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Fuel Property of Biodiesel Made from Microalgae *(Chlorella sp.)*

S.V. KELAIYA[1], P. M. CHAUHAN[2] AND S. H. AKBARI[3]

[1]Department of Bio Energy, College of FPT and BE, AAU, Anand (Gujarat), India.
[2]Department of RE and RE, CAET, JAU, Junagadh(Gujarat),India.
[3] Department of PHET, College of FPT and BE, AAU, Anand (Gujarat), India.

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ABSTRACT

Microalgae chlorella is an organism capable of photosynthesis that is less than 2mm in diameter. The biodiesel extracted from algae using chloroform/methanol extraction solvent system then undergone three different transesterification processes based on three different catalysts *viz.* Alkali catalyst, Acid catalyst and Enzymatic catalyst with two temperature (50°C and 60 °C) and with 1:5 methanol to bio-oil ratio. After transesterification using different catalysts, the fuel properties were measured. All the properties were compared with standard value of ASTM D 6751 standards. Alkali catalyst yield highest biodiesel (92 %) at 60 °C temperature. Also, the closest value of different fuel properties found at par with standard value of ASTM D 6751 standards *viz.* moisture content, carbon residue, calorific value, specific gravity, acid value, flash point, viscosity, density, viscosity were found to be 0.01%, 0.04%, 40.41 MJ/kg, 0.83, 0.23 mg KOH/g, 143.67 °C, 5.16 mm^2/s, 0.83 g/cm^3 respectively in the biodiesel which was yield by transesterification done using Alkali catalyst (0.56 % NaOH) at 60 °C temperature.

Key words: Microalgae, Chlorella sp., Biodiesel, Transesterification, Fuel properties.

INTRODUCTION

Algae (macro and micro) have been taken in consideration as a residual biomass ready to be used for energy purposes. Algae, especially microalgae, were found to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels[1]. The idea of using algae as a source of fuel is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels[4].

This work aimed to process development for production of biodiesel from microalgae, using two different extraction solvent systems to get the best result in oil extraction. Biodiesel is produced by trans-esterifying the parent oil or fat with an alcohol, usually methanol, in presence of a catalyst, usually a strong base such as sodium or potassium hydroxide or increasingly, alkoxides, acids and enzymes. The resulting product therefore can contain not only the desired alkyl ester product but also unreached starting material (TAG), residual alcohol, and residual catalyst. Glycerol is formed as by-product and separated from biodiesel in the production process, however, traces thereof can be found in the final biodiesel product. Since transesterification is a stepwise process, MAG and DAG formed as intermediates can also be found in biodiesel[6].

With the increasing interest and use, the assurance of fuel properties and quality has become of paramount interest to the successful commercialization and market acceptance of biodiesel. Accordingly, biodiesel standards have been established or are being developed in various countries and regions around the world, including the United States[2], Europe (EN 14214), Brazil, South Africa, Australia and elsewhere[6]. In ASTM D 6751 and EN 14214 are commonly used standards as reference or base for other standards, and their analysis.

MATERIALS AND METHODS

Microalgae (*chlorella sp.*), which was cultivated from locally available sources which was cultivated for the study. The investigation methodology includes procedure for properties of microalgae biodiesel and analysis of resulting data.

Experimental Design

The experiments were planned using completely randomized design (C.R.D.)[5]. The treatments consisted three levels of catalyst for transesterification, two levels of temperature and two levels of solvent oil extraction methods in which best suitable method was adopted for oil extraction. The details of treatments and parameters are given as below.

- Year/Season of experiment: 2013-2014
- Crop: Algae, *Chlorella Sp.*

Experimental Design: C.R.D.

First factor: Catalyst for transesterification

(a) Alkali catalyst (C_1): Sodium Hydroxide (NaOH)
(b) Acid catalyst (C_2): Sulphuric Acid (H_2SO_4)
(c) Biocatalyst (C_3): Lipase Crude

Second factor: Temperature for transesterification

(a) 50 ˚C (T_1)
(b) 60 ˚C (T_2)

Third factor: Solvents for oil extraction

(a) Chloroform/Methanol (S_1)
(b) Hexane/Ether (S_2)

Total Treatment combination: 6 and total no of observation (properties) is 8 from each treatment with 3 Replication.

Dependent variable

(a) Quantity of biodiesel
(b) Quality of biodiesel

The results regarding effect of solvent methods on oil recovery and effect of catalyst and temperature on biodiesel recovery were analyzed statistically[5].

