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Advances in algal biofuel production through catalysts and nanocatalysts: an integrated chemical, biochemical, and physical analysis

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Abstract

Biofuel has emerged as one of the most promising alternatives to petroleum-derived fuels, particularly in the current context of rapidly depleting fossil fuel reserves. The combustion of fossil-based fuels poses serious risks to both the environment and human health due to the release of greenhouse gases. This study aimed to assess the fatty acid methyl ester (FAME) composition, viscosity, acid number, and chemical metal elements present in algal biodiesel produced through a transesterification bioprocess. Nanoparticles (NPs) offer a promising solution to current challenges in biomass utilization. Their advantages include high selectivity, energy efficiency, effective time management, and reduced costs. Biodiesel conversion yield, along with its physical and chemical properties, was analyzed using GC–MS and a multi-element oil analyzer. The highest conversion yield of 98.9% was achieved using a mixed catalyst–nanocatalyst system (NaOH + CaO + ZnO) under optimal conditions: a 1:4 oil-to-methanol volumetric ratio, 1.0% catalyst concentration, reaction temperature of 40°C, and stirring speed of 320 rpm. A significant difference was observed in the viscosity and acid value between biodiesel produced using a single catalyst and that produced using catalyst mixtures. Furthermore, the concentrations of chemical metal elements (Na, Ca, Mg, Cu, Fe, Zn, Ag, Al, P, Pb, B, Cr, Mn, Sn, Mo, Si, Ba, Ni, Ti, and V) were lower in biodiesel derived from mixed nanocatalyst–catalyst combinations (NaOH + ZnO, NaOH + CaO, and NaOH + CaO + ZnO) compared to the single-catalyst system (NaOH). Overall, biodiesel produced using the combined catalyst and nanocatalyst approach exhibited reduced metal element content and superior yield compared to biodiesel obtained using a single catalyst. In addition, nanomaterials such as nanocatalysts have been found to be cost effective as well as stable catalysts for enzyme immobilization, thus improving biofuel synthesis.

Keywords: Biofuel, Transesterification, Nanocatalyst, Nanoparticles, Catalyst, Immobilization.

1. Introduction

It is very well known that the consumption of fossil fuels is increasing rapidly with an increase in population growth rate and urbanization, leading towards the exhaustion of petroleum-derived fuels in the near future. The limited availability of fossil fuels is a major problem around the globe. Moreover, high dependency on petroleum-derived fuels has raised many questions about its adverse effects on the environment, on the economy, and on energy saving. Therefore, significant research has been focused on the search for an alternative source that can reduce the consumption of fossil-derived fuels [1]. With the need to reduce carbon emissions as well as global warming and the dwindling reserves of crude oil, alternative fuels derived from plant materials or biomasses are an attractive source of energy [2]. The last couple of decades have seen the necessity for energy increased exponentially to fulfill the demands of industrialization and population explosion worldwide [3]. The primary energy sources are petroleum, natural gas, coal, hydro, and nuclear [3,4]. However, using petroleum-based fuels has become a significant disadvantage as it brings about atmospheric pollution, as petroleum combustion is a primary source of greenhouse gas that is believed to be causing global warming. One way to mitigate greenhouse gas emissions was to utilize biofuels instead of fossil fuels, and biodiesel was a substitute that reduced greenhouse gas emissions [5,6].

According to the American Standard and Testing Material specifications (ASTM D6751), biodiesel is a fuel comprised of mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats [3]. It is non-toxic, biodegradable, produced from renewable sources, and contributes minimal net greenhouse gases, such as CO_2 and NOx emissions and sulfur to the atmosphere [7]. It has been used alone or blended with conventional petro-diesel fuel in unmodified diesel-engine vehicles. Waste cooking oil was an economical and renewable source for biodiesel production [3]. Biodiesel fuel can be prepared from waste cooking oil, such as palm oil, soybean, canola, rice bran, sunflower, and corn oil. It can also be made from waste fish oil and chicken fat [8]. Although this can partly alleviate the dependency on petroleum-based fuel, it would fall far short of the world's demand for alternative energy.

