Bio-oil and Biodiesel as Biofuels Derived from Microalgal Oil and Their Characterization by Using Instrumental Techniques

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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 include bio-oil 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 physicochemical characteristics different from biodiesel. This is attributed to difference in their chemical characteristics. Bio-oil possesses 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 the former (30–55 wt%) as compared to around 11 % in the latter. To enhance the applicability and fuel characteristics of bio-oil, deoxygenation is done. The methods through which the amount of oxygen could be reduced are chemical (viz., hydroprocessing, cracking) and physical (viz., char removal, hot vapor filtration, liquid filtration, solvent addition) (Xiong et al. 2011). Wang et al. (2009) reported that bio-oil could be upgraded via catalytic hydrogenation, catalytic cracking, or steam reforming. It is reported that more than 400 compounds are present in bio-oil derived from fast pyrolysis of biomass. The presence of several compounds also results in a wide range of boiling points of bio-oil components. Bio-oil is said to be thermosensitive and undergoes various reactions, viz., 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 comprises mostly of water with strong acidity, poor stability, and good fluidity. The middle fraction has less water content and moderate mobility. The heavy fraction is the one having high viscosity, absence of volatile matter, black solid like appearance 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 (viz., formic and acetic) which impart it a low pH value (2–4) and make 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 to substantial loss in polar carbon compounds (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 complexity of the reactions involved in pyrolysis of biomass (Capunitan and Capareda 2013). The heating value, water content, and storage stability issues of bio-oil warrant for its upgradation (Zheng and Wei 2011). The properties of bio-oil can be improved by either physical or chemical means. The techniques for upgradation include filtration (for ash removal), solvent addition (for homogenization and reducing the viscosity of oil), emulsification of bio-oil with mineral diesel for its utilization as transport fuel or engine fuel, and other methods (Capunitan and Capareda 2013). For the upgradation 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 to yield of 61 % with water phase of 29 % and residual content of 10 %. The oxygen content of the bio-oil got 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 of that obtained via fast pyrolysis).

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2 Bio-oil and Biodiesel: Characteristics and Components

As the fuel quality of bio-oil is inferior as compared to biodiesel, they have utility in different engines. The heating value of bio-oil has been reported to be one-third of that of diesel (No. 2) due to the presence of water and oxygen. The cetane number of pyrolytic bio-oil has also been reported to be low (5.6) (Ikura et al. 2003). While bio-oil could be used in boilers and turbines that are designed to burn heavy oil for generation of electricity, biodiesel 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 the biodiesel. 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 acidity, and has low ignitability due to high structural water content. To overcome these problems, the pyrolyzed 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 as "pyrolysis oil," and the technology is termed as flash pyrolysis technology. Flash pyrolysis has been used to convert the solid biomass (wood waste, aquatic plant biomass, municipal solid waste, and agricultural and industrial residues) to potential energy resource. The composition of bio-oil varies with the type of biomass subjected to pyrolysis (due to the differences in biomass composition). The varying ratio of the major constituents in biomass, i.e., cellulose, hemicellulose, and lignin, has an impact on the quality of the bio-oil. 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 the 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 with Pd/C as catalyst has been reported in a packed bed catalytic reactor at a temperature ranging 175–300 °C and pressure 50–150 bar for its upgradation (Chaiwat et al. 2013). For an effective fast pyrolysis aimed at improving the yield of bio-oil, it is suggested that the heating rate should be high, carefully controlled temperature (at about 500 °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 has got application as fuel (as blend with mineral diesel), in the synthesis of value-added products, as feedstock in production of hydrocarbons, and for obtaining hydrogen (via steam reforming). The abundantly available lignocellulosic biomass based bio-oil can be transported to refinery units for large-scale production of fuels with commercial value, viz., hydrogen. Each of the products have numerous applications at 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 considered as a very good adsorbent.

