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APPLICATION OF ALKALINE CATALYSED TRANSESTERIFICATION BIOPROCESS IN BIODIESEL PRODUCTION FROM FRESH WATER ALGAE

A.B.M. Sharif Hossain
Programme of Biotechnology, Department of Biology, Faculty of Science, University of Hail, Hail 2440, KSA
*Corresponding Authors email: hossainsharif41@gmail.com

ABSTRACT
The experiment was conducted to evaluate the biodiesel production (yield and quality) at different alkaline and acid catalysts from fresh water algae. Algal species, Spirogyra was used to produce biodiesel by the implementation of the alkaline and acid transesterification process. A single homogenous catalyst (NaOH) and heterogeneous mixture of catalysts (KOH + NaOH + H_2SO_4) were treated to achieve a high quality biodiesel fuel that complied with the specifications of the American Standard Testing and Material (ASTM D 6751) and European Norm (EN 14214) standards. The biodiesel yield, physical and chemical properties of the produced biodiesel was investigated. The highest biodiesel yield of 95.9% was achieved dealing 1:3 volumetric oil-to-methanol proportions by 1.5% mixture of catalysts (NaOH + KOH + H_2SO_4) at 40°C reaction temperature and a stirring speed of 320 rpm. Biodiesel formation yielded a lower at the rate of 92.9% biodiesel in a single catalyst than in the mixture of catalysts (96.9%). There was no significant difference in the viscosity of the biodiesel produced between the single and mixture of catalysts. However, the total acid number and metal (Na, Ca, Mg, Cu) content differed significantly between the homogenous and heterogeneous catalysts of produced biodiesel. There was more methyl ester (biodiesel yield) of biodiesel produced in the mixture of catalysts compared to the biodiesel formed applying a single catalyst. The results demonstrated that biodiesel obtained from *Spirogyra sp.* under optimum conditions through alkaline and acid transesterification, were of good quality that could be practiced as a source of diesel fuel.

KEY WORDS: *Spirogyra*, biodiesel yield, alkaline and acid transesterification, heterogeneous mixture of catalysts

INTRODUCTION

The necessities for energy increased exponentially to mitigate the demands of industrialization and population explosion worldwide since last couple of decades. At present the main source of energy comes from petroleum, natural gas, coal, hydro, and nuclear[3]. However using petroleum-based fuels has become a major disadvantage as it brings about atmospheric pollution as petroleum combustion is a major source of greenhouse gas that is believed to be causing about global warming. One way to mitigate greenhouse gas emissions is to use biofuel in place of fossil fuels and biodiesel is one such substitute that reduces greenhouse gas emissions [4]. According to the American Standard Testing and Material specifications for biodiesel (ASTM D6751), biodiesel is defined as a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats[1]. It is non-toxic, biodegradable, produced from renewable sources and contributes a minimal amount of net green house gases, such as CO_2 and NO emissions and sulfur to the atmosphere[2]. It has been used alone or blended with conventional petro-diesel fuel in unmodified diesel-engine vehicles. One economical and renewable source for biodiesel production is used cooking oil[3]. Biodiesel fuel can be prepared from waste cooking oil, from such sources as palm oil, soybean, canola, rice bran, sunflower and corn oil. It can also be made from waste fish oil and chicken fat [5]. Although this can partly alleviate the dependency on petroleum-based fuel it would fall far short of the world's demand for alternative energy. Over the last decade, algae have emerged as one of the most promising if not the best source for biodiesel production. It has been reported that algae are the highest yielding feedstock for biodiesel[6]. They reported that algae could produce up to 250 times the amount of oil per acre compared to crops like soybean. It has also been estimated that algae can produce 7 to 31 times more oil than palm oil[7]. It is generally believed that microalgae would be the best source of algae for biodiesel production. Unlike their larger cousins, the macro algae has more oil and are much easier and faster to culture and grow. In fact, producing biodiesel from algae is now considered to be the most promising way to produce enough automotive fuel to replace current gasoline usage. Recently, it has been reported that the use of heterogeneous catalysts can reduce the processing costs associated with the use of homogeneous catalysts and many alkaline catalysts such as sodium hydroxide, potassium hydroxide, calcium carbonate rock, EST-4 and EST-10 catalysts, and Na/NaOH/γ-Al_2O_3 were studied[8]. However there is no literature reporting on biodiesel formation from the filamentous freshwater alga Spirogyra *sp*. In this study, biodiesel formation from Spirogyra was investigated to know the methylester yield using the alkaline transesterification method applying different catalysts under optimum conditions and to identify the fatty acid methyl ester. In addition to this the quality of the biodiesel produced was determined to observe if it met ASTM standards [9].