Extraction method of algae oil

In Chloroform-Methanol (2:1, v/v) Method, 10 g of dry algae was taken and mixed with chloroform-methanol (100 ml, 2:1, v/v) for 20 min. using shaker followed by the addition of mixture of chloroform/water (50 ml, 1:1, v/v) for 10 min. Then it was filtered. While in Hexane-Ether (1:1, v/v) method, 10 g of dry algae was mixed with hexane-ether (100 ml, 1:1, v/v); keep it to settle for 24 h[3] and then it was filtered.

Transesterification and biodiesel production

In this method, oil to methanol ratio was 1:5 and the amount of all three catalysts was 1 % by weight with oil. The reactions were started at two different temperatures likely 50 ˚C and 60 ˚C. After reaction took place, biodiesel and glycerin were separated by gravitational method using separating funnel. The produced biodiesel was tested for the different property measurement which are given below,

Analysis of biodiesel

The fuel properties of algal biodiesel were determined by the following methods.

Moisture Content %

As per ASTM D2709, the moisture content of extracted biodiesel was determined by calculating the loss in weight of sample using hot air oven drying method.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W2} \times 100$$

Where,
W1 = Initial weight of biodiesel
W2= Final weight of biodiesel after drying

Carbon Residue %

As per ASTM D4530, percent of carbon residue content was determined. A 10 g of biodiesel was placed in a glass vial and heated to 500°C under an inert (nitrogen) atmosphere in a controlled manner for 1 h. Calculation for the mass % carbon residue in the original sample, or in the 10 % distillation bottoms as follows:

Calculation for percent residue as follows:

$$\% \text{ carbon residue} \quad \frac{(A \times 100)}{W}$$

Where,
A = carbon residue, g, and
W = sample used, g

Calorific Value

The calorific value of biodiesel was determined using Bomb calorimeter (ASTM D240). Water equivalent (W) of calorimeter will be obtained by conducting the experiment and using the benzoic acid as standard sample having known calorific value of 'H' equal to 6319 cal/g. The value of W will be calculated by using the following formula.

$$H = \frac{\left(wt of water, ml or g or \frac{cal}{°C}\right) + \left(W, \frac{cal}{°C}\right) x temperature, °C}{weig ht of sample, g} - (E_1 + E_2)$$

Then, calorific value of biomass will be obtained by conducting the separate experiment by using the following formula and the value of water equivalent, W obtained from the above formula.

$$\text{Calorific value} = \frac{\left(wt of water, ml or g or \frac{cal}{°C}\right) + \left(W, \frac{cal}{°C}\right) * temp, °C}{weig h of sample, g} - (E_1 + E_2)$$

Where,
E1= Correction for nichrome wire, cal= wt of wire, g x calorific value, 335cal/g
E2= Correction for cotton thread, cal=wt of thread, g x calorific value 4180 cal/g

Specific Gravity

As per ASTM D4530, specific gravity of biodiesel was determined by the following equation.

$$\text{Specific Gravity at 30 °C} = \frac{A - B}{C - B}$$

Where,
A = weight in gm of specific gravity bottle with oil at 30°C
B = weight in gm of specific gravity bottle at 30°C
C = weight in gm of specific gravity bottle with water at 30°C

Acid Value

As per ASTM D664, acid value of biodiesel was determined by the following equation.

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where,
V = Volume in ml of standard potassium/ Sodium hydroxide
N = Normality of the potassium/ Sodium hydroxide solution
W = Weight in g of the sample

Flash Point

As per ASTM D93, Flash point of biodiesel was determined by Pensky Marten (Closed Cup) method.

Table 1: Production practice of microalgae

Number of days	Production volume of culture, litre	Amount of media,g	Production of wet biomass, g	Yield of microalgae (dried powder)g
0-25	20	12.5	157	36
26-40	60	25	718	158
41-55	80	37.5	1421	341
56-70	100	50	2020	505
71-85	120	62.5	2741	603
86-100	140	75	3091	711
101-115	160	87.5	3317	796
116-130	180	100	4114	905
131-145	200	112.5	3879	1125
146-160	220	125	5041	1210
Total productivity			26499	6390

Viscosity Measurement of a Liquid

As per ASTM D445, viscosity of biodiesel was determined. The liquids of known densities were allowed to flow through the capillary maintaining the same differences of levels in the limbs and the time equation which governs the flow lead to the relation:

$$\frac{h_1}{h_2} = \frac{t_1 d_1}{t_2 d_2}$$

Where, h_1 and h_2 are viscosity coefficients of the liquid and water, respectively. d_1 and d_2 are the densities of liquid and water, respectively. Knowing the value of viscosity of one liquid, one can calculate the viscosity of other liquid.

