

# A Comparative Study and Analysis of Two Types of Bio-Oil Samples Obtained from Freshwater Algae and Microbial Treated Algae

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## Abstract

Pyrolysis experiments of two types of algal biomass (Spirogyra) freshwater Algae (FWA) and microbial treated algae (MTA) were carried out in an electrically heated stainless steel reactor to determine the effects of pyrolysis temperature, pyrolysis product yields and bio-oil properties. After pyrolysis two types of bio-oil were produced, freshwater algae bio-oil (FWAB) and microbial treated algae bio-oil (MTAB). Gas chromatography/mass spectrometry (GC-MS) analysis and Fourier transform infrared spectroscopy (FTIR) has been done of both types of bio-oil samples and also a comparison is shown between these two samples and explained it with the help of graphs and tables. The bio-oil was obtained from pyrolysis in which temperature of the system was kept 25°C and then increased up to 650°C time by time. After pyrolysis the obtained bio-oil was introduced to distillation and then analyzed by GC-MS technique and FTIR, which show different peaks and results of different compounds and functional groups present in both bio-oil samples and a noticeable difference is shown between these two bio-oil samples on the basis of hydrocarbons as well as other compounds.

**Keywords:** Freshwater algae; Microbial treated algae; Pyrolysis; GC-MS; FTIR

**Abbreviations:** GC-MS: Gas Chromatography-Mass Spectrometer; FTIR: Fourier Transform Infrared Spectroscopy; FWAB: Bio-oil obtained from Freshwater Algal Biomass; MTAB: Bio-oil obtained from Microbial Treated Algal Biomass; FWA: Freshwater Algae; MTA: Microbial Treated Algae.

## Introduction

Bio-Oil, a liquid product from thermal conversion of biomass has been paid attention to provide fuels and chemicals [1]. The rising demand for energy, chemicals, and materials from sustainable resources poses challenging efforts. A pyrolytic process converts biomass feedstock into three classes of products: a solid (or char), a liquid (also known as bio-oil from pyrolysis or simply bio-oil), and a gas [2]. Biomass has been entering into our life and industries as an interesting renewable resources used to provide second generation of Biofuels or chemicals [3]. Abundant amount and CO<sub>2</sub> neutrality with low sulfur and nitrogen contents make biomass a sustainable and eco-friendly energy source [4,5]. This has been demonstrated by many researchers, the experiments on catalytic pyrolysis of biomass were generally carried out in a fixed bed reactor or fluidized bed [6,7]. On the other hand biodiesel has higher molecular weight, density, viscosity and pour point than conventional diesel fuel [8,9]. Higher molecular weight and viscosity of biodiesel causes low volatility and poor fuel atomization, injector coking, piston ring sticking and leading incomplete combustion [10] as well as it has cold flow property which is a barrier to use it in cold or chill weather [11]. anyhow the best benefit of Bio-oils is that they are preparing from renewable sources like corpse, plants, trees and residues etc. approximately 100 years ago, Rudolf Diesel tested Bio-oil as the fuel for his engine that was available with him [12,13]. Soybean oil was transesterified into ethyl and methyl esters, and comparisons of the performances of the fuels with diesel were made [14,15]. Also, methyl esters have been prepared from palm oil by transesterification using methanol in the presence of a catalyst (NaOH) or (KOH) in a batch reactor [16]. Ethan oils a preferred alcohol in the transesterification process compared to methanol because it is derived from natural agricultural products and is renewable and biologically less objectionable in the environment. The success of rapeseed ethyl ester production would mean that biodiesel's two main raw materials

would be agriculturally produced, renewable and environmentally friendly [17]. Methyl, ethyl, 2-propyl and butyl esters were prepared from canola and linseed oils through transesterification using KOH and/or sodium alkoxides as catalysts. In addition, methyl and ethyl esters were prepared from rapeseed and Sunflower oil using the same catalysts [18,19]. Biomass is a renewable source which has received attention due to various characteristics, particularly its low cost and wide availability. Biomass can be converted into bio-fuel by means of different processes, e.g., reductive combustion, liquefaction, pyrolysis and gasification [20]. The use of biomass is particularly interesting when it involves waste products such as waste vegetable oil, fruit seeds, sugarcane bagasse, sugarcane straw, rice husks, coconut fibers, and coffee grounds, which are also potential sources of energy [21]. Taking into account that bio-oil from pyrolysis contains a very complex mixture of products, its chromatographic analyses requires the coupling of these instruments with a mass selective detector to aid the identification of eluted products [22]. The mass spectrum, sometimes, is not sufficient to identify unknown compounds, due to the similarity of their spectra, thus the use of reference compounds may be an aid to overcome this problem. Furthermore, the use of analytical standards may allow quick quantification through one of several techniques, such as the internal standard method [23,24]. However bio-oils from pyrolysis may include up to several hundred of compounds [25]. Among many kinds of alternative energies, biodiesel can be directly applied to nowadays diesel engines with a benefit of low pollution.

