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Title: Bio-oil and biodiesel as biofuels derived from microalgal oil and their characterization by using instrumental techniques

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Abstract:

Microalgal oil has been a source for production of biofuels such as bio-oil and biodiesel. These two biofuels can be characterized quantitatively using advanced instrumentation techniques. Nile Red fluorescence method, PAM fluorometry, NMR, GC/GC-MS and FTIR are among the major techniques available for characterization and quantification of algal oil. NMR is a rapid and non-destructive analytical technique as it requires minimal sample preparation and even one intact algal cell can be analyzed. It can also be used for continuous monitoring of cellular composition of algal culture. NMR can be used to monitor transesterification reactions and oxidation of lipids and biodiesel components. GC has remained the most widely used analytical technique for fatty acid profile analysis. GC-MS is a destructive analytical technique as derivatization of algal oil is required owing to its poor volatility and hence involves lengthy sample preparation procedure. FTIR is a relatively inexpensive technique, and like NMR, can analyze intact cells with scanning time in the order of seconds. FTIR may offer high signal-to-noise ratio and can also be used to monitor transesterification.

Keywords: Microalgae, lipid, bio-oil, biodiesel, instrumentation

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1. Introduction

Microalgal oil has shown an immense potential in the production of biofuels. The biofuels that are derived from microalgal oil primarily includes bio-oil (also known as biocrude) and biodiesel. Bio-oil is derived from the pyrolysis of biomass, which could be further processed to obtain a variety of chemical products. Bio-oil has physico-chemical characteristics different from bio-diesel. This is attributed to differences in their chemical characteristics. Bio-oil has high acidity, high viscosity, poor heating value, and poor stability. The comparatively low quality of bio-oil as compared to biodiesel is attributed to the high oxygen content in bio-oil (30-55 wt %) as compared to around 11% in biodiesel. To enhance the applicability and fuel characteristics of bio-oil, deoxygenation is done. The methods through which the amount of oxygen can be reduced include chemical (eg, hydroprocessing, cracking) and physical (eg, char removal, hot vapor filtration, liquid filtration, solvent addition) (Xiong et al. 2011). Wang et al. (2009) reported that bio-oil could be improved via catalytic hydrogenation, catalytic cracking or steam reforming. More than 400 compounds are present in bio-oil derived from fast pyrolysis of bio-oil. This results in a wide range of boiling points of compounds. Bio-oil is said to be thermo-sensitive and undergo various reactions such as decomposition, polymerization, and oxygenation. The bio-oil obtained from fast pyrolysis has been categorized into three fractions: light, middle, and heavy. The constituent in the light fraction is primarily water with strong acidity, poor stability, and good fluidity. The middle fraction has less water content and

comparatively lower mobility. The heavy fraction appears like a black solid, and has no volatile matter and a comparatively higher heating value (Wang et al. 2009). The water content in the bio-oil has been reported to be around 15-35 wt %. The products of the fast pyrolysis include organic acids (eg, formic and acetic) that gives it a low pH value (2-4) and makes bio-oil corrosive. The removal of water from bio-oil has been a challenging task due to its miscibility with hydrophilic thermolysis products from cellulose and hemicellulose. Phase separation of water also results in substantial loss of polar carbon compounds (eg, small aldehydes, ketones, hydroxyaldehydes, few anhydrous sugars, and other compounds) (Zhang et al. 2010). Apart from this, compounds such as acids, esters, phenols, and lignin-derived oligomers are also formed due to the complexity of the reaction (Capunitan and Capareda 2013). The heating value, water content, and storage stability issues of bio-oil also warrants for its upgrading (Zheng and Wei 2011). The properties of bio-oil can be improved by either physical or chemical means. The techniques for upgrading include filtration (for ash removal), solvent addition (for homogenization and reducing the viscosity of oil), and emulsification with mineral diesel for its utilization as transport fuel or engine fuel and other methods (Capunitan and Capareda 2013). For the upgrading of the fuel, Zheng and Wei (2011) reported that reduced pressure distillation could be done to obtain distilled bio-oil from fast pyrolysis. The method resulted in a yield of 61%, with 29% water phase and 10% residual content. The oxygen content of the bio-oil was reduced to 9.2%. The comparatively lower oxygen content has been reported to enhance the heating value of distilled bio-oil to 34.2 MJ/kg (around twice that obtained via fast pyrolysis).

