10. BIODIESEL FROM *APIS MELLIFERA*

ABSTRACT:

The search for alternate and sustainable fuel for internal combustion engines which fulfils around 25% of energy needs have lead the researchers towards biodiesel which can be easily extracted from vegetable oils. Biodiesels can also be extracted from waxes, oligenius species of algae and pyrolysis oils. In the above mentioned materials waxes can be used predominantly because, they are entirely composed of esters of higher chain aliphatic alcohols. In the current study biodiesel form beeswax has been studied for its characteristics with the aid of analytical techniques such as GC-MS and FTIR spectrum. And also the properties of fuel and combustion chemistry of biodiesels have been discussed. The spectrum results have clearly shown the presence of five methyl esters of palmitic acid and oleic acid. Further the biodiesel is found to have density of 880 Kg/m^3 and calorific value of 38.5MJ/Kg with the Cetane number of 48. The analysis of the combustion of the fuel gives compromising results proving biodiesels from wax esters will be an excellent alternative to mineral diesel and will find a valuable position in list of materials that can be used for biodiesel formulation.

SCOPE OF RESEARCH /NEED OF THE STUDY

The increase in power demand to meet the requirement of the expanding population has landed up the energy sector to produce more power to satisfy the demand. As the demand is being met, still there is shortage of power which leads to the limited supply of power from grid to user called “load Shedding”. This action has not only affected the normal life of the people but also affected the economy growth. The power shortage can be compensated by providing the additional power produced from various renewable sources like windfall and solar farms. But, the fluctuations in power generation from these sources have proved to be hopeless.

The solution for solving this complex situation is by considering the conventional technique with the aid of renewable energy resources. Identifying such a type of resource directs the pathway to bio fuels. The most prominent fuel among biofuels is bio diesel. These biodiesel are produced by Trans- esterification of waxes with alcohol in presence of acid/base catalyst.

The other serious problem faced is the disposal of the waxes, which has been disposed as waste residue. Direct disposal of this waste into environment causes harmful effects to humans and animals. Thus various problem related to disposal and handling arises which makes this issues more complex. But these waxes have very good fat content which can be used as a substitute for these edible oils. These fats have same characteristic properties like of the oil and can be promising. Thus by employing the concept of "waste to energy ",this work deals with the extraction of biodiesel from the waste waxes extracted from the waste and thereby analysing, evaluating and implementing it for real time applications.

AIM AND OBJECTIVES

The following are the aims and objectives of this project:

1. **Identify a feasible method for extraction of lipids (fats) from waste waxes:** Since the waxes consists a cluster of lipids, proteins and nitrogenous compounds, an optimum method must be identified to extract maximum amount of fat from it with less residues.

2. **Optimize the parameters for an effective trans-esterification reaction:** The extraction of biodiesel from waxes is a long way process which are influenced by various parameters like molar ratio, temperature, stirring speed, viscosity, amount and type of catalyst. These parameters are linked with each other and have great impact in biodiesel production.

3. **Evaluate the performance and emission characteristics of the Biodiesel:** The extracted diesel must be analyzed using GC-MS analysis and the performance of the biodiesel must be carried. The comparison in performance of biodiesel with petro diesel will be done to identify the degree of superiority. Also the emission characteristics will be investigated by testing it on diesel engine.

DELIVERABLES OF THE STUDY

1. Understand the basic concept of waxes, phospholipids and various methods involved in the extraction of the fat from animal waste**.**
2. Implement a very feasible and cost effective method for biodiesel production optimizing all the parameters
3. Obtain the fuel combustion study for the biodiesel produced from A.Mellifera

INTRODUCTION

ANIMAL FATS

Beeswax (ceraalba) is a natural wax produced by honey bees of the genus Apis. Waxes from nature are widely classified into plant wax and animal wax in the division of animal wax, wax of particular bee species melifera of the genre Apis (common honey bee) contributes majorly to production of beeswax. Waxes are mainly composed of long chain esters of fatty alcohols and fatty acids. Waxes available from these kind of sources can be considered as a promising source for production of biodiesels by converting the long chain esters to fatty acid methyl esters (FAMEs) using the process of transesterification [1-9]. The wax is formed into "scales" by eight wax-producing glands in the abdominal segments of worker bees, who discard it in or at the hive. The hive workers collect and use it to form cells for honey-storage and larval and pupal protection within the beehive.