Biodiesel could be obtained from the 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). Silva et al. (2015) reported that the abundance of marine microalgae makes it a potential raw material for production of biodiesel. Biodiesel is considered to be clean, renewable, and biodegradable. However, the polar nature of biodiesel renders it to solubilize the metals from the container in which it is stored. There will be potential danger of release of toxic metals in the environment on the combustion of biodiesel. The biodegradable nature of biodiesel makes the fuel corrosive to the engine parts. This in turn may cause the biodiesel to go off-specification. Hence, a thorough characterization of biodiesel becomes important for its usage as a transport fuel. Hence, the characterization of biodiesel becomes important to ensure the suitability of fuel for usage in transport sector.

It is reported that the lipid profile of the majority of microalgal species is similar to that of the 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 the 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 in between oxidation stability and cold flow properties (e.g., cloud point, cold filter plugging point) of biodiesel.

As per the European specifications (EN 14214), biodiesel should posses a minimum 96.5 % of fatty acid alkyl ester to be considered for use as a transport fuel (Sharma et al. 2011). The other specifications (viz., acid value, viscosity, flash point, cetane number, etc.) are mentioned in American Society for Testing and Materials (ASTM D6751) and should be fulfilled. Nations also have their own specifications for

biodiesel. Specification for biodiesel in India comes under IS 15607. The specification for biodiesel in Germany is DIN V 51606 (Sharma et al. 2008).

3 Instruments for Characterization of Lipid in Microalgal Oil

Instrument offers an important mode for the characterization of lipid present in microalgal oil, biodiesel and bio-oil derived from microalgal biomass.

3.1 Nile Red Fluorescence Method

Nile red, a lipophilic dye, has been reported to be 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 penetrate the cell structure and diffuse into lipid droplets and fluoresces in the nonpolar environment. The fluorescence technique is reported to be rapid and can be integrated with the measurement of optical density. Hence, this allows to track the growth and lipid levels through the different stages of growth of microalgae (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) have reported quantification of neutral lipid from the yeast cell based on fluorescence. The technique has been reported to be more efficient than the conventional technique of gravimetric analysis as lesser amount of organic solvent is used. The excitation and emission wavelength for total lipid (neutral and polar) have been reported to depend on the individual organism as well as composition and content of intracellular lipids. The characteristic wavelength of excitation and emission range from 470 to 547 nm and 540 to 628 nm, respectively. The sample preparation in fluorescence method requires mixing the cell suspension with isopropanol in the solution of potassium chloride phosphate buffered saline. The limitation of Nile red fluorescence has also been reported. It has been reported that in few microorganisms, Nile red may not penetrate intracellular component of the microorganism to form the Nile red-lipid complex. Hence, separate method is required for such organisms.

3.2 PAM Fluorometry

White et al. (2011) have reported pulse amplitude modulated (PAM) fluorometry to measure the physiological stress in the microalgae and synthesis of cellular neutral lipids. It is reported that the 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. The light energy is absorbed by the chlorophyll to do photochemical work or is reemitted as heat. The energy used to do photochemical work is inversely related to the amount of fluorescence emission from chlorophyll *a*. PAM fluorometry has been reported to be a common, noninvasive, and rapid mode to measure chlorophyll fluorescence and photosynthetic performance. PAM fluorometry has earlier been used to study physiological stress, viz., temperature, salinity, nutrient, and irradiance. The dual-channel PAM fluorometer detects the variability in photosynthesis activity in photosyntheses I and II. The "maximum quantum efficiency" of PSII (Fv/Fm) is reported to be constant in the nonstressed culture and decreases when the culture is stressed. PAM fluorometry can be used to determine the stress induced by the environmental factor in the microalgae. The stress induced by iron depletion was observed to be minimal. Using the PAM fluorometer gave the extent of neutral lipid synthesis in freshwater microalgae, *Chlorella* sp. The physiological stress induced by nutrient limit and complete nutrient deprivation was accessed by decrease in rETR, Fv/Fm, and E_k values. Insausti et al. (2013) have reported estimation of various parameters (FAME, cetane number, heat of combustion, and color) in biodiesel and diesel blend using synchronous fluorescence. Szabo et al. (2014) have reported multiwavelength chlorophyll fluorescence analyses for examining the photosynthetic efficiency of *Nannochloropsis oculata* that was grown under high and low irradiance of light. The type of lipid accumulation in the algal cell was found to be influenced by the type of irradiance. The high irradiance of light resulted in high accumulation of saturated fatty acids, whereas low irradiance led to high accumulation of polyunsaturated fatty acids.

