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RESEARCH ARTICLE

Optimising a solvent system for lipid extraction from cyanobacterium *Microcystis* spp.: future perspective for biodiesel production

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Abstract: Due to unsustainability of petro derived fuels, the world is now concerned about alternative, sustainable and eco-friendly energy production. As a successful alternative energy, biodiesel plays a major role to replace petrodiesel. Cyanobacterial lipids have great potential for biodiesel production due to their rapid growth rate and renewability. The production of biodiesel from biomasses depends on upstream (lipid extraction) and downstream (trans-esterification) processes. Lipid extraction should be effective and energy efficient to reduce the production cost of commercial biodiesel production. Therefore, the present study was experimented to optimise the lipid extraction solvent system from cyanobacteria.

Cyanobacteria samples were collected from Beira Lake, Colombo where, *Microcystis* spp. are dominant (> 96 %). Solvent systems n-hexane, chloroform: methanol (2:1), n-hexane: ethanol (3:2), chloroform: n-hexane (1:1), dichloromethane: methanol (1:1), n-hexane: isopropanol (2:3) and diethyl ether were used for lipid extraction and subjected to optimisation for the lipid extraction method from the cyanobacterium *Microcystis* spp. Extracted lipid yield (g/1 g of dried biomass) for each solvent system was 1.11 wt. %, 1.61 wt. %, 1.71 wt. %, 2.15 wt. %, 3.41 wt. %, 4.33 wt. % and 1.95 wt. %, respectively where fatty acid methyl ester (FAME) compositions found to be 80.13 %, 78.27 %, 76.39 %, 29.01 %, 85.72 %, 92.39 % and 80.09 % in each system. The gas chromatographic-mass spectrometry analysis of the products revealed that the most abundant fatty acid types of *Microcystis* spp. were palmitic acid (C16:0) and its derivative such as 14-methyl pentadecanoic acid. The highest lipid yield (4.33 wt. %) and fatty acid methyl ester (92.39 %) composition were recorded from n-hexane: isopropanol solvent system.

Keywords: Fatty acid methyl esters, lipid extraction solvent systems, *Microcystis* spp.

INTRODUCTION

Fossil fuels are the major energy sources throughout the world. According to the calculation given by Shafiee and Topal (2008), fossil fuel reserves of oil, natural gas and coal will deplete within approximately 35, 37 and 107 years, respectively. Among many alternative energy sources such as solar energy, tidal energy and hydro-power, biofuels play a major role and may give a potential solution for future energy crisis sustainably.

Biofuels are carbon neutral and will not result in a net release of carbon dioxide gas to the atmosphere. Out of many biofuel types, biodiesel is the most important type that is a promising alternative to fossil fuel for compression-ignition (CI) engines (Huang *et al*., 2012). Biofuels derived from microalgae and cyanobacteria belong to the third generation biofuel type (Daroch *et al*., 2013). Thus, biodiesel from microalgae or cyanobacteria is a novel concept, which is popular among the scientific community. Cyanobacteria commonly called as blue green algae have simple cellular structures (Da Rós *et al*., 2012) and they are microscopic photosynthetic organisms found in both marine and freshwater environments (Brennan & Owende, 2010). Unlike microalgae, cyanobacteria are the dominant phytoplankton group in

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eutrophic freshwater bodies where water is polluted by nitrogen and phosphorus (Sethunge & Manage, 2013). They are prokaryotes, possessing a cell wall composed of peptidoglycan and lipopolysaccharide layers instead of the cellulose of green algae (Da Rós *et al*., 2012).

Cyanobacteria are mainly sunlight driven organisms and contain lipids, proteins and many other bioactive compounds such as pigments, carotenoids, ketones etc. (Da Rós *et al*., 2012). Out of these, lipids are considered as the responsible component for production of biodiesel (Huang *et al*., 2010; Da Rós *et al*., 2012; Mubarak *et al*., 2014). Cyanobacterial lipids mostly range within 12 – 22 carbon atoms and the lipid molecules can be either saturated or unsaturated (Huang *et al*., 2010; Mubarak *et al*., 2014). Based on the polarity of the lipid head group, lipids can be categorised into neutral or polar lipids (Halim *et al.*, 2012).

