SAARC J. Agric., 20(2): 273-281 (2022) *DOI: https://doi.org/10.3329/sja.v20i2. 63587*

ALGAL BLOOMS -A SOURCE OF OIL FOR BIODIESEL

S.J. Meghana and S.M. Murthy*

Department of Botany, Mount Carmel College, Palace Road, Vasanthnagar, Bengaluru, India

ABSTRACT

Algal biomass is good source of oils and it can be used for producing biodiesel. The present work is a study of biodiesel production from the algal blooms. *Microcystis* species was isolated from the blooms and cultured in BG-11 medium. The biodiesel was extracted using alkali catalyzed transesterification method. The trans esterified oil was collected and subjected to GC MS analysis. The FAME profiles showed the presence of unsaturated fatty acids which confirms the presence of biodiesel. The extracted biodiesel was also characterized for the physical properties like fatty acid value, saponification value, iodine value, cetane number, kinematic viscosity, pour point and cloud point.

Keywords*:* Algal bloom, Biodiesel, Cetane number, Cloud point, FAME, Pour point, Transesterification.

INTRODUCTION

Use of fossil fuels is unsustainable because of the accumulation of CO_2 in the atmosphere. (Vishwakarma et al., 2018). Producing biodiesel from algae is a method of generating biofuels to meet the global demand for vehicular fuels in the current situation. Usage of biodiesel will allow a balance to be sought between agriculture, economic development and environment. These problems can be solved by replacing the fossil fuels with some alternative renewable and sustainable biofuel (Klass, 1998).

Microalgae are photosynthetic micro-organisms that synthesize lipids and triacyl glycerol (TAG). The TAG content of some microalgae is so high and can be a promising sustainable feed stock for biodiesel production. These are considered as one of the promising alternative and renewable feed stock for biodiesel production (Thao et al., 2013).

Biodiesel is a non-petroleum derived fuel having mono alkyl esters of long chain fatty acids, which are derived from transesterification of lipids and fats. It is a renewable and eco-friendly fuel which is similar to fossil fuel (Lapuerta et al., 2008 and Kulkarni and Dalai, 2006).

* Corresponding author: dr.savithamurthy@mccblr.edu.in

Received: 22.07.2022 *Accepted: 14.11.2022*

Algal biomass is the upcoming source for sustainable energy and it will contribute to sustainable development environmentally, socially and economically. The algal oil was found to be having low melting point of their polyunsaturated fatty acids. The biodiesel produced from the algae are economically competitive with the conventional fuel (Ihsanullah et al., 2015).

In the present study, an attempt was made to extract lipids from algal blooms. The extracted lipids were subjected to direct alkali catalyzed transesterification to get biodiesel. The obtained product was subjected to GC-MS analysis to know the presence of Fatty Acid Methyl Esters (FAME). Biodiesel is biodegradable and non-toxic and can be used in place of petroleum fuel as a sustainable / renewable energy source.

The Algal blooms are excessive growth of algae in any water body. Blooms of these organisms give a green or yellow green color to the water and under certain conditions form a green scum (Carole Lembi, 2003). This formation occurs when there is excess supply of nutrients mainly nitrogen and phosphorous. The major source of these is from the untreated sewage water.

Due to the rise of fluctuating prices of fossil fuels there is a great challenge ahead to think alternative for the use of fossil fuel. Selection of feed stock for biodiesel production generally depends upon the availability and economic aspects of country. Based on the type of feedstock, biodiesel is classified as 1st generation, 2nd generation and 3rd generation. The 1st and 2nd generation biodiesel are from edible and non-edible vegetable oil respectively. The 3rd generation biodiesel is from algae.

In the present investigation, an attempt was made to study the extraction of biodiesel from algal blooms. This may help to sustain the fossil fuels as it is extracted from the natural organism which is biodegradable and non-toxic.

