Production of Biodiesel from Algal Biomass Collected from Solani River using Ultrasonic Technique

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Abstract- The present paper presents the production of biodiesel from algal biomass collected from Solani aqua duct in Roorkee. The identification of algal sample shows the presence of *Hydrodictyon recticulatum (L) Lagerheium* with few vegetative filaments of *ulotrichalean* strain in it. The algal biomass contains about 14.47 wt % of lipid. The algal biomass were collected from Solani aqua duct and washed several times with water and dried in sunlight for three days, then the algal-oil was extracted from pulverized biomass (100-125 μm particles). The most suitable conditions for the extraction of bio-oil using ultrasonic technique were found to be solvent to biomass ratio of 6, extraction time of 50 min and amplitude percentage of 70% at 0.6 cycle/sec. Under these conditions, around 51% of oil/lipid in the algal cells was extracted. The produced bio-oil was upgraded to biodiesel by transesterification using CH_3ONa as catalyst. The catalyst shows ~ 80 % conversion of bio-oil to biodiesel. A comparison of ultrasonic extraction and Soxhlet extraction showed that ultrasonic extraction provides higher extraction efficiency with shorter extraction time.

Keywords- Bio-oil; Algae; Algal biomass; Transesterification; Ultrasonic extraction; Catalyst; Biodiesel

1. Introduction

Energy crises are one of the major current issues in the world. The primary source of energy is coal, petroleum, natural gas, hydro and nuclear. The use of fossil fuels raises atmospheric pollution by releasing various gaseous pollutants such as NO_X, SO_X, CO, particulate matters and volatile organic compounds [1-2]. Further, the availability and quality of fossil fuels are gradually depleting day by day. In addition, to address these problems the world must look for alternate renewable energy with eco-friendly manner. Recently, bio-fuel is identified as alternate renewable energy source because they are biodegradable, renewable, non-toxic and significantly produces fewer emissions than petroleum based diesel when burnt [3-4].

Biodiesel can be obtained from corn, canola, jatropha seeds, lignocellulosic biomass, soybean, palm, sunflower seeds and algae etc. [5-6]. Algae is considered as one of the most promising non-food feedstocks for bio-fuel production because of their higher rate of productivity and high lipid content in some species [7]. They don't require arable land for cultivation and fresh water as well as saline water can be used for the production of algae. It has great capacity to absorb CO_2 and is not prone to photosynthetic inhibition under intense sunlight condition. The remaining algal biomass after the extraction of algal oil can be used as a high protein feed for livestock. So, the use of the remaining biomass reduces the waste production and gives value to the process.

Algae are the third generation of bio-fuel. They are the potential source of green renewable energy as they absorb atmospheric CO_2 and produce bioenergy from cellular components by carbon fixation. Algae can also be used to produce methane by anaerobic digestion process, ethanol by alcoholic fermentation as well as syngas by gasification etc. Some algae can do produce hydrogen photo synthetically [8]. Some algae can also accumulate lipids from which fatty acids can be extracted to produce biodiesel. Algae has potential ability to employ for the production of bio-fuel in an economic and environmentally sustainable manner. Algae fuel may meet our energy demand in the eco-friendly manner and this source may be available throughout the year [9-11].

Although some literature is available on production of bio-oil from algal biomass, there is still scope for identifying new strain capable of producing a good amount of lipid in their cell. Further, the efficiency of the solvent extraction commonly used for bio-oil extraction from algal biomass can be improved by the use of ultrasonic assisted extraction. However, very few literatures are available in this area.

Catalyst also plays significant role in the up gradation through transesterification of bio-oil to biodiesel. Both the lipid/oil extraction from algal biomass and its up gradation to biodiesel are relatively new concept and system is not well understood. Thus, there are few literatures on the modeling of bio-oil production and empirical models are being used to find out the correlation among process parameters and oil production. In case of ultrasonic assisted extraction, process parameters like solvent to biomass ratio, extraction time and amplitude percentage can influence the performance of oil production. However, there is hardly any literature on the modeling of ultrasonic assisted algal oil extraction.

In the present paper natural algal biomass collected from Solani aqua duct Roorkee (29° 52' N, 77° 53' E) has been investigated for the production of algal oil/bio-oil and its up-gradation to biodiesel. The bio-oil has been produced by an ultrasonic extraction method, which has further been upgraded to biodiesel through catalytic transesterification method using CH_3ONa as catalyst. The correlation among various operating parameters such as solvent to biomass ratio, extraction time and amplitude (%) and the yield of bio-oil production has been developed. The upgraded bio-oil (biodiesel) has been characterized to assess its quality. Algal oil extraction using ultrasonic extraction and soxhlet extraction technique has been compared.