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Nowadays, micro and macroalgae have emerged as one of the most promising, if not the best, sources for biodiesel production. It has been reported that algae are the highest-yielding feedstock for biodiesel [9]. They noted that algae can produce up to 250 times the amount of oil per acre compared to crops like soybeans. It has also been estimated that algae can produce 7 to 31 times more oil than palm oil [10]. The best algae source for biodiesel production is generally believed to be the microalgae with nano-catalysts. Unlike their larger cousins, the macroalgae have more oil and are much easier and faster to culture and grow. Producing biodiesel from algae is now considered the most promising way to make enough automotive fuel to replace current gasoline usage [11]. The use of nanotechnology and nanomaterials in biofuel research has risen as a promising tool in providing cost-effective techniques to improve production quality. There are multiple advantages to using nanoparticles (NPs) over other sources for biofuel synthesis due to their size and unique properties such as the high surface area to volume ratio and special attributes such as a significant extent of crystallinity, catalytic activity, adsorption capacity, and stability [12,13]. Carbon nanotubes and metal oxide nanoparticles are generally used as nano-catalysts for biofuel production because of their additional properties which aid in high potential recovery [14, 15]. Therefore, the research work was undertaken to investigate the biodiesel formation from algae using catalysts (NaOH) and a mixture of base catalysts and nano-catalysts (NaOH+ZnO, NaOH+CaO and NaOH+CaO+ZnO) as well as to evaluate the chemical metal elements of produced biodiesel using the catalyst and nano-catalyst-based transesterification bioprocess.

2. Materials and methods

Algae (*Spirogyra sp*) were collected from the Yamuna River, New Delhi, India. The macroalgae were cultured and cared for in the aquaculture pond, maintaining all necessary environmental and production factors. The sample has been identified in Department of Environmental Botany, Jamia Hamdard University, New Delhi, India.

2.1. Oil extraction

Oil from the algae was extracted following the method established and modified [11]. The algae sample was ground in a motar and pestle and semi-dried in the oven at 40°C for about 30 minutes (Figure 1A). After cooling down the semi-dried sample, hexane and diether (10 x volume of sample) were added to extract the oil. The mixture was then kept aside for 72 hours to settle down. The biomass was collected after filtration and weighed (Figure 1B).

The extracted oil was evaporated in a rotary vacuum evaporator to remove hexane and ether. Subsequently, the catalysts, 1% of NaOH alone, 1% of NaOH+CaO, 1% of NaOH+ZnO, and 1% NaOH+CaO+ZnO nano-catalyst in methanol, were poured into the algal oil in a 1:4 volume ratio of algae to methanol in a conical flask. The conical flask was shaken for 3 hours on an electric shaker at 320rpm. The transesterification reaction process is shown below (Figure 2).

After shaking, the solution was kept for 24 hours to settle down the biodiesel. The biodiesel was carefully separated from the sediments by using a separating flask. The biodiesel formed was washed with 10% water until clean and dry-washed using sodium sulfate to remove soap/foam formation. The biodiesel was then dried at 40°C in an oven for 2 hours (Figure 2).

2.2. Biodiesel analysis

The biodiesel produced was analyzed using standard procedures to determine whether it met the specifications laid out by the American and European standards, ASTM D6751 and EN14214. GC-MS was used to identify the fatty acid methyl ester (FAME). GC-MS service utilized advanced instrumentation, including the Agilent 7890A Technologies, USA, GC for analysis of fatty acids. A GC-MS analyzed FAME, peaks of interest elute by 16 minutes, but a 24-minute bake-out at 320˚C is required to elute heavy components. Using the 7890A system's backflush capability, with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. Helium was used as carrier gas. All chromatographic separation were performed on a capillary column (TRB-FFAP; Technokroma), having specifications: lenth; 30 m, i.d; 0.35 mm, thickness; 0.250 µm, treated with polyethylene glycol. 1 µl sample and standard were injected into the CG column. MS scanning was performed from 85 to 380 m/z. GC-MS solutions software provided by the supplier was used to control the system and to acquire the data.