MATERIALS AND METHODOLOGY

Materials and methods

Methanolysis

The wax from honey bees majorly consists of myricylpalmitate and cerotic acid at the ratio of 6:1. Apart from this it contains mono esters of long chain (30-32 carbon chains) aliphatic alcohols with palmitic acid and oleic acids. The detailed chemical composition of the beeswax is given in the table (1).

Table 1.Chemical composition of bees wax. [4]

Composition	%
acid esters	4
cerotic acid	4.4
lauryl palmitate	2
lignoceric acid	1
Melissic acid	2
Montanic acid	2.6
myricylpalmitate	23
myricylcerotoate	12

The biodiesel from bee wax is extracted by two stage transesterification process. Initially the solid wax is purified and treated with methanol and concentrated hydrochloric acid (HCl) at the ratio of 1:2:0.04 (oil: methanol: acid) for two hours at a temperature 65^0C. The treated wax is then heated to a temperature of 85^0C and maintained for 30 minutes in order to remove excess methanol. The treated wax is then subjected to transesterification reaction with 0.02% (by weight) of sodium hydroxide (NaOH) as catalyst at the molar ratio of 1:0.25 in reaction chamber maintained at 60^0C. The reactant mixture is left for 8-12 hrs for

settlement of fatty alcohols (FA's). The clear bio diesel layer from the top is removed and further processed to remove methanol and catalyst [3, 4].

Spectrometric studies

Gas chromatography and mass spectrometry (GC-MS)

GC-MS is a technique for quantitation of organic compound. This technique puts together the feature of gas chromatogram and mass spectrometer to analyse the given sample. Gas chromatogram is used to differentiate the sample into individual component using temperature controlled capillary column. Mass spectrometer provides the molecular weight and structural formula of the compounds by measuring and comparing the unique mass spectrum (m/z) of the fragment with NIST standard library [14]. In the present work, JOEL GC MATE II gas chromatogram- mass spectrometer with data system, double focussing is used. It has a resolution of 6000 Daltons and max calibrated mass of 1500 Daltons with 3 different source options viz. electron impact (EI) with beam intensity of 70eV, chemical ionization (CI) and fast atom bombardment (FAB). The detailed description of the GC-MS apparatus used is given in table (2)

Table 2: specification of JOEL GC MATE II mass spectrometer.

Description	**Range**
Mass range	2-1000 amu
Resolution	0.1 amu

Ion source temperature	350^{o}C
Transfer line temperature	100-400^{o}C
Quadrupole temperature	100-200^{o}C
Ionization energy	5-200eV
Ionization current	1-300μA

Fourier transformed infra-red (FTIR) spectrometer

Infra-red spectrometry is a technique adopted to find out the absorption frequencies of the sample in infra-red region of electromagnetic spectrum. Organic molecules undergoes constant vibration due to stretching and bending vibrations of C-H, C-O and C=O present in the molecule. The vibrations of different pairs have characteristic frequency, if the sample containing the organic compound is exposed to frequency of the vibration of the molecule and due to resonance, the amplitude of the vibration is increased. By finding the wave length of the radiation absorbed, the type of the bond present can be determined. For the current study Perkin Elmer spectrum 1 FTIR spectrometer from sophisticated analytical instrumental facility (SAIF), Indian institute of technology (IIT), Madras is used which is having a scan range of MIR 450-4000cm^{-1} with resolution of 1.0 cm^{-1}. The detailed description of the FTIR spectrometer used is given in the table (3).

