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Alkaline and Acid Catalyzed Transesterification Bioprocess in Biodiesel Preparation from Fresh Water Algae

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ABSTRACT

The study was carried out to investigate the biodiesel yield and quality with different alkaline and acid catalysts from algae. Algal species, *Spirogyra* was used to produce biodiesel applying the alkaline and acid transesterification process. A single homogenous catalyst (KOH) and heterogenous mixture of catalysts (KOH+NaOH+H₂SO₄) were treated to obtain 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 and the physical and chemical properties of produced biodiesel were evaluated. The highest biodiesel yield of 96.9% was achieved dealing 1:3 volumetric oil-to-methanol proportions by 1.5% mixture of catalysts (NaOH+KOH+H₂SO₄) at 40°C reaction temperature and a stirring speed of 320 rpm. Biodiesel formation yielded a lower at the rate of 94.9% 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 heterogenous 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 showed that biodiesel obtained from *Spirogyra* sp., under optimum conditions through alkaline and acid bioprocess transesterification was of good quality that could be practiced as a source of diesel fuel.

Key words: *Spirogyra*, biodiesel yield, alkaline and acid transesterification, heterogenous mixture of catalysts

INTRODUCTION

The need for energy increased exponentially to meet 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 (Kulkarni and Dalai, 2006). 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 (Goldemberg, 2000).

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 (Vincecate, 2006). It is non-toxic, biodegradable,

produced from renewable sources and contributes a minimal amount of net green house gases, such as CO₂ and NO emissions and sulfur to the atmosphere (Bouaid *et al.*, 2007). 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 (Kulkarni and Dalai, 2006). 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 (Hossain *et al.*, 2007). 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 (Khan *et al.*, 2009). 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 7-31 times more oil than palm oil (Ramachandra *et al.*, 2009). It is generally believed that microalgae would be the best source of algae for biodiesel production. Unlike their larger cousins, the macroalgae 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 (ASTM., 2002).

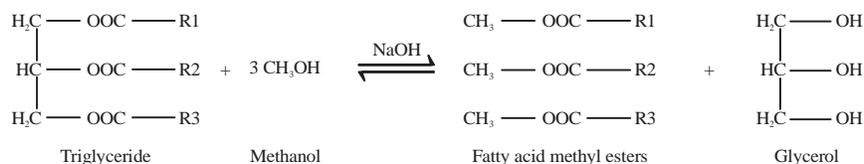
Recently, it has been reported that the use of heterogeneous catalysts can reduce the processing costs associated with the use of homogeneous catalysts and many alkaline catalysts such as sodium hydroxide, potassium hydroxide, calcium carbonate rock, EST-4 and EST-10 catalysts and Na/NaOH/ γ -Al₂O₃ were studied (Trakarnpruk and Porntangjitlikit, 2008). However, there are few literatures reported on biodiesel formation from the filamentous fresh water algae. So, the objectives of the present research were undertaken to investigate the methyl ester yield (biodiesel) by using the alkaline and acid transesterification bioprocess as well as to identify the fatty acid methyl ester from algal biodiesel.

MATERIALS AND METHODS

Materials: 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 Santhanam (2004) and modified by Sharif Hossain *et al.* (2008). The algae sample was ground in a motor and pestle and semi-dried in the oven at 80°C for 30 min (Fig. 1). After cooling down the semi-dried sample, hexane and diether (10X volume of sample) were added to extract the oil. The mixture was then kept aside for 72 h 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 KOH alone and 1.5% of NaOH and KOH and H₂SO₄ 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 h using an electric shaker at 320 rpm.

The transesterification reaction process is shown below:



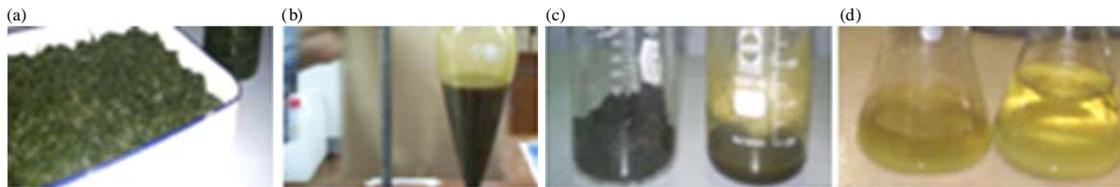


Fig. 1(a-d): Oil extraction and biodiesel yield from *Spirogyra* sp., algae (a) Algae, (b) Oil extraction, (c) Extracted oil and (d) Biodiesel (I: Single, II: Mixture)

After shaking, the solution was kept for 24 h 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°C in an oven for 2 h. 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. The 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 Integrated Solutions Ltd (ISL) software version 2.1. Total acid value was determined by titration with KOH and phenolphthalein as an indicator.

