Duel Bio-Fuel and Bio-Solvent as Biodiesel and Bio-Ethanol from Algal Oil and its Biomass Residues Using Bioprocess Technology

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ABSTRACT

Biomass is plant or animal derived source of material that can be converted into different forms of bioenergy and biosolvent using different biotechnological procedures. Biomass is biodegradable, sustainable, economic sources as well as renewable and outstanding energy and biomaterial resource for the manufacturing industries like bio-plastics, bio-film, biomaterials, bio-chemicals, bio-fuels, bio-electricity, bio-solvent in the agro-industry, pharmaceuticals, biomedical and bioengineering aspect. The study was conducted to investigate the biodiesel and bioethanol yield and quality with different alkaline-acid catalysts and fermentation technology using algal oil extracted from algae and its biomass residues. Algal species, *Spirogyra* was used to produce biodiesel from algal oil applying the alkaline and acid transesterification process and bioethanol from algal biomass residues. In biodiesel production, single homogenous catalyst (KOH) and heterogenous mixture of catalysts (KOH+NaOH+H₂SO₄) were treated to obtain a high quality biodiesel fuel. The highest biodiesel yield of 96.9% was achieved dealing 1:4 volumetric oil-to-methanol proportions by 1.5% mixture of catalysts (NaOH+KOH+H₂SO₄). The lowest biodiesel yield was found 95.8% by 1.5% of single catalyst (KOH). 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 content differed significantly between the homogenous and heterogenous catalysts of produced biodiesel. In bioethanol production, bioethanol yield was found (15%) higher in the cellulase enzyme than in lipase enzyme (11%) fermentation. The results conclude that biodiesel and bioethanol obtained from algal oil and its residual biomass were of good quality that could be practiced as a source of diesel and bioethanol fuel and solvent.

Key words: *Spirogyra*, biodiesel yield, bioethanol, alkaline and acid transesterification, mixture of catalysts, cellulase, lipase

INTRODUCTION

Biomass is biodegradable, sustainable, economic sources as well as renewable and outstanding energy and biomaterial resource for the manufacturing industries like bio-plastics, bio-film, biomaterials, bio-chemicals, bio-fuels, bio-electricity, bio-solvent in the agro-industry, pharmaceuticals, biomedical and bioengineering (Sharif Hossain et al., 2008). The importance of bioenergy, biomaterial, fermenter and solvent are increasing exponentially to meet the demands of industrialization and population explosion worldwide since last couple of decades (Sharif Hossain et al., 2008). 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).

It is non-toxic, biodegradable, produced from renewable sources and contributes a minimal amount of net greenhouse gases, such as CO₂ and NO emissions and sulfur to the atmosphere (Vincecate, 2006; Bouaid et al., 2007, 2009). It has been used alone or blended with conventional petro-diesel fuel in unmodified diesel-engine vehicles.

One of the economical and renewable sources 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 and Boyce, 2009). Although, this can partly alleviate the dependency on petroleum-based fuel it would fall far short of the world’s demand for alternative energy.

From the last decade, algae have emerged as one of the most promising 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. 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).

Bioethanol can be produced from fruit residues like pineapple, banana peel, apple and mango fruit residues and other sources like algal biomass by the use of yeast, cellulase, lipase and amylase fermentation bioprocess technology (Sharif Hossain et al., 2008; Hossain and Boyce, 2009). Ethanol has been produced for thousands of years and is one of the oldest organic processes utilized by the people appreciating its intoxicating properties. It was produced by the fermentation of carbohydrates and large volumes of ethanol are still produced by this fermentation of agricultural raw materials (biomass). The raw materials can be starch, cellulose, lignocellulose and sugar based feed stocks, such as corn grain or sugar cane, or cellulosic feed stocks such as grass, wood, or fruit waste biomass, crop residues (Solventis Limited, 2013).

However, there are a few literatures reported on biodiesel from the Spirogyra sp., algal oil and but no literature found yet on bioethanol as solvent formation from the Spirogyra sp., algal residual biomass. The objectives of the study were undertaken to evaluate the methylester and bioethanol yield using the alkaline and acid transesterification and fermentation bioprocess technology and to identify the quality of the biodiesel and bioethanol as standards.

MATERIALS AND METHODS
Biodiesel production part
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.
Fig. 1(a-b): (a) Oil extraction and biodiesel yield from algae and (b) Bioethanol production by fermentation bioprocess (algae) (oil extraction) biodiesel (I: single, II: mixture)

**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 (10 x volume of sample) were added to extract the oil. The mixture was then kept aside for 72 h to settle down (Fig. 1a). 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 1:4 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:

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. 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, Ca, Cu, Pb) content. Viscosity was measured in centi stokes (cSt) 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.

Bioethanol production part

Raw material and enzyme: Algae residual biomass was collected after extraction the algal oil. Samples were kept until fully soften after mixing the distilled water in cupboard, at room temperature. Cellulase and lipase enzymes were obtained from the Biotechnology laboratory, University of Hail, Saudi Arabia and subjected to rehydration process with the addition of 10% distilled water and warmed at 40°C in water bath for 15 min.

Sample preparation and measurements: The biomass was pulverized separately. The 50 g of sample was filled into 500 mL Schott bottle. The sample pH value was measured by using pH meter (Hanna) and set to be at pH 5.8. This pH was standardized to all samples tested.

Fermentation: Prepared cellulose and lipase (1.5 g L\(^{-1}\)) were poured into 500 mL Schott bottle containing 50 g of slurry sample and was shaken well. Batch fermentation of sample was conducted in the incubator for 72 h at room temperature. The experiments for all parameters tested were done in triplicates.

Filtration: After 72 h of incubation, samples were taken out from incubator and then were filtered by filter paper and extracted samples were evaporated by vacuum evaporator and bioethanol was collected.