Due to high photosynthetic efficiency, high biomass production, rapid lipid accumulation (Halim *et al.*, 2012) and fast growth rate than other conventional crop products, oil rich cyanobacteria are considered as a good source of carbon neutral biodiesel production (Miao & Wu, 2006; Huang *et al*., 2010). Depending on the species, conditions and other parameters, cyanobacteria produce many different kinds of lipids, hydrocarbons and other complex oils (Metzger & Largeau, 2005; Guschina & Harwood, 2006; Huang *et al*., 2010). All algal oils are not satisfactory for the production of biodiesel, but neutral fatty acids such as palmitic acids are desirable for biodiesel production (Chisti, 2007; Da Rós *et al*., 2012).

Most of the recreational freshwater bodies in Sri Lanka are being eutrophicated and the genus *Microcystis* is the dominant and well distributed cyanobacterium (Sethunga & Manage, 2013). In the Beira Lake, *M. aeruginosa* is the dominant cyanobacteria species where *M. wesenbergii* and *M. incerta* are co-dominant species. Idroos *et al.* (2014) recorded that *Microcystis* spp. increase their biomass more than 95 % in the Beira Lake throughout the year.

According to Huang *et al*. (2010) the production cost is generally high for biodiesel, and the cost of raw materials (fat or oil) and downstream processing (trans-esterification) can be considered as two main components of the production cost of biodiesel. Therefore, an efficient solvent system for lipid extraction from cyanobacteria is more important to chemical based upstream processes in commercial biodiesel production to reduce the production cost. Thus, the present study was carried out to optimise an efficient solvent system for lipid extraction from *Microcystis* spp.

METHODOLOGY

Sampling

Microcystis bloom samples were collected from the Beira Lake from March to August in 3 sampling occasions, and 100 L of bloom samples were collected into plastic containers and transported to the laboratory.

Harvesting and extraction pre-treatments

Bloom samples were filtered through a 1 mm^2 wire mesh to remove debris and were concentrated using the electrolytic foam floating (EFF) apparatus. Approximately 10 – 12 h was taken for the concentration process of 20 L of bloom sample through the EFF apparatus.

The concentrated bloom samples were dried under sunlight for 3 – 4 d until complete removal of water. After drying, the biomass was subjected to fine grinding using a mortar and pestle. Then the powdered biomass was stored under cold conditions (5 $^{\circ}$C) until lipid extraction was performed (Halim *et al.*, 2011).

Lipid extraction from cyanobacteria

Lipid extraction was carried out using 7 different solvent systems of n-hexane, chloroform: methanol (1:2), n-hexane: chloroform (1:1), dichloromethane (DCM): methanol (1:1), isopropanol: n-hexane (3:2) and diethyl ether to assess the lipid content of the *Microcystis* biomass.

Dried *Microcystis* powder (20.00 g) was weighed and placed in the round bottom flask of the soxhlet apparatus at 80 $^{\circ}$C, and 100 mL of solvent was added in order to get *Microcystis* dry biomass: solvent in 1:5 (W/V) ratio (Fajardo *et al*., 2007). Extraction was carried out for 10 hrs and the procedure was repeated 3 times for each solvent system. After that, the mixture was re-dissolved in each solvent system for lipid extraction. The dissolved lipid fraction was subjected to washing with water in order to remove contaminants such as pigments and non-lipid contaminants. The washing process was continued until the lipid dissolved organic layer becomes clear. Then, the purified lipid product was subjected to drying process using the rotary evaporator (Halim *et al*., 2011). Dried lipids were mixed with 20 mL of n-hexane and stored at 5 $^{\circ}$C to reduce the chemical and photo oxidation reaction (Halim *et al*., 2011).

