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Research Article

PRODUCTION AND CHARACTERIZATION OF BIODIESEL USING *AZOLLA PINNATA*

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ABSTRACT

Biodiesel is a renewable substitute for neat diesel. Algae is identified as a potential source to produce biofuels. In the present work, Azolla pinnata macro algae has been grown and their evaluation of growth was done and it was used for producing biofuels by trans-esterification process. Its characterization study like Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography (GCMS) reveals the feasibility of algal based biofuel. The physico-chemical properties such as Flash point, Fire point, Calorific value, Cloud point, Pour point and Viscosity have been evaluated. The results were very close to conventional diesel.

INTRODUCTION

In the recent years, there is a huge demand for unconventional energy resources. Even though various substitutes have arrived, in order to achieve sustainable fuel alternative, much more are needed (Marchetti, *et al*., 2007). Now-a-days, as a replacement to conventional fossil fuels, Biodiesel is gaining great relevance. The use of biodiesel creates more advantages (Demirbas, 2009; Van, 2005) due to its compatible physical and chemical properties with conventional diesel, biodiesel can be directly used in diesel engines, eliminating engine modifications (Subramanian, *et al*., 2005). Properties like biodegradability, renewability and non-toxicity makes biodiesel more eco-friendly (Fangrui and Milford, 1999).

Edible oil seed crops can be used for the generation of bio diesel. They are known as first generation biodiesel feed stocks and the second generation feed stocks including non-edible seed crops created special relevance for biodiesel production (Indhumathi, *et al*., 2014). The third-generation feed stock, focuses on macro algae. High photosynthetic efficiency and higher biomass production are the advantages while using macro algae (Tredici, 2010). Macro algae doesn't need any land and can grow anywhere, even in brackish saline water, using CO_2 fixation, macro algae can grow rapidly and convert solar energy to chemical energy. This method is now being considered as a viable method for making biodiesel. The generation of bio diesel is supported by the fatty acid profile of macro algal oil. The tremendous oil production capacity of macro algae attracts the usage of algal oil. Azolla, a free-floating water fern is another fast-growing plant, which can grow in the absence of nitrogen (N) in water (Muradov, *et al*., 2014).

MATERIALS AND METHODS

Azolla pinnata Biomass Production

In this research work, *Azolla pinnata* was obtained from Regional fodder centre, Tamilnadu, India. It algae was cultivated in a pit of size 2.6 m × 2 m × 0.30 m deep at Mepco Schlenk Engineering College, Sivakasi, India. Well grown pinnata was harvested partly from the pit every 5th or 6th day, since sewage waste water contain enormous amount of phosphorous and nitrogen that enhance the growth of algae. CO_2 concentration in air like 200 ppm also supplied for its fast growth. This

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pit was subjected to a temperature of 26°C/ 32°C and photosynthetic photon flux density of 50 µm/ m^2s was provided by three fluorescent lamps for 14 hours photo period [Muradov et al., 2014].

Investigation of Growth

(i) Dry cell method: The growth of *Azolla pinnata* was evaluated by dry cell weight method, a known amount of azolla biomass from the growing medium was taken for evaluation. To remove the salt traces in the biomass, it was thoroughly cleansed with purified water. Pre-weighed glass fiber filter was used for filtering the biomass and the same was dried at 60°C for a night in oven and weighed for dry cells in it (Zhu and Lee, 1997).

(ii) Chlorophyll-a content: Growth pattern of *Azolla pinnata* was studied through the chlorophyll-a content in it for the entire duration of 21 days. The procedures for estimation and extraction of chlorophyll-a content was carried out according to the procedure (Chinnasamy, *et al.*, 2010).