2. Materials and Methods

2.1. Materials

All the chemicals and reagents used in the present study are of AR grade and obtained from S.D. Fine Chemicals Limited, Mumbai, India.

2.2. Procedure

Procedure for the collection and pretreatment of algal biomass, extraction of bio-oil, model development, up-gradation of bio-oil to biodiesel and their characterization are described below.

2.2.1. Collection, Identification and Pre-Treatment of Algae Biomass

The algal biomass were collected from Solani Aqua duct (29° 52' N, 77° 53' E) in the month of December 2013 and the collected algal biomass were washed with tap water to remove the sand, dirt and other unwanted organic matter. Systematic authentication of the native algal sample was carried out to identify the algal species present in the algal biomass. The washed wet biomass was then spread on the net and kept in sunlight for three days to evaporate water, followed by drying in an oven at 80 °C for twenty-four hours and moisture content was reduced to 20% from 84%. The dry biomass were pulverized and sieved to obtain particle size 100-125 microns. The algae powder was packed in high density polyethylene bags and stored at room temperature for use in extraction experiments.

2.2.2. Determination of Lipid Content in Algal Biomass

Modified Bligh and Dyer Protocol [9] used to find out the lipid content in the algal biomass as detailed in Soni et al., [12]. The algal oil content (g of oil per g of dry algae) was calculated by using Eq. (1).

$$\text{Algal oil content (\%)} = \frac{(\text{Mass of (flask+algal oil)[g]- Mass of empty flask[g]})}{\text{Mass of dry algal biomass[g]}} \times 10 \quad (1)$$

2.2.3. Oil Extraction

Extraction of oil from algal biomass was done by mixing a particular amount of biomass with a certain amount of solvent (hexane and water) followed high intensity ultrasound waves. The extraction was carried out at 0.6 cycle/sec and at room temperature. The ranges for various operating parameters during the oil extraction method are shown in Table 1. The extracted liquid was a mixture of algal oil, hexane and disrupted biomass.

The mixture of solvent, oil and disrupted biomass was centrifuged at 8000 rpm for 5 min to allow a total separation of solid (bottom layer) and liquid (top layer). The extracted liquid was a mixture of algal oil and solvent which was transferred into the flask. The extracted liquid (solvent and oil) was evaporated in a vacuum evaporator to release solvent from lipid. In this way lipid and recovered solvent were obtained separately. Oil extraction (%) was calculated using Eq. (2).

$$\text{Oil extraction (\%)} = \frac{\text{Mass of (flask+ oil)-Mass of empty flask}}{\text{Mass of dry algal biomass} \times (\text{\% algal oil content})} \times 100 \quad (2)$$

2.2.4. Model Development

Optimization and modeling of the algal oil extraction were done using Design Expert software version 6.2. Box-Behnken model for three factors (solvent to biomass ratio, amplitude percent and extraction time) with three levels, each was used to fix the experimental conditions as shown in Table 1. Various levels are solvent to biomass ratio (5, 6, 7), amplitude percent (60, 70, 80) and extraction time (40, 50, 60 min) and a set of 17 experimental conditions was obtained. Using the concept of Design of experiment (DOE) the experimental data was processed through regression to develop an empirical model correlating process variables with % algal oil extraction.

Table 1. Experimental design for bio-oil extraction from algal biomass

Run	A: Solvent to Biomass Ratio	B: Amplitude (%)	C: Time (min)
1	6	60	60
2	6	60	40
3	6	70	50
4	7	70	60
5	6	70	50
6	7	60	50
7	7	80	50
8	6	80	60
9	7	70	40
10	6	70	50
11	5	70	40
12	5	70	60
13	6	70	50
14	5	60	50
15	6	80	40
16	6	70	50
17	5	80	50

Percentage error was computed as per Eq. (3)

$$\% \text{ Error} = \frac{(\text{Actual value - Predicted value}) \text{ of algal oil (\%)}}{\text{Actual value of algal oil (\%)}} \times 100 \quad (3)$$

2.2.5. Up-gradation of Algal Bio-Oil to Biodiesel

Up-gradation of algal oil was done through trans-esterification process using a homogeneous catalyst (CH_3ONa). Approximately 5ml of algal bio-oil was mixed with 20ml of methanol and 1ml of sodium meth oxide as catalyst. Thereafter, the reaction was carried out in a sonicator for 50 min and after mixing, the solution was kept to settle the biodiesel and sediment layers for 16 hr and separate the biodiesel [11], which then gone through GC-MS analysis.

2.2.6. Characterization of Algal Bio-Oil and Biodiesel

Properties like viscosity and density were found out as per the specified methods in IS-15607:2005 and compared with the standard data. FT-IR Spectrometer Model: Nicolate Avatar 370 DTGS, USA was used to find out the presence of functional groups in the biodiesel with sample to KBr ratio of 1:10.