2.3. Analytical condition

Thermo-Finnigan Trace DSQ contained a Hewlett-Packard-5 (5%-phenyl-methyl-polysiloxane) column with a dimension of 30 m x 0.32 mm and a 0.25 µm film thickness. The auto-injector was applied in the spitless mode at 220°C, helium was used as a carrier gas at a flow rate of 1.5 mL/min. 1 µL of sample was injected using a Thermo Scientific TriPlis RSH autoinjector, the oven temperature was at 80°C for 1 min and then it was increased to 200°C at a rate of 10°C/min [16]

An Atomic Emission (AE) spectroscopic Multi-element Oil Analyzer was used to determine the metal content Na, Ca, Mg, Cu, Fe, Zn, Ag, Al, P, Pb, B, Cr, Mn, Sn, Mo, Si, Cr, Ba, Ni, Ti & V).

2.4. MOA condition

MOA (MOA 11 plus, USA) was designed for metals and fuel analysis using atomic emission (AE) spectroscopy. A nuclear emission spectrometer employing the rotating disk electrode (RDE) technique was used following the temperature of MOA, which was 30°C (60% R.H.). The dimensions were 89 x 81 x 65 cm (LHD), and the power was 1.5 kW [16]. Viscosity was measured in centrifugation stokes (cSt) at 40°C using a Houillion viscometer with ISL (Integrated Solutions Ltd) software version 2.1. The total acid value was determined by titration with KOH and phenolphthalein as an indicator.

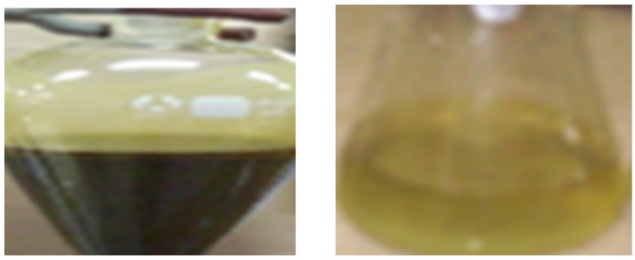


Fig. 1. Photograph shows the oil extraction and biodiesel-yielding process from algae (A. Algae Oil extraction, Algal biodiesel).

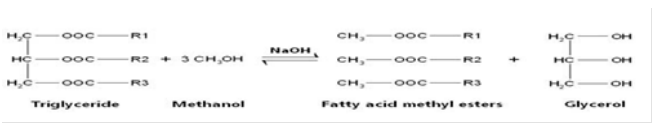


Fig. 2. Reaction shows transesterification process in biodiesel.

3. Results

3.1. Effect catalysts on biodiesel formation and quality

As shown in Table 1, biodiesel yield from *Spirogyra sp* was greater when a mixture of nanocatalysts was used than when a single catalyst was employed. The amount of biodiesel produced when the heterogenous mixture of catalyst and nanocatalysts (NaOH+CaO+ZnO) was used amounted to a 98.9% conversion. When only sodium hydroxide (NaOH) was used, the conversion was lower at 89.7%. Looking at the viscosities of the two biodiesels formed, which partially represents its quality, it was observed that it was lower in the mixture of catalysts and nanocatalyst reaction than a single of catalysts used (Table 1). The viscosity values were 3.4 cSt when the NaOH catalyst was used and 3.0, 2.6 & 2.1 when NaOH+ZnO, NaOH+CaO and NaOH+CaO+ZnO nano-catalysts mixtures were used, respectively. It has been seen in Table 1, that the TAN value was much higher (0.38 mgKOH/g) in the biodiesel made using NaOH alone compared to the using NaOH+ZnO, NaOH+CaO and NaOH+CaO+ZnO mixture of nanocatalysts where the values obtained were 0.34, 0.23 and 0.18 mgKOH/g respectively.