High environmental friendliness and sustainability and it contain neither sulfur, nor aroma. These facts lead biodiesel to enhance more complete combustion and less emission of particulate matter [26,27]. Recently, catalytic pyrolysis has aroused a great interest for the

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advantages of operating at atmospheric pressure and the lack of need for hydrogen [28,29]. Recently, increasing attention has been paid to the recovery of chemicals from bio-oil, providing chemicals compared to fuels could make recovery of even small concentrations viable. As we all know, most of oxygen-containing organic compounds, such as phenols, ketones and alcohols are value-added chemicals used in industry and life [30].

## Experimental

### Preparation of FWA and other reagents

The FWA was first converted to powder form then it was dried in fresh air and sunlight until 10% moisture remained, the fine powder FWA was introduced into a plastic jar. The plastic jar was closed through lid in such a way that it became air tight. The jar was kept at room temperature for 3 days, then the sample was used as FWA biomass sample and finally this WFA biomass sample was introduced to pyrolysis in the presence of catalyst calcium oxide.

### Preparation of MTA and other reagents

The FWA was first converted to powder form and then carried out in a cylindrical plastic jar provided with an air tight lid. Fine powder FWA was put into the plastic jar and one liter tap water was added to it and mixed well. Then plastic jar was closed through lid in such a way that it became air tight. The jar was kept at room temperature and after 7 days rhizopus appeared on its surface, it was due to the addition of tap water which believed to contain microbes. Then everyday water was sprayed on its surface and mixed well, this process was also maintained for 7 days. Then the sample was dried and used as MTA biomass sample in the presence of same catalyst (Cao).

### Production of bio-oil (FWAB and MTAB) from FWA and MTA biomass through pyrolysis

The bio-oil was produced from the pyrolysis of FWA and MTA. A round block shaped structure of sample was made inside the filter paper from biomass (filter paper as side wall of the sample block to kept the biomass tight) while the weight of this sample was kept 150 grams, also some amount of sand was added to the mixture for some purposes (as heat resistant). After preparation of this sample block, it was fixed inside the stainless steel reactor which was further connected to other condensation chambers which are shown in the diagram in Figures 1-6. The temperature of the reactor was increased from 25-650°C time by time, through which biomass was converted to biogas and then the biogas was condensed in condensation chamber as bio-oil (FWAB and MTAB). The condensed fractions from condensation chamber were collected and introduced to fractional distillation. The bio-oil obtained at the optimum conditions was separated into fractions by distillation. The bio-oil was separated into four fractions including residue. These fractions were found in the temperature range of 65-200°C and the residue of distillation. The first fraction was obtained in the temperature range of 65- 100°C, the second fraction was collected in the range 100-150°C, the third fraction was found in the range of 150-180°C, the fourth fraction was at 180- 200°C. The first fraction of FWAB and MTAB is flammable while the remaining all fractions are nonflammable. However the residue of the fractional distillation was a sticky organic material and believed as combustible.

### GC-MS characterization of the FWAB and MTAB after pyrolysis

The GC-MS analysis Table 1 was carried out in order to find out the compounds present in both types of bio-oil samples. The main peaks identification of compounds has been performed by using NIST mass