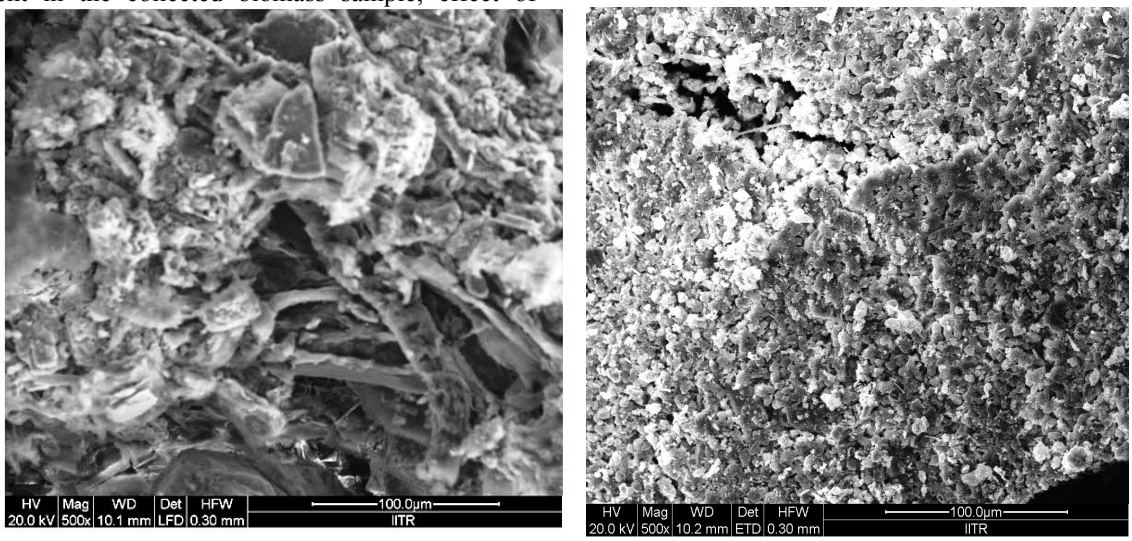
Analysis of biodiesel was performed through a GC-MS Perkin-Elmer Mass Spectrophotometer Clarus SQ 8T model and Gas Chromatographer Clarus 680 model equipped with GC Column specification of (HP-1, 30m X 0.25mm X 0.25 μm). Sample injection took place at 70^{o}C oven temperature and was hold for 3 min. Then temperature increased up to 280°C at 10°C min^{-1} and hold for 6 min. The GC-MS interface temperature was set at 220°C and helium was used as a carrier with a flow rate of 1.2 mL/min.

3. Results and Discussions

Identification of algal species, oil content of the native algae present in the collected biomass sample, effect of process parameter on bio-oil production, characterization of bio-oil and its up-graded products are described below.

3.1. Identification and Oil Content of the Native Algae

The algal species found in the Solani aqua duct are *Hydrodictyonreticulatum (L) Lagerheim*, green algae, which belongs to the Phylum: Chlorophyta, Order: Chlorococcales, Family: Hydrodictyaceae, Genus: *Hydrodictyon and* Species: *reticulatum.* It is thallus, macroscopic and composed of cylindrical cells, which are adjoined at their ends to form a cylindrical net with 5 or 6 sided meshes. Trace amount of *ulotrichalean* strain was also found in the algal sample. The oil content of the algal biomass determined as per Bligh and Dyer method is found as 14.4 wt. %. Figure 1 (a) and Figure 1 (b) show the SEM image of algal biomass before and after oil (lipid) extraction. This evidences the morphological changes of algal biomass.



(a) **(b)**

Fig.1. SEM of algal biomass (a) before algal oil extraction (b) after algal oil extraction

3.2. Response Surface Methodology for Optimization of Ultrasonic Extraction Conditions

The ultrasonic extraction conditions for algal oil extraction from algal biomass were optimized using a standard Response surface methology (RSM) based on the Box–Behnken design (BBD). By design of experiment the algal oil extraction (%) varied from 27.82% to 50.68%. The maximum value for algal oil extraction has been obtained using solvent to biomass ratio 6, for 50 min using the amplitude 70 %. By applying multiple regressions on the experimental data obtained from the experiment, the response variable data and the test variable are correlated by following second order polynomial equation given in terms of coded factor:

$$\text{Algal oil extraction \% (Y)} = 50.68+5.64\times A+3.53\times B+1.83\times C-8.69\times A^2-6.84\times B^2-4.11\times C^2+1.43\times AB+1.06\times AC \quad (4)$$

The significance of the developed quadratic model has been assessed based on the correlation coefficient. The R^2 value for Eq. (4) is found to be 0.9989 which is relatively high and close to unity which shows that there is a good agreement between the experimental and the predicted data from the quadratic model. The adequacy of the quadratic model is further verified using analysis of variance (ANOVA) given in Table 2. From the ANOVA analysis, the F-value of 889 and P-value < 0.0001 shows the response surface of the quadratic model is significant as well as all independent variables (A, B, C), interaction terms (AB, AC) and three quadratic terms (A^2, B^2, C^2) have a significant effect on Y (P- value < 0.05).