Statistical analysis: Least Significant Difference (LSD) test was done to observe significant differences at 5% level (0.05) within the replicates. Standard error was also determined.

RESULTS AND DISCUSSION

Algal oil extraction: Table 1 shows the quantity of oil extracted from the algal biomass. From 2.0 kg of algae, 0.16 L of algal oil was obtained which represented 8.9% oil of the biomass. The dry weight of the algal biomass was 1.0 kg which amounted to 50% of the total fresh biomass. The oil extracted was subsequently utilized for the transesterification 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 (Sharif Hossain *et al.*, 2008; Macedo, 1992; Chisti, 2007).

Effect of homogenous and heterogenous 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 (Sharif Hossain *et al.*, 2008). Most researchers generally agree that the optimum conditions are an alcohol (methanol) to oil ratio of between 3:1-6:1, at the temperature of 40°C and a stirring speed of around 320 rpm (Zeng *et al.*, 2008). These conditions were employed in this study with the variation of using a single (KOH/homogenous) catalyst and a mixture of catalysts (heterogenous) (NaOH+KOH+H₂SO₄).

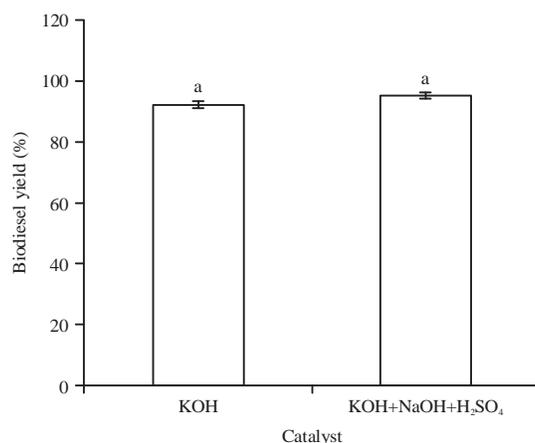


Fig. 2: Effect of catalysts on biodiesel yield (Reaction conditions, Temperature: 40°C, catalyst KOH and mixture (KOH+NaOH+H₂SO₄): 1.5 wt.%, RPM: 320, Reaction time: 180 min). Same letters represent that there is no significant difference at 5% level (p<0.05) by LSD

Table 1: Measurement of dry weight, extracted oil and biomass of algae

Algae	Dry weight	Extracted oil	Biomass/sediment
<i>Spirogyra</i> sp.	2.0 (kg)	161.9 (g)	1839.1 (g)
	20%	8.5%	91.9%

Table 2: Fatty acid methyl ester and conversion rate were determined from fresh water 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 C ₁₈ H ₃₆ O ₂ CH ₃ C ₁₈ H ₃₄ O ₂ CH ₃	Palmitic acid	51.00
	2	21.28	C ₁₈ H ₃₂ O ₂ CH ₃	Stearic acid	4.00
	3	24.47	Trace	Oleic acid	20.91
	4	24.96		Linoleic acid	19.00
				Unidentified	-
Mixture of catalyst (KOH+NaOH+H ₂ SO ₄)	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	Palmitic acid	51.90
	2	21.18	Trace	Stearic acid	4.50
	3	24.26		Oleic acid	21.00
	4	24.64		Linoleic acid	19.50
				Unidentified	-
					96.9

As shown in Fig. 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+H₂SO₄) were used amounted to a 96.9% conversion on a volumetric basis. When only natrium hydroxide was used the conversion was slightly lower at 94.9%. 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 Fig. 4, the TAN value was much higher in the biodiesel made using KOH alone compared to the mixture of NaOH, KOH and H₂SO₄.