MATERIALS AND METHODS

Algal blooms were collected from Jakkur lake, near Yelahanka, Bengaluru, Karnataka, India. The predominant species in the algal blooms was identified as *Microcystis*, *Ocillatoria* and *Aphanozimenon* species.

Culturing and Maintenance

Algae collected from algal blooms were cultured on Bold's medium. *Microcystis* species were separately isolated and cultured on BG-11 medium and were maintained under 16/8 light/dark cycles with a light intensity of 1500 lux.

Figure 1. Algal Bloom

Figure 2. Dry algal biomass

Oil Extraction

The collected algal blooms were sun dried for 3-4 days and stored in air tight containers for further use. The dried algal biomass was powdered and total lipids were extracted from the powder using Bligh and Dyer (1959) method. Five grams of dried algal biomass was taken and homogenized in 50ml of distilled water. From this, 25ml of homogenized sample was taken in a separating funnel and a mixture of chloroform and methanol in the ratio of 1:2 was added. The solution was allowed to separate after vigorous swirling. The upper organic phase was collected and 25ml of methanol was added to it. The solution was centrifuged at 1500 rpm for 10 minutes and lower organic phase was recovered. The obtained total lipids was measured and percentage yield was calculated (Fig.3).

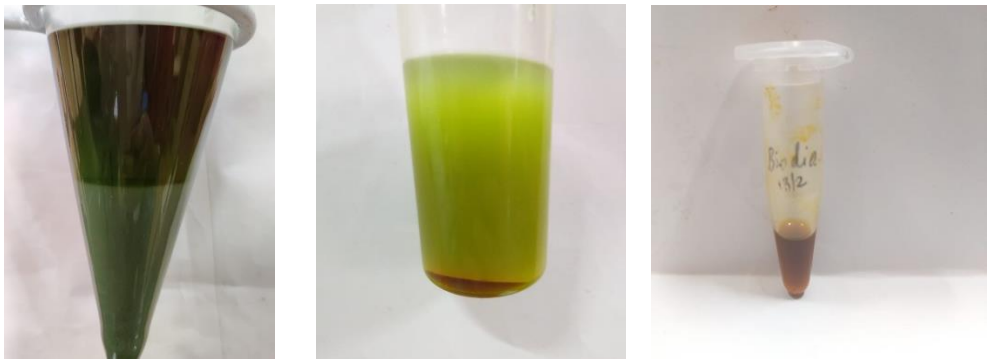

Figure 3. Process of biodiesel production

Yield of algal oil

Yield of the oil was calculated as the fraction of weight of the oil produced per kg of dried algal mass from which the oil was extracted (Leung and Guo, 2006).

$$Oil\ yield\ = \frac{oil\ extracted}{dried\ algal\ biomass\ taken}\ X\ 100$$

Transesterification

Twenty five ml of the extracted oil was heated in a water bath to a temperature of 45ºC. Sodium methoxide solution was prepared by dissolving 0.25g of NaOH pellets in 10ml of methanol. This solution was mixed with warm oil and stirred thoroughly on a magnetic stirrer for 90 minutes. After that the reaction mixture was transferred to a separating funnel and left as such for 24 hours. After settling the upper phase was collected in a beaker, which is the crude biodiesel. The collected biodiesel was measured and washed with warm water to remove the alkali catalyst and other impurities. This is then warmed to around 70ºC for an hour to get rid of the wash water (Meher et al., 2004).

GC MS Analysis

The purified biodiesel was subjected to GC-MS analysis (Varian 450GC) The column used was CP-SIL C18 with a dimension of 30m x 0.25mm x 0.25µm. The detector used was Flame ionization detector. Nitrogen gas was used as the mobile phase. The flow rate was 1ml/minute. The injector temperature was 220ºC and the detector temperature was 250ºC. NIST library was used to identify the compounds.-The fatty acids were identified by comparing the retention times with those of standard fatty acids. Their composition was calculated based on the corresponding peak areas in the chromatogram.