The three dimensional response surface plots obtained by the multivariate analysis approach show the effect of individual process parameters with respect to other variables.

Figures 2 (a) and 2 (b) show the response surface plots of the relationship between solvent to biomass ratio, amplitude percent, extraction time and their effect on the yield of algal oil extraction. From Figure 2 (a), it is evident that the yield of algal oil extraction increases as the amplitude increases and goes to the maximum then decreases and similar result shows for solvent to biomass ratio at time of 50 min and Figure 2 (b) shows the variation of yield of algal oil extraction w.r.t time and solvent to biomass ratio at an amplitude of 70 %. As time and solvent to biomass ratio increase, the yield of algal oil extraction increases up to time 50-55 min and solvent to biomass ratio of 6-6.5, then decreased as shown in the Figure 2 (b).

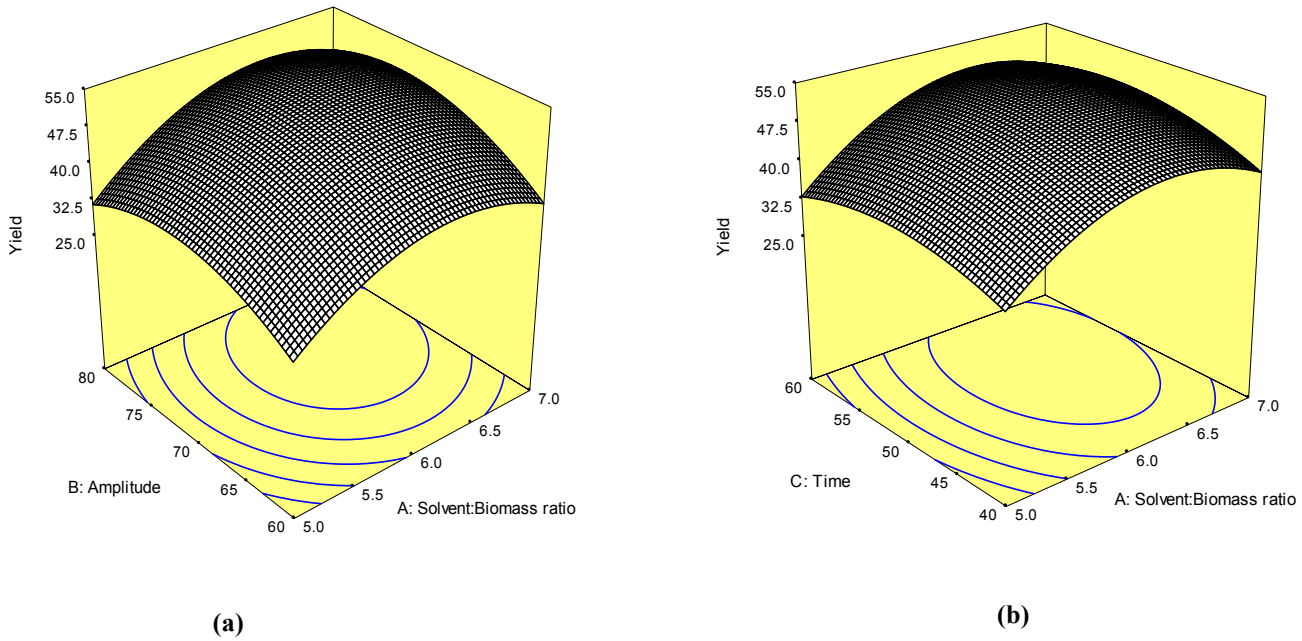
The process parameters are optimized by a point prediction method in the Design–Expert software. The optimized parameters obtained from this software are 6.2, 53.5 min and 73.6 for solvent to biomass ratio, extraction time and amplitude percent, respectively. The maximum predicted algal oil extraction (%) is 52.2% under these optimal conditions. For operational convenience, the optimal parameters are 6, 50 min and 70 for solvent to biomass ratio, extraction time and amplitude percent, respectively.

Figure 3 shows the plot between the predicted and actual values of algal oil extraction (%) and error % varies between -1.5 to 1.5.