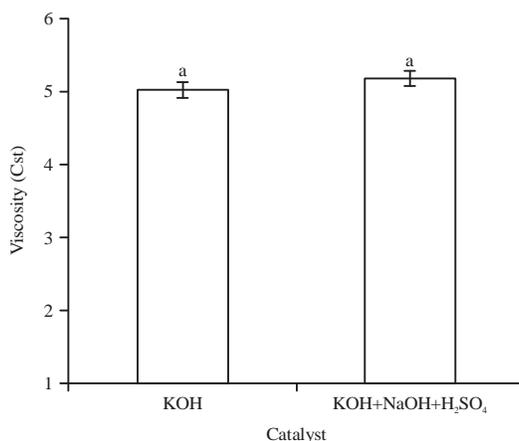


Fig. 3: Effect of different catalysts on viscosity (cSt) (Reaction conditions, Temperature: 40°C, Oil: Methanol: 1:3, Catalyst: KOH and mixture (KOH+NaOH+H₂SO₄), RPM: 320, Reaction time: 120 min). Vertical bars indicate SE (n = 3). Same letters represent that there is no significant difference at 5% level (p<0.05) by LSD

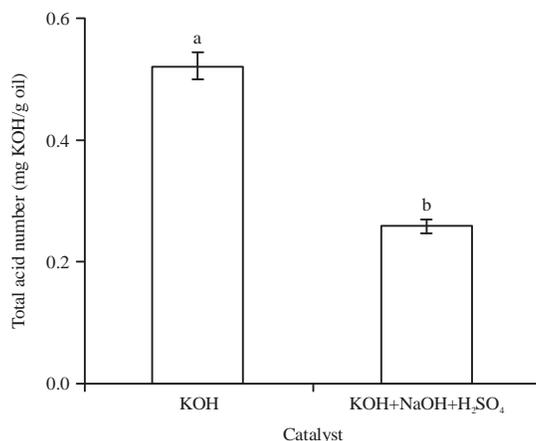


Fig. 4: Effect of different catalysts on total acid number (mg KOH/g oil) (Reaction conditions: Temperature: 40°C, Oil: Methanol: 1:3, Catalyst: KOH and mixture (KOH+NaOH+H₂SO₄), RPM: 320, Reaction time: 120 min). Vertical bars indicate SE (n = 3). Same letters represent that there is no significant difference at 5% level (p<0.05) by LSD

Xu *et al.* (2006) stated that biodiesel was produced by microalgae *Chlorella protothecoids* using transesterification bioprocess technology. The heterotrophic *C. protothecoids* contained the crude lipid content of 55.2%. The result showed that large volume of microalgal oil was efficiently extracted from the *Chlorella protothecoids* by using n-hexane and then converted into biodiesel by acidic transesterification and got higher conversion rate which was similar method to present research study. The biodiesel was characterized by a high heating value of 41 MJ kg⁻¹, a density of 0.864 kg L⁻¹ and a viscosity of 5.2×10⁻⁴ Pa (at 40°C). The method has great potential in the industrial production of liquid fuel from microalgae, *Chlorella protothecoids* as well as *Spirogyra* sp. Since lipid content was higher in *Chlorella protothecoids* than in *Spirogyra* sp., that

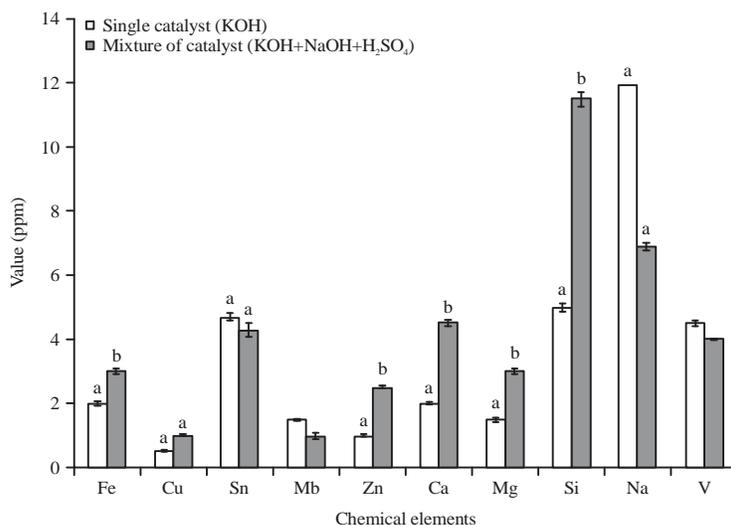


Fig. 5: Chemical elements determination at single catalyst (KOH) and mixture of catalyst (KOH+NaOH+H₂SO₄)

