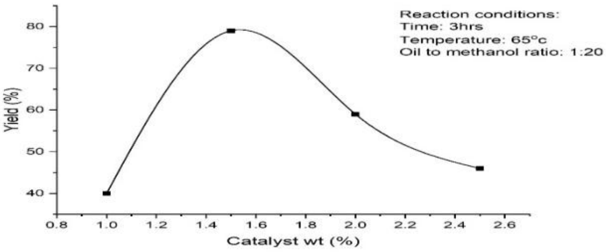


Fig.4(b) Effect of reaction temperature on biodiesel yield

(Fig. 4(b).Origin was utilized as the primary design tool)

Fig. 4(c) Effect of oil to methanol molar ratio on biodiesel yield

(Fig. 4(c).Origin was utilized as the primary design tool)

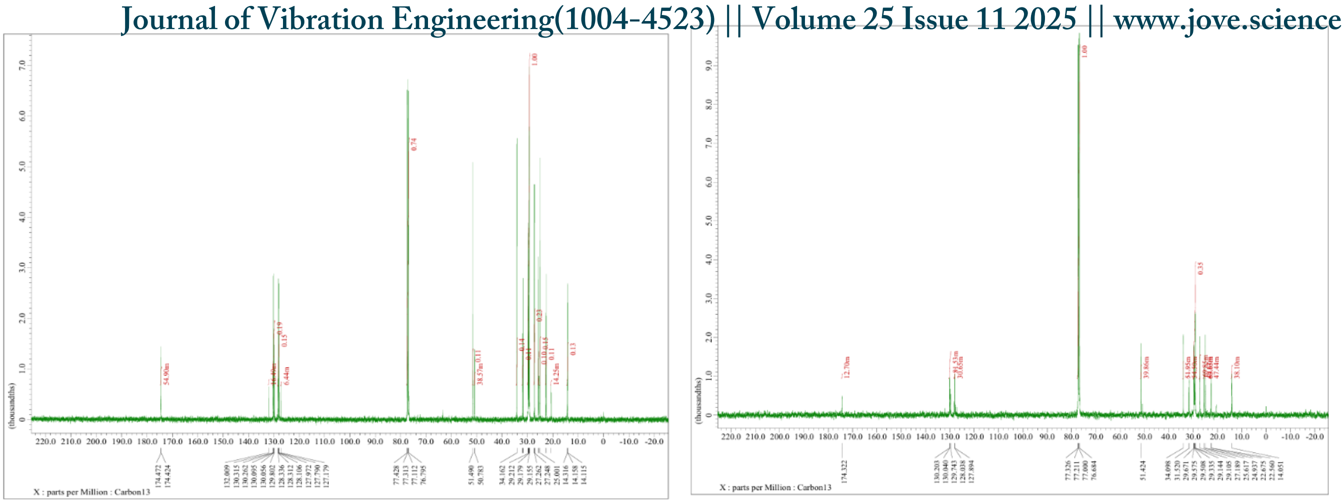
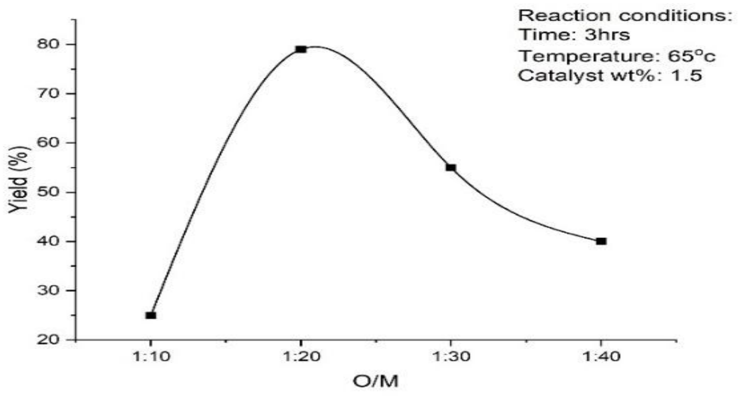


Fig. 4(d) Effect of catalyst wt% on biodiesel yield

(Fig. 4(d).Origin was utilized as the primary design tool)

