AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Biodiesel Production from Microalgae-*Chlorella Sorokoniana*

[1]Hesam Kamyab, [2]Chew Tin Lee, [1]Mohd Fadhil Md Din, [3]Shaza Eva Mohamad, [1]Ponraj Mohanadoss, [1]Ameer Badr Khudhair, [1]Anahita Maslahaiti Roudi

[1]*Inistitute of Environmental Water Resources and Management (IPASA), Water Research Alliance, Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, Johor 81310, Malaysia*
[2]*Faculty of Chemical Engineering, Department of Bioprocess Engineering, Universiti Teknologi Malaysia, Johor81310, Malaysia*
[3]*Faculty of Bioscience and Bioengineering, Universiti Teknologi Malaysia, Johor 81310, Malaysia*

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form 8 March 2014
Accepted 10 March 2014
Available online 2 April 2014

Keywords:
Microalgae; Chlorella sorokoniana; Biodiesel; saturated fatty acid

ABSTRACT

In recent years, high value lipid extraction in order to convert into a biodiesel product was potentially investigated among various microalgae strains. As a proof, in this research study, a significant amount of triacyl glyceride from *Chlorella sorokiniana* was obtained. Moreover, effective parameters such as pH, temperature and light intensity were assessed thoroughly. The petroleum fuels are limited and depleting due to increase in consumption and cause environmental problems. Microalgae are discussed as a source for the production of befouls. Therefore, biodiesel is the only substitute fuel attainable as it is technically feasible, economically competitive, environmentally acceptable and easily available to fulfill the increasing demands for energy. This research was conducted to extract of lipid from *Chlorella sorokiniana* and characterization of fatty acid composition by Gas chromatography requirements. Transesterification process was carried out t o produce methyl esters. After 15-17 days, at the end of the exponential phase of growth, the total contents of the lipids were extracted and determined. The extracted fatty acids were first esterified and then identified using GC analysis. The presence of several types of fatty acid methyl esters (FAMEs) and saturated fatty acids were identified by using microalgae, *Chlorella sorokoniana.* The result shows that the extracted lipid shows in main composition of suitable fatty acid present in the microalgae was identified as palmitic acid profile for biodiesel, ranging from 16-18 carbon lengths. This strain can be an ideal candidate for biodiesel production because of its saturated fatty acid content.

To Cite This Article: Kamyab, H., Lee, C.T., Din, M.F.M, Mohamad S. E., P., Khudhair, A.B.˙ Maslahaiti Roudi, A., Biodiesel Production from Microalgae-*Chlorella Sorokoniana* ***Aust. J. Basic & Appl. Sci.,*** *8(3): 140-145, 2014*

INTRODUCTION

For environmental and worldly sustainability of the world, renewable and carbon-neutral biofuels are necessary (Patil *et al*., 2008). Compared to conventional energy sources, alternative possibilities have only a minor economic base .Today; optimization of production processes is the main reason for increasing research activities to this area. Biodiesel is diesel fuel that is either vegetable oil or animal fat based and is the constituents of long chain alkyl esters (Pintoa *et al*., 2005). Furthermore, distinguished as mono alkyl ester, biodiesel is usually produced by chemically- reacting lipids and alcohol. The properties of the individual alkyl esters have a considerable influence over the properties of biodiesel. Hence to augment the fuel's properties, it would be appropriate to supplement the alkyl esters with the beneficial properties of fuel. Future improvement of the biodiesel properties can be carried out through genetic engineering of parent oils, which may ultimately result in a fuel enriched with certain fatty acids such as oleic acid (Pintoa *et al*., 2005).There are certain reasons as to why biodiesel is a desirable energy resource. First, biodiesel is an energy resource that is not only renewable but can also be sustainable, supplied and substitutes the dwindling petroleum resources. The petro-diesel reserves are expected to be exhausted in less than 50 years at the present rate of consumption(Sheehan etal.,1998).Another reason is that biodiesel has no net increased release of carbon dioxide and very low sulfur content, and is thus environmentally friendly(Antolin *et al*., 2002;Vicente *et al*.,2004.).Third, biodiesel is a practical renewable-energy source whereas fossil-fuel supply is dwindling, which may hike up future fuel price seven more (Cadenas and Cabezndo, 1998). Biodiesel is non-toxic and biodegradable alternative fuel that is obtained from renewable sources (Hossain *et al*., 2008).In many countries, biodiesel is produced mainly from soy beans. The carbon chain of the diesel oil molecule is a kin to plant oil, whereby diesel oil contains about 15carbons and plant oil contains 14–18carbons (Guan Hua *et al*.,2010).The most prevalent fatty acids that can