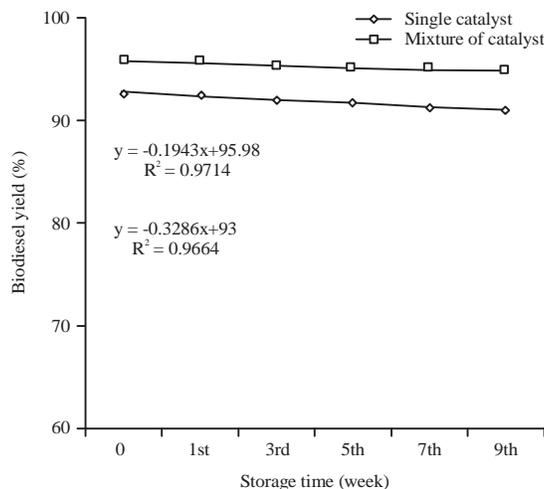


Fig. 6: Correlation between Biodiesel yield and storage time in different catalysts. Biodiesel yield (Reaction conditions, Temperature: 40°C, RPM: 320, Reaction time: 180 min)

is why biodiesel yield was less in *Spirogyra* sp., compared to the *Chlorella* sp., sometimes it may be different due to the catalyst and transesterification methods used in the production of biodiesel. Cheng *et al.* (2009) observed that 82% biodiesel was produced from *Chlorella protothecoids* which contained 44% lipid content and conversion rate was less than *Spirogyra* sp.

Biodiesel metal content analysis and biodiesel storage: As shown in Fig. 5, overall the sodium and silicon content was higher than the iron, copper, stantium, 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 stantium, molybdenum and vanadium content were similar in the homogenous and heterogenous catalyzed biodiesel. Figure 6 showed

a positive correlation between storage time and amount of biodiesel. This result showed that with increasing storage time, the amount of biodiesel showed a slightly declining trend.

It has been reported that the lipid content for macroalgae can vary from 1.3-7.8% on a dry weight basis and under heterotrophic conditions the lipid content can be higher in algae (Mansour *et al.*, 2005). It has also been reported that macroalgae (seaweeds) contain lipid content of 1.3-7.8% (dw) and reported to be very high, up to 51% of total fatty acids (Pohl and Zurheide, 1979). Recently, it has been reported that seaweeds (*Sargassum muticum*) contain about 5.5% oil on a fresh weight basis (Nye *et al.*, 1983). In addition to this, it was reported that biodiesel could be produced from *Spirogyra* sp. and *Oedogonium* sp., by using catalyst NaOH (Sharif Hossain *et al.*, 2008; Santhanam, 2004). Their results showed that the methyl ester yield was higher in *Oedogonium* sp., than in *Spirogyra* sp. Here, it was 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 (Caramujo *et al.*, 2008).

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 (Basu and Norris, 1996). 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 Free Fatty Acid (FFA) content, diglycerides and monoglycerides, using calcium and barium acetate as catalysts (Rose and Norris, 2002). 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 (Rose and Norris, 2002). They reported that methyl ester was higher in KOH alone than in combination of barium and calcium acetate (Rose and Norris, 2002). It was also reported that the FAME yield increased when heterogeneous superacid catalysts, $\text{SO}_4^{2-}/\text{ZrO}_2$, were used (Fu *et al.*, 2009).

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 (Kalam and Masjuki, 2002). Figure 6 showed that with increasing storage time, the amount of biodiesel showed a slightly declining trend. This could be due to the biodiesel becoming more viscous. It was reported that increasing biodiesel storage time could increase the acid value, peroxide value and viscosity, although the iodine value decreased (Bouaid *et al.*, 2009).

CONCLUSION

It can be concluded that biodiesel can be produced in significant amount from the freshwater algae *Spirogyra* sp., even though its lipid content is comparatively lower (8-10%) than other algae or microalgae. In addition, it can be concluded that a mixture of catalysts can enhance and produce a slightly higher biodiesel yield with a lower TAN value and the biodiesel produced from *Spirogyra* sp., was of a good quality meeting the international ASTM D6751 and EN14214 standards.

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