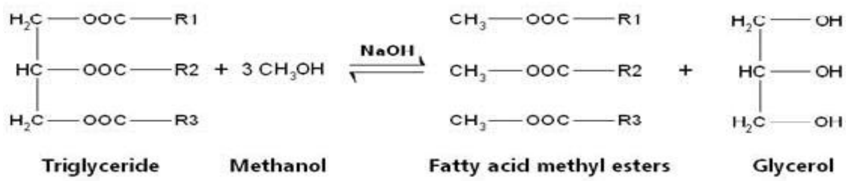
MATERIALS & METHODS

Algae (*Spirogyra sp*) were collected from the surrounding area of University of Hail (Fig. 1). The samples were identified by the Phycology laboratory, Biological Sciences, Faculty of Science, University of Hail, Hail, Saudi Arabia.

Oil extraction

Oil from the algae was extracted following the method established by [10] and modified by[11]. The algae sample was ground in a motor and pestle and semi-dried in the oven at 80^0C for 30 minutes (Fig. 1). 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 (Fig. 1). The biomass was collected after filtration and weighed (Fig. 1). The extracted oil was next evaporated in a rotary vacuum evaporator to remove hexane and ether. Subsequently the catalysts, 1.5% of NaOH alone and 1.5% of NaOH and KOH and H_2SO_4 combined in methanol, were poured into the algal oil in a 1:3 volume ratio of algae to methanol in a conical flask. The conical flask was then shaken for 4 hours using an electric shaker at 320rpm. The transesterification reaction process is shown below:

FIGURE 1. Photographs Show the oil extraction and biodiesel yield from Spirogyra sp. algae (Algae) (Oil extraction) Extrated oil Biodiesel (I: single, II: mixture)



After shaking, the solution was kept for 24 hours to settle down the biodiesel and sediment layers clearly. The biodiesel was carefully separated from the sediments by using a separating flask. The quantity of sediment (glycerol, pigments, *etc*.) was determined. The biodiesel formed was washed with 10% water until it was clean and dry washed using sodium sulphate to remove soap/foam formation. The biodiesel was then dried at 40^0C in an oven for 2hours. Biodiesel yield was determined volumetrically.

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. An atomic emission (AE) spectroscopic multi-element oil analyzer (MOA) was used to determine the metal (Na, Mg, Zn, Ca, Fe, Cu, Si, Mo, Sn and V) content. Viscosity was measured in cSt (centi stokes) at 40°C using a Houillion viscometer with ISL (Integrated Solutions Ltd) software version 2.1. Total acid value was determined by titration with KOH and phenolphthalein as an indicator.

Statistical analysis

Least Significant difference Test (LSD) was done to observe significant differences at 5% level (0.05) within the replicates. Standard error was also determined.

RESULTS

Algal oil extraction

Table 1 shows the quantity of oil extracted from the algal biomass. From 2.0 kg of algae, 0.16L of algal oil was obtained which represented 8.1 % oil of the biomass. The dry weight of the algal biomass was 2.0 kg which amounted to 50% of the total fresh biomass. The oil extracted was subsequently utilized for the trans esterification reaction to produce biodiesel. It was well documented in the literature that algal oil could be utilized for biodiesel fuel production and has been used in diesel engines [11,12,13].

