

Research Article



A Novel Gum Arabic Coated Magnetic Fe₃O₄ Nanoparticle Catalysed Trans-Esterification of *Eichhornia Crassipes* Oils for the Production of Biodiesel

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Accepted on: 10-04-2015; Finalized on: 31-05-2015.

ABSTRACT

The present research work is about the elicitation and characterisation of lipids from *eichhorniacrassipes*. The plant oil was extracted using soxhlet extraction method. The physical properties (viscosity and density) of the oil were calculated and found as 0.902 g/ml and 0.90 g/cm³ respectively and also the chemical parameters like acid value, saponification value and free fatty acid of the bio-oil showed that, it can be used for the biodiesel production. The bio-oil was characterised using GC-MS (Gas Chromatography coupled with Mass Spectroscopy). Based on the GC-MS Spectral data, totally eight fatty acids were identified using GC-MS NIST library. These fatty acids can be converted into FAME using Gum arabic coated magnetic Fe₃O₄ nanoparticles. The produced bio-diesel samples were characterised using GC-MS spectral data.

Keywords: Biodiesel, Non-renewable resources, vegetable and animal oils, water hyacinth (*Eichhornia crassipes*), lipids.

INTRODUCTION

The fossil fuels are being used inaccurately by humankind and this type of energy cannot be supplied to all energy requirements. Fossil fuels mainly emit CO₂ which will rapidly increase the global climate¹. Hence the people begin to search for new alternative energy sources and technologies to save the world climate. Increasing demand of global energy consumption, mainly used by heavy industries, is the reason for the search of new sources to supply energy demand for the whole world. The importance of using alternative energy sources is increasing globally. Using renewable energies in different sectors, it is possible to decrease greenhouse gas emissions prevent environmental pollution and increase the domestic production for energy and energy systems. Instead of petrol, new and renewable energy resources such as biodiesel produced from natural resources could be considered^{2,3}.

Water Hyacinth (*Eichhornia crassipes*) is a monocotyledonous freshwater aquatic plant belonging to the family Pontedericeae related to the lily family (Liliaceae). In the developing countries, it is used in traditional medicine and even used to remove toxic elements from polluted water bodies. Since water hyacinth is an aquatic plant, it do not compete with land resources used in arable food crop cultivation and thus are an incentive factor when it comes to biofuel production⁴. Due to the presence of low lignin content in water hyacinth, the cellulose and hemicellulose in them are more easily converted to fermentable sugar thus resulting in enormous amount of utilizable biomass for the biofuel industry⁵. A new method of extracting ethanol by saccharification with diluted sulphuric acid, and hastening the process by yeast⁶. The structural and

chemical properties of grasses in relation to biofuels conversion and observed that carbohydrate bioconversion is limited by associated lignins and low molecular weight phenolic acids⁷.

Singh⁸ reported that the daily average productivity of water hyacinth was 0.26 ton of dry biomass per hectare in all seasons. It is considered to be an effective biomass, especially for use as a raw material for bio-ethanol production. However, only a few reports exist on the production of bioethanol from water hyacinth⁹. Nigam used a process involving saccharification of the water hyacinth biomass with diluted sulphuric acid for 7h, followed by heating at 100 C for 15 min. In a study by Mishima, the water hyacinth was hydrolyzed with 1 % (w/v) NaOH at room temperature for 12 h, followed by the slow addition of 31 % (w/v) H₂O₂ so that the final concentration of H₂O₂ reached 1 % (w/v); the reaction was then allowed to continue for another 12 hour¹⁰. The pre-treated water hyacinth was autoclaved at 121°C for 20 hours, followed by saccharification with an enzyme for 96 hour. Mishima also found that bioethanol generating capacity of water hyacinth can be compared to that obtained from agricultural waste, thus is a potential new crop for biofuel production and an employment generating industry¹¹. In the present study, the bio-oil obtained from the leaves of water hyacinth was characterized using GC-MS. Based on the spectral results, the bio-oils will be converted further into bio-diesel using a novel enzymatic catalyst.