Corresponding Author: Chew Tin Lee, Faculty of Chemical Engineering, Department of Bioprocess Engineering, Universiti Teknologi Malaysia, Johor81310, Malaysia
E-mail: ctlee@utm.my.com, Tel: +607 5538005

be found in biodiesel are palmitic, stearic, oleic and linolenic acid (Knothe,2008).As for microalgae, it usually contains long chain fatty acids (C_{20}–C_{22}) and the fatty acid content varies from species to species (Meireles *et al*.,2003).There are many varieties of microalgae each species has a different proportion of lipids (fats),starch and proteins. Depending on this proportion the microalgae can be used to produce oil for bio crude or if, the variant contains more carbohydrates and less oil, it can be fermented to make ethanol or biogas. It is interesting to note, however, that some microalgae strains or variants contain up to 50 percent lipids making them very suitable for the production of liquid fuels (Chisti, 2007).The main goals of microalgae oil production are high lipid yield and high biomass productivity, which can reduce the traditional and current production costs (Rodolfietal., 2009).The quality of biodiesel will depend upon the composition of the fatty acid methyl esters (Yoo*et al*., 2010).

Methods:

Cultivation of microalgae:

The green algae *Chlorella sorokiniana* (UTEX 1602) was obtained from the culture collection of Algae, University of Texas, Austin, TX, U.S.A. The algae were maintained in the modified Bristol's medium. The stock of *Chlorella sorokiniana* was cultured in Proteose medium. A Proteose medium contains a sufficient amount of carbon, vitamins, salts and other nutrients, which are vital for microalgal growth (Mata *et al*., 2010).A volume of 10-ml *Chlorella sorokiniana* was inoculated into100-mlfreshculture and was then expanded up to 5L along with temperature, light and nitrogen concentration.

Proteose medium Preparation:

In 900 mL of distilled water, the following ingredients $NaNO_3$(2.94mM), $CaCl_2 \cdot 2H_2O$(0.17mM), $MgSO^4 \cdot 7H^2O$(0.3 mM), K_2HPO_4(0.43 mM), KH_2PO_4(1.29 mM), NaCl(0.43mM) were dissolved sequentially by stirring continuously. One gram of proteose peptone was then dissolved, and the volume was increased to one liter after adjusting pH to 6.8.

Analytical methods:

The fresh water microalgae *Chlorella sorokiniana* was cultured in the Proteose medium. The cultures were maintained under a fluorescent lamp with light intensity of 22.25 µmol.m-2. S-1for 16hrs light: 8hrs dark which was controlled automatically. The pH of the medium was 7and the temperature was 27^OC. Gentle manual swirling was applied for once per day. The lipid extracted was detected through GC analysis (Knothe, 2008).For this experiment, five standards of common fatty acids present in biodiesel were used, which are palmitic acid (C_{16}:0), heneicosanoic acid (C_{21}:0), stearic acid (C_{18}:0), oleic acid (C_{18}:1), and linoleic acid (C_{18}:2). The identification of fatty acids was performed by comparison with the retention time of standards (D'Oca *et al*., 2011).

Discussion:

The effect of percentage inoculums as starter culture:

Based on the sub culturing method the ratio of microalgae corresponds to be medium that gives optimum growth of micro algal cells identified by culturing the microalgae, the sample of 1:10 (10%v/v) was prepared by adding 10 ml of microalgae into 90 ml of Proteose medium. After one week, the microalgae were observed using a microscope (Fig. 1&2).

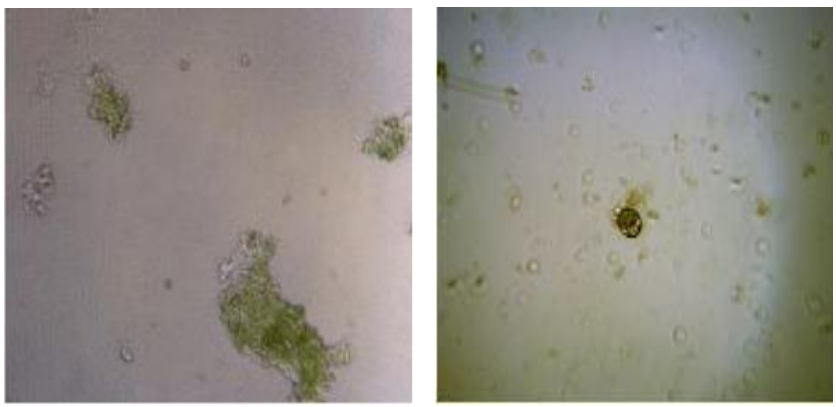

Fig. 1&2: Thesample of 1:2microalgae,*Chlorellasorokiniana*under 200 × Magnification.