3.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a very important instrumental technique for qualitative and quantitative analyses of fatty acids and its derivatives. It is a rapid analytical tool offering authentic and reproducible results. Isotopes having a nonzero spin are NMR active. ^{1}H and ^{13}C remain the most widely used isotopes, having two different spin states (α and β). Naturally, the orientation of the nuclei does not follow a pattern and their orientation remains random. However, in the presence of an applied external magnetic field, the orientation of the nuclei is not random in space, and they remain either aligned with or against the applied magnetic field. As it is energetically more stable (lower energy "α state"), most of the nuclei align with the magnetic field. The energy gap

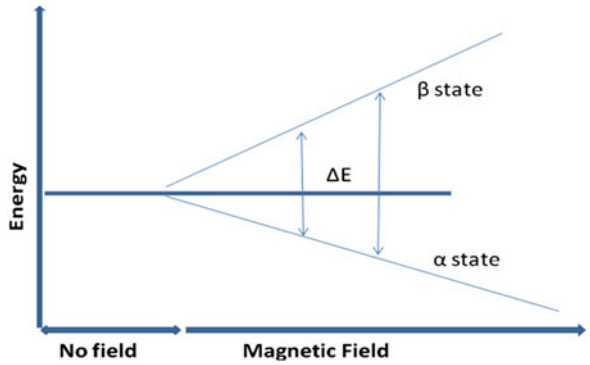

Fig. 7.1 Nuclei spin state splitting in the presence of an external magnetic field

between the two spin states is minimal and increases with the strength of the applied magnetic field (Fig. 7.1). This energy gap corresponds to the energy of radio waves.

In the presence of an applied field, nuclei tend to behave as a tiny bar magnet and generate its own magnetic field, which opposes the external magnetic field. Different nuclei of even the same isotope present in different chemical environment experience different values of net effective magnetic field as they generate local magnetic field of variable strength (B*net* = B*applied*-B*local*). When irradiated with radio waves, NMR active nuclei absorb energy corresponding to energy gap between the spin states, and this promotes nuclei to higher energy state. As being in higher energy state is energetically unstable, the nuclei tend to jump back to lower energy state simultaneously giving off radio waves that it had absorbed. Emitted radio waves 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 Fig. 7.2.

Since different NMR spectrometers have different magnetic field strength, all signals are recorded relative to a standard, TMS {tetramethylsilane: (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 TAGs). Table 7.1 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 et al. 2001). TD-NMR is cheaper as it works under low magnetic field (uses permanent magnet). ^{31}P NMR in combination with ^{1}H or ^{13}C can help differentiate between neutral triacylglycerol which is best suited lipid type for biofuel production and

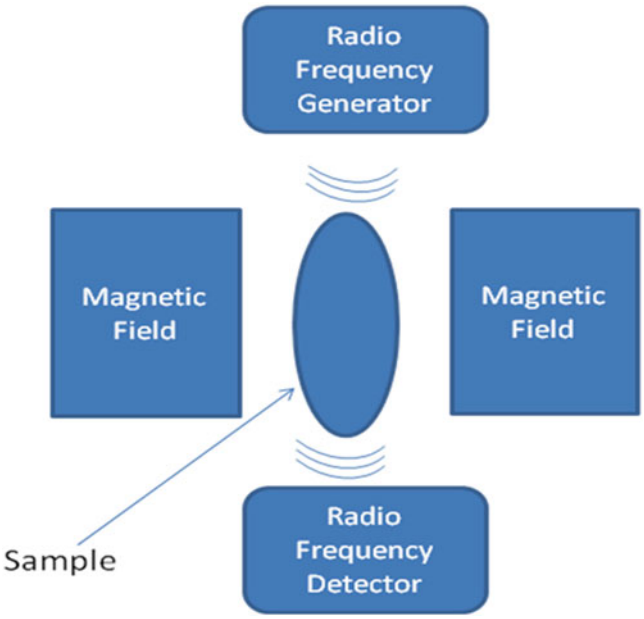

Fig. 7.2 Components of NMR spectrometer

Table 7.1 Typical chemical shift (δ) values for different lipid classes

Lipid class	^{1}H NMR (δ:ppm)
Triacylglycerol (TAG)	4.34
Total fatty acid	2.35
Phospholipids and glycolipids	4.53–4.38