Biodiesel formation via alkaline transesterification from the oil of biological origins under different conditions, such as temperature, stirring speed, catalysts, and alcohol-to-oil ratios has been well studied and reported in the literature [11]. Most researchers generally agree that the optimum conditions are alcohol (methanol) to oil ratio of between 3:1 to 6:1, a temperature of 40°C, and a stirring speed of around 320 rpm [16]. These conditions were employed in this study with the variation of using a single base KOH catalyst, a mixture of catalyst and nano catalysts (NaOH+MgO, NaOH+CaO), and NaOH+MgO+CaO nano catalyst. Table 2 shows the standard values of biodiesel properties as shown by American and European biodiesel standards in ASTM D6751 and EN14214.

Table 2 shows the different types of methyl ester and their conversion rates determined by GC-MS. Fatty acid methyl ester (FAMES) was found higher in the mixture of catalya nd nanocatalyst than the catalyst alone. It was observed 4 methyl esters (methyl esters (methyl palmitate, methyl linoleate, methyl oleate and methyl stearate) in the single catalyst (NaOH), 5 methyl esters (methyl palmitate, methyl linoleate, methyl oleate, methyl stearate and methyl arachidonate) in the NaOH+ZnO and NaOH+CaO mixture of nanocatalyst and 6 methyl esters (methyl palmitate, methyl linoleate, methyl oleate, methyl stearate, methyl arachidonate and methyl eicosapentaenate in NaOH+CaO+ZnO mixture of nanocatalyst. The biodiesel produced was composed mainly of 16 and 18-carbon fatty acids in the case of a single catalyst. However, it was found 20 carbon fatty acids in the case of a mixture of catalyst and nanocatalysts. The conversion rate was higher (palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, and linoleic acid methyl ester) mixture of catalysts than in a single catalyst. Standard values of biodiesel properties as shown by American and European biodiesel standards in ASTM D6751 and EN14214 (Table 3).

Biodiesel chemical metal element content analysis, as shown in Table 4, the silicon (Si), phosphorus (P), stannum (Sn), manganese (Mn), boron (B) and calcium (ca), contents were higher than the iron, copper, molybdenum, zinc, magnesium, and other contents in the case of single and mixture of catalyst and nanocatalyst. Furthermore, the amount of iron, copper (Cu), zinc (Zn), magnesium (Mg), phosphorus (P), silver (Ag) and silicon (Si) was lower in the biodiesel formed from the mixture of catalysts and nano-catalysts compared to the single catalyst (Table 3).

It had been reported that macroalgae **(**seaweeds**)** contained lipid content of 1.3-7.8% (dw) and were very high**,** up to 51% of total fatty acids [13]. It was reported that seaweeds (*Sargassum muticum*) contain about 5.5% oil on a fresh weight basis [15]. In addition to this, it was reported that biodiesel can be produced from *Spirogyra sp* and *Oedogonium sp.* by using catalyst NaOH [16]. Their results showed that the methyl ester yield was higher in *Oedogonium* sp than in *Spirogyra sp*. [17].

3.2. Statistical analysis

The least Significant Difference Test (LSDT) was employed to observe significant differences at a 5% level (0.05) within the replicates. Standard error was also determined.

4. Discussion

Various catalysts have been used in the alkaline transesterification method to produce biodiesel and reported the transesterification occurred of used oil to produce biodiesel using an alkaline and acid catalyst [18]. It was reported the transesterification of two types of used oils, namely partially hydrogenated soybean oil and margarine with methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, and 2-ethoxyethanol. Subsequently, they developed a process to produce alkyl esters from feedstocks that have a high FFA content, diglycerides, and monoglycerides, using calcium and barium acetate as catalysts [19]. It was reported that the use of two catalysts, such as NaOH and a combination of barium and calcium acetate, for the preparation of methyl esters from waste cooking oil exhibited higher yield in the combination of catalysts than in a single catalyst [20]. It was also reported that the FAME yield increased when heterogeneous superacid catalysts, SO_4^{2-}/ZrO_2, were used [21]

Moreover, the quality characteristics of the biodiesel produced from the *Spirogyra sp.* fell well within the standard limits (Table 2) established in the American and European biodiesel standards as in ASTM D6751 and EN14214 respectively, the biodiesel obtained from homogenous catalyst had a slightly higher acid value (TAN) of 0.4 &3.9 mg KOH/g compared to biodiesel produced from heterogenous catalysts 0.25 & 0.2 mg KOH/g. The TAN value is an important measurement of the free fatty acids present as high amounts of acid can cause corrosion of the engine and shorten its lifespan [22-24].