TABLE 1. Measurement of dry weight, extracted oil and biomass of algae

Algae	Dry weight	Extracted oil	Biomass/sediment
Spirogyra sp	2.0 (kg)	161.87 (g)	1838.13 (g)
	50%	8.1%	91.9%

Effect of homogenous and heterogeneous catalysts on biodiesel formation and quality

Biodiesel formation via alkaline transesterification from oil of biological origins under different conditions, such as temperature, stirring speed, catalysts and alcohol to oil ratio has been well studied and reported in the literature [11]. Most researchers generally agree that the optimum conditions are an alcohol (methanol) to oil ratio of between 3:1 to 6:1, at the temperature of 40^0C and a stirring speed of around 320 rpm[14]. These conditions were employed in this study with the variation of using a single (NaOH/homogenous) catalyst and a mixture of catalysts (heterogenous) ($NaOH + KOH + H_2SO_4$). As shown in Figure 2, biodiesel yield from *Spirogyra sp* was greater when a mixture of catalysts was used compared to when a single catalyst was employed. The amount of biodiesel produced when the heterogenous catalysts ($NaOH + KOH + H_2SO_4$) were used amounted to a 96.9% conversion on a volumetric basis. When only natrium hydroxide was used the conversion was slightly lower at 92.9%. Table 2 showed the different types of methyl ester and their conversion rates. Conversion rate was higher (palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester and linoleic acid methyl ester) mixture of catalyst than in single catalyst. The viscosity of the biodiesel formed, which partially represents its quality, was higher in the single catalyst reaction compared to the mixture of catalysts (Fig. 3). The viscosity values were 5.01, when single catalyst was used and 5.16 when mixture of catalysts was used. Another important characteristic of fossil fuel or biofuel that is monitored for quality is its total acid number (TAN). A high acid value can be corrosive to engines. As shown in Figure 4, the TAN value was much higher in the biodiesel made using NaOH alone compared to the mixture of NaOH, KOH and H_2SO_4.

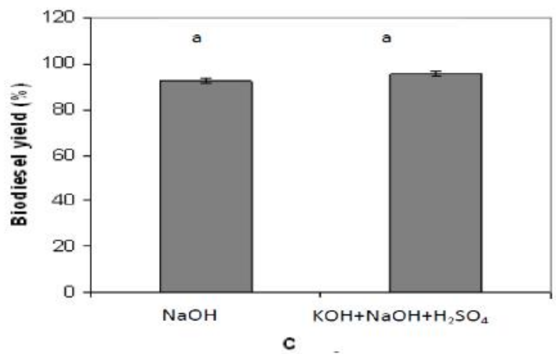


FIGURE 2. Effect of catalysts on biodiesel yield (Reaction conditions: temperature = 40°C, catalyst NaOH and mixture ($KOH+NaOH+H_2SO_4$) = 1.5 wt. %, RPM = 320, reaction time = 180 minutes). Same letters represent that there is no significant difference at 5% level ($P < 0.05$) by LSD.

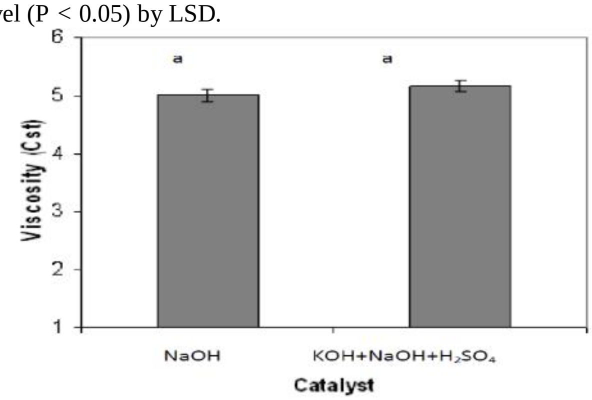


FIGURE 3. Effect of different catalysts on viscosity (cSt) (Reaction conditions: temperature = 40°C, oil: methanol = 1:3, catalyst = NaOH and mixture ($KOH+NaOH+H_2SO_4$), RPM = 320, reaction time = 120 minutes). Vertical bars indicate SE (n = 3). Same letters represent that there is no significant difference at 5% level ($P < 0.05$) by LSD.

