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POTENTIAL UTILIZATION OF *MICROCYSTIS* SP. FOR BIODIESEL PRODUCTION: GREEN SOLUTION FOR FUTURE ENERGY CRISIS

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Abstract – There is a growing concern on the use of alternative sustainable energy source to overcome the energy crisis. Hence, biodiesel from cyanobacteria has drawn the attention of the scientific community. The present study was carried out to find potential utilization of *Microcystis* bloom for biodiesel production. *Microcystis* bloom samples were collected from Beira Lake, Sri Lanka and Soxhlet extraction method with Isopropanol: n-hexane (3:2) solvent system was employed to extract lipids. In the present study,biodiesel B6 blend was prepared and the properties of B6 blend were compared with ASTM D 7467(American Society for Testing and Materials standard for B6 – B20 Biodiesel blends)and Ceylon Petroleum Corporation specifications for no: 02-grade auto diesel to confirm the fuel properties and the usability respectively. Biodiesel B6 blend was subjected to determination of fuel properties and density at 15^oC (ASTM D 1298/ 4052), Viscosity Kinematic at 60^oC (ASTM D 445), Calorific value (Gross) (ASTM D240), Cloud point (ASTM D 2500), Lubricity (HFRR wear sear dia at 60^oC) (ASTM D 6079), Sulphur content (ASTM D 4294) and CFPP (Cold Filter Plugging Point) value (ASTM D 6371) were 831.00kg/m^3, 2.83cSt., 11180.00kcal/kg, 6^{o}c, 405.00μm, 2310.00ppm and 4^oC. The results revealed that biodiesel B6 blend complied with ASTM standards for lubricity, kinematic viscosity and sulfur content (S5000). The fuel properties of kinematic viscosity, density, sulfur content and CFPP value complied with the Ceypetco auto diesel specifications and the energy content of the blend was higher than Ceypetco auto diesel specifications. The results of the present study revealed that the *Microcystis* bloom can be utilized as a potential candidate to produce biodiesel.

INTRODUCTION

Energy is of significant importance to both economic and social development (Zhang *et al.*, 2011). The main energy resources in the world are coming from fossil fuels such as petro oil, coal and natural gas that contribute more than 85% of the world energy needs (Huang *et al.*, 2012). Plants and animals which died hundreds of millions of years ago are responsible for the formation of petroleum (Huang *et al.*, 2012). Their organic matter decomposition forms petroleum after millions of years and these resources have very high economic value because these are non-renewable energy sources which cannot be rapidly regenerated naturally on equal levels of its consumption. Fossil fuel reserves depletion time for oil, natural gas,and coal of approximately 35, 37 and 107 years respectively (Gülüm and Bilgin, 2015; Shafiee and Topal, 2008).

The emission produced by the combustion of fossil fuels, contribute to the air pollution and subsequently for the global warming (Agarwal, 2007; Zhou and Thomson, 2009). Due to the decline of petro fuels with the current rate of consumption, the rapid development of modern industries, limited availability (Chisti, 2007) and the global warming and pollution due to petroleum-derived greenhouse gases (GHGs) (Hoekman *et al.*, 2012), world concern is focused on alternative, sustainable and eco-friendly energy production since about two decades (Halim *et al.*, 2011). Thus biofuels are considered to be the best available alternative energy sources for future requirements (Atadashi *et al.*, 2012; Liu *et al.*, 2012). Mainly biofuels are carbon neutrals that will not result in the net release of carbon dioxide gas to the atmosphere that is accumulated as a consequence of burning fuel. Other than that non-toxicity and renewability of biofuels ensure the environment and economic

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sustainability (Chisti, 2007; Xu *et al.*, 2006).

Biodiesel is a renewable fuel type that consisting of fatty acids methyl esters (FAMEs)(Hoekman *et al.*, 2012). As an oxygenated fuel, biodiesel generally contains 10 wt.% of the oxygen that is improve burning efficiency, reduction of particulate matter, carbon monoxide and other gaseous pollutants (Huang *et al.*, 2012), and biodiesel appears to be an attractive and successful energy source for several reasons. Including that it could be sustainably supplied, have low sulfur content (Lang *et al.*, 2001) high flash point and biodegradability (Ma and Hanna, 1999). Therefore biodiesel considered as a successful alternative for petrodiesel for comperssion-ignition (CI) engines (Knothe and Razon, 2017).