Table 2. Analysis of variance for second order polynomial

Source	Sum of Squares	Degree of freedom (df)	Mean Square	F-value	p-value prob>F	Remarks
Model	1041.49	8	130.19	889.38	< 0.0001	Significant
A-Solvent: Biomass Ratio	254.70	1	254.70	1740.03	< 0.0001	Significant
B-Amplitude	99.83	1	99.83	681.99	< 0.0001	Significant
C-Time	26.72	1	26.72	182.53	< 0.0001	Significant
A^2	318.05	1	318.05	2172.82	< 0.0001	Significant
B^2	197.06	1	197.06	1346.27	< 0.0001	Significant
C^2	70.99	1	70.99	485.01	< 0.0001	Significant
AB	8.21	1	8.21	56.08	< 0.0001	Significant
AC	4.47	1	4.47	30.56	0.0006	Significant
Residual	1.17	8	0.15			
Lack of Fit	1.17	4	0.29			
Pure Error	0.00	4	0.00			
Cor Total	1042.66	16				

$R^2 = 0.9989$, Adj $R^2 = 0.9978$, Pred $R^2 = 0.9830$,
Adeq Precision=83.609



(a) **(b)**

Fig.2. Response surface for variation in algal oil yield (%) **(a)** w.r.t Solvent: Biomass ratio and Amplitude (%) at Time = 50 min **(b)** w.r.t Solvent: Biomass ratio and Time (min) at Amplitude (%) = 72.

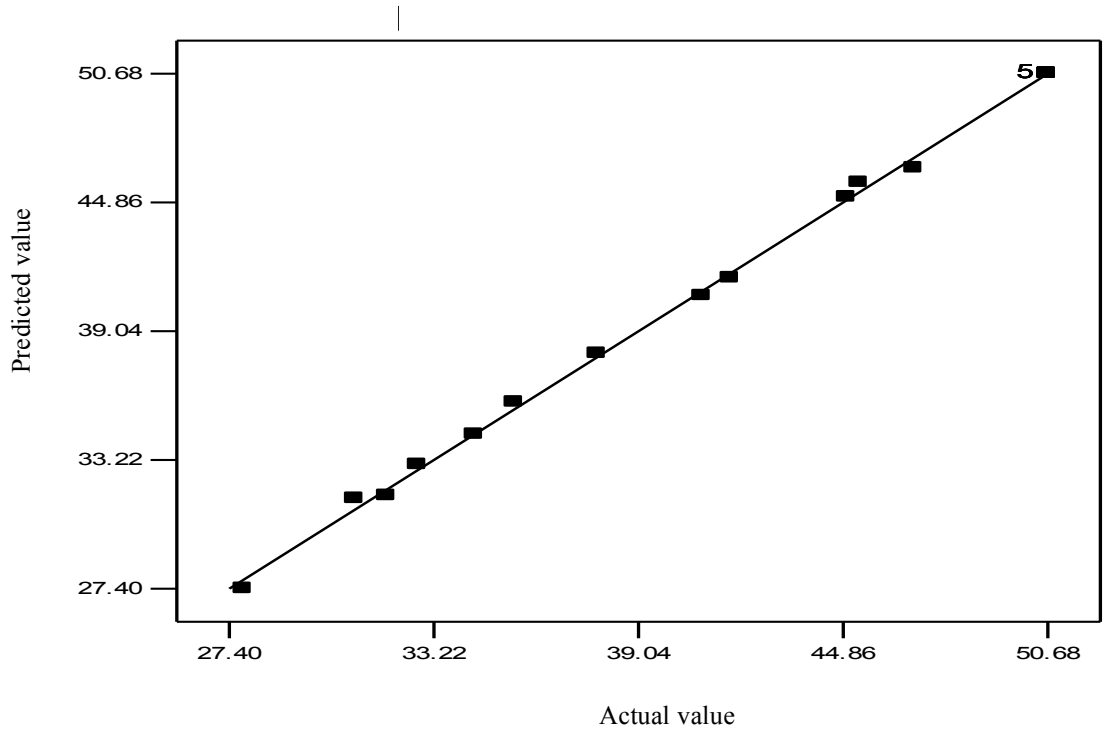


Fig. 3. Predicted value of algal oil extraction (%) vs. Actual value of algal oil extraction (%)

3.3. Up gradation of Bio-Oil to Biodiesel and Its Characterization

Up gradation of bio-oil to biodiesel is performed through transesterification as reaction shown in the following Figure 4.