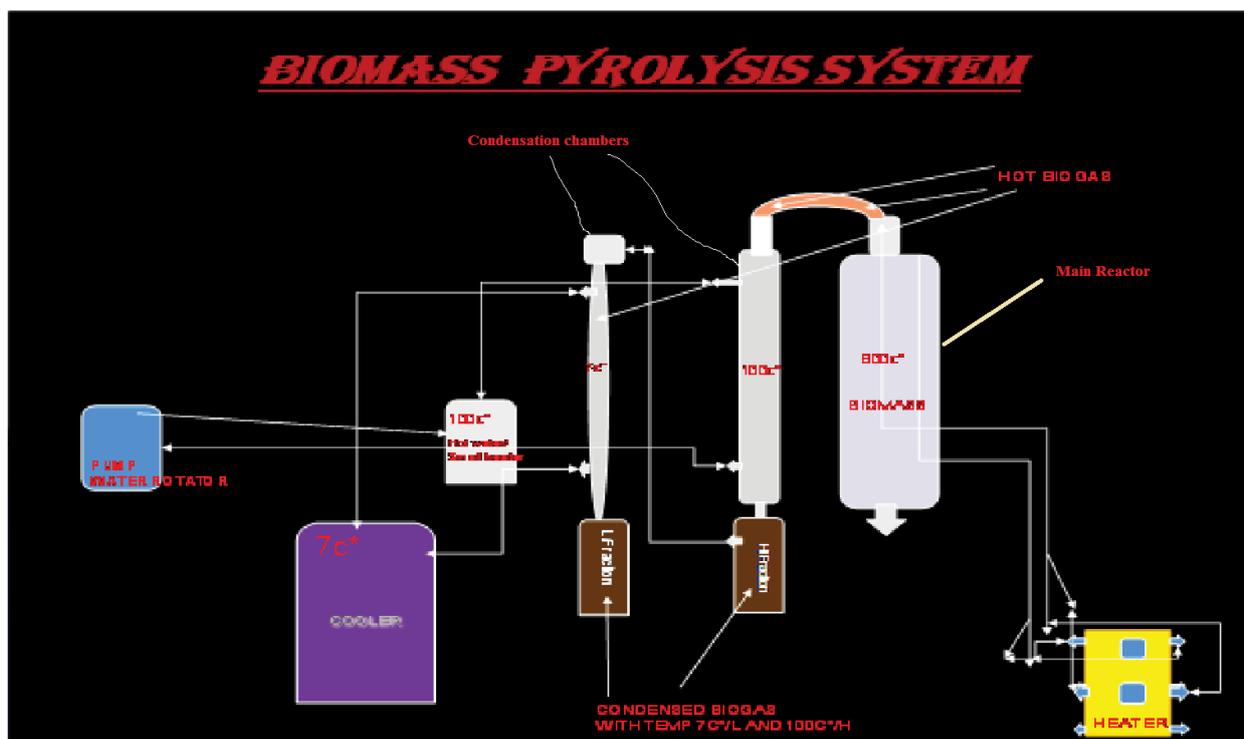


Figure 1: The biomass pyrolysis system.

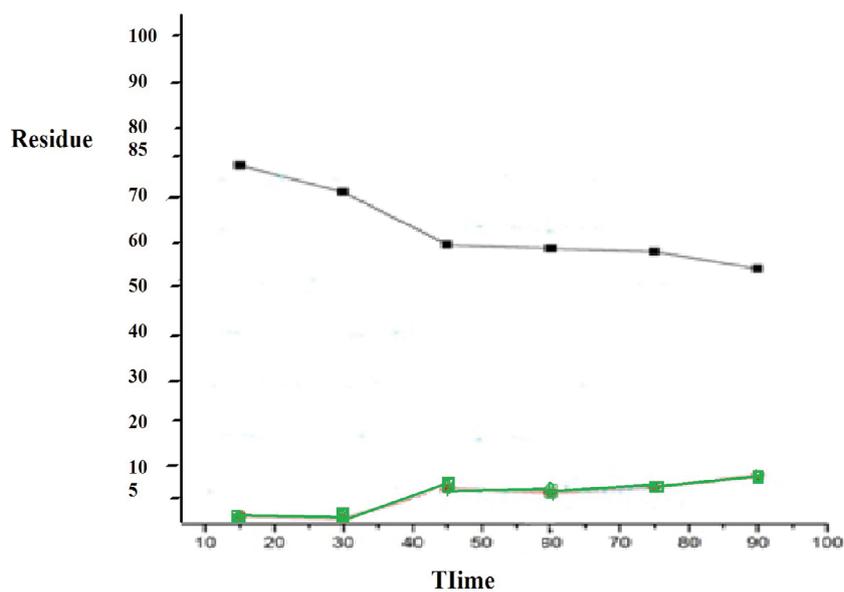


Figure 2: Shows the relationship between Residues and time during pyrolysis.