Density at 15 °C

As per ASTM D4052, specific gravity of biodiesel was determined. Density of biodiesel was determined by the following equation.

$$\text{Density of biodiesel} = \frac{W}{V}$$

Where,
W= weight in gm of biodiesel at 15 °C
V = volume in ml of pycnometer bottle at 15°C

RESULTS AND DISCUSSION

Production of microalgae

Initially in open condition, 20 liters of water was taken in to 200 lit. Capacity tank in which previously prepared culture (4 lit.) and media was added. Whole system was kept for 25 days for the growth of chlorella algae. The rate of production algae was 1.5 g/lit/day. Production data was given in Table 1.

Quality analysis of algal bio-diesel

Unlike the previous studies, the present study also evaluated two independent variables: type of catalyst, reaction temperature. Three types of catalyst were used: Alkali catalyst, Acid catalyst

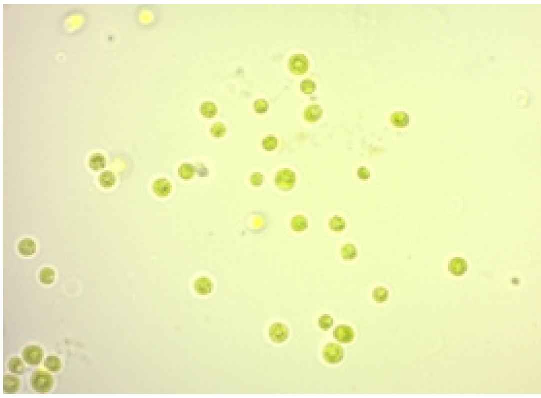

Fig. 1: Microscopic photograph of microalgae

Fig. 2: Pure microalgae culture (*Chlorella sp.*)

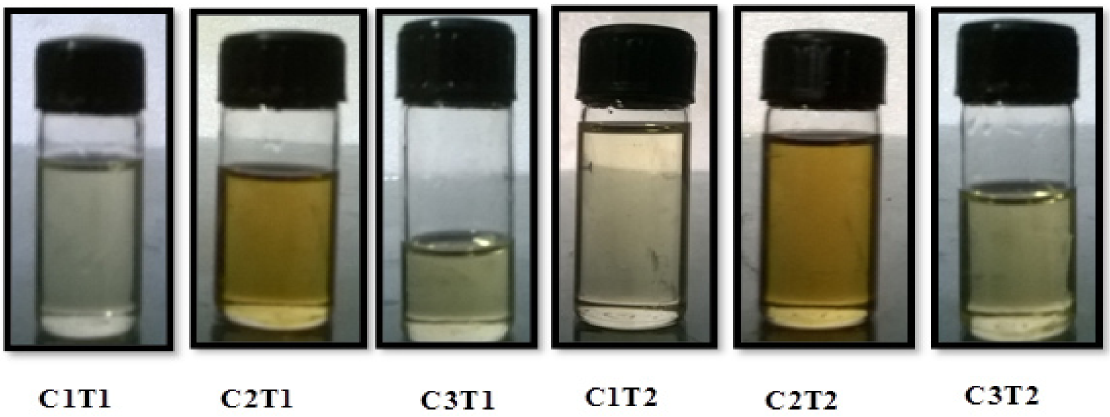

Fig. 3: Biodiesel samples with different treatments

Table 2: Effect of reaction temperature (T) and catalyst (C) on fuel properties of biodiesel from microalga

Property		Moisture content (%)	Carbon Residue (%) (MJ/kg)	Calorific value	Specific gravity	Flash point (°C)	Acid value (mgKOH/g)	Viscosity (mm^2/s)	Density (g/cc)
Catalyst	Base (C1)	0.01	0.04	40.11	0.86	137.00	0.27	5.68	0.86
	Acid (C2)	0.03	0.06	41.09	0.90	114.83	0.41	7.04	0.90
	Enzyme(C3)	0.03	0.02	40.31	0.86	142.50	0.22	5.66	0.86
	S.Em ±	0.00	0.00	0.11	0.00	0.67	0.01	0.01	0.00
	C.D. at 5%	0.01	0.01	0.35	0.01	2.05	0.02	0.04	0.01
Temperature	Temp.(T1)	0.02	0.04	40.29	0.89	127.00	0.32	6.52	0.89
	Temp.(T2)	0.02	0.04	40.72	0.86	135.59	0.28	5.73	0.86
	S.Em ±	0.00	0.00	0.09	0.00	0.54	0.01	0.01	0.00
	C.D. at 5%	NS	NS	0.29	0.01	1.68	0.02	0.03	0.01
C×T	S.Em ±	0.00	0.00	0.16	0.01	0.94	0.01	0.02	0.01
	C.D. at 5%	NS	0.01	0.50	0.02	2.91	0.03	0.06	0.02
	C.V. %	23.14	10.07	0.69	1.15	1.24	5.42	0.55	1.15