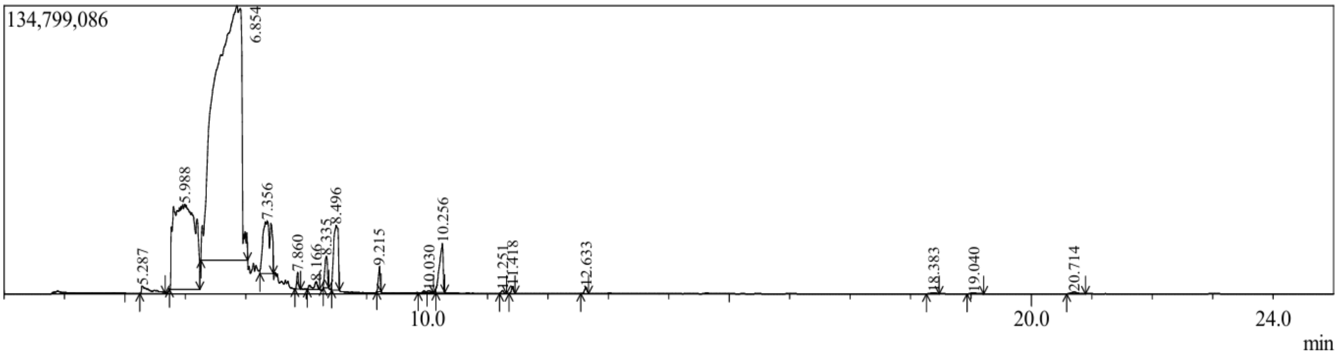


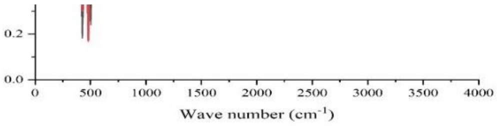
Figs. 5(a)&(b)^{13}C NMR analysis of biodiesel produced from with and without UV pretreated Microalgae oil

(Fig. 5 (a)&5 (b).Origin was utilized as the primary design tool)

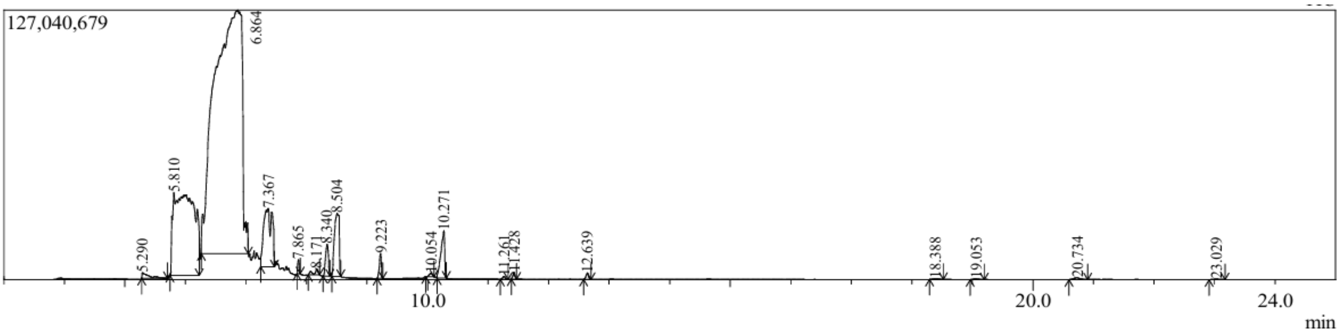
Fig. 6 Comparison of FTIR analysis of biodiesel produced from with and without pretreatment of feedstock

(Fig. 6. Origin was utilized as the primary design tool)





Figs. 7(a)& 7(b)GC-MS analysis of biodiesel produced from with and without UV pretreated



feedstock

(Fig. 7 (a)&7(b).Origin was utilized as the primary design tool)

Table-1. Comparison of Fuel properties of biodiesel with ASTM/EN standards:

Property	**Micro Algae Biodiesel**	**ASTM/EN standards**
Flashpoint(°C)	149	>93
Density(kg/m^3)	870	860-900

Kinematic viscosity(mm^2/s)	4.54	1.9-6.0
pH	9	8-9
Specific gravity	0.87	0.86-0.9
Color	Yellow	Yellow or orange
Acid value (mgKOH/g)	0.2	<0.5
Cetane	52	>47
Refractive index	1.446	1.447-1.48
Calorific value (MJ/kg)	45.26	>37
Ash content (%)	0.016	<0.02

Table 2: Comparison of GC-MS peak relative areas of the identified compounds in biodiesel produced from with and without UV pretreatment of feedstock:

SNO	Compounds	Biodiesel (with UV) Area%	Biodiesel (without UV) Area%
1	Hexadecanoic acid, methyl ester	0.41	0.68

2	7,10-octadecadienoic acid, methyl ester	18.10	18.86
3	7-hexadecenoic acid, methyl ester	68.80	68.39
4	13-docosenoic acid, methyl ester	5.08	-
5	Docosanoic acid methyl ester	0.21	4.44
6	Tricosanoic acid methyl ester	0.73	0.75
7	Tetracosanoic acid methyl ester	2.74	2.18
8	Pentacosanoic acid methyl ester	3.32	1.02
9	Hexacosanic acid methyl ester	0.16	3.117
10	z-7-tetradecenal	0.11	-
11	Squalene	0.07	0.08
12	8,11,14-docosatrienoic acid, methyl ester	-	0.12

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