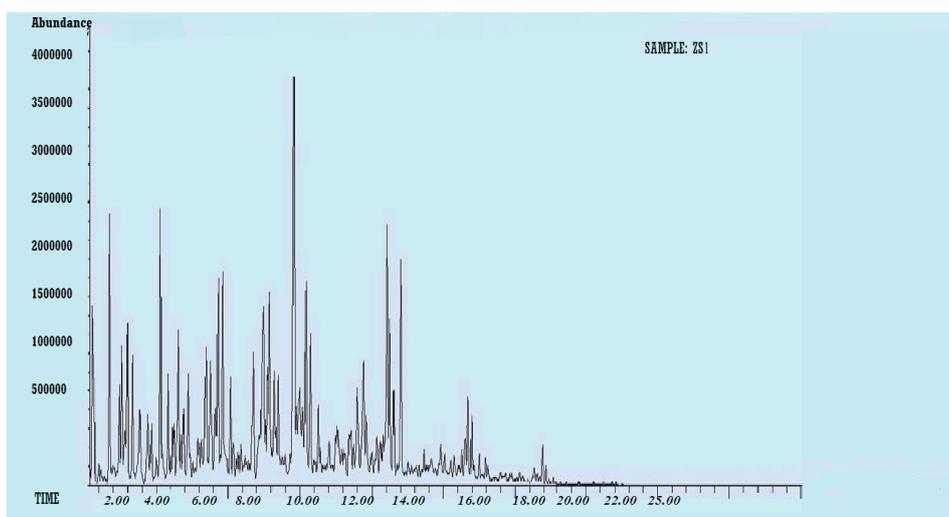


Figure 3: GC-MS Chromatogram of the FWAB.

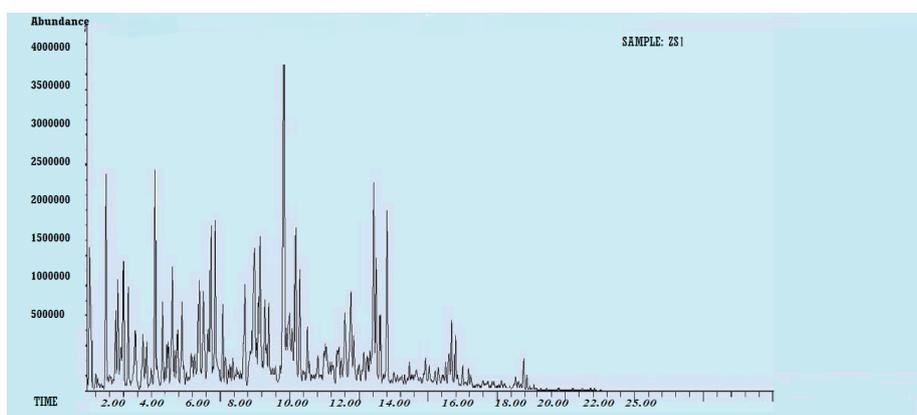


Figure 4: GC-MS Chromatogram of the MTAB.

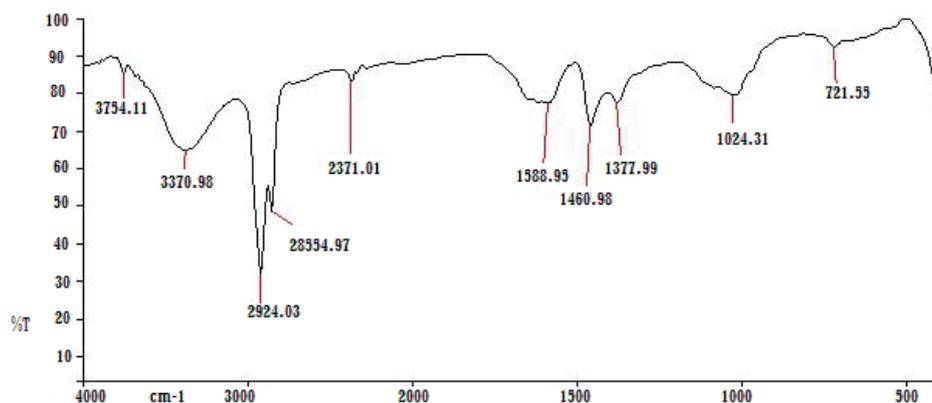


Figure 5: FTIR Spectrum of FWAB.