Lipid trans-esterification

Trans-esterification processes of lipid fractions were done by a modified method of the fatty acid methylation procedure given in Halim *et al*. (2011). The dried lipid was re-dissolved in 20 mL of hexane in order to enhance the reaction kinetics during trans-esterification. Roughly 1:5 volume ratio of cyanobacterial oil to methanol was added. As lipids and methanol are not miscible (Moser, 2009), a two-step protocol was used for the methylation process of all the extracted lipids, and all the reagents were added in stoichiometric excess (Halim *et al.*, 2011). In the first step H_2SO_4 was used as the acid catalyst for methylation of free fatty acids (FFAs) and in the second step potassium methoxide (KCH_3O) was used as the base catalyst to methylate the acylglycerols. Five milliliters of conc. H_2SO_4 was added to the lipid methanol mixture and heated at 50 ^oC with moderate agitation for 2 h. During the period, the evaporated methanol was replenished. Then 25 wt. % KCH_3O solution was prepared by dissolving 25.00 g of KCH_3O in 127 mL of methanol and added dropwise to the gently stirred lipid methanol mixture until pH 12 was attained. The mixture was heated at 55 ^oC and moderately agitated for 2 h, and evaporated methanol was frequently replenished.

After trans-esterification was completed, trans-esterified fatty acids were re-dissolved in n-hexane. Then the dissolved fatty acid methyl esters (FAMEs) were washed with water to remove methylation by-products, methanol, remaining catalysts, etc.

GCMS screening of FAMEs

Two hundred micro liters of pure FAME was dissolved in 800 µL of n-hexane prior to gas chromatographic-mass spectrometry (GCMS) screening. The FAME composition was analysed by GCMS, Agilent USA model 7890 GC with Agilent 19091S-433HP-5MS capillary column (30 m × 250 µm × 0.25 µm).

Two microliters of sample was injected in split mode with 100:1 split ratio and split-flow in 50 $mLmin^{-1}$. The initial oven temperature was maintained at 160 ^oC for 10 min, and then the temperature was ramped to 190 ^oC for 5 min with 3 $^oCmin^{-1}$ rate. Finally, the oven temperature was raised to 280 ^oC with 10 $^oCmin^{-1}$ rate and the temperature was maintained for 14 min. Helium (He) was used as the carrier gas and total run time was 45 min.

Optimising a solvent system

Lipid yields, volume of FAME products, relative percentage of total FAME and relative percentage of saturated and unsaturated FAME of different solvent systems were the major criteria for optimisation. Properties of the most available methyl esters, such as carbon chain length, chain structure and saturation or unsaturation of the FAME molecule of all solvent systems were assessed to select the most appropriate solvent system for further experiments.

RESULTS AND DISCUSSION

In the present study, the highest lipid yield (4.33 wt. %) was recorded from n-hexane: isopropanol solvent system, whereas the lowest lipid yield (1.11 wt. %) was recorded from the n-hexane solvent system (Figure 1). The second highest lipid yield was recorded from DCM: methanol solvent system, which was 3.14 wt. %. In the present study lipid yield varied from 1.1 wt. % to 4.33 wt. % (Figure 1).

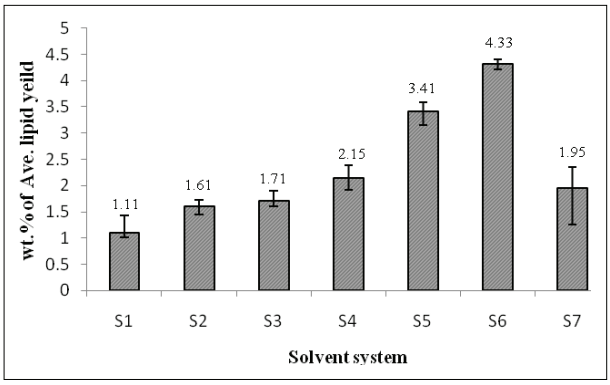

Figure 1: Percentages of average lipid yields (wt. % of lipid g/ 1 g dried biomass). Error bars represent the minimum and maximum of the data set.
S1 – n-hexane; S2 – chloroform: methanol (2:1); S3 – n-hexane: ethanol (3:2); S4 – chloroform: n-hexane (1:1); S5 – DCM: methanol (1:1); S6 – n-hexane: isopropanol (2:3); S7 – diethyl ether.

The total lipid composition of microalgae and cyanobacteria vary with many parameters, such as the species, environment conditions, nutrient availability and phase of life cycle, etc; especially in logarithmic growth phase they have higher lipid contents than the same species, which are in stationary phase (Dunstan *et al*., 1993).