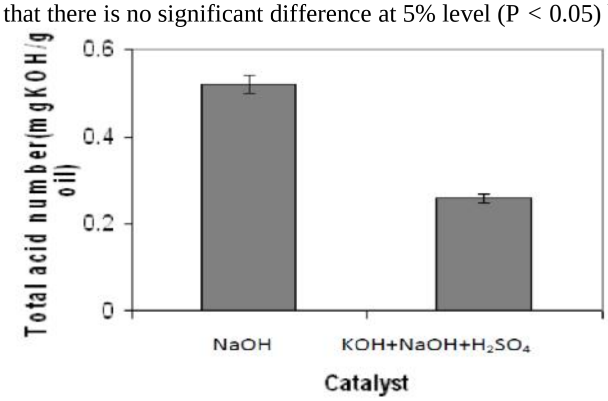


FIGURE 4. Effect of different catalysts on total acid number [mgKOH/g oil] (Reaction conditions: temperature = 40°C, oil: methanol = 1:3, catalyst = NaOH and mixture ($KOH+NaOH+H_2SO_4$), RPM = 320, reaction time = 120 minutes). Vertical bars indicate SE (n = 3). Same letters represent that there is no significant difference at 5% level ($P < 0.05$) by LSD.

TABLE 2. Fatty acid methyl ester and conversion rate were determined from fresh ater algae by using GC-MS.

Algal biodiesel sample	Peak serial	RT (min)	Fatty acid Methyl ester	Fatty acid	% Relative
Single catalyst (KOH)	1	17.36	$C_{16}H_{32}O_2$ CH_3	Palmitic acid	49.0
	2	21.28	$C_{18}H_{36}O_2$ CH_3	Stearic acid	3.0
	3	24.47	$C_{18}H_{34}O_2$ CH_3	Oleic acid	18.1
	4	24.96	$C_{18}H_{32}O_2$ CH_3	Linoleic acid	17.0
			Trace	Unidentified	--
					92.9%
Mixture of catalyst (KOH + NaOH)	1	17.28	$C_{16}H_{32}O_2$ CH_3	Palmitic acid	51.5
	2	21.18	$C_{18}H_{36}O_2$ CH_3	Stearic acid	3.5
	3	24.26	$C_{18}H_{34}O_2$ CH_3	Oleic acid	19.3
	4	24.64	$C_{18}H_{32}O_2$ CH_3	Linoleic acid	17.5
			Trace	Unidentified	--
					96.9%