Use of biodiesel reduces the release of sulfur and carbon monoxide by 30% and 10% respectively and also it can reduce 90% of air toxicity and 95% of lung cancer compared to the common petro-diesel sources (Huang *et al.*, 2010). Therefore, uses of biodiesel not only sustain the economic aspects but also protect the environment from the toxic gases. Most importantly, biodiesel must comply with the ASTM (American Society of the International Association for Testing and Materials) biodiesel standard D 6751 which is the American standard for using biodiesel while Europe Union provide separate standards for biodiesel in vehicle use (EN 14214) and biodiesel which use as heating oil (standard EN 14213) (Chisti, 2007).

However, the production cost is generally high for biodiesel (Huang *et al.*, 2010). The cost of raw materials (fat or oil) and the cost of downstream processing can be considered as two main components of the biodiesel production cost. The cost of raw materials accounts for 60% to 75% of the total cost of the biodiesel fuel (Krawczyk, 1996). Though there might be large amounts of low-cost oils and fats available in as restaurant waste and animal fats, the major problem of the use of these low-cost oils and fats is they often contain large amounts of free fatty acids (FFA) which are difficult to convert them into biodiesel through the trans-esterification process (Demirbas, 2003).

Cyanobacteria and microalgae consider as a good source for renewable biofuel production (Vimalarasan *et al.*, 2011). As third generation biofuel types, microalgal and cyanobacterial renewable biofuels include methane (biogas) that is produced through anaerobic digestion of algal biomass (Spolaore *et al.*, 2006). These microalgae and cyanobacteria photobiologically produced biohydrogen(Akkerman *et al.*, 2002; Ghirardi *et al.*, 2003; Melis, 2002; Mubarak *et al.*, 2014) bio-char (Mubarak *et al.*, 2014) and, lipids which provide the great potential feedstock for biodiesel synthesis (Chisti, 2007).

The carbon atoms of microalgal and cyanobacterial fatty acids mostly range from 12-22 in number and are main constituents of either neutral or polar lipid molecules that can be either saturated or unsaturated type (Huang *et al.*, 2010; Mubarak *et al.*, 2014). Neutral, saturated lipid molecules such as acylglycerols (tri-, di, mono-glycerols) are involved in high energy storage and are the main materials (Liu *et al.*, 2012; Huang *et al.*, 2010), which are desirable for commercial-scale biodiesel production (Halim *et al.*, 2012). All most all the unsaturated fatty acids of cyanobacterial and microalgal are cis-isomers and never exceed 6 double bonds along with the carbon chain (Medina *et al.*, 1998).

In Sri Lanka, most of the freshwater bodies are being eutrophicated and cyanobacterium *M. aeruginosa, M. wesenbergii,* and *M.incerta* are the dominant species which were recorded in more than 97% in Beira Lake (Idroos and Manage, 2014). Other species such as *Pediastrum duplex, Spirulina* sp., *Actinastarum* sp., *Scenedesmus armatus, Coelastrum* sp. and *Chlorococcus* sp. strains are also presented in the lake water in low numbers.

Compared with algae, cyanobacteria have a simpler fatty acid composition. Most of the fatty acids of *Microcystis* are saturated (about 50%) and major saturated fatty acids are palmitic acid (C16:0), lauric acid (C12:0) and myristic acid (C14:0) (Da Rós *et al.*, 2012). The predominant unsaturated fatty acid in *Microcystis* are oleic acid (C18:1) and linoleic acid (C18:2) and it was detected that erucic acid (C22:1), linolenic acid (C18:3) and palmitoleic acid (C16:1) as well in lower amounts which are favorable for quality biodiesel production (Da Rós *et al.*, 2012). Therefore, the present study was focused on to determine the potential utilization of *Microcystis* bloom as an alternative biofuel.

MATERIALS AND METHODS

Sampling

Microcystis bloom samples were obtained from the Beira Lake and about 100 L of bloom samples were collected into plastic containers and transported to the Centre for Water Quality and Algae Research, Department of Zoology, University of Sri Jayewardenepura.

Bloom harvesting and pre-treatment

About 100 L of bloom samples were filtered through a 1 mm^2 wire mesh to remove suspended large substances and were concentrated using electrolytic foam floating (EFF) apparatus. The EFF apparatus was made with modifications to combine the mechanisms of electrolytic flocculation and electrolytic floatation which are electrophoresis harvesting methods.