Griffiths and Harrison (2009) reported that the lipid content of cyanobacteria was about 5 – 13 wt. % with an average of 8 wt. %. The highest lipid yields from *Chlorella* sp. (11.9 wt. %), *Isochrysis galbana* (8.9 wt. %), *Botryococcus braunii* (28.6 wt. %), *Phaeodactylum*

tricornutum (6.3 %) and *Chlorococcum* sp. (6.8 %) were recorded in other countries as well (Guckert *et al*., 1988; Grima *et al*., 1994; Fajardo *et al*., 2007; Lee *et al*., 2010; Halim *et al*., 2011). In the present study, it was found that lipid yields of *Microcystis* spp. varied from 1.1 wt. % to 4.33 wt. %. The lipid yield of the present study was relatively lower than the recorded values for other algae. Most of the researches on biodiesel production from microalgae has been carried out under controlled systems while in the present study, *Microcystis* bloom samples were collected from Beira Lake, which is subjected to different environmental variations such as light, temperature, pH, carbon dioxide and nutrient content (Manage, 2009; Idroos *et al*., 2014). Further Da Rós *et al*. (2012) recorded that cell growth, biomass productivity and lipid content of microalgae depend on some important factors such as light, temperature, pH, carbon dioxide and nutrient concentration. Therefore, this could be one of the reasons which influence the reduction of lipid yield in the present study.

Table 1: Volume of FAME products related with different solvent systems

Solvent system	S1	S2	S3	S4	S5	S6	S7
Produced FAME volume (mean vol.± SD) (mL)	2.73 ± 1.08	6.20 ± 0.36	7.10 ± 0.40	20.20 ± 1.7	6.97 ± 0.80	13.56 ± 0.21	1.5 ± 0.80

As shown in Table 1 the highest FAME volume 20.20 ± 1.7 mL was obtained from S4 where n- hexane: chloroform (1:1) solvent system was used. The lowest volume 1.5 ± 0.80 mL was recorded from S7 solvent system where di-ethyl ether was used as the solvent. The final volumes [means (mL) ± SD] of FAME products of S1, S2, S3, S5 and S6 solvent systems were detected as 2.73 ± 1.08, 6.20 ± 0.36, 7.10 ± 0.40, 6.97 ± 0.80 and 13.56 ± 0.21, respectively.

Twelve types of methyl esters were detected by GCMS screening from all solvent systems. They were identified as dodecanoic acid methyl ester (C13:0), tridecanoic acid methyl ester (C14:0), pentadecanoic acid 14-methyl methyl ester (C17:0), hexadecanoic acid methyl ester (C17:0), 7-hexadecenoic acid methyl ester (C17:1), 7, 10, 13- hexadecatrienoic acid methyl ester (C17:3), octadecanoic acid methyl ester (C19:0), 7-octadecenoic acid methyl ester (C19:1), 13-octadecenoic acid methyl ester (C19:1),15-octadecenoic acid methyl ester (C19:1), 9,12 – octadecadienoic acid methyl ester (C19:2) and 11,14- eicosadienoic acid methyl ester (C21:2) (Xu *et al*., 2006; Da Rós *et al*., 2012).

Out of the detected FAMEs, hexadecanoic acid methyl ester and pentadecanoic acid 14-methyl methyl ester were the major FAME products (Figure 2). Under threshold value 14, the highest FAME percentage was recorded as 92.39 % from the solvent system 6, where the n-hexane: isopropanol (2:3) system was used for lipid extraction, and total FAMEs were expressed as hexadecanoic acid methyl esters. The second highest percentage (85.72 %) was recorded from the S5 solvent system where dichloromethane: methanol (1:1) was used for lipid extraction and the most abundant FAMEs were pentadecanoic acid 14-methyl methyl esters. Further, relatively higher FAME percentages of the solvent systems S1, S2, S3 and S7 were recorded as 80.13 %, 78.27 %, 76.39 % and 80.09 %, respectively (Figure 2). The lowest FAME percentage (29.01 %) was recorded from S4 solvent system when n-hexane: chloroform (1:1) was used for lipid extraction and the recorded FAME type was hexadecanoic acid methyl ester (Figure 2).