Characterization of Biodiesel

The physical properties like Iodine value, Saponification value and values of Free Fatty Acid, Cetane number (ASTM D 6751) Cloud point (ASTM D 2500), Pour point (ASTM D 97) and Kinematic viscosity (ASTM D445) of the prepared biodiesel were determined following standard methods. The values of these parameters fell within the limits of ASTM standards for a good biodiesel.

Determination of Acid value/ Free Fatty Acid (FFA)

2g of the oil was taken and a neutral solvent was prepared by adding equal amount of petroleum ether and ethanol. 50ml of neutral solvent was added to the oil. The mixture was stirred continuously for 20-25minutes.0.1M of KOH (0.3g of KOH in 50ml of ethanol) was prepared and was poured into a burette. To that w3 drops of phenolphthalein indicator as added and was titrated against 0.1M KOH solution. Pink colour persists for about 15 seconds which indicated the end point (AOAC,1999 and Indhumathi et al., 2014).

$$AV = \frac{56.1\ X\ AxN}{W\ oil}$$

$$FFA = \frac{AV}{2}$$

where, V= Volume of standard alkali used;

N= Normality of standard alkali used;

W = Weight of the oil used;

A= Volume of standard ethanol KOH used.

Determination of Iodine value

To determine the iodine value, 0.25g of the oil was taken into a 250ml conical flask. To that 10ml of chloroform and 30ml of Hannus solution were added. The solution was incubated for 30 minutes with constant vigorous shaking. 10ml of 15% potassium iodide and 100ml of distilled water were added and the mixture was titrated against 0.1N sodium thiosulphate till yellow color and then 2drops of 1% starch was added and the titration continued till the blue color disappears (AOAC 1999).

$$Iodine\ value = \frac{(blank\ value - sample\ value)\ X\ N\ of\ sodium\ thiosulphate\ X\ 0.127}{weight\ of\ the\ oil} X100$$

Determination of Saponification value

1g of the oil was weighed in a conical flask and to this 12.5ml of 0.5N ethanolic KOH was added. This solution was kept in the water bath for 1 hour. It was cooled and 2-3 drops of phenolphthalein indicator was added. The mixture was titrated against 0.5N HCl until the pink colour disappeared (AOAC 1999).

$$Saponification\ value = \frac{(56.1(V1 - V2)\ X\ N\ of\ HCl}{weight\ of\ the\ oil}$$

where, V_1 = Volume of HCl used by the blank;

V_2 = Volume of HCl used by the sample.

Determination of Cetane Number

The CN of the fuel is an important parameter which responsible for the delay period. It is defined as the percentage by volume of normal cetane in a mixture of normal cetane and alpha methyl naphthalene (ASTM D 613).

$$CI = 346.3 + \left(\frac{5458}{SV}\right) - (0.225\ X\ IV)$$

Determination of Cloud Point and pour point

The cloud point and pour point determination was carried out at Karnataka Soaps and Detergents Limited (KSDL), Yeshwanthapur. Cloud point is a test to characterize the low temperature performed of bio diesel. It is defined as the temperature at which a cloud or haze appears in the fuel under prescribed test condition. 6ml of the biodiesel sample was taken in a beaker and in a mortar and pestle in the ratio 1:1 ice cubes and NaCl was taken and crushed. The sample containing beaker was placed in the mortar and pestle and the temperature was noted regularly until the biodiesel forms a cloud. Sample of biodiesel was kept in a freezer and then heated to melt. The temperature at which the fuel starts to pour is taken as the pour point (ASTM D2500 and ASTM D97).

Determination of kinematic viscosity

A viscometer was placed in a water bath with a known temperature and left as such for 30 minutes. Then the sample was added to the viscometer and allowed to remain in the bath till it reached the temperature. The sample was allowed to flow freely and the time was noted and the viscosity can be measured according to the ASTM D445.