Optimization of microalgal growth:

Under optimized conditions, microalgae can be induced to accumulate substantial quantities of lipids thus contributing to a higher oil yield (Sheehan *et al*., 1998). Not only organic carbon, vitamins, salts and other nutrients are vital for microalgal growth, but also equilibrium between operational parameters such as oxygen, carbon dioxide, pH, temperature, light intensity and byproduct removal are besides important (Mata *et al*., 2010).In this research, culture of *Chlorella sorokiniana* was grown under well balanced optimized condition (Fig.3) in order to give higher yield of lipid. The cultures were maintained under a fluorescent lamp with light intensityof22.25 µmol.m-2.s-1 for 16 hours light.

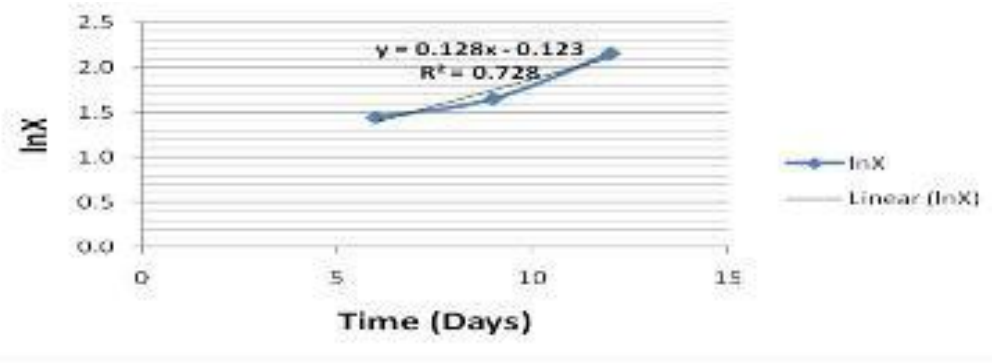

Fig. 3: Growth profile of *Chlorella sorokoniana.*

Biomass collection:

After15days of cultivation period, 2L of a culture medium was harvested to obtain biomass from the microalgal culture by transforming the pellet layer of dried powder to be used for lipid extraction process later (Fig.4). The additional centrifugation process was carried out to obtain pellet and the remaining pellet was mixed with the supernatant.

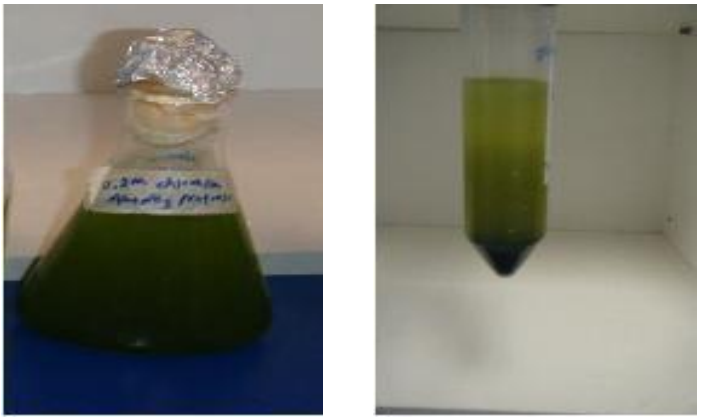

Fig. 4: Biomass from the culture and supernatant after centrifuge.

Effect of solvent extraction:

For this study chloroform/methanol, mixture was used to extract lipid instead of hexane (Miaoand Wu, 2006).Our results using hexane as solvent for extraction of lipids resulted in poor yield (data not shown) so chloroform/methanol mixture was employed throughout this study. For further extraction, the pellets were dried in freeze drier (Mahesar *et al*., 2008) (Fig.5&6). The dried biomass was weighed and recorded as shown in Table1.

Lipid extraction:

The lipid present in dried biomass was extracted using oven extraction method via methanol. After the extraction, it was shown that the control culture produced the lowest amount of lipid compared with the optimized condition. The results of extracting lipid in duplicates are shown in Table2.

Transesterification:

Transesterification of lipid which is biodiesel oil preparation yielded two products: methyl esters biodiesel oil and glycerin. Glycerin is extremely challenging to analyze by GC, but because excessive amounts in

biodiesel products can cause problems during storage or in the engine, and it is necessary to monitor glycerin levels to ensure less contamination with the biodiesel.

Fig. 5: Freeze dryer machine used in this experiment to dry biomass.