MATERIALS AND METHODS

Preparation of Water Hyacinth

The water hyacinth (10 kg) was collected from the Arani Lake which is 54 km away from VIT University. The raw materials were dried for a period of three days and then



the roots have been cut into small pieces and leaves are separated which are kept in oven for about three hours at 60°C. These dried leaves are made into powder with the help of grinder. Nearly 500 grams of the powder was collected and stored.

Extraction and Quantification of Bio-oil

The Bio-oil was extracted by Soxhlet extractor using hexane as solvent. Bio-oil from the solvent mixture was separated and dried in a rotary evaporator in order to remove the excess solvent. The oil content was measured gravimetrically and expressed in percentage by using following formula:

$$\text{Oil Content \%} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Weight of the sample in gram}} \times 100$$

Characterization of Bio-oil using Analytical Methods

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250 μ I.D., 0.25 μ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2 minutes, then ramp at 20°C per minute to 300°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode). A JEOL GC Mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-20001 software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan. Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. The free fatty acid content (Acid number or Acid value) in the oil was estimated by Cox and Pearson (1962) method. The degree of unsaturation of lipid (Iodine value) and the average molecular weight of the triacylglycerols (saponification value) were calculated according to William Horowitz (ed) (1975).

Catalyst Preparation

Synthesis of Magnetic Nanoparticles coated with Gum Arabic

Magnetic Fe₃O₄ nanoparticles were prepared by co-precipitation method. Take the round bottom flask 2.33g of FeCl₃·6H₂O and 0.86 g of FeCl₂·4H₂O were dissolved in 100 ml deionized water under nitrogen and 10 ml 25% NH₃·H₂O was added to the solution drop wise under vigorous stirring at 70°C. After the colour bulk solution turned to black, and then the magnetite precipitates were washed several times with deionized water and once with ethanol and separated by magnetic decantation. For

surface coating with gum arabic, magnetic nanoparticles were suspended in 25 ml distilled water containing about 100 mg of gum arabic powder, under mechanical stirring, and the mixture was ultra-sonicated for 30 minutes. This method allowed the gum arabic to mix thoroughly in the sample. After sonication, the sample was washed several times with water to remove untreated polymer. The solid product was isolated by magnet separation and dried under vacuum.

Lipase Preparation

Lipase was incubated with surfactants CTAB (cationic) which coat the hydrophobic pocket of the lipase and stabilize its open form. It is worth mentioning that the coexisting of surfactant molecules in the reaction surfactant solution does not affect enzymatic Trans-esterification in organic media. Surfactant lipase was prepared using cationic (CTAB) Lipase (1 g), suspended in 100 ml of 1mM phosphate buffer (pH 7), and 0.1% CTAB were added to reaction vessel and mixed thoroughly. Each sample mixture was sonicated in an ultrasonic bath for 20 min followed by incubation for 24 h at 4°C. After incubation, precipitates were collected by centrifugation at 250 rpm (4°C), washed with 1mM phosphate buffer solution (pH 7) and dried under reduced pressure. A white powder was obtained and the yield was about 15%.

Lipase Immobilization

Gum Arabic coated magnetic nanoparticles (100 mg) were suspended in 2 ml of 25% (w/v) glutaraldehyde solution and were incubated at 20°C for about 2 hours. Particles were washed several time with water and dried. For lipase immobilization, 200 mg of surfactant coated lipase and 100 mg support material were mixed with 50 ml of 1mM phosphate buffer (pH-7). The mixture was incubated at 20°C for about 3 hours for immobilization. After reaction, immobilized lipase was recovered by a magnet and washed several time with distilled water. The immobilized lipase was dried overnight under vacuum by vacuum freeze drier.

Trans-esterification of Water Hyacinth Oil

The trans-esterification of eichhornia crassipes oil was carried out using magnetic nanoparticles immobilized lipase enzyme as a biocatalyst. The Lipid and Methanol was mixed with a molar ratio of (1:10) and then the reaction was stirred at 90°C for about 5 hours. The completion of the reaction was monitored using TLC.