2. Bio-oil and Biodiesel: Characteristics and components

As the quality of bio-oil is low compared to bio-diesel, they have utility in different engines. The heating value of bio-oil has been reported to be one-third that of diesel (No. 2) due to the presence of water and oxygen. Ikura et al. (2003) reported that the Cetane number of pyrolytic bio-oil is low (5.6). While bio-oil could be used in boilers and turbines that are designed to burn heavy oil for generation of electricity, bio-diesel is usually used as fuel in compression ignition (CI) engines to run transport vehicles and can be used either in neat form or blended form (because of its good solubility with mineral diesel). Bio-oil is considered to be immiscible with the hydrocarbons present in bio-diesel. Solubilization of bio-oil with mineral diesel could be achieved by addition of surfactants as emulsifiers (Zheng and Wei 2011). Bio-oil produced by fast pyrolysis is highly viscous, possesses high acidic and has low ignitability due to high structural water content. To overcome these problems, the pyrolysed bio-oil is emulsified. The cost of the emulsification is governed by the type of surfactant used (Ikura et al. 2003).

Bio-oil is also named 'pyrolysis oil' and the technology is termed as flash pyrolysis technology. Flash pyrolysis has been used to convert solid biomass (wood waste, aquatic plant biomass, municipal solid waste, and agricultural and industrial residue) to potential energy resource. It is also reported that different biomass (owing to difference in composition) produce different yields. The varying ratio of the major constituents in biomass i.e. cellulose, hemi-cellulose, and lignin has an impact on the quality of the biomass. Pyrolysis conditions also have a strong impact on the formation of end products (Tessarolo et al. 2014). Catalysts are used to deoxygenate the bio-oil and to simultaneously derive useful products from lignin. Pyrolysis is an old practice in which biomass is thermally decomposed in the absence of oxygen to produce solid, liquid, and gaseous products (Capunitan and Capareda 2013). Filtration technique has a

potential to remove ash from bio-oil. Capunitan and Capareda (2013) suggested the fractional distillation of bio-oil derived from corn stover to facilitate the separation of components. The heavy fraction that constituted around 53% showed improved properties and composition that could be either upgraded further or blended with other liquid fuels. Continuous hydrotreatment of pyrolysis bio-oil occurs with Pd/C as catalyst in a packed bed catalytic reactor at a temperature ranging from 175-300^{o}C and pressure 50-150 bar (Chaiwat et al. 2013). For an effective fast pyrolysis, the heating rate should be high, carefully controlled temperature (500^{o}C), short vapor residence times (usually below 1 s), rapid removal of char from the reaction environment, and rapid cooling of the pyrolysis vapors (Alvarez et al. 2014). Bio-oil can be used as fuel (as blend with mineral diesel), in the synthesis of value-added products, as feedstock in the production of hydrocarbons, or as steam reformed to produce hydrogen. The lignocellulosic biomass can be transported to refinery units for large-scale production of fuels with commercial value such as hydrogen. Each of the products has numerous applications at the industrial level. The char could be utilized as fuel to provide heat to the process of pyrolysis. Alternatively, char could also be carbonized to obtain activated carbon that is a good adsorbent.

Biodiesel could be obtained from microalgal oil by conversion of triglycerides present in it to fatty acid alkyl ester via transesterification. Apart from biodiesel, long chain hydrocarbons could also be derived from microalgal oil (Vidyashankar et al. 2015). Because of their high abundance, conversion of marine microalgae to biodiesel is particularly of interest (Silva et al. 2015). Biodiesel is considered to be clean, renewable, and biodegradable. However, the polar nature of biodiesel renders it to solubilize metals from the container in which it is stored. The biodegradability nature of biodiesel makes the fuel corrosive to the engine parts. This, in turn,

may cause the biodiesel to go off-specification. There will be potential danger of release of toxic metals in the environment on the combustion of biodiesel. Hence, a thorough characterization of biodiesel becomes important for its usage as a transport fuel, and the characterization of biodiesel becomes important to ensure the suitability of fuel for usage in transport sector.

The lipid profile of the majority of microalgal species is similar to that of plant-derived vegetable oils and is suitable for the production of biodiesel. Microalgae have the capability to synthesize and accumulate neutral lipids that are considered to be most suitable for the production of biodiesel (Swarnalatha et al. 2015). Among the lipid profile present in microalgal oil, the saturated and monosaturated fatty acids are preferred (Sepúlveda et al. 2015). This could be due to the fact that they provide a better compromise between oxidation stability and cold flow properties (eg, cloud point, cold filter plugging point) of biodiesel.