RESULTS AND DISCUSSION

The fatty acid composition of algal bloom was evaluated and the results were depicted in Table 1. The fatty acid profile of algal strains generally influenced by growth conditions. Different algal based oil, their fatty acid profile will be different (Suresh et al., 2014, Duran et al., 2019). The fatty acid composition of the oil is important for determining the fuel properties (Chaudhary et al., 2014).

There are different methyl esters in the biodiesel and the chromatogram showed several compounds with various retention times. The fatty acid ester composition of the extracted biodiesel was shown in Table 1. The fatty acid profile comprises of myristic acid, palmitic acid, lauric acid, arachidic acid, stearic acid, oleic acid and many more. The presence of saturated fatty acids indicates good quality biodiesel (Duran et al., 2019).

Table1. Fatty acid methyl ester profile of algal biodiesel

Sl. No	Fatty acids	Area (%)
1	Myristic acid	2.80
2	Pentadecanoic acid	2.51
3	Palmitic acid	7.81
4	Arachidic acid	0.88
6	Lauric acid	0.86
7	Heptadecanoic acid	0.72
8	Tetradecanoic acid	2.80
9	Oleic acid	0.42
10	Stearic acid	4.69

It was observed that the oil extracted from the algal blooms contained both saturated and unsaturated fatty acids. The percentage of unsaturated fatty acid were more compared to saturated fatty acids (Table 1). The ideal biodiesel is suggested to have the presence of Palmitic acid, Stearic acid and Myristic acid.

In the present study, the unsaturated fatty acids were more compared to saturated fatty acid. Similar kind of observations were made by Chinnasamy et al. (2010) in the algal oil using GC. The microalgal lipids derived from *Chlorella vulgaris*, *Scenedesmus maxima*, *Dunaliella tertiolecta* were also showed more percentage of unsaturated fatty acids (Gouveia and Oliveira, 2009).

The quality of the biodiesel was analyzed by comparing its physical properties like iodine value, saponification value, kinematic viscosity, cloud point, pour point and cetane number (Table 2). All the parameters were in the range of biodiesel ASTM standards (Srivastava et al., 2000) indicating the quality of the biodiesel. Higher cetane number is another parameter which also indicates the quality of the biodiesel and also good alternative fuel (Ihsanullah et al., 2015).

In the present work, CN was found to be 49 which higher than that of the ASTM D 6751 standard limits. The CN was found to be 51 in the study conducted by Enwereuzoh et al. (2020). Similar CN value was observed by Indhumathi et al. (2014) in their study also.

High cetane number is an indication of good ignition quality. The value of cetane number is increasing with increased carbon chain length. Ignition properties are better with increasing CN in general (Meher et al., 2004). Higher the CN, lesser will be the ignition delay time (Knothe, 2006). Higher the molecular weight of the fatty acid esters in the biodiesel, higher will be the cetane number (Ramirez-Verduzco et al., 2012).

Table 2. Algal biodiesel characteristics

Characteristics	Algal Biodiesel	ASTM D 6751standards
Acid value mgKOH/g	0.56	<500
Cloud point^{o}C	-5	-3 - -15
Pour point^{o}C	-3	-5 - -15
Kinematic viscosity mm^2/s	3.5	1.9 – 6
Iodine value mg I_2/g	15.24	<115
Cetane Number	49	≥47

CONCLUSION

Biodiesel can be used as an alternate fuel which will be obtained from renewable biological source like algal bloom. The polyunsaturated fatty acids present in the algal oil have very low melting point which is very good parameter to consider for production of biodiesel. Algal bloom was successfully used to extract the biodiesel in two steps oil extraction and transesterification. The quality of biodiesel was analyzed by GC and few physical tests, which showed the parameters were in close agreement with the biodiesel ASTM standards. The present study shows that the 3rd generation biodiesel obtained from algal bloom biomass is having the required characteristics as per the ASTM standards and this can be used in place of the conventional petro products.