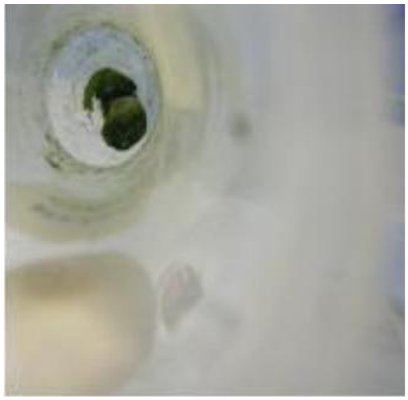

Fig. 6: The biomass obtained after freeze drying.

Table 1: The amount of biomass collected from 500ML culture.

Weight of beaker (g)	Weight of beaker with Algal powder (g)	Algal powder/ biomass (g/L)
102.24	102.336	0.0966
102.24	102.368	0.1280
102.24	102.783	0.540
102.24	102.332	0.0925

$$\text{Weight of biomass (g/L)} = \frac{[\text{weight of beaker(g)} + \text{algal biomass(g)}] - [\text{weight of beaker(g)}]}{3}$$

Table 2: The amount of lipid extracted from methanol method.

Weight of GC vial (g)	The Weight of the GC vial with lipid Extracted (g)	Extracted lipid (g/L)
2.742	4.244	1.502
2.742	4.328	1.586

$$\text{Weight of biomass (g/L)} = \frac{[\text{weight of beaker (g)} + \text{algal biomass (g)}] - [\text{weight of beaker (g)}]}{3}$$

Before GC was run, the mixture of catalyst and methanol was initially prepared and were kept for 24 hours. The process was carried out with 1g of crude biomass oil and 0.01g of 95% sodium hydroxide which was added as a catalyst. Then 0.217g methanol was poured into a conical flask as to ensure the transesterification process to occur. The conical flask containing solution was shaken for 2hours by electricshakerat300rpm. After shaking the solution, it was kept for 24 hours to settle into biodiesel and sediment layer clearly. The biodiesel was poured into separating funnel. The biodiesel was separated into two layers: to a player via yellowish or light brown color which was kept and the lower layer which was glycerol and discarded. The upper organic phase was washed seven times with acidic water (used dilution HCL 5%) and was later subjected for GC analysis.

Gas chromatography analysis of biodiesel:

The fatty acid component lipids were analyzed by GC. It is necessary to convert them to low molecular weight non-polar derivatives, such as methyl esters before analysis in GC. The high sensitivity of chromatographic analysis procedures, small amounts of material (usually less than1mg), maybe all that was required and most of the procedures described below are on this scale.

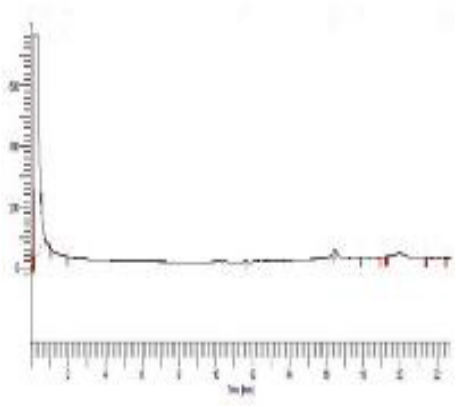

Fig. 7: Chromatograms for sample 1.

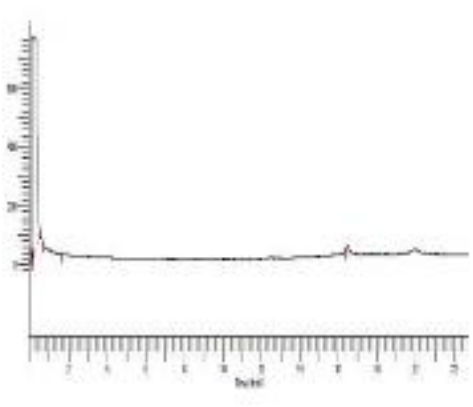

Fig. 8: Chromatograms for sample 2.

Fig.7&8 shows the chromatogram of methyl ester from the optimized sample and highest retention achieved was at16.5 minute, which showed the presence of palmitic acid (C_{16}:0). *Chlorella* species are found to contain shorter chain fatty acids, mainly 16–18 carbon length, which is ideal for biodiesel production and in *Chlorella sorokiniana*, palmitic acid was commonly dominant. Palmitic acid and also stearic acid content is known to be the most common fatty acids contained in biodiesel (Li *et al*., 2009), are present in this strain. Highly saturated fatty acids give an excellent cetane number and oxidative stability to biodiesel (Amini *et al*., 2011).