According to international specifications (EN 14214), biodiesel should posses a minimum 96.5 % of fatty acid alkyl ester to be considered for transport fuel (Sharma et al. 2011). Other specifications (ie, Acid value, Viscosity, Flash point, Cetane number, etc.) mentioned in the American Society for Testing and Materials (ASTM D6751) should be fulfilled (Sharma et al. 2012). Nations also have their own specifications for biodiesel. Specification for biodiesel in India comes under IS 15607, and specification for biodiesel in Germany is DIN V 51606 (Sharma et al. 2008).

3. Instruments for characterization of lipid in microalgal oil

Instruments offer an important mode for the characterization of lipid present in microalgal oil and biodiesel and bio-oil derived from microalgal lipids.

3.1. Nile Red fluorescence method

Nile red, a lipophilic dye, has been used to assess the relative quantity of lipid in strains of algae, yeast, fungi, and mammalian cells. The live cell is incubated in the dye, often along with a solvent, and fluorescence is recorded using a spectrophotometer. Fluorescence occurs when the dye penetrates cell structure and diffuses into liquid droplets and fluoresces in the non-polar environment. The Fluorescence technique is rapid and can be integrated with the measurement of optical density, which allows tracking the growth and lipid levels through the different stages of microalgae growth (Higgins et al. 2014). Fluorescence technique serves as a fast screening method for the characterization of lipids derived from a microscopic species. Poli et al. (2014) reported quantification of neutral lipid from the yeast cell based on fluorescence. The technique is more efficient than the conventional technique of gravimetric analysis because less organic solvent is used. The excitation and emission wavelength for total lipid (neutral and polar) depends on the individual organism as well as composition and content of intracellular lipids. The characteristic excitation and emission wavelengths range from 470 to 547 nm and 540 to 628 nm, respectively. Sample preparation in the Fluorescence method requires mixing the biotic cell suspension with isopropanol in the solution of potassium chloride phosphate buffered saline. In a few microorganisms, Nile Red may not penetrate intracellular components of the microorganism to form the Nile Red-lipid complex. Hence, separate methods may be adopted for an individual organism.

3.2. (PAM) fluorometry

Pulse Amplitude Modulated (PAM) fluorometry can be used to measure physiological stress in microalgae and synthesis of cellular neutral lipids. Fluorescence technique is a tool that could be utilized to examine energy metabolism in photosynthetic cells and interactions between carbon and nutrient assimilation in microalgae. Light energy is absorbed by chlorophyll to do photochemical work or is reemitted as heat. The energy used to do photochemical work is inversely related to amount of fluorescence emission from chlorophyll *a*. PAM fluorometry is a common, non-invasive, and rapid mode to measure chlorophyll fluorescence and photosynthetic performance. PAM fluorometry has been used to study physiological stress caused by variances in temperature, salinity, nutrient concentration, and irradiance. The dual channel PAM fluorometer detects the variability in photosynthetic activity in Photosystems I and II. White et al. (2011) reported that the maximum quantum efficiency of PSII (Fv/Fm) was constant in non-stressed cultures and decreased when the cultures were stressed. Stress induced by iron depletion was observed to be minimal. Using the PAM fluorometer gave the extent of neutral lipid synthesis in the freshwater microalgae, *Chlorella* sp. The physiological stress induced by nutrient limitation and complete nutrient deprivation was accessed by decreases in rETR, Fv/Fm, E_k values.

3.3. **Nuclear Magnetic Resonance (NMR) Spectroscopy**

NMR Spectroscopy is an important instrumental technique for qualitative and quantitative analysis of fatty acids and their derivatives. It is a rapid analytical tool offering authentic and reproducible results. Isotopes having a non-zero spin are NMR active. ^{1}H and ^{13}C remain the most widely used isotopes, having two different spin states (+½ and ½). In the presence of an applied external magnetic field, orientation of the nuclei are not random in space and they remain

either aligned with, or against, the applied magnetic field. As it is energetically more stable (lower energy; also called 'α state'), most of the nuclei align with the magnetic field. The energy gap between the two spin states is minimal and increases with the strength of the applied magnetic field (Figure 1). This energy gap corresponds to the energy of radiowaves.