Table 3: Property of biodiesel with different processes

Properties	Units	C_1T_1	C_1T_2	C_2T_1	C_2T_2	C_3T_1	C_3T_2	ASTM 6751	Methods and Reference
Density	g/cc	0.89	0.83	0.91	0.9	0.84	0.87	0.86-0.9	D 4052
Viscosity	mm²/s	6.2	5.16	7.18	6.9	5.14	6.17	1.9-6.0	D 445
Specific gravity	—	0.89	0.83	0.91	0.9	0.84	0.87		D 4052
Acid value	mgKOH/g	0.32	0.23	0.42	0.4	0.21	0.23	d"0.50	D 664
Flash point	°C	130.33	143.67	112.33	117.33	138.33	146.67	130.0 min	D 93
Moisture content	%(m/m)	0.01	0.01	0.03	0.02	0.03	0.04	d"0.05	D 2709
Carbon Residue	%(m/m)	0.04	0.04	0.06	0.05	0.02	0.03	d"0.05	D 4530
Calorific value	MJ/kg	39.82	40.41	41.59	40.59	41.14	39.48	NR	D 240

NR=Not reported in Specification by ASTM and ES or IS

•C_2T_2: Acid catalyst + Temperature 60°C; •C_3T_1: Enzymatic catalyst + Temperature 50°; •C_3T_2: Enzymatic catalyst + Temperature 60°C; •C_1T_1: Alkali catalyst + Temperature 50°C; •C_1T_2: Alkali catalyst + Temperature 60°C; •C_2T_1: Acid catalyst + Temperature 50°C

and Enzymatic catalyst; two levels of reaction temperature were used: 50 °C and 60 °C. All the experiment combinations were carried out three times in order to determine the consistency of the results and to assess the experimental errors. The statistical data is given in Table 2 and comparison of the properties of algal biodiesel prepared by different methods is given in Table 3.

Analysis of Biodiesel

Effect of temperature (T) and catalyst type(C) on different properties of Biodiesel

An appraisal data on effect of catalyst type and temperature on different properties of biodiesel is presented in Table 3. Properties like moisture content, carbon residue, calorific value, specific gravity, acid value, flash point, viscosity and density were found to be significant with change in catalyst type. Whereas significant properties with change in temperature were calorific value, specific gravity, acid value, flash point, viscosity and density. As well as the combined effect of the interaction between catalyst and temperature (C x T) on carbon residue was found to be significant while calorific value, specific gravity, acid value, flash point, viscosity and density were found to be highly significant while carbon residue and moisture content were found to be non-significant with change in temperature and the combined effect of the interaction between catalyst and temperature (C x T) on moisture content was found to be non-significant shown in Table 2.

The relationship in between different biodiesel properties and different treatment combinations of biodiesel were shown in Fig. 4.

Process development for algal bio-diesel production

For the process development of biodiesel from microalgae, represents review of various reaction parameters and other factors which affects the production of algal biodiesel. From all above discussion it has been observed that the reaction parameters which affect the production of microalgae based biodiesel are temperature, catalyst concentrations, physical properties and composition, Purity of Reactants, Mixing Intensity ,type of catalyst used and other factors affects which are Free Fatty Acid (FFA) and moisture level Contains in feedstock. The experiment was conducted for the effect of catalyst type and temperatures and transesterification processes on production of biodiesel from microalgae as described above. As the result shows that Alkali catalyst transesterification process is suitable for the production of biodiesel from microalgae because of the highest biodiesel production and fuel properties of that treatment combination is match with ASTM satandards.