FAME components of tridecanoic acid methyl esters (1.59 %), 7-hexadecanoic acid methyl esters (1.54 %), octadecanoic acid methyl esters (3.59 %), 7- octadecanoic acid methyl esters (5.42 %), 13- octadecanoic acid methyl esters (2.5 %), 15- octadecanoic acid methyl esters (2.62 %) and 9,12- octadecadienoic acid methyl esters (4.99 %) were detected in S1 solvent system as minor components. In S2 solvent system, octadecanoic acid methyl esters (7.67 %) and 11, 14- eicosadienoic acid methyl esters were detected. Dodecanoic acid methyl esters, 7, 10, 13- octadecatrienoic acid methyl esters and octadecanoic acid methyl esters were recorded in S7 at 0.19 %, 7.68 % and 7.08 %, respectively as minor components (Figure 2).

Many experiments on mixed-solvents (polar and non-polar solvent mixture) have given higher lipid extraction than the other tested solvent systems. Most of the polar lipids were assumed to be the major contaminant in the extracted lipids, which cause some problems in esterification process (Halim *et al*., 2011) and utilising of biodiesel.

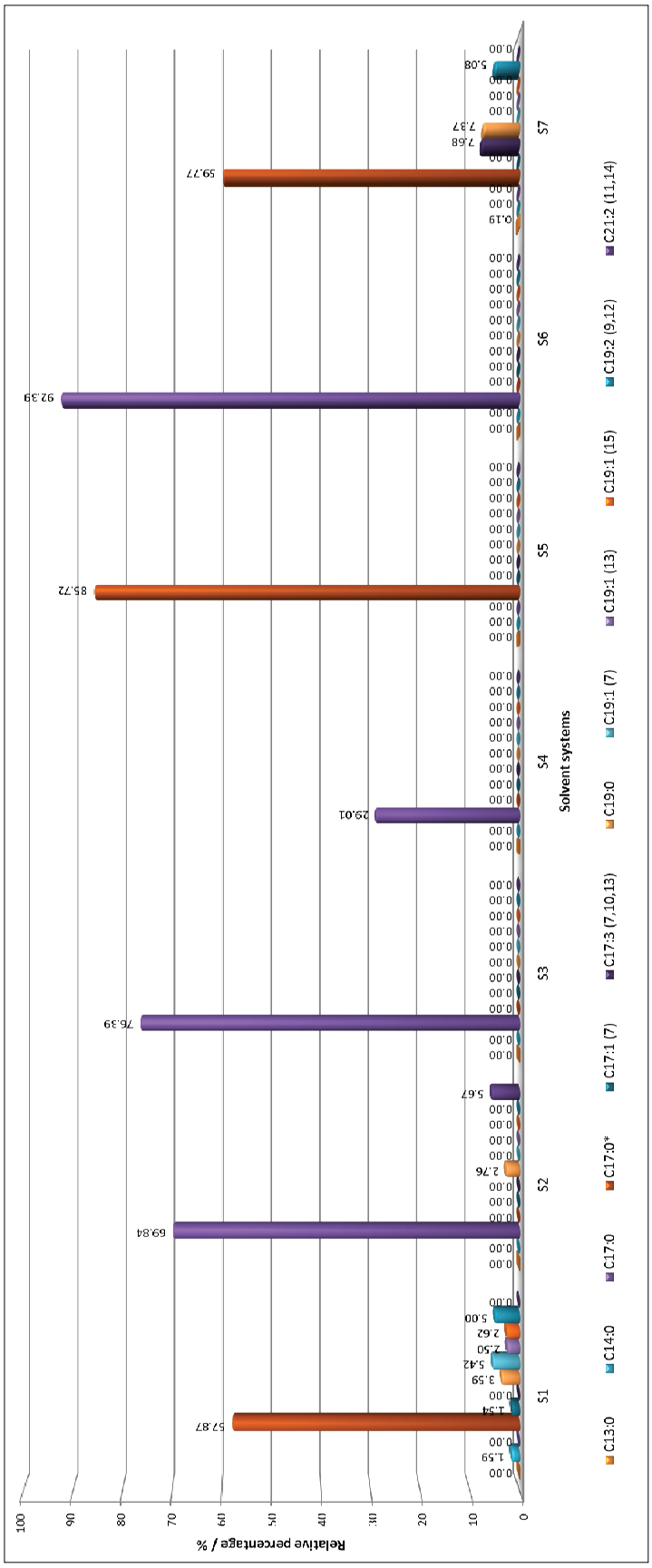

Figure 2: Availability of FAME composition (under GCMS threshold value 14) of different solvent systems
S1 – n-hexane; S2 – chloroform: methanol (2:1); S3 – n-hexane: ethanol (3:2); S4 – chloroform: n-hexane (1:1); S5 – DCM: methanol (1:1); S6 – n-hexane: isopropanol (2:3); S7 – diethyl ether
dodecanoic acid methyl ester (C13:0); tridecanoic acid methyl ester (C14:0); pentadecanoic acid 14-methyl methyl ester (C17:0) where one carbon group (CH3-) attached to the carbon chain of the fatty acid, hexadecanoic acid methyl ester (C17:0); 7-hexadecenoic acid methyl ester (C17:1); 7, 10, 13- hexadecatrienoic acid methyl ester (C17:3); octadecanoic acid methyl ester (C19:0); 7-octadecanoic acid methyl ester (C19:1); 13-octadecanoic acid methyl ester (C19:1); 15-octadecanoic acid methyl ester (C19:1); 9,12– octadecadienoic acid methyl ester (C19:2); 11, 14- eicosadienoic acid methyl ester (C21:2)