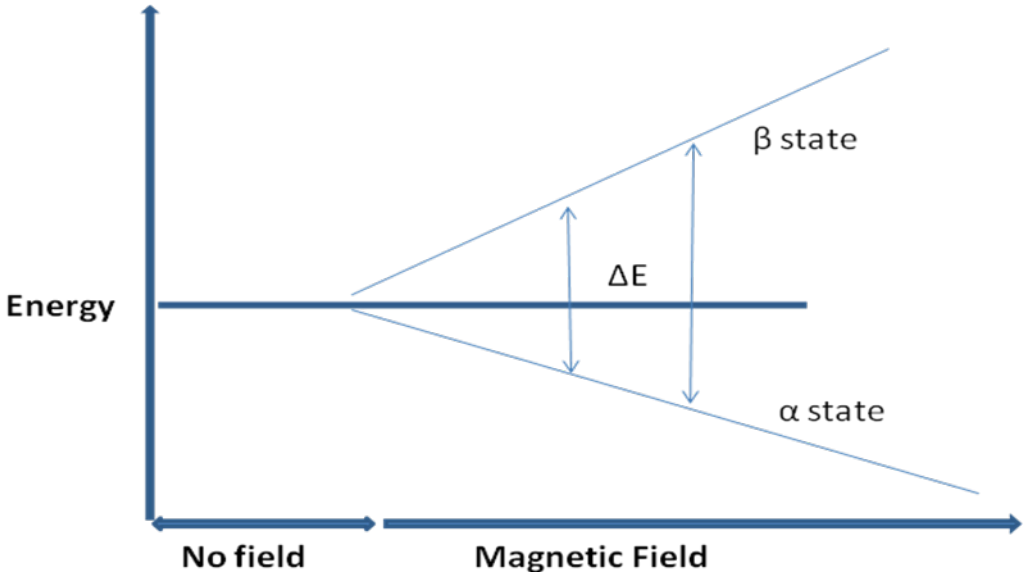

Figure 1: Nuclei spin state splitting in presence of an external magnetic field.

In the presence of an applied field, nuclei tend to behave as a tiny bar magnet and generate their own magnetic field, which opposes the external magnetic field. In different chemical environments, different nuclei of even the same isotope experience different values of net effective magnetic field as they generate local magnetic fields of variable strength (B*net*=B*applied*-B*local*). When irradiated with radiowaves, NMR-active nuclei absorb energy corresponding to the energy gap between the spin states and this promotes nuclei to a higher energy state. Being in higher energy state is energetically unstable, so the nuclei tend to jump back to a lower energy state simultaneously, giving off radiowaves that they absorbed. Emitted radiowaves have variable frequencies and each frequency serves as a fingerprint for a particular chemical species present in a given chemical environment. Typical components of an NMR instrument are shown in Figure 2.

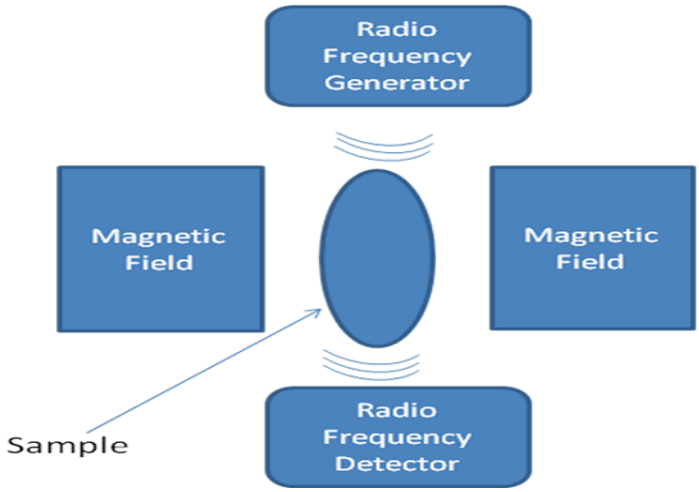

Fig. 2: Components of an NMR spectrometer.

Because different NMR spectrometers have different magnetic field strength, all signals are recorded relative to standard TMS {Tetramethyl silane: (Si $(CH_3)_4$)} and the scale used is called the Delta (δ) Scale and values are reported in ppm. One major advantage offered by NMR spectroscopy is its ability to analyze intact and bulk algal biomass and thus avoids lengthy and inefficient oil extraction procedures. The methylene peak signal intensity is generally used as an indicator of lipid presence (generally Triacylglycerols:TAGs). Table 1 shows characteristic chemical shift values (^{1}H NMR) for different types of lipid. Time Domain (TD) NMR is an alternative to classical NMR (^{1}H, ^{13}C, ^{31}P NMR, etc.) which is based on the difference in relaxation times of hydrogen nuclei present in the different phases of the sample analyzed (Todt 2001). TD-NMR is cheaper as it works under low magnetic field (using a permanent magnet). ^{31}P NMR, in combination with ^{1}H or ^{13}C, can help differentiate between neutral triacylglycerol,(the best suited lipid type for biofuel production) and polar phospholipids (constituting a major fraction of cell membrane and considered unsuitable for biofuel production).