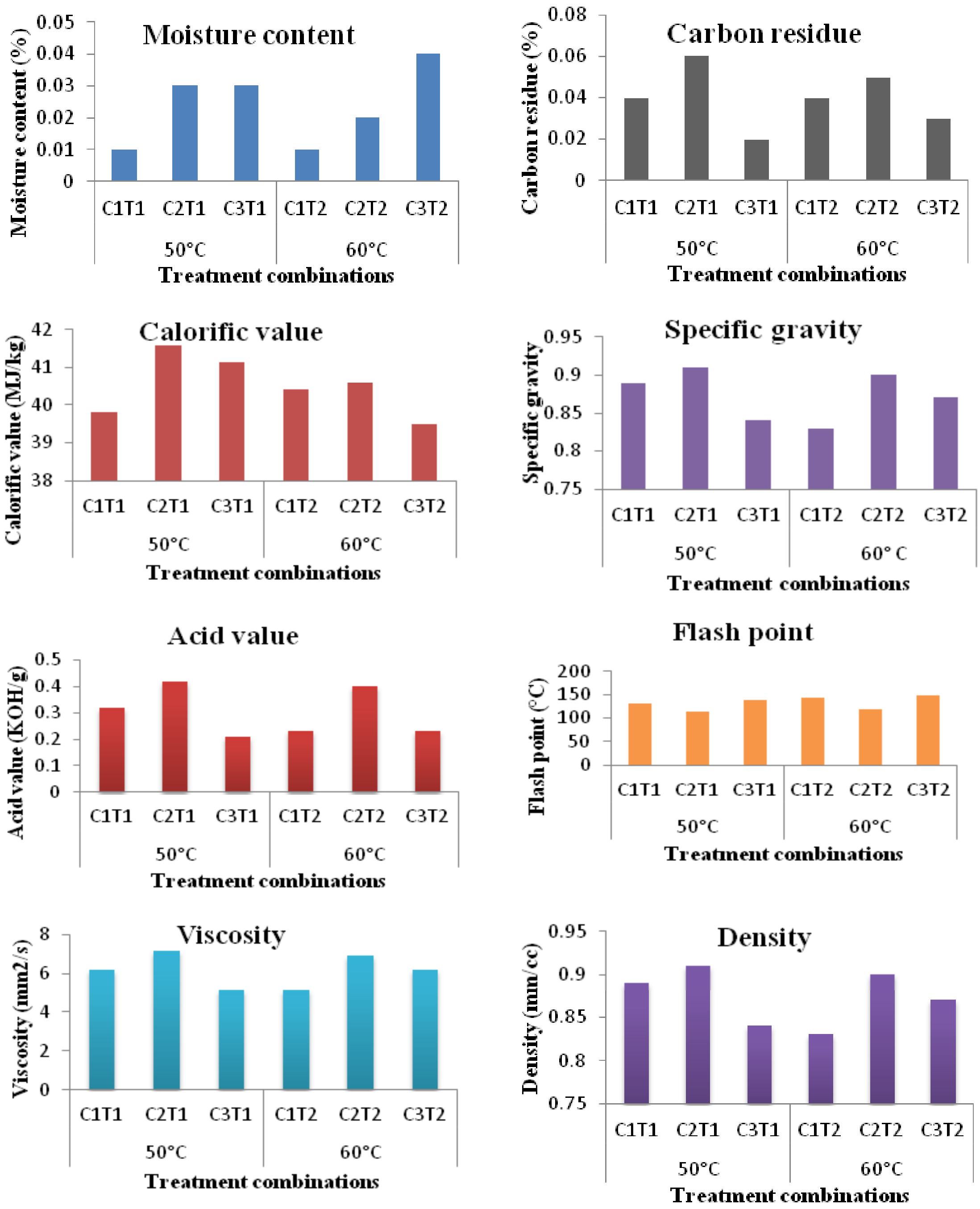

Fig. 4: Effect of temperature (T) and catalyst type(C) on different properties of Biodiesel

Hence the results obtained from these two methods are depicted in Table 3 and the values of different properties of biodiesel were compared with ASTM standards.

CONCLUSION

To develop the process for biodiesel production from microalgae, transesterification of microalgae oil was carried out. For that three different transesterification processes were employed in which three different catalysts, two temperature level and two methods for oil extraction were undertaken.

The results of transesterification process obtained from the study shows that the biodiesel yield was found to be 92 % for alkali catalyst method. Further this sample was analyzed for various fuel properties such as moisture content, carbon residue, calorific value, specific gravity, flash point, acid value, viscosity and density. These properties were compared with ASTM D 6751 standards.

The major conclusions were drawn during the process development of biodiesel from microalgae, as follows.

1. Chlorlla sp., as microalgae for the biodiesel production is used because it can be easily available in fresh water. It also have high oil percentage depends on cultivation practices.
2. The prepared biodiesel was analyzed for different fuel properties *viz.* moisture content, carbon residue, calorific value, specific gravity, acid value, flash point, viscosity, density, viscosity and their values found to be 0.01%,0.04%, 40.41 MJ/kg, 0.83, , 0.23 mgKOH/g, 143.67 °C, 5.16 mm^2/s, 0.83 g/cm^3 respectively. All the properties were found to be closed to the standard value of ASTM D 6751 standards.
3. In the process development for the biodiesel production, the highest yield was obtained in the Alkali catalyst process which was 92 % at the 60 °C temperature and 0.56 % NaOH.

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