ACKNOWLEDGEMENT

Authors are grateful to the management of Mount Carmel Educational Institutions, Bengaluru, Karnataka, India for all the support to carry out this research work.

REFERENCES

AOAC. (1999). Official methods of analysis, 16th Edn. Association of Official Analytical Chemists, Washington.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8): 911-917.

Carole A Lembi. (2003). Control of nuisance algae In: Fresh water algae of North America. (Ed)John, D. Wehr., and Robert, G, Sheath. Academic Press, Pp. 804-834.

Chaudhary, R., Khattar, J.I.S. and Singh, D.P. (2014). Microalgae as feedstock for biofuel: Biomass yield, lipid content and fatty acid composition as selection criteria. International Journal of Electrical Power & Energy Systems, 1: 62-71.

Chinnasamy, S., Bhatnagar, A., Hunt, R.W. and Das, K.C. (2010). Microalgae cultivation in a waste water dominated by carpet mill effluents for biofuel applications. *Bioresource Technology*, 101: 3097-3105.

Duran, S.K., Kumar, P. and Sandhu, S.S. (2019). Prospect of algal biodiesel as a fuel in engine. *International Journal of Engineering and Advanced Technology*, 8(6): 2739- 2744.

Enwereuzoh, U., Harding, K. and Low, M. (2020). Characterization of biodiesel produced from microalgae grown on fish farm waste water. *SN Applied Sciences*, 2: 970.

Gouveia, L. and Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, 36: 269-274.

Ihsanullah, Shah, S, Ayaz, M, Ahmed I., Ahmad, N. and Ahmad, I. (2015). Production of biodiesel from algae. *Journal of Pure and Applied Microbiol*ogy, 9(1): 79-85.

Klass, D.L. (1998). Biomass for Renewable Energy, Fuels, and Chemicals. Academic Press, San Diego, 1-651.

Kulkarni, M.G. and Dalai, A.K. (2006). Waste Cooking Oil-An Economical Source for Biodiesel, a Review. *Industrial & Engineering Chemistry Research*, 45: 2901-2913.

Indhumathi, P., Shabudeen Syed, P.S. and Shoba, U.S. (2014). A method for production and characterization of biodiesel from green micro algae. *International Journal of Bio Science and Biotechnology*, 6(5): 111-122.

Knothe, G. (2006). Analyzing biodiesel: standards and other methods. *Journal of American Oil and Chemical Society*,83(10): 823 – 833.

Lapuerta, M., Armas, O. and Rodriguez, J. (2008). Effect of biodiesel fuels on diesel engine emissions. *Progress in Energy and Combustion Science*., 37(2): 198-223.

Leung, D.Y.C. and Guo, Y. (2006). Transesterification of neat and used frying oil: Optimization for biodiesel production. *Fuel Processing Technology*, 87(10): 883-890.

Meher, L.C., Vidyasagar, D. and Naik, S.N. (2004). Technical aspects of biodiesel production by transesterification -A review. *Sustainable Energy Reviews*, 10: 248-268.

Ramerez-Verduzco, L.F., Rodriguez -Rodriguez, J.E. and del Jaramillo-Jacob, A.R. (2012). Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition. *Fuel*, 91: 102-111.

Srivastava, A. and Prasad, R. (2000). Triglycerides based diesel fuels. *Renewable and Sustainable Energy Reviews*, 4: 111-133.

Suresh, A., Praveen Kumar, R., Thangaraj, R., Oscar, F.L. and Baldev, N. (2014). Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Disease*, 4: 979-984.

Thao, N.T.P., Tin, N.T. and Thanh, B.X. (2013). Biodiesel production from microalgae by extraction – transesterification method. *Waste Technology*, 1(1): 6-9.

Vishwakarma, R. Dhar, D.W. and Pabbi, S. (2018). Formulation of minimal nutritional medium for enhanced lipid productivity in *Chlorella* sp. and *Botryococcus* sp. using response surface methodology. *Water Science and Technology*, 77(6): 1660 -1672.