Gao (2008) used TD-NMR to rapidly quantify the lipid content of *Chlorella protothecoides* and obtained better correlation for TD-NMR(R^2=0.9973) than the Nile Red staining method (R^2=0.9067) when compared to traditional gravimetric analysis based on solvent extraction procedure. Gelbard (1995) used ^{1}H NMR to determine the yield of biodiesel produced by transesterification of rapeseed oil with methanol. Yield determination was based on disappearance of signal from a proton located adjacent to the methylene group in triacylglycerol and appearance of signal due to the proton in the alcohol moiety of the methyl ester. Dimmig (1999) used ^{13}C NMR to study turnover and transesterification kinetics of oil derived from rapeseed with methanol and reported that acylglycerol formation from triacylglycerol (slowest) was the rate-determining step. Points of unsaturation (double bonds) on individual fatty acid molecules can be used to assess the oxidation of biodiesel on prolonged storage in the presence of air, and upon oxidation changes in NMR spectra peaks that can be identified and assessed easily (Knothe 2006). There are several advantages and limitations associated with NMR spectroscopy (Table 2).

Table 1. Typical Chemical shift (δ) values for different lipid classes (Nuzzo 2013)

Lipid Class	**^{1}H NMR (δ:ppm)**
Triacylglycerol (TAG)	4.34
Total Fatty Acid	2.35
Phospholipids and Glycolipids	4.53-4.38

Table 2. Advantages and Disadvantages of NMR

ADVANTAGES OF NMR	DISADVANTAGES OF NMR
1. Ability to analyse intact algae(Beal 2010) 2. Structural Details can be obtained 3. Non Destructive analysis 4. Lower maintenance Cost 5. Lower analysis time 6. Easy sample preparation 7. Ability to distinguish between polar and neutral lipids when combined with ^{31}P NMR 8. Allows for continuous monitoring of oil content in growing cells 9. Automated Technique 10. Require very small sample volumes 11. Ability to measure TAG content across all oleaginous microalgae species producing triacylglycerides. 12. TD-NMR is an extremely fast technique (Gao 2008) 13. High reproducibility 14. Can be used as an online technique for continuous lipid analysis, under different stages and cultivation conditions, as live cells can be analyzed directly. 15. Can be used to study oxidation of lipids and biodiesel (Knothe 2006)	1. Expensive instrument 2. Relatively new technique 3. Use of ^{13}C NMR requires large sample volume, because of its lower natural abundance(Davey 2012) 4. Although more accurate, compared to TD and ^{1}H NMR, ^{13}C NMR is time taking.(Beal 2010) 5. TD-NMR provides limited qualitative results. (Beal 2010)

3.4. Gas chromatography–mass spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is a powerful analytical technique that combines the separation power of gas chromatography with the analytical power of mass spectrometry. GC-MS thus overcomes the shortcomings and limitations of either technique when used separately.

3.4.1. Gas Chromatography (GC)

Gas chromatography is the most widely used analytical tool for fatty acid profile analysis. Like any other chromatographic technique, GC involves a mobile phase and a stationary phase. The mobile phase is a non-reactive gas (also known as carrier gas) and a stationary phase (which is usually a liquid coated on to a solid surface). Before the sample enters the stationary phase, which is kept in a thermostatic oven in the form of a coiled column, it is heated to vaporize the sample components. The sample is heated in the injection port which is situated upstream of the stationary phase, at a temperature which is about 50˚C higher than the average boiling point of the components. The vaporized mixture is then carried along the stationary phase by a carrier gas. The various components of the sample have different affinity for the stationary phase at a given temperature and therefore elute out of the column at different rates. Temperature programming is often required for sample components having a wide range of boiling points. Thus, different sample components spend different retention times in the GC system before they come out of the column one by one. Based on the retention time, peak integration values of individual sample components' qualitative and quantitative details can be obtained.

One major limitation of GC is its inability to analyze non-volatile (within the temperature range of the system) sample components. Fatty acids and triacylglycerols (TAGs) are relatively less volatile and cannot be analyzed directly by GC. These compounds therefore require derivatization into some other compound having higher volatility before they can be analyzed by GC. Fatty acids and TAGs are converted into their respective esters prior to their analysis by GC. GC separation can be time consuming if the temperature gradient is low, but it provides high-resolution results. Higher temperature gradients, on the other hand, can be fast but provide poor resolution.

3.4.2. **Mass spectrometry** (MS)

Mass spectrometry requires samples of high purity, which is provided by GC. After being separated by GC the individual sample components enter into a mass spectrometer one by one. The individual sample components are first ionized and fragmented into parent and daughter ions (Figure 3).