Nuzzo et al. (2013)

polar phospholipids constituting a major fraction of cell membrane and is considered unsuitable for biofuel production.

Gao et al. (2008) used TD-NMR to rapidly quantify the lipid content in *Chlorella protothecoides* and obtained better correlation for TD-NMR(R^2 = 0.9973) than Nile red-based staining method (R^2 = 0.9067) when compared to traditional gravimetric analysis based on solvent extraction procedure. Gelbard et al. (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 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 et al. (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) as the rate determining step. Points on 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 can be identified and assessed easily (Knothe 2006). There are several advantages and limitations associated with NMR spectroscopy, as shown in Table 7.2.

Table 7.2 Advantages and disadvantages of NMR

Advantages of NMR	Disadvantages of NMR
1. Ability to analyze intact algae (Beal et al. 2010)	1. Expensive instrument
2. Structural details can be obtained	2. Relatively new technique
3. Non-destructive analysis	3. Use of ^{13}C NMR requires large sample volume, because of its lower natural abundance (Davey et al. 2012)
4. Lower maintenance cost	4. Although more accurate, compared to TD and ^{1}H NMR, ^{13}C NMR is time taking (Beal et al. 2010)
5. Lower analysis time	5. TD-NMR provides limited qualitative results (Beal et al. 2010)
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 et al. 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)	

3.4 Gas Chromatography–Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is a powerful hyphenated analytical technique which combines the separation power of gas chromatography with analytical power of mass spectrometry. The combination of GC-MS overcomes the shortcomings and limitations of either technique individually and provides a powerful analytical technique. Typical components of a hyphenated GC-MS system are shown in Fig. 7.4.

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. In GC, the mobile phase is a nonreactive gas (also known as carrier gas) and a stationary (which is usually a liquid coated onto a solid surface) phase. Before the sample enters the stationary phase which is kept in a thermostatic oven in the form of a coiled column, it is heated so as to vaporize all 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 sample 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 wide range of boiling points. Thus, different

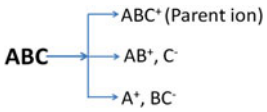

Fig. 7.3 Pattern of ionization and fragmentation

sample components spend different amount of time (retention time) in the GC system before the come out of the column one by one. Based on the retention time values and peak integration values of individual sample components, qualitative and quantitative details can be obtained.

One major limitation of GC is its inability to analyze nonvolatile (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 usually converted into their respective esters prior to their analysis by GC. GC separation can be time-consuming if temperature gradient is low but can provide high resolution result. Higher temperature gradient on the other hand can be fast but provides 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 mass spectrometer one by one. First of all the individual sample components are ionized and fragmented into parent and daughter ions by high energy electrons (Fig. 7.3).