Table 3: specification of Perkin Balmer spectrum one FTIR spectrometer

Description	**Range**
Wave length range	7800-350cm^{-1} with KBr beam splitter
Resolution	0.5cm^{-1}-64cm^{-1}
Wavelength accuracy	0.1cm^{-1} at 1600cm^{-1}
Signal to noise ratio for KBr	30000/1 rms, 6000/1 p-p for a 1 minute

optics	measurement.
Available OPD velocities	0.1,0.2,0.5,1 and 2cms^{-1}

Combustion chemistry

Combustion is a set of instantaneous chemical reaction between hydrocarbon fuel and oxygen to liberate energy by oxidizing carbon to carbon di oxide and hydrogen to water. To understand the essence of combustion the properties of fuel such as stoichiometric air to fuel ratio, adiabatic flame temperature, higher heating value and Cetane number of the fuel must be studied.

Stoichiometric air to fuel ratio

Stoichiometric air to fuel ratio is actual air required for 1 mole of fuel to undergo complete combustion. Considering $C_{N_C}H_{N_H}O_{N_O}$ which undergoes oxidation under atmospheric air to form combustion products the combustion reaction can be given as

$$C_{N_C}H_{N_H}O_{N_O} + N_{air}(\mathrm{O_2} + 3.776\,\mathrm{N_2}) \rightarrow pCO_2 + qH_2O + (3.776 \times N_{air})N_2 \quad (1)$$

Considering carbon, hydrogen, oxygen and nitrogen balance in above equation we can find out the moles of air required for complete combustion of one mole of fuel as

$$N_{air} = \frac{4N_C + N_H + 4N_O}{8} \quad (2)$$

Where N_{air} is amount of air required for complete combustion of 1 mole of any hydrocarbon fuel that satisfies with the equation (8). N_C, N_H and N_O are number of carbon, hydrogen and oxygen molecules present in the fuel respectively. Based on mass spectrometric studies the molecular formulae of the bees wax is found out as $C_{18}H_{35}O_2$. In the

case of biodiesel from beeswax the number of carbon, hydrogen and oxygen will be 18, 35 and 2 respectively. Therefore, stoichiometric air fuel requirement for bees wax biodiesel will be 12.825:1. Normally diesel engines are known as excess air engine since, it takes 1.5 times more than stoichiometric air requirement than the actual fuel required to burn one mole of fuel and the actual air required for complete combustion of fuel will be 19.237:1 at the air equivalence ratio of 0.667.

Adiabatic flame temperature

Adiabatic flame temperature is the maximum temperature attained when one mole of fuel is completely burnt with stoichiometrically correct amount of air. Considering first law of reacting system, for steady flow systems state the rate of net energy transfer the system in the form of heat, work or mass should be equal to the rate of net energy transfer out of the system in the form of heat, work or mass (Equ (3) and (4)).

$$\dot{E_{in}} = \dot{E_{out}} \tag{3}$$

$$Q_{in} + W_{in} + \sum N_r (\overline{h_f^o} + \bar{h} + \overline{h^o})_{\mathrm{r}} = Q_{out} + W_{out} + \sum N_p (\overline{h_f^o} + \bar{h} + \overline{h^o})_{\mathrm{p}} \tag{4}$$

Where N_p and N_r are total mass flow rate of reactants and products respectively. And $\overline{h_f^o} + \bar{h} + \overline{h^o}$ Is total enthalpy h for reactants with subscript r and products with subscripts p.

$$Q_{in} + W_{in} + \sum N_r H_{\mathrm{r}} = Q_{out} + W_{out} + \sum N_p H_{\mathrm{p}} \tag{5}$$

For analysis it is considered than one mole of reactant makes N_r equal 1. Since adiabatic combustion is taken into account and considering that no work is given into the system to initiate combustion W_{in} and Q_{in} is taken as zero. So equation (5) will become,

$$Q - W = \sum N_p H_{\mathrm{p}} - \sum N_r H_{\mathrm{r}} \tag{6}$$

The enthalpy can be represented as a function of temperature.

$$h(\mathrm{T}) = \int_{T_o}^{T} C_p dT + u(\mathrm{T_o}) + RT_o \tag{7}$$

Here $u(\mathrm{T_o}) + RT_o$ is enthalpy of the system at ambient temperature $\mathrm{T_o}$. Therefore, equation 7 becomes

$$h(\mathrm{T}) = \int_{T_o}^{T} C_p dT + h(\mathrm{T_o}) \tag{8}$$

Here $\mathrm{C_p}$ is the specific heat of the working substance which is determined experimental. From equation 8 taking adiabatic flame temperature as a function of specific enthalpy of reactants and products.