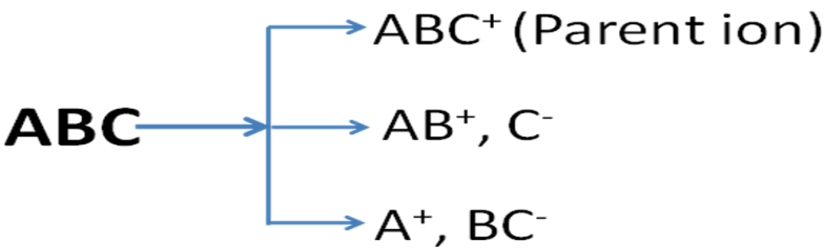

Figure 3. Pattern of Ionization and fragmentation.

These ions are accelerated towards the detector by creating an electrical potential. These daughter ions are then deflected by a magnetic field from their path depending on their mass-to-charge ratio, where daughter ions having a lower ratio are deflected more than ions having a higher ratio. By varying the strength of the magnetic field, all of the daughter ions can be

brought to the detector. The detector donates electrons to the positively charged ions, thereby neutralizing the ions and creating an electron deficit in the metal. The electrons shuffle along to fill the gap and thus create current, which is amplified and recorded. The largest peak (base peak) in mass spectrum denotes ions having the highest number and is given a numerical value of 100, while intensities of all other ions are expressed relative to the base peak. The parent ion represents the mass of the parent molecule from which all other ions are derived. The molecule fragmentation process follows a definite pattern that depends on bond strength and stability; thus, relative abundance of ions help elucidate structure of the molecules. Mass spectrometry is a destructive analytical technique. Methyl esters coming out of a GC column are ionized and fragmented using high energy electrons. Based on the pattern of fragmentation and mass-to-charge (m/e) of individual fragments. the parent molecule can be identified. GC remains the most extensively used analytical technique for qualitative and quantitative analysis of fatty acid methyl esters derived from lipids. Suitability of vegetable oil as biodiesel feedstock is greatly dependent on the constituent fatty acid profile. Identification of individual fatty acids is dependent on a library database or use of internal standards. Although GC is the most widely used analytical technique for analyzing biodiesel, there can be ambiguities related to individual compound identification due to problems such as signal overlap and baseline drift (Knothe 2001). GC coupled with MS can potentially eliminate any ambiguity related to the nature of material coming out of a GC column as mass spectra for individual compounds is inherently unique (Knothe 2001).

Qin (2012) used GC-MS for studying the characteristic fatty acid profile of deacidified *Pistacia chinensis* oil. Barman (2012) carried out an algal oil screening study for determining suitability as biodiesel feedstock based on algal oil derived from 21 algal taxa using GC-MS

dependent fatty acid profiling. Omotoso (2011) used GC-MS in a comparative suitability analysis of *Jatropha* oil and Palm oil as biodiesel feedstock based on individual fatty acid profiles and yields under identical transesterification conditions.

GC and GC-MS have been widely used in the characterization and identification of the fatty acid alkyl esters and their amount in biodiesel. The easy ambient sonic-spray ionization mass spectrometry has been reported be a direct, simple, and rapid method with high specificity and sensitivity for determining the Iodine value of biodiesel. The common method for assessing the level of unsaturation in biodiesel is done by determining the Iodine value. An alternative and direct method for the determination of unsaturation has been suggested by Fernandes et al. (2014) using a technique known as 'Easy Ambient Sonic-Spray Ionization Mass Spectrometry' (EASI-MS). The technique has been reported to be effective in quantitative analysis of unsaturated FAME in biodiesel obtained from various feedstocks such as Soybean, Canola, and Jatropha. The calibration curve plotted for the FAMEs was reported to show good linearity (correlation coefficient > 0.99) and spike recovery (ranging from 76-127%, with a relative standard deviation range of 5.1-17.7%). Fernandes et al. (2014) reported that the method was fast and accurate.