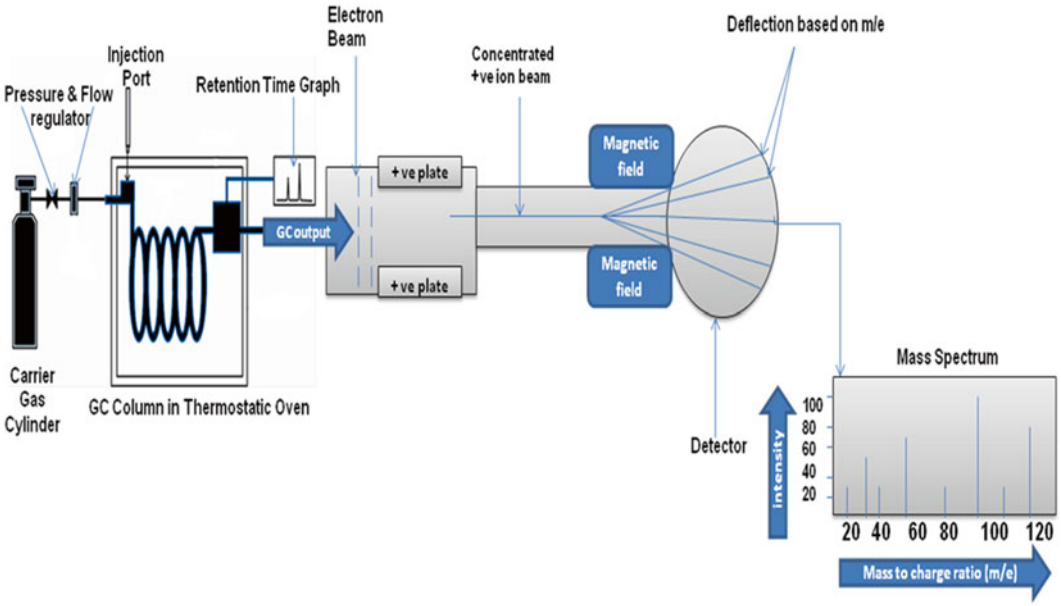

Fig. 7.4 Schematic diagram of GC-MS

These ions are accelerated toward the detector by creating an electrical potential. These ions are then deflected by a magnetic field from their path depending on their mass-to-charge ratio, in which the ions having lower mass-to-charge ratio are deflected more than ions having higher mass-to-charge ratio. By varying the strength of the magnetic field, all the ions can be brought to the detector (Fig. 7.4). The detector which consists of a metal box donates electrons to the positively charged ions thereby neutralizing the ions and creates 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 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 fragmentation process of molecules follows a definite pattern which depends on bond strength and stability, and thus relative abundance of ions helps elucidate structure of the molecules. Mass spectrometry is a destructive analytical technique. Methyl esters coming out of a GC column are ionized and fragmented by using high-energy electrons, and based on the pattern of fragmentation and 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 of 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 overlapping 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 et al. (2012) used GC-MS for studying the characteristic fatty acid profile of deacidified *Pistacia chinensis* oil. Barman et al. (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 et al. (2011) used GC-MS in a comparative suitability analysis of *Jatropha* oil and palm oil as biodiesel feedstock based on individual fatty acid profile and yield under identical transesterification condition.

Gas chromatography and gas chromatography coupled with mass spectrometry have been widely used in the characterization and identification of the fatty acid alkyl ester and its amount in biodiesel. The common method for assessing the level of unsaturation in biodiesel is 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 called "easy ambient sonic-spray ionization mass spectrometry" (EASI-MS). The easy ambient sonic-spray ionization mass spectrometry has been reported to be direct, simple, and rapid, along with high specificity and sensitivity in determination of the iodine value of biodiesel. The technique has been reported to be effective in quantitative analysis of unsaturated fatty acids in biodiesel obtained from various feedstocks (soybean, canola, and Jatropha). As per the European standard (EN 14214), biodiesel must have atleast 96.5% of fatty acid methyl esters

Table 7.3 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	1. Oil extraction is required
2. Hyphenated technique of separation and analysis	2. Derivatization is required (usually to ester) Lin et al. (2012)
3. High resolution	3. Longer analysis time
4. Wide range of column types (depending on polarity) and detectors available allow high selectivity depending on the sample composition	4. Destructive analysis
	5. High speed only at the expense of quality signal-to-noise ratio and resolution
5. Minor components can be quantified with higher accuracy (Knothe 2001)	6. Only volatile and thermally stable compounds can be analyzed
	7. Factors such as signal overlapping and baseline drift can affect accuracy (Knothe 2001)

(FAME) content. The calibration curve plotted for the FAME was reported to show good linearity (correlation coefficient > 0.99) and spike recovery (ranging from 76 to 127 %). The relative standard deviation was reported to range from 5.1 to 17.7 %. Fernandes et al. (2014) report the method to be fast with a high accuracy. Table 7.3 lists various advantages and limitations of a GC-MS system.