The concentrated bloom samples were dried under sunlight for 3-4 days in order to remove water completely from the sample. After drying, the biomass was ground using a mortar and pestle. Then the dried powered biomass was stored under cold condition (5^0C) until lipid extraction was performed.

Lipid extraction and lipid Transesterification

Soxhlet extraction method was used for lipid extraction. According to Madusanka and Manage (2016), Isopropanol: n-hexane (3:2) solvent system was used to extract lipids from 160.00 g of dried powdered *Microcystis* biomass. 800 ml of isopropanol: n-hexane mix solvent system was used in 1:5 (W/V) biomass to thesolvent ratio as given in Fajardo *et al*. (2005). Trans-esterification processes of lipid fractions were done by a modified method of the fatty acid methylation procedure as given in Halim *et al*. (2011).

Biodiesel B6blend preparation and quality analysis

Pure FAMEs (biodiesel) were used to prepare biodieselB6 blends. 6 ml of biodiesel was blended with 94 ml of grade No. 2 diesel (Auto diesel) purchased from Petroleum Corporation, Sri Lanka to obtain 100 mL of biodiesel-diesel blends.

Determination of Quality parameters of Biodiesel blends

Following quality parameters of biodiesel B6 blend were measured. Fuel properties of Density at 15^oC (ASTM D 1298/ 4052), Viscosity Kinematic at 60^oC (ASTM D 445), Calorific value (Gross) (ASTM D240), Flash point (ASTM D 93), Cloud point (ASTM D 2500),Lubricity (HFRR wear sear dia@ 60^oC) (ASTM D 6079), Sulphur content (ASTM D 4294) and CFPP (Cold Filter Plugging Point) value (ASTM D 6371) tests were carried out at refinery laboratory of Ceylon Petroleum Corporation (Ceypetco), Sapugaskanda, Kelaniya.

The tested properties of B6 biodiesel blend of *Microcystis* were compared with ASTM D 7467 standards for confirmation of the fuel properties and specifications forno: 02-grade auto diesel (given by Ceylon Petroleum Corporation – Sri Lanka), to assess the usability of biodiesel blend for compression-ignition (CI) engines without modifications.

RESULTS AND DISCUSSION

In order to get high lipid content from cyanobacteria, an efficient solvent system should be selected for the extraction process. However, if the feedstock contains a significant amount of free fatty acids cause to reduce the quality of the product (Knothe & Razon, 2017). It was noted that n-hexane/ isopropanol mixture is highly selective for neutral lipids and their inability to extract polar lipids such as glycolipids in chloroplasts and phospholipids in cell membrane ensured the selectivity for neutral lipids (Gukert *et al.,* 1988; Madusanka and Manage, 2016). Therefore, n-hexane/isopropanol solvent system was selected to extract lipids from *Microcystis* biomass for biodiesel blend preparation.

The results of fuel properties of *Microcystis* biodiesel B6 blend is given in Table 1 and it clearly shows that the biodiesel blend complied with ASTM standards for lubricity, kinematic viscosity and sulfur content (S5000). Moreover, the fuel properties of kinematic viscosity, density, sulfur content and CFPP value complied with the Ceypetco auto diesel specifications. The energy content of the blend was higher than Ceypetco auto diesel specifications. However, the flash point of biodiesel blend did not comply with either ASTM standards or Ceypetco specifications for auto diesel.

Various structural features such as the presence of heteroatoms, chain length, and unsaturation influence the lubricity of biodiesel (Knothe and Steidley, 2005b). According to the ASTM standards, the product did not exceed the maximum level, 520 μm. However, the obtained value was as high as 405 μm that might be due to the presence of higher percentages of saturated FAMEs in the biodiesel product.