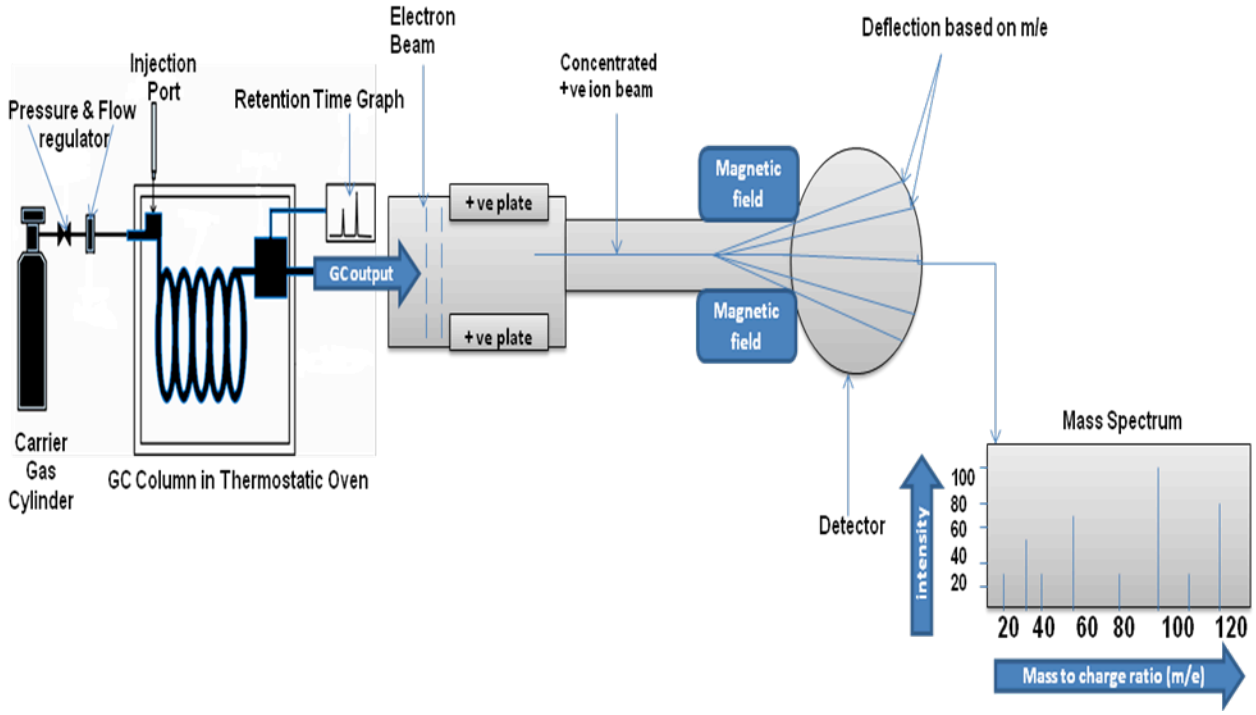

Fig. 3: Schematic diagram of GC-MS

Table 3. Advantages and disadvantages of GC-MS

ADVANTAGES OF GC-MS	DISADVANTAGES OF GC-MS
1) Most widely used analytical techniques for fatty acid profile analysis 2) Hyphenated technique of separation and analysis 3) High resolution 4) Wide range of column types (depending on polarity) and detectors available allow high selectivity depending on the sample composition	1) Oil Extraction is required 2) Derivatization is required (usually to ester) Lin (2012) 3) Longer analysis time 4) Destructive analysis 5) High speed only at the expense of quality signal to noise ratio and resolution 6) Only volatile and thermally stable compounds can be analyzed.

5) Minor components can be quantified with higher accuracy (Knothe 2001)	7) Factors such as signal overlapping and baseline drift can affect accuracy (Knothe 2001).

3.5. Fourier Transform Infrared Spectrometer (FT-IR)

An infrared spectrometer subjects the sample under analysis to infrared radiation of sufficient energy to cause bonds in the molecule to vibrate. Each type of functional group has a characteristic frequency at which its constituent bonds undergo vibration. Fourier Transform Infrared (FTIR) is a special type of infrared spectrometry using a polychromatic infrared radiation instead of a monochromatic radiation, and thus all wavelengths are detected and measured at the same time. An infrared spectrum serves as a fingerprint of a sample in which absorption peaks correspond to the frequency of radiation responsible for bond vibrations. A major component of a FTIR spectroscope is the interferometer consisting of a beamspliter, which divides the infrared radiation from the source into two beams and creates an optical path difference (OPD) between the beams(Figure 4). The beams are later recombined to produce repetitive interference signals that are measured by a detector as a function of OPD. As it passes through the sample the interference signal obtains spectral information of the sample components. The interference signal is recorded in the form of an interferogram, which is subsequently decoded by a mathematical operation called Fourier Transform. Different types of lipids absorb infrared radiation at different wavelengths and their simultaneous detection is facilitated by FTIR spectrometer. FTIR is a very fast analytical technique, completing a wide spectral analysis within seconds. Intact algal cells can also be analyzed directly, and thus facilitates continuous monitoring of cell composition and effects of metabolic control of cellular

composition. Nitrogen starvation is known to enhance lipid accumulation in microalgae and has been confirmed based on several analytical techniques including FTIR (Dean 2010).