According to Da Rós *et al*. (2012) major saturated fatty acids of *Microcystis* was palmitic acid (C16:0) and lauric acid (C12:0), and such acids were recorded as 24.34 % and 13.21 %, respectively and myristic acid (C14:0) was recorded as low as 6 %. The predominant unsaturated fatty acids were oleic acid (C18:1) and linoleic acid (C18:2), which represented 26.88 % and 12.53 %, respectively. However, erucic acid (C22:1), linolenic acid (C18:3) and palmitoleic acid (C16:1) were detected in low percentage up to 3 %.

The trans-esterified products of S3, S4, S5 and S6 in which lipids were extracted from n-hexane: ethanol (3:2), n-hexane: chloroform (1:1), DCM: methanol (1:1) and n-hexane: isopropanol (2:3) systems, respectively, resulted only saturated FAMEs (100 %). When S1 solvent system was used with n-hexane solvent system 78.68 % of saturated FAMEs, 15.08 % of mono-unsaturated FAMEs and 6.23 % of poly-unsaturated FAMEs were obtained. The lipid products obtained from chloroform: methanol (2:1) solvent mixture (S2) had 92.76 % of saturated FAMEs and others were poly-unsaturated FAMEs that were available as 7.24 % from the total FAMEs. In S7 solvent system, 84.07 % from the total FAMEs were saturated and other 15.93 % of FAMEs were poly-unsaturated (Table 2).

Results of the present study revealed that the saturated and unsaturated FAME composition was more or less similar to that recorded by Da Rós *et al*. (2012).

Table 2: Saturated and unsaturated FAME compositions of solvent systems

	Percentage from total FAME*			
		Saturated	Unsaturated FAME	
Solvent system	Total FAME % *	FAME	Mono-unsaturated	Poly-unsaturated
S1	80.13	78.68	15.08	6.23
S2	78.27	92.76	ND	7.24
S3	76.39	100.00	ND	ND
S4	29.01	100.00	ND	ND
S5	85.72	100.00	ND	ND
S6	92.39	100.00	ND	ND
S7	80.09	84.07	ND	15.93

* - FAME screening carried out under GCMS threshold value 14

ND – not detected

S1 – n-hexane; S2 – chloroform: methanol (2:1); S3 – n-hexane: ethanol (3:2); S4 – chloroform: n-hexane (1:1); S5 – DCM: methanol (1:1); S6 – n-hexane: isopropanol (1:1); S7 – diethyl ether