To determine the chlorophyll-a content in the pinnata, a known amount of algal biomass was taken and then grounded with known amount of methanol. The mixture was maintained in the water bath for 1/2 hour at 60°C to extract the chlorophyll-a from *A. pinnata*. After the said time, the suspension was filtered with whattman glass fiber and then it was tested for its chlorophyll-a content spectrophotometrically using UV visible photo spectrometer (Perklin Elmer lambda). The formula for determining Chla (Sumanta, *et al*., 2014) was used

$$\text{Chl a (ug/ml)} = 16.72\, A^{665.2} - 9.16\, A^{652.4}$$

Where $A^{665.2}$ and $A^{652.4}$ are the absorbance value of algal biomass and methanol suspension at 665.2 nm and 652.4 nm respectively.

Oil Extraction followed by Trans-esterification

After pulverizing the well dried *Azolla pinnata*, Algae biomass of sufficient quantity was subjected to soxhlet extraction using chloroform-methanol (2:1 v/v) as a solvent at 56°C [Sumanta et al., 2014]. Excess solvent in the crude lipids were separated from oil by distillation process. Then these lipids were subjected to Trans esterification process for biodiesel production. To change di glycerides (DG), fatty acids (FA), triglycerides (TG) and mono glycerides (MG) to fatty acid methyl esters (FAMEs) methanol along with conc. H_2SO_4 were added to the crude lipids and maintained at 60 °C for 1 h with continuous magnetic stirring in the reactor during the process at center for Nano science and technology, Mepco schlenk engineering college, Sivakasi. (Salehzadeh, *et al*., 2014).

After complete mixing of the crude lipids and Trans esterification agent, the mixture was allowed to cool at room temperature, then the mixture was transferred to separating funnel for 24 hrs.

Water Wash and Drying

Biodiesel obtained through Transesterification process contain impurities like methanol, glycerine, catalyst and soaps & these impurities affects the quality of biodiesel. To remove these impurities warm water of about 45°C is mixed with Transesterified biodiesel, to allow soluble material (soaps), catalyst and other impurities to dissolve with water and to be settled to the bottom of the vessel. After removing impurities present in the Trans esterified biodiesel, sometimes it showed cloudy colour. This means there is trace amount of water in it. It was heated gently to 100°C until all moisture present was evaporated (Nautiyal, *et al.*, 2014).

CHARACTERIZATION OF BIODIESEL

Pure Biodiesel produced through Trans esterification process was investigated by Fourier transform infrared spectroscopy and Gas chromatography.

Fourier Transform-Infrared (FTIR) Spectroscopy

In the present study, FTIR Spectrum was used to ensure the completion of Trans esterification process of algae oil and methanol through FTIR Spectroscopy which is available in Mepco Schlenk Engineering College. By analyzing the functional groups and its vibrations, quality and important fatty acid methyl ester of biodiesel could be predicted.

GAS-Chromatography

GC-MS analysis was used to analyse the fatty acid methyl ester profile present in the bio diesel. Since fatty acid have very similar properties like fossil fuel, it is necessary to identify the chemical composition of the biodiesel for its suitability towards replacement of fossil fuel. 1 ml of algal biodiesel was passed through column of Perkin Elmer gas chromatogram mass spectrometer and helium was used as carrier gas of flow rate 1.0 mL/min. Peaks obtained for sample was identified by comparing with Supelco TM 37 Component fame mix standard of the biodiesel.

Measurement of Fuel Properties

Properties of *Azolla pinnata* biodiesel was determined according to ASTM standard for biodiesel. Determined results were compared with neat diesel. Following physico chemical properties were determined for the synthesized biodiesel: Viscosity (Brook field Viscometer), Flash and Fire point (Pensky Marteins apparatus), Cloud and Pour point (cloud and pour point apparatus) and Calorific Value (Bomb Calorimeter).

RESULTS AND DISCUSSION

Evaluation of Growth

The species was kept in the presence of sewage waste water for 24 days and the growth pattern has been shown in (Fig. 1 and 2). From the growth pattern, it is observed that the chlorophyll-a content is gradually increased with time and this indicates the algae growth. Also for 24 days the growth of this species was monitored using the dry cell weight content method. By chlorophyll-a method growth of the algae in the due course of period is monitored as 0.136 ug/ml, 0.201 ug/ml, 0.267 ug/ml, 0.815 ug/ml, 1.113 ug/ml and 1.225 ug/ml for 4, 8, 12, 16, 20 and 24 days respectively.