Studies done to analyze kinematic viscosity of B6 blend (2.83 cSt) is complied with the ASTM D 7467 standard (1.9-4.1 cst) as well as with the auto diesel

Table 1. Fuel properties of *Microcystis* biodieselB6 blend

Parameter	Test method	Units	Limits of ASTM D7467 standard	B6 biodiesel blend from *Microcystis* lipids	Auto diesel**
Biodiesel content	ASTM D7371	% Vol.	6-20	6	-
Lubricity	ASTM D6079	µm	520 max	405	ns
Viscosity	ASTM D445	cSt	1.9-4.1	2.83	1.5 – 5
Flash point	ASTM D93	^{0}C	52 min	<30	60 min
Density	ASTM D1298/4052	kg/m^3	ns	831.0	820 – 860
Sulfur content*	ASTM D4294	wt.%		0.231	0.300
S15			0.0015 (15 ppm)	(2310 ppm)	(3000 ppm)
S500			0.0500 (500 ppm)		
S5000			0.5000 (5000 ppm)		
Gross calorific value	ASTM D240	kCal/ kg	ns	11180	10500
Cloud point	ASTM D2500	^{0}C	ns	6.0	ns
Cold flow properties (CFPP)	ASTM D6371	^{0}C	ns	4.0	10 max

ns – Not specified
* - The fuel grades S15, S500, and S5000 refer to the maximum sulfur content allowed in the fuel expressed in ppm by weight and represent the maximum sulfur content of .0015 wt.%, .05 wt. % and .5 wt. % respectively.
** - According to Ceypetco Product Specifications for Auto diesel (www.ceypetco.gov.lk)

specifications (1.5-5.0 cSt). Kinematic viscosity is mainly considered in determining the use of biodiesel as alternative fuel instead of neat oils or fats (Knothe and Steidley, 2005a). Several structural features influence the kinematic viscosities of FAMEs, such as chain length, the degree of unsaturation, double bond orientation, vand type of ester head group etc. (Knothe, 2006). Higher kinematic viscosities may lead to operational problems such as engine deposits (Knothe and Steidley, 2005a). The kinematic viscosities of FAMEs are much lower than their fatty acids and slightly higher than petrodiesel.

Flashpoint is considered as a safety limitation (Huang *et al.*, 2012) and serves to restrict the amount of alcohol in the biodiesel fuel (Moser, 2009). As noted in Hoekman *et al.* (2012), the flash point is inversely related to the fuel volatility. In the present results, the flash point of the B6 blend (<30^{o}C) was lower than ASTM D 7467 standards (55^{o}C) and auto diesel specification (60^{o}C). According to Knothe and Steidley (2005a), biodiesel when contaminated with methanol, even with a small fraction (<1%), it will fail to meet the minimum flash point specified in relevant fuel standards (Knothe and Steidley, 2005a).Therefore contaminants such as methanol, water, catalyst, glycerol, FFA, soap,and metals etc. may cause to lower the flash point. In the present study, a small fraction (6%) of biodiesel was added to grade No: 02 diesel (auto diesel). The results indicated the presence of contaminations in the final product. Therefore proper purification of the product is very important in downstream processes.

The density of biodiesel is directly affected by the relative composition of available FAMEs (i.e- higher FAME chain length shows lower biodiesel density)(Hoekman *et al.*, 2012). The tested biodiesel B6 blend mostly consisted of hexadecanoic acid methyl esters in which the molecular weight is 270.41g/mol. As a lower blend, the density of B6 blend is more or less similar to grade no: 02 diesel oil (Table 1). The density of fuel oil is not documented as a fuel property in ASTM D 7467 standards. However, the density of obtained *Microcystis* B6 biodiesel blend (831.0 kg/m^3) complies with the Ceypetco specification for the density of grade no: 02 diesel (820-860 kg/m^3). As cited in Hoekman *et al.* (2012), FAMEs with higher densities leading to lower viscosities.

As a mid-level (B6-B20) biodiesel blend, B6 contains a small fraction (6%) of biodiesel when compared to the available petro-diesel fraction (94%). According to the Ceypetco specification, the maximum sulfur limit of auto diesel was 0.3 wt. % (3000 ppm). Thus, the results obtained from the present study revealed that sulfur content does not exceed the maximum level of Ceypetco specification. However, the sulfur content of the product did not comply with the sulfur limits of the ASTM S15 and S500 grades of biodiesel B6 blends (0.0015%wt. and 0.05 %wt.). The results only complied with the maximum limit of ASTM S5000 grade (0.5 %wt.). Therefore, it can be assumed that available sulfur content may have resulted from petro diesel which was used to prepare the blend.