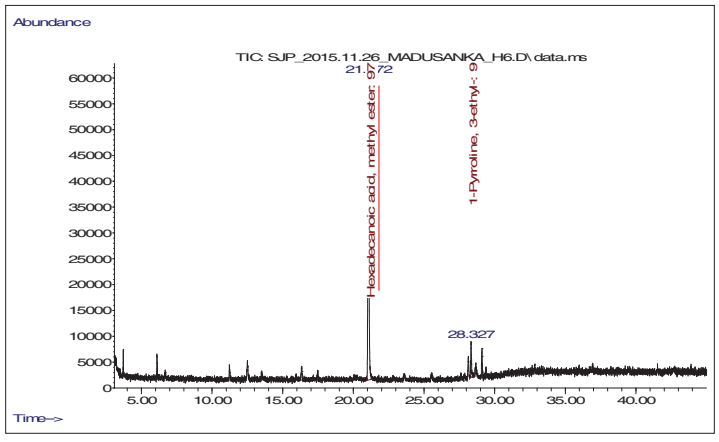

Figure 3: GC-MS for S6 solvent system which used n-hexane: isopropanol (2:3) for lipid extraction

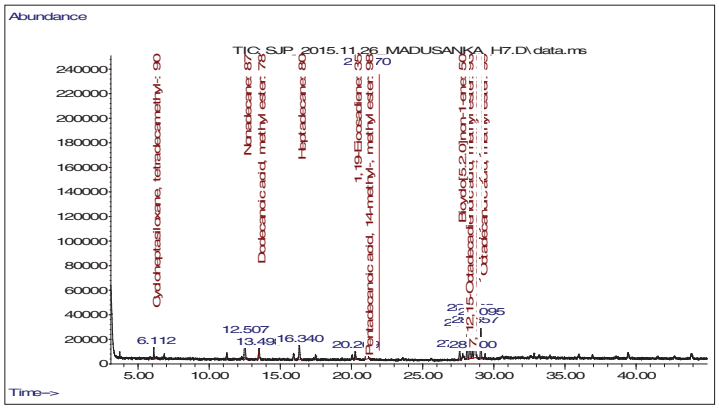

Figure 4: GC-MS for S7 solvent system which used diethyl ether for lipid extraction

The dominant saturated FAME types of *Microcystis* were methyl esters of palmitic acid and its derivatives (hexadecanoic acid methyl esters and pentadecanoic acid 14-methyl methyl esters). Both hexadecanoic acid methyl esters and pentadecanoic acid 14-methyl methyl esters have seventeen carbon atoms and thirty-four hydrogen atoms. Therefore, both compounds have the same molecular weight (Figures 3 and 4).

Although these FAMEs have some characters in common, they vary in their chain length, carbon atom and hydrogen atom arrangements. Pentadecanoic acid 14-methyl methyl ester has a methyl group on the fourteenth carbon atom in the hydrocarbon chain. Therefore, hexadecanoic acid methyl ester hydrocarbon chain length is higher than pentadecanoic acid 14-methyl methyl ester. Thus, the most abundant FAME type in S1, S5 and S7 solvent systems were found to be pentadecanoic acid 14-methyl methyl esters. It was found that hexadecanoic acid methyl ester was abundant in S2, S3, S4 and S6 solvent systems.

In the present study, pure non-polar solvents such as n-hexane and diethyl ether were used in S1 and S7 systems, respectively. Other five solvent systems were mixed solvent systems and among these, S2, S3, S5 and S6 consisted of polar/non-polar solvent mixtures, which contained chloroform: methanol (2:1), n-hexane: ethanol (3:2), DCM: methanol (1:1) and n-hexane: isopropanol (2:3), respectively. S4 solvent system contained a mixture of two non-polar solvents; chloroform: n-hexane (1:1). For optimising a solvent system for lipid extraction, lipid yield of each solvent system, FAME composition, availability of co-extractions, and other physical and chemical parameters were considered.

It is significant that higher FAME compositions result in higher volumes of biodiesel. A higher FAME composition is a key requirement to a quality biodiesel product. Figure 5 shows the mean FAME volumes and FAME percentages of the solvent systems. The circled areas represent the mean volumes, which were obtained from each solvent system. S1, S2, S3, S5, S6 and S7 achieved high FAME percentages (under GCMS threshold value 14), while S4 recorded a relatively low FAME percentage. Relatively high volumes were recorded from S4 and S6 while S7 solvent system had a low FAME yield. Although, S4 solvent system has a high FAME mean volume, it showed the lowest FAME percentage. S6 solvent system showed a relatively high FAME volume while achieving the highest FAME percentage from all solvent systems.