FTIR Spectra Analysis

FTIR Spectrum was used to identify the functional group present in the Trans esterified biodiesel. To confirm the conversion of extracted oil to biodiesel this study was done. FTIR Spectra of azolla biodiesel (Fig. 3) reveals well absorbed region of 800-500cm^{-1}, 1800 - 1000cm^{-1} and 3500-3000cm-1. The peaks which appeared around 2921 and 2812 cm^{-1} are due to presence -CH_2-stretching vibration of the alkanes compound. The fatty acid methyl ester exhibits its appearance at 1156 cm^{-1} and 1031 cm^{-1}. C=O stretching vibration of methyl ester moiety was found at 1741 cm^{-1} while -CH_2-peaks appeared at 722 cm^{-1}.

The above results showing the presence of broad band spectra confirmed the functional group which was present in neat diesel is also present in the biodiesel. The peak appeared at 1741 cm^{-1} in algal biodiesel was

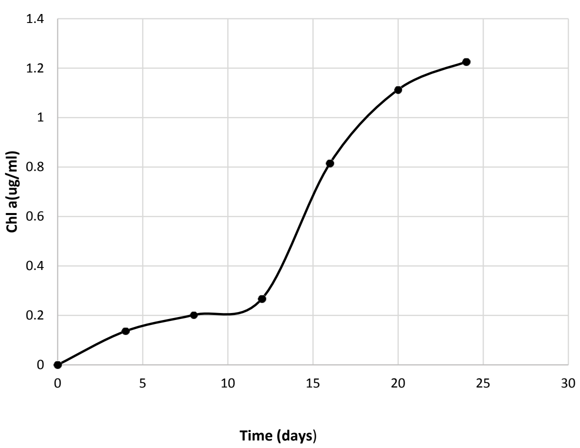

Fig. 1 Chlorophyll-a content in *A. pinnata*.

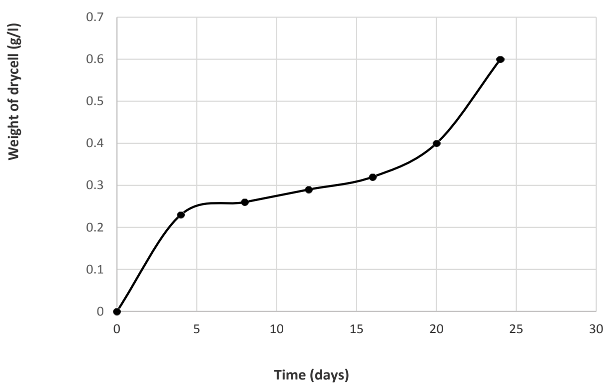

Fig. 2 Dry cell weight of *A. pinnata*.

compared with the FTIR spectra of Diesel and found the absence of 1741cm^{-1} (i.e., Carbonyl groups). Diesel and Biodiesel showed its peaks at 2924 cm^{-1} and 2853 cm^{-1} confirming the presence of aliphatic hydrocarbon (Fig. 3 and 4).

Fatty Acid Methyl Ester Distribution (FAME)

To identify the chemical composition in the *A. pinnata* biodiesel Gas chromatography (GC) study was used. Supelco™ standard peak and their retention time was compared with the chromatogram peak of *A. pinnata* Biodiesel to quantify and identify the peaks. GC analysis reveals the composition of FAME (Fatty Acid Methyl Ester) and confirmed the presence of seven Saturated (Single bond) and Unsaturated (double bond) acids. These saturated fatty acid methyl ester (Butyric acid, mystric acid, palmitic acid, stearic acid and magaric acid) possess good oxidative stability and ignition property, whereas unsaturated fatty acid methyl ester (oleic acid, linoleic acid, eicosapantaenoic acid and docosahexaenoic acid) possess low (or) decreased energy content and poor oxidative stability due to presence of double bond atoms. These unsaturated fatty acids affect the cold behavior of biodiesel such as cloud and pour point. Table 1 shows FAME Composition in *A. pinnata* Biodiesel.