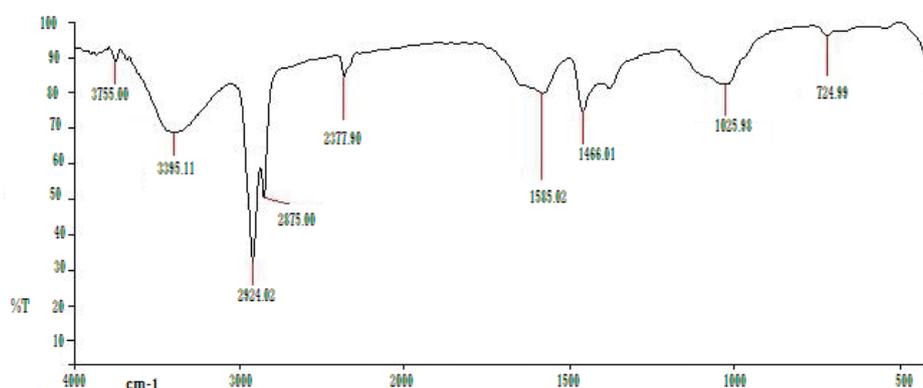


Figure 6: FTIR Spectrum of MTAB.

spectral data base. Both FWAB and MTAB were complex mixture and contain different classes of hydrocarbons and other compounds, which are shown in the Tables 2 and 3 respectively.

Table 2 is the FWAB's table which contains different compounds. The GC-MS results indicate the presence of different compounds which includes azole, aromatic amines and nitriles, carbohydrate, alkenes, alkanes and many others. The most abundant compound 2,3,5-trimethyl pyrazole 19.09% accounted for the total bio-oil. The azoles including 3,4,5-trimethyl-pyrazole, pyrrolidinone etc. and aromatic amine and nitrile, these compounds are assumed to be derived from protein degradation. While the 2,3, 4-trimethyl-d-xylose derived from the hydrolysis of cellulose. The alkenes class compound 3, 7,11, 15-tetramethyl-2-hexadecene is probably generated from the conversion of unsaturated fatty acid in algal cells. Long chain compound Pentadecane accounted 6.10% of the total oil, which contributes better combustion property of the algal fuel.

Table 3 is the MTAB's table in which different compounds are shown. The GC-MS analysis results indicate the presence of different classes of hydrocarbons and other compounds which includes azoles, azene, nitriles, and long chain aliphatic compounds and some others. The different % composition of every organic compound in the fuel is listed in the Table 3. The most abundant compound 2-pyrrolidinone accounted for 25.20% of the total oil. The azoles and azene which indicate the class of nitrogen containing heterocyclic compounds such as 3,4,5-Trimethyl pyrazole, 2-pyrrolidinone, 1,2,4,5-Trimethyl-

1H-imidazole, belongs to azole class while the piperidinone belong to azene class. These compounds were supposed to be produce due to the degradation of protein in algal biomass. The tetradecanenitril and Hexadecaneperoxoic are long chain aliphatic compounds containing nitrile and ester functional group. The tetradecanenitrile and Hexadecaneperoxoic acid accounted 14.10% and 14.26% respectively of the total bio-oil, which contribute better combustion property of the algal fuel.

## Results and Discussion

### Theoretical bases of the pyrolysis of biomass/Spirogyra

Spirogyra is blue green algae which is a cellulosic biomass, and is considered as third generation bio-fuel. It is used for the preparation of liquid and gaseous fuels. It contains small quantity of the lignin. Its growth and multiplication is very rapid. The pyrolysis of the cellulosic biomass gives water soluble oxygenated compounds. It is thought that the cellulosic biomass may manufacture methanol, anhydrosugars, furans and aldehydes compounds. These compounds can be used as fuel if appropriately utilized. These products are formed along with large quantity of water therefore need treatment to use as fuel. The product of pyrolysis also contains oil like liquid which is believed that it is composed of compounds containing less oxygen contents. These are partially soluble in the aqueous mixture. Different pre-treatment procedures are used in this work to increase the oily products and to decrease the contents of organic acids as well as other reactive

S. No	Temperature (°C)	Weight of biomass (g)	% Residue	% Liquid	% Gases
1	250	120	92.89	7.01	0.103
2	350	120	70.23	20.11	9.66
3	450	120	62.77	25.99	11.24
4	550	120	57.10	26.97	15.93
5	650	120	53.10	34.16	12.84

Table 1: Important data of Temperature for Pyrolysis of FWA and MTA.