3.5 Fourier Transform Infrared Spectrometer (FTIR)

An infrared spectrometer subjects the sample under analysis to infrared radiation having sufficient energy to cause bonds in the molecule to vibrate, and 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 in which a polychromatic infrared radiation is used instead of using 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. Interferometer is the major component of an FTIR spectroscope. The interferometer divides the infrared radiation from the source into two beams and creates an optical path difference (OPD) between the beams. The beams are later recombined to produce repetitive interference signals which 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. The interferogram is subsequently decoded by a mathematical operation called Fourier transform. Figure 7.5 represents FTIR instrument schematically.

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 and completes a wide spectra analysis within a matter of seconds. Intact algal cell can also be analyzed directly and thus facilitates continuous monitoring of cell

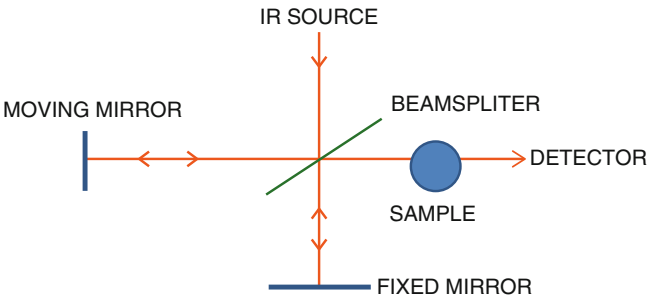

Fig. 7.5 Schematic diagram of FTIR

composition and effects of metabolic control of cellular composition (Dean et al. 2010). Nitrogen starvation is known to enhance lipid accumulation in microalgae, and this has been confirmed by several researchers based on several analytical techniques including FTIR.

Dean et al. (2010) studied *C. reinhardtii* and *S. subspicatus* under variable availability of nitrogenous fertilizers and reported high absorption at 1740 cm^{-1} characteristic of lipids for algae grown under nitrogen-starved conditions. Giordano et al. (2001) based on his carbon allocation pattern analysis on intact *Chaetoceros muelleri* in response to optimized nitrogen availability reported diversion of carbon from other biomolecules (carbohydrate, protein) and chlorophyll toward lipids, producing IR spectra with enhanced absorption at 1740 cm^{-1}. According to Ivanoiu et al. (2011), the presence of broadband signal between 2500 and 3300 cm^{-1} can be used as an indication of 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 (Bergougnou et al. 2009). The advantages and disadvantages of FTIR are presented in Table 7.4.

4 Conclusions

The biofuels, viz., bio-oil and biodiesel, find application as fuel in transport sector. The two biofuels should adhere to the national/international specifications for their usage. The

Table 7.4 Advantages and disadvantages of FTIR

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

characterization of the biofuels involves advanced analytical techniques that include Nile red fluorescence method, PAM fluorometry, NMR, GC-MS, and FTIR as the major instrumental techniques available for qualitative and quantitative analyses of algal oil. Each of these methods has their own advantages and limitations. Therefore, the selection of a particular method is subjective and depends on different factors, viz., cost, time, sample preparation, and accuracy and precision desired, among others. Among the various techniques available, NMR offers characteristic chemical shift values for individual components along with its structural details in relatively short time span. It can also differentiate between neutral and polar lipids. NMR has emerged as a powerful technique in the characterization of the biofuels, biodiesel. It takes a very short time to quantify the amount of fatty acid alkyl ester present in the biodiesel. Another similar technique, i.e., TD-NMR, is rapid but offers limited qualitative details. Hyphenated technique of GC-MS offers high resolution and signal-to-noise ratio with a wide range of column and detector types to choose from depending on the requirement, but its sample preparation procedure is complicated and time taking as the sample requires derivatization. Another useful technique, FTIR uses a polychromatic IR radiation and thus facilitates simultaneous detection of different functional groups present within a matter of seconds. It can also be used to directly analyze freeze-dried algal biomass.

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