Regarding viscosity, which is an important trait since it measures the resistance of the biodiesel to flow and can affect the operation and performance of fuel injection engines, both the biodiesels produced showed significant differences in viscosity

Table 1. Determination of biodiesel conversion yield, total acid number and viscosity of algal biodiesel. Means followed by the same letter do not differ significantly at 5% level by the Least Significance test (LSD) test. Mean± SE (n=4).

Parameters	Biodiesel conversion yield (%)	Total acid No. (TAN) (mgKOH/g oil)	Viscosity (cSt)
NaOH	89.7±0.03c	0.38±0.02a	3.4±0.05a
NaOH+ ZnO	94.5±0.04b	0.34±0.02a	3.0±0.01a
NaOH+CaO	95.6±0.03b	0.23±0.01b	2.6±0.04ab
NaOH+CaO+ZnO	98.9±0.02a	0.18±0b	2.1±0.02b

Table 2. Fatty acid methyl ester and conversion rate were determined from freshwater algae by using GC-MS.

Algal biodiesel sample	Peak serial	RT (min)	Fatty acid Methyl ester	Fatty acid	% Relative
Single catalyst (NaOH)	1	17.36	$C_{16}H_{32}O_2$ CH_3 $C_{18}H_{36}O_2$ CH_3 $C_{18}H_{34}O_2$ CH_3 $C_{18}H_{32}O_2$ CH_3 Trace	Palmitic acid	50.4
	2	21.28		Stearic acid	3.4
	3	24.47		Oleic acid	18.4
	4	24.96		Linoleic acid	17.5
				Unidentified	--
					89.7%
Mixture of catalyst (NaOH +CaO)	1	17.28	$C_{16}H_{32}O_2$ CH_3 $C_{18}H_{36}O_2$ CH_3 $C_{18}H_{34}O_2$ CH_3 $C_{18}H_{32}O_2$ CH_3 $C20H_{32}O_2$ CH_3	Palmitic acid	51.5
	2	21.18		Stearic acid	3.5
	3	24.26		Oleic acid	18.3
	4	24.64		Linoleic acid	17.4
	5	22.24		Arachidonic acid	4.9
					95.6%
Mixture of catalyst (NaOH +ZnO)	1	17.23	$C_{16}H_{32}O_2$ CH_3 $C_{18}H_{36}O_2$ CH_3 $C_{18}H_{34}O_2$ CH_3 $C_{18}H_{32}O_2$ CH_3 $C20H_{32}O_2$ CH_3	Palmitic acid	50.5
	2	21.19		Stearic acid	4.0
	3	24.36		Oleic acid	18.2
	4	24.54		Linoleic acid	17.2
	5	22.44		Arachidonic acid	4.6
					94.5%
Mixture of catalyst (NaOH +CaO+ZnO)	1	17.29	$C_{16}H_{32}O_2$ CH_3 $C_{18}H_{36}O_2$ CH_3 $C_{18}H_{34}O_2$ CH_3 $C_{18}H_{32}O_2$ CH_3 $C20H_{32}O_2$ CH_3 $C20H_{30}O_2$ CH_3	Palmitic acid	51.6
	2	21.18		Stearic acid	3.8
	3	24.35		Oleic acid	18.7
	4	24.37		Linoleic acid	17.6
	5	22.34		Arachidonic acid	4.3
	6	22.32		Eicosapentaenoic acid	2.9
					98.9%

Table 3. Standard values of biodiesel properties as shown by American and European biodiesel standards in ASTM D6751 and EN14214.