Viscosity

Viscosity is a measure of resistive flow of liquid, it is one of the important parameter for biodiesel, because viscosity of fuel should be in optimum range otherwise it affects the efficiency and combustion. The viscosity of Bio diesel of *Azolla pinnata* was found out to be 4.3 mm^2/s. *Azolla pinnata* biodiesel showed its viscosity was within the limits of ASTM D6751. (Table 2).

Flash Point

The lowest temperature at which the volatile material starts to liberate ignitable vapour in air up on placing

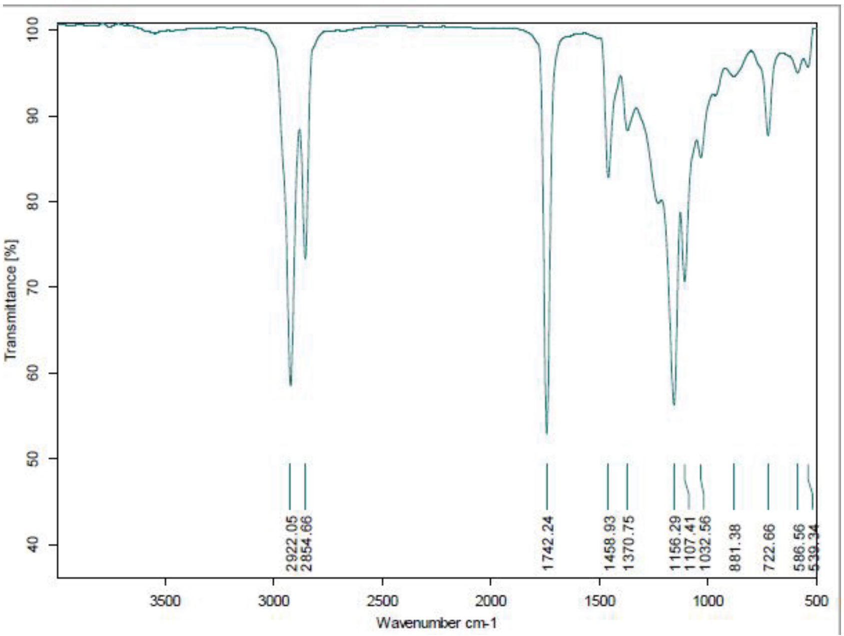

Fig. 3 FTIR of Algae biodiesel.

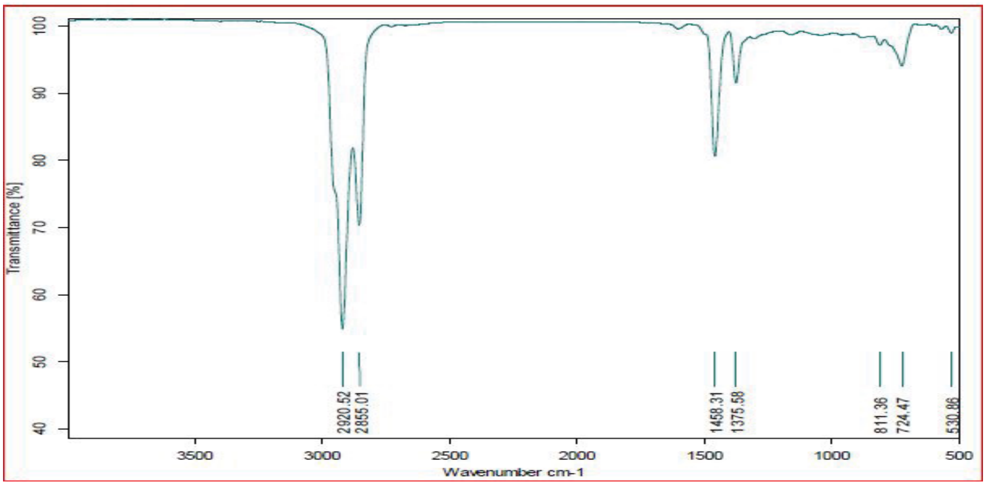

Fig. 4 FTIR of neat diesel.