$$f(\mathrm{T}) = \mathrm{H_p}(\mathrm{T}) - H_r(\mathrm{T_r}) \tag{9}$$

Here $\mathrm{H_p}$ is specific enthalpy of products $\sum H_p h_p$ and T is final temperature of products initially it is assumed as the highest possible temperature and the exact value will be obtained by iterating the value of the obtained results by solving equation 9 using Newton-Raphson iterative procedure given below. $\mathrm{H_r}$ is specific enthalpy of the reactants $\sum H_r h_r$ and $\mathrm{T_r}$ is temperature of the reactants.

Results of Gas Chromatography and Mass Spectrometry

The graph below shows the fragments present in the beeswax biodiesel. The gas chromatogram plot shown in the figure (1) shows the presence of the various esters at the various retention time varying from 16min to 22 minutes of the gas chromatogram operation. This graph shows five distinct peaks which are due to characterization of different esters. The

mass spectrum results are compared with standards of NIST standards to find out the nature of the ester present.

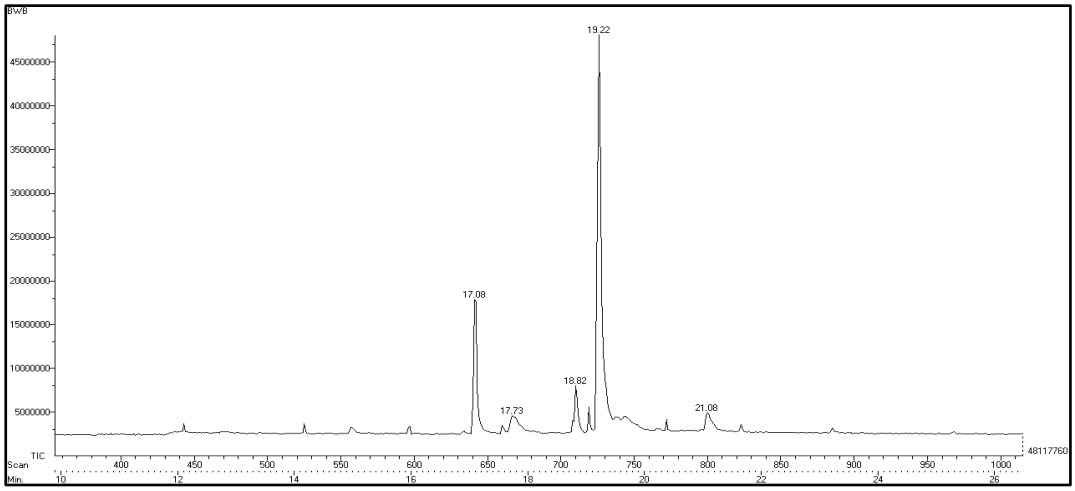

Figure 1: Gas Chromatogram of BWB

\Even though, the reaction of the waxes shows the formation of esters of Certoic acid, lignoceric acid and Montanic acid, those mass fragments are not seen in the mass spectrometry. Instead, mass fragments detected are methyl esters of palmitic acid and oleic acid the reason for this scenario can be majorly explained with the help of reaction kinematics since the amount of palmitic esters in the raw bees wax is predominantly high the higher yield of biodiesel would be only obtained only if all of the esters of palmitate is taken in to conversion this predominantly shows around 29% of the total volume of the esters present in biodiesel. The esters of oleic acid are seen in abundance of around 60%. The temperature at which the reaction proceeded is the main reason for the formation of the esters of oleic acid. In order to maintain wax in the liquid state the reaction has to be proceeded in the temperature of 60^{o} C and duration of 2 hrs that leads the domination of the reverse equilibrium in a esterification of acids to esters this is the reason for the absence of the acid esters in the biodiesel from bees wax however, the percentage composition of the acids are

comparatively lesser than that of esters in beeswax therefore, their contribution to the total yield of the biodiesel will be less.