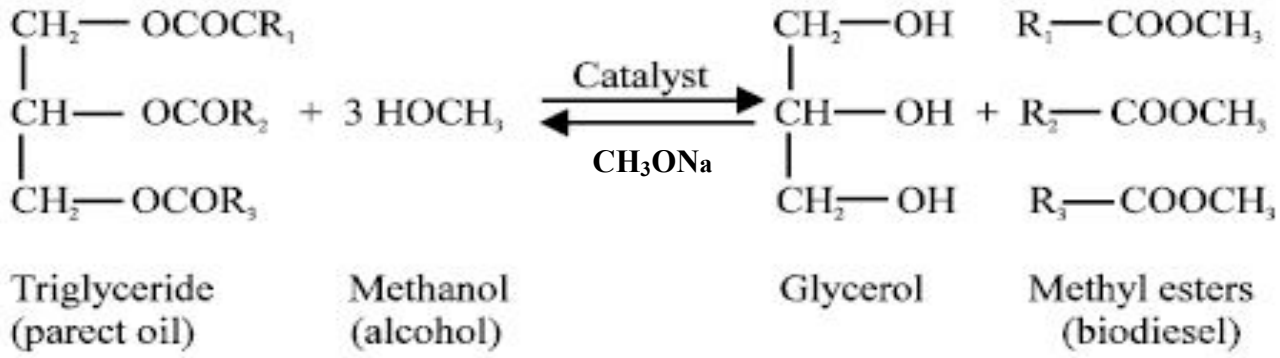
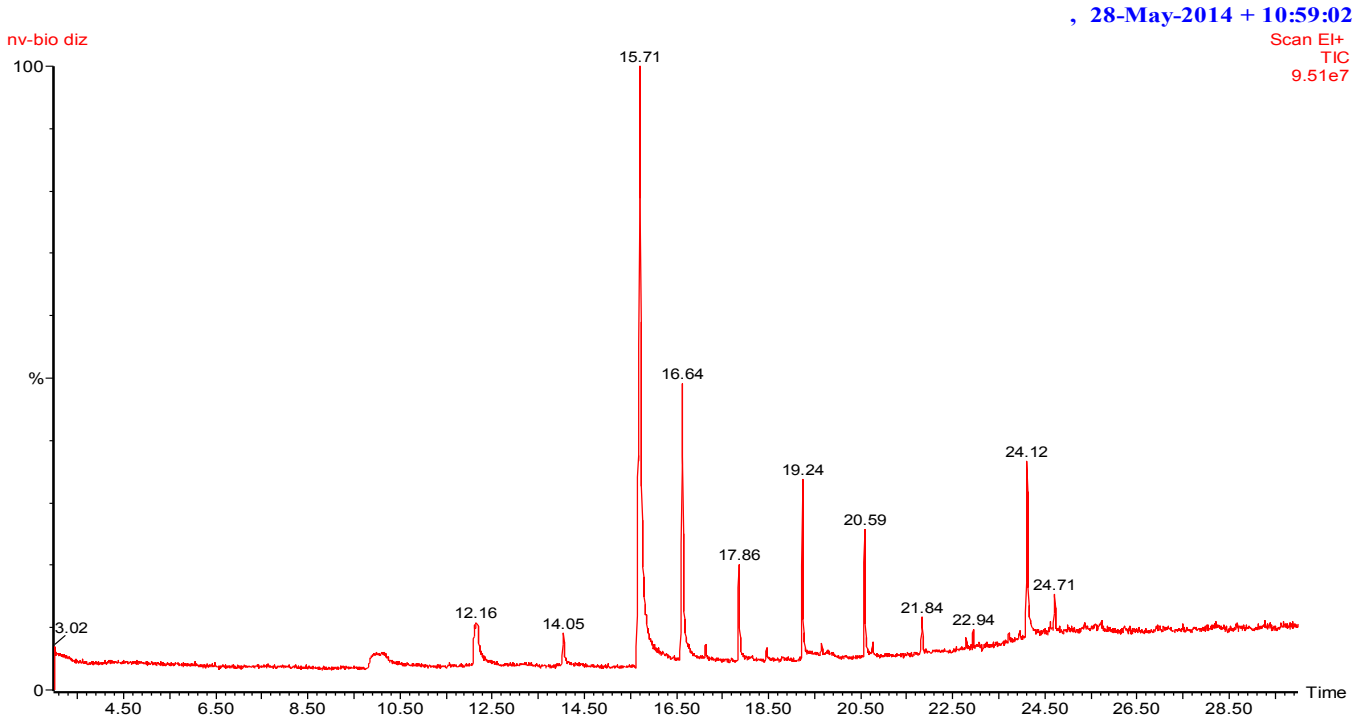


Fig.4. Transesterification reaction

The application of ultrasonication for the production of biodiesel from algal bio-oil is not limited to the extraction of algal oil from algae. Biodiesel is made from algae bio-oil using a catalyst and alcohol by a chemical conversion process known as transesterification. Ultrasonication improves the mixing of reactant and also increases the chemical reactivity. Consequently, this process reduces the time required for the chemical conversion up to 90%, leading to a whole new perspective for biodiesel production. The residence time of approximate one hour is sufficient for complete conversion. The separation of glycerine and biodiesel is done by using a centrifuge. The other benefits of this process include more complete transesterification of the tri-glyceride molecules (Algal oil) into biodiesel and require less amounts of alcohol and catalyst so this reduces the production costs and improves the environmental effect. Ultrasonic reactors enhance the cavitational shear to the mixing process. This gives much smaller droplets of methanol, which results in improved methanol and catalyst utilization [13].