Conclusion:

The results from this study showed that *Chlorella sorokiniana*, according to its fatty acid content is an ideal candidate for biodiesel production. The freeze drying method seemed to be the best lipid extraction method that can be applied for the selected strain of microalgae. The lipid extraction method gave a higher yield of lipid and was much easier in identifying a fatty acid composition of *Chlorella sorokiniana*. The extracted lipid displayed a suitable fatty acid profile for biodiesel, ranging from16–18 carbon lengths with palmitic acid found predominantly in the lipid. Thus, making *Chlorella sorokiniana* was one of the possible candidates for biodiesel production.

REFERENCES

Amini, S.R., N. Montazeri - Najafabady, M.A. Mobasher, S. Hoseini- Alhashemi and Y. Ghasemi, 2011. Chlorellasp: A new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor. Appl Energy, 88: 1-4.

Antolin, G., F.V. Tinaut, Y. Briceno, V. Castano, C. Perez, A.I. Ramirez, 2002. Optimisation of Biodiesel Production by Sunflower Oil Transesterification. Bioresour.Technol., 83: 111.

Cadenas, A. and S. Cabezndo, 1998. Biofuel as Sustainable Technologies: Perspectives for Less Developed Countries. Technol Forecast Social Change, 58: 83-103.

Chisti, Y., 2007. Biodiesel from Microalgae, Biotech adv., 25: 294-306.

D"Oca, M.G.M., C.V. Vegas, J.S. Lemoes, E.K. Miyasaki, J.A. Moron-Villarreyes, E.G. Primel and P.C. Abreu, 2011. Production of FAMEs from several microalgal lipidic extract sand direct transesterification of Chlorella pyrenoidosa, Biormass Bioenergy, 35: 1533-1538.

GuanHua, H., C. Feng, W. Dong, Z. Xue Wu and C. Gu, 2010. Biodiesel production by microalgal biotechnology. Applied Energy, 87: 38-46.

Hossain, A.B.M.S., A. Salleh, A.N. Boyce, P.C. Howdhury and M. Naqiuddin, 2008. Biodiesel fuel production from algae as renewable energy. Am. J.of Biochem. Biotech, 4: 250-254.

Knothe, G., 2008. Designer' biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties. Energy Fuel, 22: 1358-1364.

Li, Y., M. Horsman, B. Wang, N. Wu and C.Q. Lan, 2009. Effects of nitrogen sources on cell growth and lipid accumulation of green alga", J. Appl. Phycol., 21: 493-507.

Mahesar, S.A., K. Sherazi, A. Abro, M.I.B. Kandhro, Hanger, F.R. Van deVoort and J. Sedman, 2008. Application of microwave heating for the fast extraction of fat content from the poultry feeds. Talanta, 75: 1240-1244.

Mata, T.M., A. Antonio, A. Martins, S. Nidia and S. Caetano, 2010. Microalgae For Biodiesel Production And Other Applications. Renewable and Sustainable Energy Review, 14: 217-232.

Meireles, L.A., A.C Guedes and F.X. Malcata, 2003. Lipid Class Composition Of The Microalga PavlovaLutheri: Eicosapentaenoic and Docosahexaenoic Acids, J. Agri. Food Chem., 51: 2237-41.

Miao, X. and Q. Wu, 2006. High quality biodiesel production from heterotrophic microalgal oil. Bioresour Technol., 97: 841-846.

Patil, V., K.Q. Tran, H.R. Giselrød, 2008. Towards sustainable production of biofuels from microalgae. Int. J. Mol. Sci., 9: 1188-1195.

Pintoa, A.C., L.N. Guarieiroa Lilian, J.C. Rezendea Michelle, M. Ribeiroa Núbia, A. Torresb Ednildo, A. Lopesc Wilson, A. Pereirac Pedro and B. de. Andrade Jailson, 2005. Biodiesel: An Overview. J. Braz. Chem. Soc., 16: 1313-1330.

Rodolfi, L., G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnol Bioeng, 102: 100-12.

Sheehan, J., T. Dunahay, J. Benemann and P. Roessler, 1998. A look back at the U.S. Department of Energy‟sAquatic Species Program: Biodieselfrom Algae. Close-Outreport. National Renewable Energy Lab, Department of Energy, Golden, Colorado, U.S.A.

Vicente, G., M. Martinez, J. Aracil, 2004. Integrated Biodiesel Production: A Comparison of Different Homogeneous Catalysts Systems.Bioresour. Technol., 92: 297.

Yoo, C., S.Y. Jun, J.Y. Lee, C.Y. Ahn and H.M. Oh, 2010. Selection of microalgae for lipid production under high levels carbon dioxide, Bioresour Technol., 101: 71-4.