The gross calorific value represents the higher value of the heat of combustion (Knothe and Steidley, 2005a) which is chemical energy released by the combustion of a unit fuel mass and the fuels with higher heating values are more efficient with smaller engines (Gülüm and Bilgin, 2015). The heat of combustion is directly proportional to chain length. Longer chains contain more carbons with a similar number of oxygen atoms that increase the gross calorific value and lower carbon to hydrogen ratios (more hydrogen) exhibit greater energy content (De Oliveira *et al.,* 2005). The saturated FAMEs have lower C: H ratios while unsaturated FAMEs of similar chain length have higher C: H ratios. According to Ceypetco specifications, the gross calorific value of auto diesel is 10500 kcal/kg (43.94 MJ/kg) and the gross calorific value of *Microcystis* FAMEs B6 blend was 11180 kcal/kg (46.81 MJ/kg) which was higher than auto diesel. According to De Oliveira *et al*. (2005), the heat of combustion of soya bean biodiesel B20 blend was 10468.45 kcal/kg (43.83 MJ/kg) which is less than the result of the current study. Therefore, the results revealed that the addition of biodiesel with petro diesel; even by a small fraction, may increase the gross calorific value of fuel product by approximately 6.47 %. Hexadecanoic acid methyl esters ($C_{17}H_{34}O_2$) which have lower C: H ratio (1:2). The availability of higher percentage of saturated FAMEs could be the reason to find a high gross calorific value. Thus the results revealed that blending of biodiesel in diesel (6%) increase the gross calorific value (energy content) by 6.45%.

Both cloud point and cold flow properties (CFPP – cold filter plug point) are parameters of low-temperature operability. ASTM D 7467 does not state any particular limit for cloud point and CFPP, while Ceypetco specification has given 10^0C as the maximum temperature for auto diesel. Cloud point is defined as the temperature which larger crystals fuse together and form agglomerations which prevent the pouring of fluid (Knothe and Steidley, 2005a).

The CFPP is defined as the lowest temperature at which a particular volume of biodiesel or biodiesel blend completely flows under vacuum through a wire mesh filter screen within the 60s (Knothe and Steidley, 2005a). The CFPP is considered as a more reliable parameter of lower temperature operability than cloud point (Knothe and Steidley, 2005a). The lower temperature behaviors of biodiesel and biodiesel blends are influenced by structural features such as the degree of unsaturation, chain length, theorientation of double bonds and type of ester head group of FAMEs (Hoekman *et al.,* 2012; Knothe and Steidley, 2005a). Cis-unsaturated fatty acid methyl esters often have advantageous cold flow properties. In contrast to saturated chains which pack rapidly upon temperature decrease to form tight semi-crystalline structures, cis-unsaturated fatty acids are prevented from forming regular molecular packing due to the bends imposed by the cis-double bonds and consequentially freeze at a lower temperature (Lang *et al.,* 2001; Mathews and Van Hlode, 1996).

According to the Minowa *et al.* (1995), a good quality biodiesel was synthesized from *Dunaliella tertiolecta* and the viscosity and calorific value was 1.5 – 3.3 cSt and 36 MJ/kg respectively. Moreover, high-quality biodiesel from *Chlorella protothecoides* and was characterized by a viscosity (at 40^0C) of 5.2 cSt., a high heating value (calorific value) of 41 MJ/kg and a density of 864 kg/m^3 (Xu *et al.*, 2006). Similarly, in the present study, a viscosity (at 40^0C) of 2.83 cSt, a density of 831 kg/m^3, and a calorific value of 46.81 MJ/kg were obtained from *Microcystis* based biodiesel. Therefore, the biodiesel produced using *Microcystis* bloom compiles with required standards of a biofuel and this B6 blend could be effectively used as a biofuel following optimum purification.

CONCLUSION

Biodiesel B6 blend obtained from *Microcystis* oil was characterized by a relatively high heating value, a favorable density, an optimum viscosity and an optimum lubricity and most of the tested parameters were found to comply with both ASTM D7467 and Ceypetco specifications for No: 02 diesel oil. Results provide evidence that blending of biodiesel with the grade no: 02 diesel oil enhances the fuel properties (i.e. gross calorific value).

Currently, there is no potential utilization of *Microcystis.* However, the present study reveals that the biodiesel production from *Microcystis* oil is technically feasible and this will be a successful alternative energy source that can be used to overcome the future energy crisis.

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