Figure 5 shows the GC spectrum of biodiesel. Different molecules present in the biodiesel enter into the MS for detection of molecular mass at different time as per the retention time in the GC column [14]. Various compounds present in the biodiesel as evident from peaks on the spectrum as shown in Figure 5 are summarized in Table 3.

In Figure 6 FT-IR of biodiesel shows nine different absorption bands over the wave number of 4000-500 cm^{-1}. The bands in between 3500-3000 cm^{-1} are due to (O-H) functional groups (carbohydrates and protein stretching). The two absorption band at 2926 cm^{-1} and 2856 cm^{-1} are assigned to the asymmetric and symmetric stretching of C-H and could be attributed to C-H aliphatic axial deformation in CH_2 and CH_3 groups [15]. Band at 1740 cm^{-1} is associated with vibration of C=O shows ester groups, primarily from lipids and fatty acids [16]. Peak at ~1449 cm^{-1} is due to the presence of methyles and proteins of functional group (C-O) and stretching of (COO-) groups at ~1380 cm^{-1}. Peak at ~1150 cm^{-1} shows the presence of cellulosic compounds associated with (C-N). Two bands of were particular interest, and the region from 1200-950 cm^{-1} associated with (C-O-C) stretching of polysaccharides [16]. Table 5 shows the properties of bio-oil and biodiesel. Figure 7 shows the photograph of bio-oil and biodiesel.



Retention Time

Fig. 5. GC spectra of biodiesel

Table 3. List of compounds found in GC-MS spectra of upgraded algal oil/biodiesel

Sl. No.	Compound	Formula	Molecular Weight	Retention Time
1	ANILINE, N-(3',3'-DIPHENYLSPIRO[FLUORENE -9,2'-OXETAN -4-YLIIDENE)	$C_{33}H_{23}ON$	449	12.05
2	1,5-DIPHENYL-2H-1,2,4-TRIAZOLINE-3-THIONE	$C_{14}H_{11}N_3S$	253	14.16
3	BENZENE, 1-METHYL-3,5-BIS[(TRIMETHYLSILYL) ESTER	$C_{13}H_{24}O_2Si_2$	268	15.71
4	1, 4-CYCLOHEXADIENE, 1, 3, 6-TRIS (TRIMETHYL SILYL)	$C_{15}H_{32}Si_3$	296	16.04
5	BENZENE, 1-(1,1-DIMETHYLETHYL)-4-(2-ETHOXYETHOXY)	$C_{14}H_{22}O_2$	222	17.86
6	N-METHYL-1-ADAMANTANEACETAMIDE	$C_{13}H_{21}ON$	207	19.24
7	ARSENOUS ACID, TRIS (TRIMETHYLSILYL) ESTER)	$C_9H_{27}O_3Si_3As$	342	20.59
8	SILICIC ACID, DIETHYL BIS (TRIMETHYLSILYL) ESTER	$C_{10}H_{28}O_4Si_3$	296	24.12