S. No	Name of compounds	Compounds Formula	Retention Time	% composition
1	2,2-dimethylpropiophenone	C <sub>11</sub> H <sub>14</sub> O	2.12	2.05
2	3,4,5- tri-methyl pyrazole	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub>	2.97	19.09
3	2-pyrilidinone	C <sub>4</sub> H <sub>7</sub> NO	2.41	8.05
4	1-H-imidazole1,2,4,5-tetramethyl	C <sub>7</sub> H <sub>12</sub> N	3.43	14.25
5	3-methylbutanenitrile	C <sub>5</sub> H <sub>9</sub> N	3.75	2.10
6	2,3,4-trimethyl-d-xylose	C <sub>6</sub> H <sub>16</sub> O <sub>5</sub>	3.93	14.09
7	Benzonitrile, 4-methyl	C <sub>8</sub> H <sub>7</sub> N	4.35	5.16
8	Pyrrole	C <sub>4</sub> H <sub>5</sub> N	4.45	2.05
9	Pentadecane	C <sub>15</sub> H <sub>32</sub>	6.21	6.10
10	Benzenamine,N-(1-methyl-2-propyl)	C <sub>9</sub> H <sub>13</sub> N	6.35	5.23
11	Benzocyclobuten	C <sub>8</sub> H <sub>8</sub>	7.52	2.04
12	Phenol	C <sub>6</sub> H <sub>6</sub> O	9.75	1.43
13	2-hexadecene3,7,11,15-tetramethyl	C <sub>20</sub> H <sub>40</sub>	9.92	4.09
14	Hexadecanenitrile	C <sub>16</sub> H <sub>31</sub> N	10.59	9.02
15	2-methoxy-3H-azepine	C <sub>7</sub> H <sub>9</sub> NO	11.07	2.15
16	1-chloro-3-fluorobenzene	C <sub>6</sub> H <sub>4</sub> ClF	12.55	2.11

Table 2: GC-MS shows the compounds present in FWAB after pyrolysis.

S. No	Name of compounds	Compounds Formula	Retention Time	% Composition
1	Isobutyronitrile	C <sub>4</sub> H <sub>7</sub> N	2.33	2.75
2	2-pyrilidinone	C <sub>4</sub> H <sub>7</sub> NO	2.424	25.20
3	3,4,5-trimethyl pyrazole	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub>	2.939	9.25
4	2-piperidinone	C <sub>5</sub> H <sub>9</sub> N	3.289	13.30
5	1H-imidazole. 1,2,4,5-tetramethyl	C <sub>7</sub> H <sub>12</sub> N	3.420	14.16
6	Hexadecane-peroxoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>	3.557	14.26
7	2-methylpyrazine	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub>	5.91	2.10
8	1,4-pentadien-3-one	C <sub>5</sub> H <sub>8</sub> O	6.45	2.50
9	2,5-dimethylpyridine	C <sub>7</sub> H <sub>9</sub> N	8.38	1.40
10	Tetradecanenitrile	C <sub>14</sub> H <sub>27</sub> N	10.590	14.10
11	1-methyl-3-vinyl-1H-pyrazole	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	11.93	1.22

Table 3: GC-MS table of the compounds present in MTAB after pyrolysis.

compounds, as oxygenated and nitrogenated species, because they not only lead to high corrosiveness and acidity, but also set up many obstacles to applications.

### Infrared spectroscopy (IR) results of FWAB

In Table 4, Results of FWAB indicate that (C-H) alkanes stretching at 2924.09 cm<sup>-1</sup> and intense broad peak and axial at 3000 cm<sup>-1</sup>. While alkanes bending occurs at 1359.2 cm<sup>-1</sup> and the peak intensity is variable. The (O-H) is also a weak broad peak is due to the presence of water content in bio-oil or may be due to alcohol which appears at 3456.44 cm<sup>-1</sup>. The strong intensity due to stretching vibration of ether appears at 1238.30 cm<sup>-1</sup>. The aromatic C=C stretching at 1556.55 cm<sup>-1</sup> its intensity

is medium weak multiple band The alkene = C-H stretching at 3080.32 cm<sup>-1</sup> and its intensity is also medium while the alkenes stretching occur at 1645.28 cm<sup>-1</sup> and the intensity of peak is variable. It is concluded that the presence of different functional group of compounds indicate the presence of hydrocarbons in the fuel and also the (O-H) broad peak indicate the presence of water contents in bio-fuel. The broad O-H peak was due to the interaction (hydrogen bonding) among the water contents. This oil sample consists of Alkanes, Alkenes, ether, alcohols and aromatics.