RESULTS AND DISCUSSION

The bio-oil content in the *eichhornia crassipes* was found to be 65%. The quality of the bio-oil was analysed using various analytical methods. The free fatty acid content was found as 45.3 mg KOH/g. The iodine value and saponification value was also calculated and found as 98.4 mg I₂/g and 170.4 mg/g respectively. The density and viscosity of the oil sample was 0.902 g/ml and 0.90 g/cm³ respectively. Similarly, saponifiable lipids and free fatty acids can be used for the conversion of biodiesel by



trans-esterification process and so it is vital to find out the type of fatty acids present in the bio-oil using GC-MS.

Gas Chromatography and Mass Spectrometry (GCMS) analysis has been performed on the extracted sample. The spectrum of the sample spread from 450 to 850 m/z values and the relative abundance of the resulted components varies from 10000000 to 110000000. Figure 1 and Table 1 shows the fatty acids content present in the oil of eichhornia crassipes and it was found that there are about 34.82% of 9-Octadecenoic acid and 23.9% of 8-

octadecenoic acid, methyl esters. The mass spectroscopy of different obtained fatty acids and fatty acid methyl esters have been shown. From the graphs we can find that the chemical and bonding structure of the fatty acids and fatty acid –methyl (or) ethyl esters. This shows that the obtained sample of eichhornia crassipes consist of plenty of fatty acids, which are the important features in the production of bio diesel. Based on the GC-MS spectrum of the different obtained constituents and their chemical structures were identified.