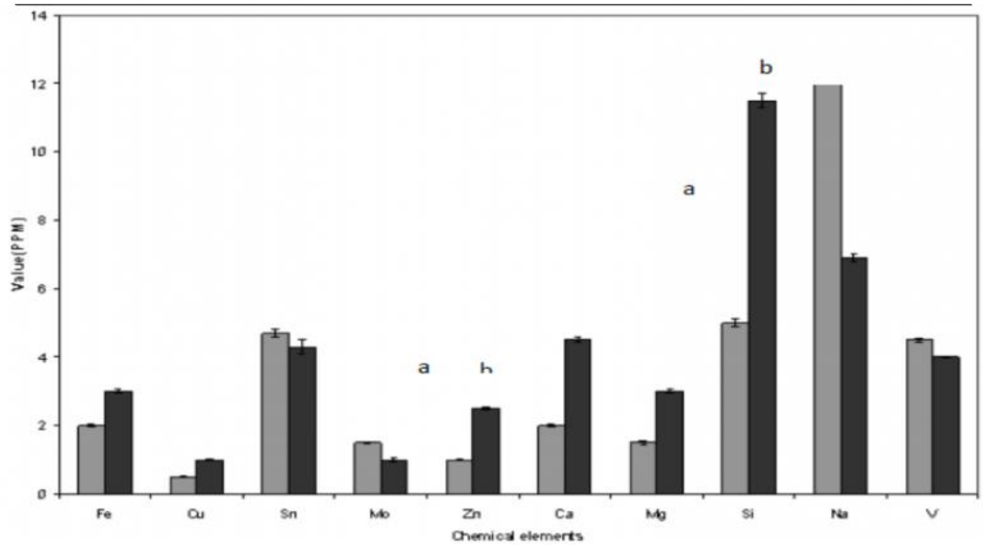


FIGURE 5. Metal content of biodiesel produced under optimum condition in contrast with standard value. Group I metals (Na + K), Standard method, EN 14214 (5.0mg/kg max.)
Group II metals (Ca + Mg), Standard method, EN 14214 (5.0mg/kg max.), P content Standard method, EN 14214 (10.0mg/kg max.). Trace amount or no interfere of Zn, Cu, Mn, Fe, CO, Pb, Cr metal. Standard method, EN 14214. Same letters represent that there is no significant difference at 5% level ($P < 0.05$) by LSD..

Biodiesel metal content analysis and biodiesel storage

As shown in Figure 5, overall the sodium and silicon content was higher than the iron, copper, stanium, molybdenum, zinc, calcium, magnesium and vanadium content. Furthermore the amounts of iron, copper, zinc, calcium, magnesium and silicon were higher in the biodiesel formed from the mixture of catalysts compared to when a single catalyst was used. In addition to this, the stanium, molybdenum and vanadium content were similar in the homogenous and heterogenous catalyzed biodiesel. This result showed that with increasing storage time, the amount of biodiesel showed a slightly declining trend. Table 3 showed the American and European biodiesel standards as in ASTM D6751 and EN14214.

DISCUSSION

It has been reported that the lipid content for macro algae can vary from 1.3-7.8 % on a dry weight basis and under heterotrophic conditions the lipid content can be higher in algae[15]. It has also been reported that macro algae (*seaweeds*) contain *lipid content* of 1.3-7.8% (dw) and reported to be very high, up to 51% of total fatty acids [16]. Recently it has been reported that seaweeds (*Sargassum muticum*) contain about 5.5% oil on a fresh weight basis [17]. In addition to this, it was reported that biodiesel could be produced from *Spirogyra sp* and *Oedogonium sp.* by using catalyst NaOH[11]. Their results showed that the methyl ester yield was higher in *Oedogonium sp.* than in *Spirogyra sp.* Here we reported that the oil content in *Spirogyra sp* was 8.04 % of its total biomass. This figure was about similar to the values reported previously for other freshwater algae[18]. 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 a non-alkaline (acid) catalyst[19]. It was reported that the transesterification occurred 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 esters from feedstocks that have a high FFA (free fatty acid) content, diglycerides and monoglyerides, using calcium and barium acetate as catalysts [20]. More recently, it has been compared the use of two catalysts, such as KOH and a combination of barium and calcium acetate, for the preparation of methyl esters from waste cooking oil [20]. They reported that methyl ester was higher in KOH alone than in combination of barium and calcium acetate [20]. It was also reported that the FAME yield increased when heterogeneous superacid catalysts, SO_4^{2-}/ZrO_2, were used[21]. Furthermore, 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, albeit the biodiesel obtained from homogenous catalyst had a slightly higher acid value (TAN) of 0.5 mg KOH/g compared to biodiesel produced from heterogenous catalysts. 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. With regard to 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 very similar viscosity values which were well within the ASTM standard range of 1.9 – 6.0 centistokes (cSt.) (Table 2). The metal content in both the 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[22].

CONCLUSION

It can be concluded that biodiesel can be produced in significant amount from the freshwater algae *Spirogyra sp*. In addition, it can be concluded that a mixture of catalysts can enhance and produce a slightly higher biodiesel yield. The biodiesel produced from *Spirogyra sp*. was of a good quality meeting the international ASTM D6751 and EN14214 standards.

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