Dean et al. (2010) studied *C. reinhardtii* and *S. subspicatus* under variable availability of nitrogen fertilizers and reported high absorption at 1740 cm^{-1}, which is characteristic of lipids for algae grown under nitrogen-starved conditions. Wood (2001) based his carbon allocation pattern analysis on intact *Chaetoceros muelleri* cells in response to optimized nitrogen availability. He reported diversion of carbon from other biomolecules (eg, carbohydrate and protein) and chlorophyll towards lipids, producing IR spectra with enhanced absorption at 1740 cm^{-1}. According to Ivanoiu et al. (2011), the presence of a broad band signal between 2500-3300 cm^{-1} indicates presence of free fatty acid and moisture in algal oil. The methyl peak ($O\text{-}CH_3$) at 1436 cm^{-1} reflects the methyl esters of all types and can be used to monitor conversion of triglycerides into biodiesel via transesterification (Bergougnout et al. 2009).Table No. 4 lists some of the advantage and limitations of FTIR.

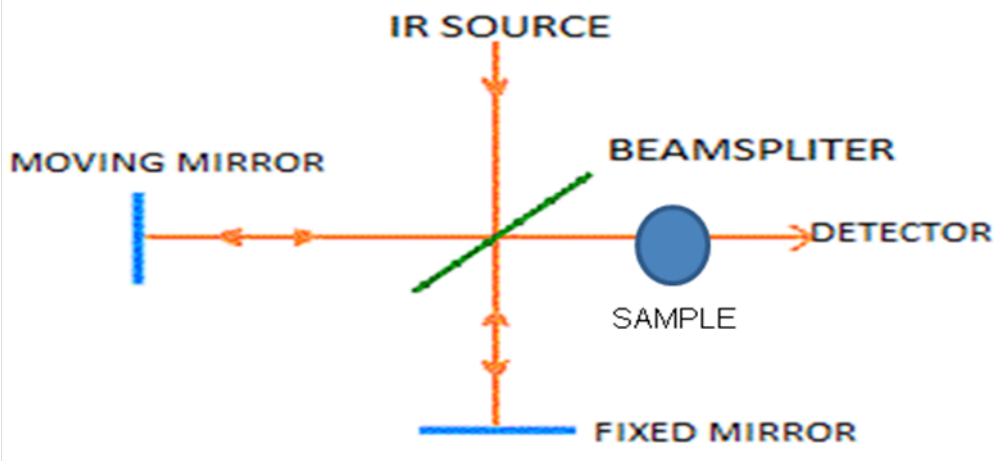

Fig. 4: Schematic diagram of FTIR

Table 4. Advantages and disadvantages of FTIR

ADVANTAGES OF FTIR	DISADVANTAGES OF FTIR
1) Intact algal cells can be analyzed (Dean 2010) 2) Non Destructive analysis 3) Relatively Lower Scanning time 4) Higher signal to noise ratio 5) Relatively inexpensive 6) Wide spectra analysis 7) Easy maintenance 8) Deivitization is not required 9) Tolerant to a limited level of variation in the samples (Han 2011) 10) Very little sample preparation requirement (Han 2011)	1) Only IR active molecules can be analyzed 2) Solvents must be transparent in the spectral region of interest 3) Requires freeze dried algal samples (otherwise oil extraction is required) 4) Requires exogenous lipid standards. 6) possibly requires preparation of separate calibration curves for varying algae species

Conclusions:

Biofuels such as bio-oil and biodiesel can be used as transport fuels as long as they adhere to national/international specifications for their usage. Characterization of the algal feedstock biofuels for qualitative and quantitative analysis involves advanced analytical techniques such as Nile Red fluorescence, PAM fluorometry, NMR, GC-MS, and FTIR. Each of these methods has advantages and limitations. The selection of a particular method is subjective and depends on different factors such as cost, time, sample preparation, accuracy, and precision desired. NMR has emerged as a powerful technique in the characterization of biodiesel because it takes only a short time to quantify the amount of fatty acid alkyl ester present in the biodiesel. It can also

differentiate between neutral and polar lipids. Another similar technique, TD-NMR, is rapid but offers limited qualitative details. GC-MS offers high resolution and a signal-to-noise ratio with a wide range of column and detector types to choose from depending on requirements, but its sample preparation procedure is complicated and time consuming because the sample requires derivatization. Another useful technique, FTIR, uses polychromatic IR radiation and thus facilitates simultaneous detection of different functional groups present within seconds. It can also be used to directly analyze freeze-dried algal biomass.

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