Table 1. FAME composition in *A. pinnata* biodiesel

S. no	Fatty acid methyl ester	Structure
1.	Butyric acids	C4:0
2.	Capric acid	C10:0
3.	Lauric acid	C12:0
4.	Mystric acid	C14:0
5.	Palmitic acid	C16:0
6.	Margaric acid	C17:0
7.	Stearic acid	C18:0
8.	Oleic acid	C18:1
9.	Linoleic acid	C18:2
10.	Eicosapantaenoic acid	C20:5

open flame is called flash point at which sudden intense flash will occur. For safe transportation and storage of flammable liquid it is necessary to determine flash point. Flash point determined for

Table 2. Comparison of *A. pinnata* biodiesel with ASTM

S. no	Properties	Test Method	ASTM D6751 Bio diesel standard	*Azolla pinnata* biodiesel value
1.	Flash Point (°C)	ASTM D93	100-170	108
2.	Fire point (°C)	ASTM D93	106-180	120
3.	Viscosity (cP)	ASTM D6751	1.9-6	4.3
4.	Calorific Value (MJ/Kg)	EN 14213	Min 35	38.2
5.	Cloud point (°C)	ASTM D2500	-3 to 15	8
6.	Pour point (°C)	ASTM D97	-5 to 10	3

pinnata biodiesel was 108°C and found to be within limits of ASTM D93. (Table 2).

Fire Point

The lowest temperature at which the sample gives off sufficient vapour upon introducing the open flame, the flame continuously for at least 5 seconds is called fire point. For every 1°C rise in temperature open flame was placed over the cup of the Pensky Martin apparatus at proper interval to determine the fire point. The fire point of the *A. pinnata* biodiesel was about 120°C and found to be within limits of ASTM D93 (Table 2).

Cloud Point and Pour point

To assess behavior of biodiesel in cold environment could and pour point were determined. The lowest temperature at which sample form hazy cloud wax at the bottom of the beaker is called cloud point, whereas pour point is the lowest temperature at which sample becomes semi solid or lowest temperature at which sample becomes semi solid or loses its flow characteristics. The cloud and pour of the pinnata biodiesel were about 8°C and 3°C (Table 2).

Higher Heating Value

Energy packed in a fuel can be measured by burning specific quantity of it and determining the heat produced by it. This can be expressed in joules per kilogram. If the fuel has higher value of calorific value engine performance will be good. The calorific value determined for *Azolla pinnata* biodiesel is 38.2 MJ which is slightly less than diesel (Table 2).

CONCLUSION

In this present work, an attempt has been made to extract biodiesel from *Azolla pinnata* macroalgae. The algae was grown in sewage wastewater, which is rich in nitrogen and phosphorous.

- In FTIR Spectroscopy test, the various functional group present in the biodiesel were identified which was also present in Neat Diesel. The presence of Fatty acid methyl ester confirmed the conversion of oil in to biodiesel.
- For presence of saturated and unsaturated fatty acids using Gas chromatography (GC-MS). It showed the presence of FAME, in which eight were saturated and three were unsaturated fatty acids. This reveals the suitability of the synthesized bio-diesel to be used as fuel or fuel blender.
- The calorific value of biodiesel was 38.2 MJ/Kg, which is comparable with the diesel calorific value of 44 MJ/Kg. Hence, by blending with normal diesel, an optimum calorific value can be obtained.
- The synthesized biodiesel showed a dynamic viscosity of 4.3 cP whereas normal diesel is having a viscosity of 3.06 cP.
- The synthesized biodiesel showed a cloud point of +8°C and a pour point of +3°C, thereby confirming with ASTM D2500, ASTM D97standards.
- The synthesized biodiesel showed a flash point of 108°C and fire point of 120°C, thereby ensuring the ASTM D93.

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