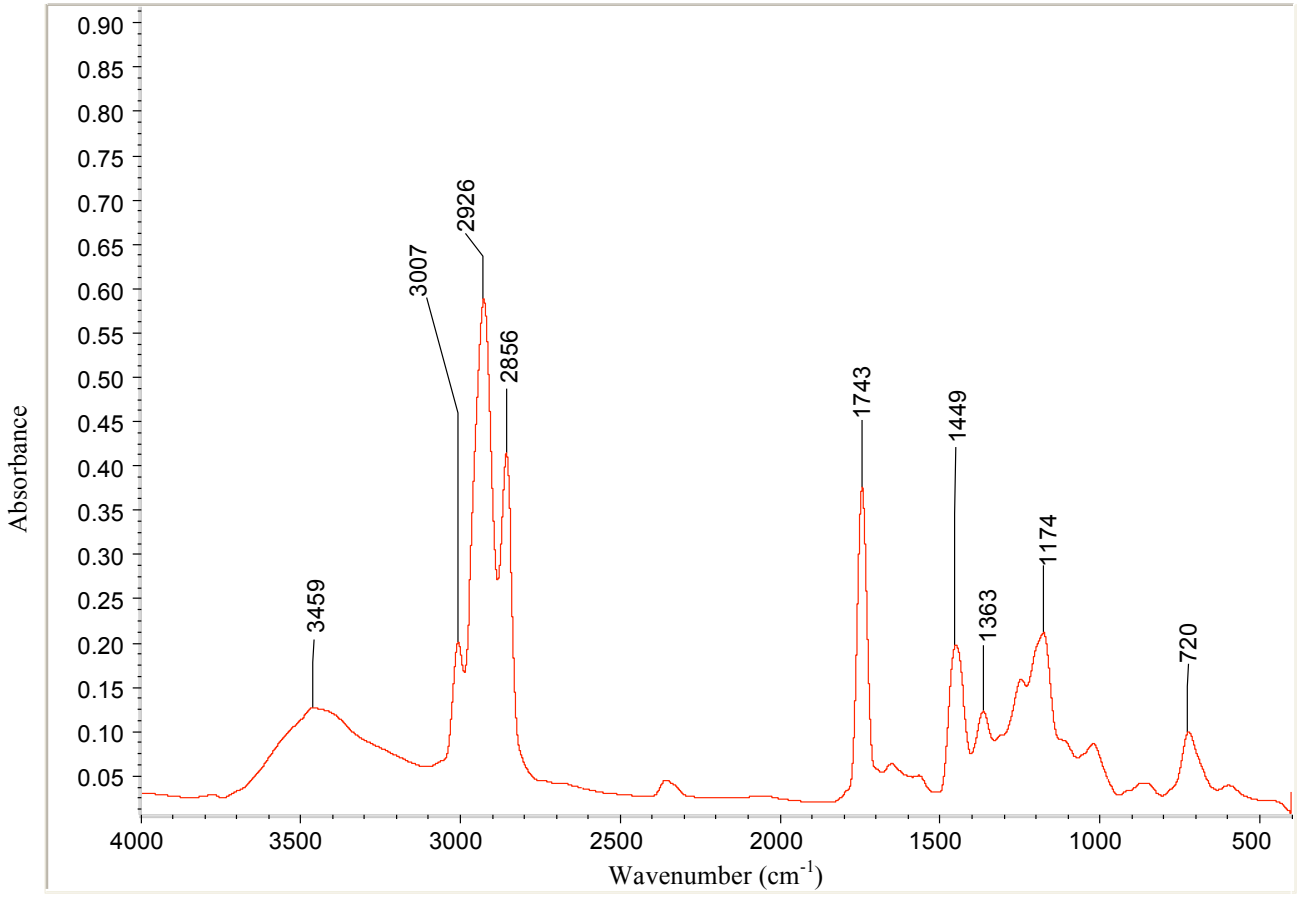


Fig. 6. FT-IR of biodiesel

Table 5. Properties of bio-oil & biodiesel

Property	Bio-oil	Biodiesel	Standard Biodiesel IS-15607:2005
Density (g/l)	904.67	875.67	860-900
Viscosity (mm^2/sec)	27.45	5.4	2.5-6.0

(Bio-oil)

(Biodiesel)

Fig. 7. Photograph of bio-oil and biodiesel

3.4. Comparison of Soxhlet and Solvent Extraction Methods

Soxhlet extraction was performed previously [17] using the same algal strain to extract oil from algae and process condition such as the extraction time of 12 hr and extraction temperature of 50°C. Under these conditions, the algal oil extraction was found to be 42%. Whereas it is found to be 51% using ultrasonic extraction method under process condition such as solvent to biomass ratio of 6, extraction time of 50 min and amplitude percentage of 70%. Hence, it is clear that ultrasonic extraction is superior to soxhlet apparatus and reduces extraction time remarkably.

Intense sonication of liquid produces sound waves that propagate into the liquid media, which results in alternating high pressure and low pressure cycles. During the low pressure cycle, high intensity tiny vacuum bubbles form in the liquid. When the bubbles attain a certain size, they collapse violently during a high pressure cycle, which is called cavitation [18]. The high pressure cycles of the ultrasonic waves support the diffusion of solvents into the cell structure. As ultrasound breaks the cell wall mechanically by the cavitation shear forces, it assists the transfer of lipids from the cell into the solvent [19-20].

4. Conclusion

1. *Hydrodictyon reticulatum (L) Lagerheim* is the predominant algal species in the sample collected from Solani aqua duct, Roorkee. The biomass contain 14.47 wt. % of lipid/oil in its cell.
2. Under experimental domain the most suitable conditions for extraction of bio-oil is achieved under following conditions such as solvent to biomass ratio of 6, extraction time of 50 min, the amplitude percentage of 70 % and algal oil extraction is 51%.
3. The empirical model gives prediction on the % extraction of bio-oil with $\pm$ 1.5% error limit under the experimental domain.
4. Ultrasonic extraction has high extraction efficiency than soxhlet extraction.

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