Bioethanol yield: Bioethanol yield was determined by the measurement of bioethanol by the Refractometer, Japan. Viscosity and acid value were determined following the method mentioned in the above.

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, 0.16 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, 1999; 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
Table 1: Measurement of dry weight, extracted oil and biomass of algae

| Parameters             | Weight (%) |
|------------------------|------------|
| Spirogyra sp.          |            |
| Dry weight             | 2.0 (kg)   | 20.0 |
| Extracted oil          | 178 (g)    | 8.9  |
| Biomass/sediment       | 1822 (g)   | 91.1 |

Table 2: Fatty acid methyl ester and conversion rate were determined from fresh water algae by using GC-MS

| Algal biodiesel samples | Peak serial | RT (min) | Fatty acid methyl ester | Fatty acid | Relative (%) |
|------------------------|-------------|----------|-------------------------|------------|--------------|
| Single catalyst (KOH)  | 1           | 17.26    | C_{16}H_{32}O_{2}CH_{3}  | Palmitic acid | 51.0         |
|                        | 2           | 21.38    | C_{18}H_{36}O_{2}CH_{3}  | Stearic acid | 5.0          |
|                        | 3           | 24.49    | C_{18}H_{34}O_{2}CH_{3}  | Oleic acid  | 19.0         |
|                        | 4           | 24.95    | C_{18}H_{32}O_{2}CH_{3}  | Linoleic acid | 20.8       |
|                        | Trace       |          |                         | Unidentified | -            |
| Total                  |             |          |                         |             | 95.80        |
| Mixture of catalyst    | 1           | 17.27    | C_{16}H_{32}O_{2}CH_{3}  | Palmitic acid | 51.5         |
| (KOH+NaOH+)            | 2           | 21.19    | C_{18}H_{36}O_{2}CH_{3}  | Stearic acid | 5.5          |
|                        | 3           | 24.25    | C_{18}H_{34}O_{2}CH_{3}  | Oleic acid  | 19.3         |
|                        | 4           | 24.66    | C_{18}H_{32}O_{2}CH_{3}  | Linoleic acid | 20.6       |
|                        | Trace       |          |                         | Unidentified | -            |
| Total                  |             |          |                         |             | 96.90        |

RT: Reaction time

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).

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 (KOH) 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 potassium hydroxide was used the conversion was slightly lower at 95.8%. Table 2 show 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
KOH KOH+NaOH+H$_2$SO$_4$

Catalyst

Fig. 3: Effect of different catalysts on viscosity (Reaction conditions: temperature = 40°C, oil: methanol = 1:3, catalyst = KOH and mixture (KOH+NaOH+H$_2$SO$_4$), 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 (Reaction conditions: temperature = 40°C, oil: methanol = 1:3, catalyst = KOH and mixture (KOH+NaOH+H$_2$SO$_4$), 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.

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 H$_2$SO$_4$.

**Biodiesel metal content analysis and biodiesel storage:** As shown in Table 3, the magnesium and calcium contents were higher than copper and sodium content in the biodiesel formed from the mixture of catalysts compared to a single catalyst (KOH) used.
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Table 3: Chemical elements determination at single catalyst (KOH) and mixture of catalyst (KOH+NaOH+H2SO4)

| Biodiesel          | Na   | Ca    | Mg    | Cu    | Pb |
|--------------------|------|-------|-------|-------|----|
| KOH                | 0.5<sup>a</sup> | 2.0<sup>a</sup> | 1.8<sup>a</sup> | 0.3<sup>a</sup> | 0  |
| KOH+NaOH+H2SO4    | 0.9<sup>b</sup> | 4.4<sup>b</sup> | 4.2<sup>a</sup> | 0.5<sup>a</sup> | 0  |

<sup>a,b</sup>Same letters represent that there is no significant difference at 5% level (p<0.05) by LSD, Na: Sodium, Ca: Calcium, Mg: Magnesium, Cu: Copper and Pb: Lead

Table 4: Bioethanol yield and properties determination

| Bioethanol      | Bioethanol yield | Viscosity | Acid value |
|-----------------|------------------|-----------|------------|
| Cellulase enzyme| 15.4<sup>a</sup> | 4.5<sup>a</sup> | 0.3<sup>a</sup> |
| Lipase enzyme   | 11.2<sup>b</sup> | 5.0<sup>a</sup> | 0.4<sup>a</sup> |

<sup>a,b</sup>Same letters represent that there is no significant difference at 5% level (p<0.05) by LSD

Bioethanol yield: From the Table 4, it has been shown that bioethanol yield was found (15%) higher in the cellulase enzyme than in lipase enzyme (11%) fermentation. Cellulase enzyme showed higher yield than lipase enzyme in the fermentation of algal biomass. Viscosity and acid value were followed as standard.

DISCUSSION

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 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). 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 (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, SO<sub>4</sub><sup>2-</sup>/ZrO<sub>2</sub>, 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<sup>−1</sup> compared to biodiesel produced from heterogeneous 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).

From the Table 4, it has been explained that biethanol yield was found (15%) higher in the cellulase enzyme than in lipase enzyme (11%) fermentation. It might be due to the more speed occurred and enhanced more activation in the fermentation in the case of cellsulase compared to the lipase. There might be more cellulose fiber existed in the algae residual biomass.

CONCLUSION
It can be concluded that biodiesel and bioethanol can be produced in signficant amount from the freshwater algae. 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. The biodiesel produced from Spirogyra sp., was of a good quality meeting the international ASTM D6751 and EN14214 standards. Bioethanol may be used as biofuel as well as bio-solvent in the pharmaceutical and cosmetic industry. In addition, cellulose enzyme showed better activation in producing bioethanol as biofuel and bio-solvent than lipase enzyme.

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