### Infrared spectroscopy (IR) results of MTAB

The IR results are shown in the Table 5. Indicates that there is

Functional group	Type of vibration	Characteristics Absorption (cm <sup>-1</sup> )	Intensity
C-O (Ether)	Stretch	1238.30	Strong
-C-H (Alkane)	Bending	1359.82	Variable
C=C (Aromatic)	Stretch	1556.55	Medium-weak, multiple bands.
C-H (Alkane)	Stretch	2924.09	Strong
=C-H (Alkene)	Stretch	3080.32	Medium
O-H (Alcohol)	Stretch (H-bonded)	3456.44	Strong, broad
Alkene	Stretch	1645.28	Variable

Table 4: Characteristics IR Absorption frequencies of organic functional groups of FWAB.

Functional group	Type of vibration	Characteristics Absorption (cm <sup>-1</sup> )	Intensity
C-O (Ether)	Stretch	1242.16	Strong
-C-H (Alkane)	Bending	1373.32	Variable
C=C (Aromatic)	Stretch	1444.68	Medium-weak, multiple bands.
C=C (Alkene)	Stretch	1643.35	Variable
C-H (Alkane)	Stretch	2920.23	Strong
=C-H (Alkene)	Stretch	3088.03	Medium
O-H (Alcohol)	(stretched-bonded)	3412.08	Strong broad

Table 5: Characteristics IR Absorption frequencies of organic functional groups of MTAB.

a broad peak in the region of 3200 cm<sup>-1</sup> to 3600 cm<sup>-1</sup> because this spectrum is the water soaked and microbial treated sample in which due to the hydrolysis of cellulose sugar is formed. The residue sample indicates that (C-H) alkanes stretching at 2920.23 cm<sup>-1</sup> and intense broad peak and axial at 3000 cm<sup>-1</sup>. While alkanes bending occurs at 1373.32 cm<sup>-1</sup> and the peak intensity is variable. The strong intensity due to stretching vibration of ether appears at 1242.16 cm<sup>-1</sup>. The aromatic C=C stretching at 1444.68 cm<sup>-1</sup> its intensity is medium weak multiple band. The alkenes stretching at 1643.35 cm<sup>-1</sup> and the intensity of peak is variable. While the stretching of alkenes at 3088.03 cm<sup>-1</sup> the intensity of peak is strong and broad. It was concluded that the different functional groups indicate the presence of different classes of hydrocarbons.

### Catalytic optimization

Numerous reaction were carried out in which the amount of catalyst was remained the same while biomass was varying to choose a suitable ratio of catalyst with biomass at which maximum liquid oil while less amount of % residue is obtained. At different ratio of catalyst and biomass the pyrolysis reactions were carried out, finally 1:10 of catalyst and biomass respectively in both FWA and MTA, was found proper for good product yield of pyrolysis.

### Conclusions

Pyrolysis of algal biomass was performed in a stainless steel reactor to investigate the effects of pyrolysis temperature, particle size and catalyst on product yields. As we have used two samples of Algae (FWA and MTA) in this study, that's why it is clear in results, tables and figures that there is a considerable difference between these two bio-oil samples. The microbial action converts the cellulosic biomass into compounds of low oxygen contents. Anyhow spirogyra as a biomass for bio-oil production was found very useful due to its unignorable effect on the products of pyrolysis. In addition to the pre-treatment the catalyst (CaO) was found more efficient in increasing the amount of the bio-oil product in both cases (FWA and MTA). Pyrolysis of algal biomass is a promising process for both renewable fuel production

and environment. Our future work will be extraction of important chemicals like Phenols from these bio-oil and also formulation (10%-20%) of these bio-oil with common diesel oil to use it in a diesel engine, the engine will shows us particulate matter (PM) emission, output power and efficiency of these bio-oil.

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