4.2 FTIR spectroscopy results

The Fourier transformed infra-red spectrometry results shown in the figure (7) shows the presence of esters, aliphatic compounds and alkyl groups with the traces of C-O stretch and O-H stretch.

The figure.2 depicts the presence of alcohols (O-H) stretch in the peak of 3387 cm^{-1}, the esters of the carboxylic acids are shown as peaks from 2952 cm^{-1} to 2843 cm^{-1} the peak at the wavelength of 2169 cm^{-1} shows the presence of the double bond between two carbon molecules. The peak in the region of 1207 cm^{-1} to 1648 cm^{-1} shows the presence of the double bond between carbon and oxygen which can be of esters of carboxylic acid.

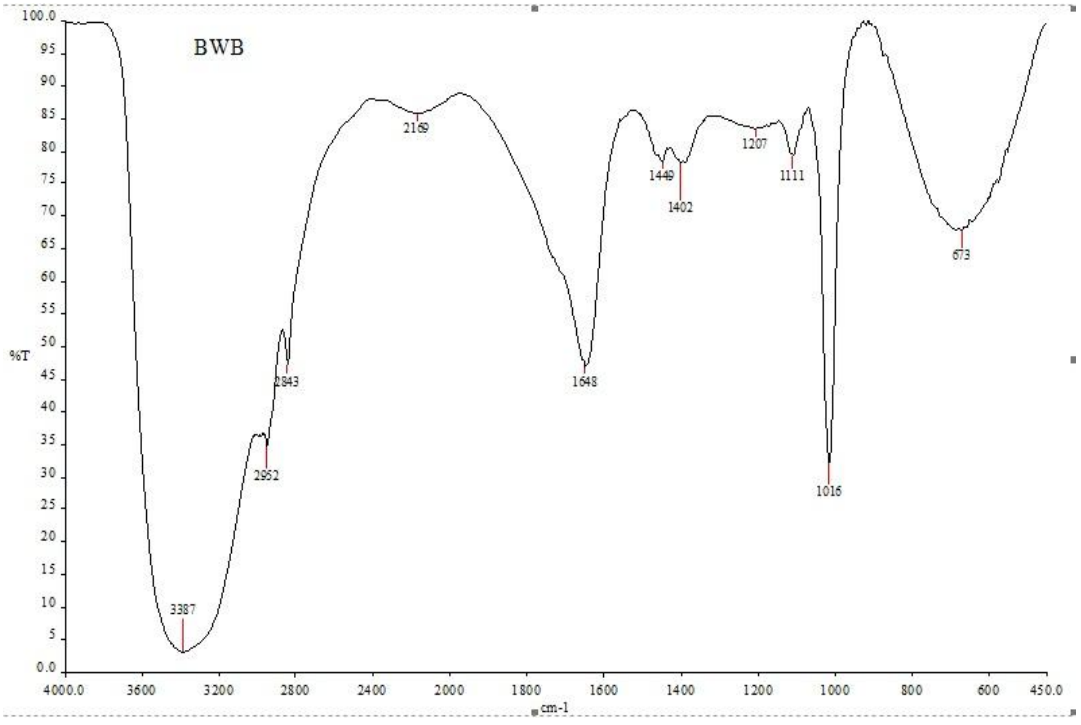

Figure 2. Fourier transformed infra-red spectra

4.3. Properties of biodiesel

The properties of the biodiesel are studied with respect to diesel. BWB showed higher kinematic viscosity at 40^{o}C, which was determined as 3.5 mm^2/s whereas for straight diesel, the kinematic viscosity was noticed to be 2.6 mm^2/s. Similarly Cetane number of the BWB was found to be 48 whereas the value for the straight diesel lies between 46 and 53. The calorific value of BWB, is found to be 38.5MJ/Kg which is found to be lesser than straight petro-diesel. Density of the BWB is found to be 880 Kg/m^3 which was higher by 2.3% than straight .

Table 6:Physio- chemical properties of biodiesel

Property	**Diesel**	**BWB**
kinematic viscosity at 40^{o} C (mm^2/s)	2.6	3.5
Cetane number	51	48
Calorific value(MJ/Kg)	45.5	38.5
Density(Kg/m^3)	860	880