Lipid extractability (the lipid solubility within a particular solvent system) depends on the total energy of cohesion between lipid molecule and solvent system divided into three major components; atomic dispersion (van der Waals) interactions, molecular dipolar interactions and molecular hydrogen bonding (electron exchange) interactions. As pointed out by Halim *et al.* (2012), n-hexane:isopropanol solvent system has a lower relative energy difference (RED) according to Hansen solubility parameters.

Use of non-polar solvents mixed with polar solvents increased the lipid extractability from cyanobacteria, and n-hexane:isopropanol mixture was highly selective for neutral lipids because they are not able to extract polar lipids such as glycolipids in chloroplasts and phospholipids in cell membrane, which ensured the selectivity for neutral lipids (Guckert *et al*., 1988). In the present study,

it was observed that the n-hexane:isopropanol solvent system was the most efficient solvent system to extract lipids from *Microcystis* spp.

Extraction pretreatments are very important to remove water content of the biomass. According to Mubarak *et al*. (2014), moisture content more than 5 % in biomass reduce the oil extraction yield and increase the free fatty acid fraction of oil. Therefore, the *Microcystis* biomass was dried under solar light to reduce the water content and it also reduced energy consumption for the process. Although minimum operational cost was beneficial, time consumption and resulting higher free fatty acids (FFA) (Balasubramanian *et al*., 2013) in biomass were the major drawbacks of this technique.

According to literature, lipid extraction from *Chlorococcum* sp. was enhanced with the inclusion of isopropanol as a co-solvent, which resulted in increasing the total lipid yield of pure n-hexane system by more than 300 % [total lipid yield of pure n-hexane system was 0.015 g/g dried microalgal biomass and final total lipid yield of n-hexane:isopropanol (1/1 v/v) system was 0.068 g lipid/g dried microalgal biomass] (Halim *et al*. 2011). Similarly, in the present study, the total lipid yield obtained from pure n-hexane system was 0.011 g/g from dried *Microcystis* biomass, while total lipid yield from n-hexane:ethanol (1/1 v/v) and n-hexane:isopropanol (2/3 v/v) were 0.017 and 0.043 g lipid/g dried microalgal biomass, respectively. Thus, the present study revealed that the inclusion of ethanol and isopropanol as co-solvents improves the total lipid yield of pure n-hexane system by more than 150 % and 350 %, respectively. Further, it was found that mixture of non-polar solvents (n- hexane:chloroform) resulted in the lowest FAME percentage following the highest volume, indicating the presence of co-extractions.

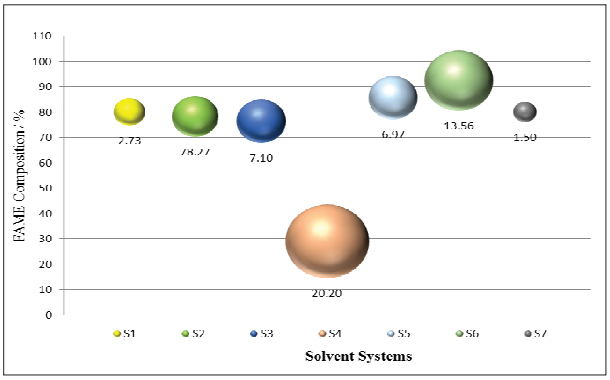

Figure 5: Comparison of FAME composition and volume of final product of each solvent system.
S1, S2, S3, S4, S5, S6 and S7 are solvent systems where S1 – n-hexane; S2 – chloroform: methanol (2:1); S3 – n-hexane: ethanol (3:2); S4 – chloroform: n-hexane (1:1); S5 – DCM: methanol (1:1); S6 – n-hexane: isopropanol (1:1); S7 – diethyl ether. (The area of circle represents the mean volume)

CONCLUSION

Out of seven solvent systems, n-hexane: isopropanol solvent system showed high selectivity to extract neutral lipids and could obtain 92 %. Genus *Microcystis* mostly consists of palmitic acid (hexadecanoic acid) or its derivatives (pentadecanoic acid 14-methyl). Inclusion of polar solvents with non-polar solvents increases the extractability of lipids from the *Microcystis* biomass. The results of the present study revealed that *Microcystis* bloom can be used to produce biodiesel as an alternative energy source.

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