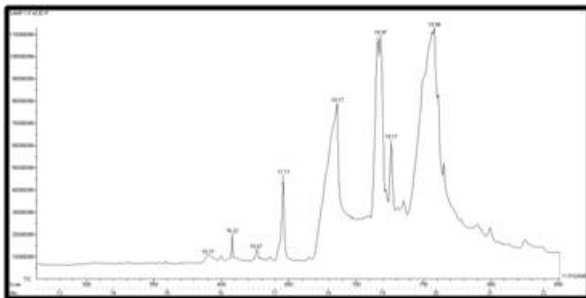


Figure 1: Fatty acid profile of eichhornia crassipes

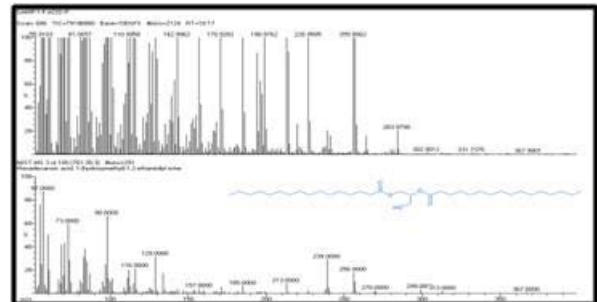


Figure 2: GCMS of hexadecanoic acid

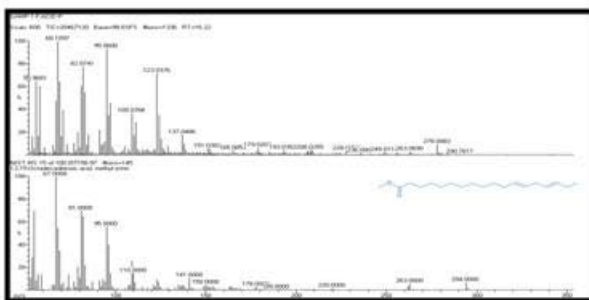


Figure 3: GCMS of Octadenoic acid methyl ester

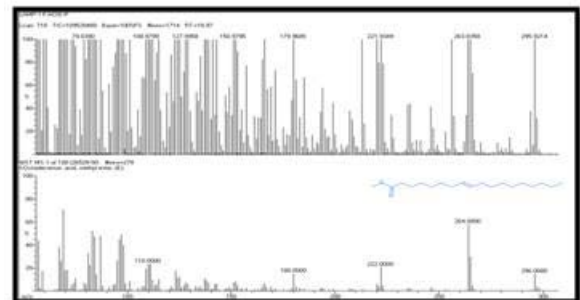


Figure 4: GCMS of Octadecanoic acid methyl ester

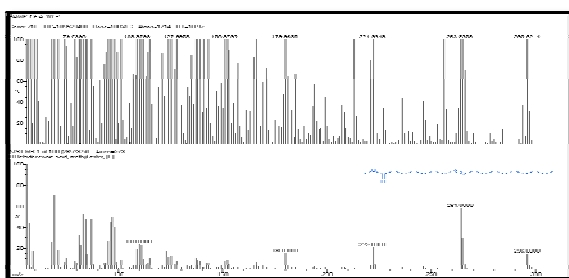


Figure 5: GCMS spectra of Heptadecanoic acid methyl ester

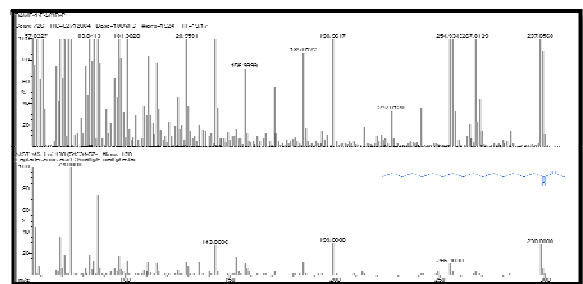


Figure 6: GCMS spectra of 9-Hexadecenoic acid methyl ester

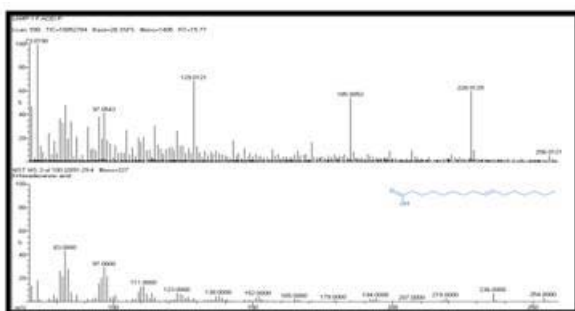


Figure 7: GC-MS Spectra of Cyclopropaneoctanoic acid

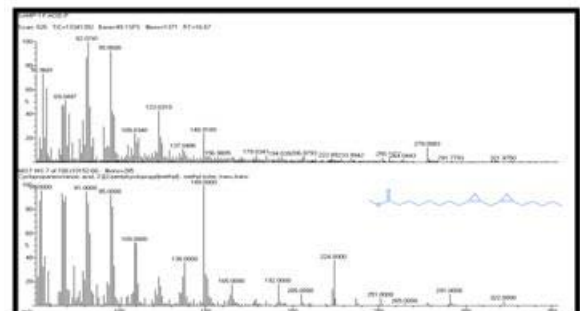


Figure 8: GCMS spectra of pentadecanoic acid methyl ester

Table 1: Represents the lipids present in eichhornia crassipes

Peak No.	RT (Min)	Compound Name	Peak area (%)	Peak Area
1	15.77	9-Hexadecenoic acid	1.46	1652175.24
2	16.22	12,15-Octacecadienoic acid	1.95	2206672.42
3	16.67	Cyclopropaneoctanic acid	1.23	1391901.06
4	17.17	Pentadecanoic acid	9.28	10501497.45
5	18.17	Hexadecanoic acid	22.41	25359758.38
6	18.97	8-Octadecenoic acid, methyl ester	23.9	27045882.43
7	19.17	Heptadecanoic acid	4.95	5601553.06
8	19.98	9-Octadecenoic acid	34.82	39403247.96

CONCLUSION

Eichhornia crassipes were spreading on the surface of the water bodies. These plants are considered to be as a waste material in the environment. We had used eichhornia crassipes for the production of biodiesel using enzymatic trans-esterification. GC-MS spectral data clearly confirm the presence of eight fatty acids.

These fatty acids are only responsible for the production of bio-diesel. The extracted lipids were converted into biodiesel using a novel Gum arabic coated magnetic Fe₃O₄ nanoparticles. The percentage yield of the produced biodiesel was calculated 86.74%.

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Source of Support: Nil, **Conflict of Interest:** None.