Property	Unit	Standard Method	Value according to standard methods
Viscosity	cSt at 40°C	ASTM D 6751	1.9 - 6.0
Acid number	mgKOH/g oil	ASTM D 6751	0.50 max
Group I Metals (Na^+ K)	mg/kg	EN 14214	5.0 max
Group II Metals (Ca^+ Mg)	mg/kg	EN 14214	5.0 max
Phosphorus content	mg/kg	EN 14214	10.0 max

Table 4. Chemical metal elements analysis from algal biodiesel. Trace amount or no interfere of Zn, Cu, Mn, Fe, CO, Pb, Cr metal. Standard method, EN 14214. Means followed by the same letter do not differ significantly at 5% level by the Least Significance test (LSD) test. Mean± SE (n=4).

Chemical Metal Elements	NaOH (mg/kg)	NaOH+ ZnO (mg/kg)	KOH + CaO (mg/kg)	KOH+ CaO+ZnO (mg/kg)
Na	3.3±0.03a	3.2±0.02a	3.2±0.02a	2.3±0.01b
Ca	3.7±0.03a	3.6±0.03a	2.5±0.03b	2.0±0.01b
P	4.5±0.05a	4.1±0.03a	4.0±0.06ab	3.8±0.03b
Mg	2.4a±0.01a	2.6±0.03a	1.7±0.02b	1.4±0.01b
Cu	0.7±0.02a	0.6±0.01a	0.36±0.01b	0.30±0.02b
Fe	2.1±0.02a	2.1±0.03a	1.7±0.02b	1.5±0.02b
Zn	2.2±0.01a	2.1±0.02a	0.7±0.01b	0.5±0.01b
Ag	1.2±0.02a	1.0±0.0a	0.65±0.02b	0.55±0.02b
Al	1.45±0.01a	1.1±0.02a	0.62±0.025b	0.16±0.01b
Pb	0	0	0	0
B	3.1±0.01a	2.2±0.02a	1.3±0.031b	0.11±0.01b
Cr	2.2±0.03a	2.1±0.02a	1.1±0.013b	0.22±0.021b
Mn	3.3±0.03a	3.1±0.03a	2.0±0.021b	1.7±0.01b
Sn	3.6±0.04a	3.2±0.04ab	3.0±0.2b	2.45±0.03b
Mo	1.69±0.02a	1.4±0.02a	1.1±0.04b	0.41±0.03b
Si	3.9±0.04a	3.8±0.05a	3.2±0.2ab	2.0±0.05b
Cr	0.8±0.02a	0.7±0.02a	0.3±0.03b	0.22±0.02b
V	2.0±0.03a	1.8±0.03a	1.5±0.4b	1.0±0.04b
Ba	0.7±0.02a	0.6±0.02a	0.4±0.03b	0.3±0.01b
Ni	0.5±0.03a	0.45±0.01a	0.2±0.02b	0.11±0.01b
Ti	0.31±0.01a	0.3±0.03a	0.22±0.02b	0.15±0.005b

values which were well within the ASTM standard range of 1.9 – 6.0 centistokes (cSt.) (Table 3). The chemical metal element in both (catalyst and nano-catalyst-based) biodiesels also showed values well within the ASTM D6751 and EN14214 standards although some variations in amounts were observed in both the homogenous and heterogenous catalyzed biodiesels [25-27]. It was reported that almost all of the metal elements followed the standards of ASTM except lead (Pb) which showed toxicity to the microalgae [28-30]. However, current results exhibited all elements followed the ASTM D6751 and EN14214 and there was no harmful value found for the freshwater algae.

The findings showed that using a combination of traditional catalysts and nanocatalysts, freshwater algae can yield significant amounts of biodiesel. Notably, mixed catalyst systems (NaOH+ZnO, NaOH+CaO, and NaOH+ZnO+CaO) greatly increased the yield of biodiesel while lowering the content of undesirable chemical elements and total acid number (TAN). The NaOH+ZnO+CaO catalyst–nanocatalyst combination turned out to be the most successful of these. This mixed nanocatalyst system produced biodiesel of exceptional quality that met international fuel standards such as ASTM D6751 and EN 14214.

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Declaration
The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics
NA

Consent
Authors read and approved the final manuscripts.

Data availability statement
This study did not report any data.

Conflicts of interest
Author declare no competing interests with the work presented in the manuscript.

Author Contributions
All the works has been done by AAC.

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