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EXTRACTION OF LIPIDS FROM MUNICIPAL SEWAGE SLUDGE FOR PRODUCTION OF BIODIESEL VIA TRANSESTERIFICATION PROCESS

BHARATHI.P*, KARTHIGA.R, SHAMLI.M , VIVETHA.K AND SUBATHRA.M

Department of Biotechnology, Karpaga Vinayaga College of Engg & Technology.

ABSTRACT

Biodiesel also called as fatty acid methyl esters (FAME) has become more attractive because of its environmental benefits. Biodiesel is the most promising renewable, biodegradable, alternative fuel is produced from various lipids sources. Here the municipal secondary sewage sludge is taken as potential sources of lipids. The potential of using sludge from municipal waste water treatment plant as feedstock for lipid is investigated because of disposal problems occurring due to increase of urbanization and industrialization. The secondary or activated sludge composed of suspended solids and microbial cells containing phospholipids produced during aerobic biological treatment. In this study, analyses were done on lipid extraction and biodiesel production from dried secondary sludge. A maximum of lipids was extracted using hexane and methanol as solvent from secondary sludge. FAME is obtained from triglycerides by acid – catalyzed transesterification using sulfuric acid. Once the biodiesel is produced it is analyzed by gas chromatography – mass spectrometry(GC-MS). GC-MS method is used for determination of fatty acid, esters,alcohol, mono,di,tri glycerides obtained. The analysis of FAME revealed fatty acids composition with predominance of palmitic acid, steric acid, oleic acid and linoleic acid.

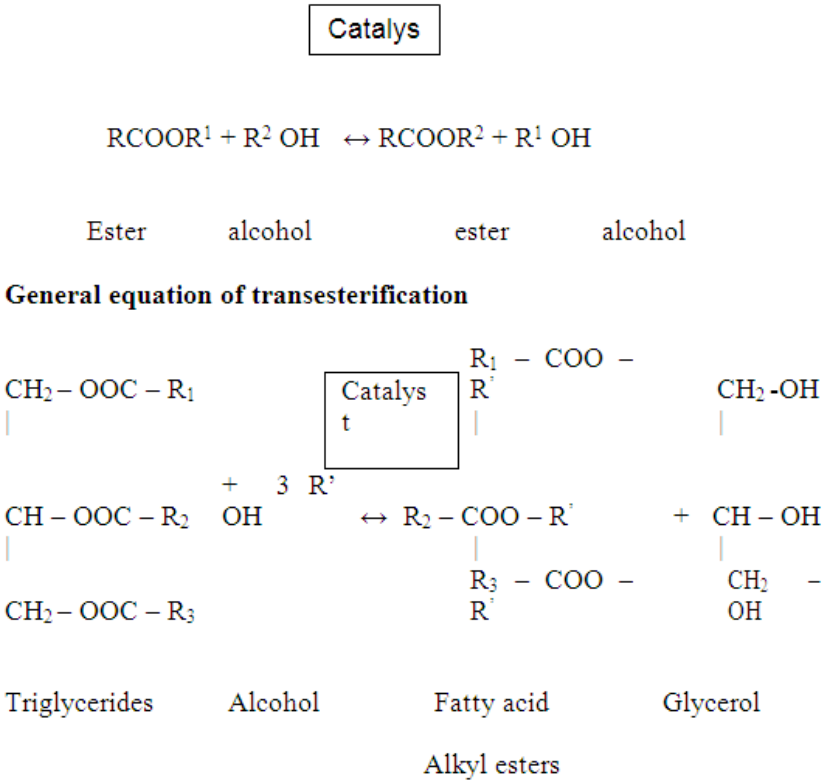
KEY WORDS: Biodiesel, Secondary Sludge, acid- catalysed transesterification, GC-MS.

BHARATHI.P
Department of Biotechnology,
Karpaga Vinayaga College of Engg & Technology

***Corresponding author**

INTRODUCTION

With the rapid development of the society, the over –consumption of the energy has lead to energy crisis."Sustainable development" has been the leading concept for our country and even the world, which also asked us to find renewable and environment-friend energy[1].Diesel fuels have an essential function in the industrial economy of a developing country and used for transport of industrial and agricultural goods and operation of diesel tractor and pump sets in agricultural sector. Economic growth is always accompanied by commensurate increase in the transport. The high energy demand in the industrialized world as well as in the domestic sector and pollution problems caused due to the widespread use of fossil fuels make it increasingly necessary to develop the renewable energy sources of limitless duration and smaller environmental impact than the traditional one. This has stimulated recent interest in alternative sources for petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available[2].Sewage sludge is the waste formed during treatment of waste water. These waste water treatment plant produces primary and secondary sludge. This sludge consist a variety of organic matter and microorganisms. The primary sludge is a combination of floating grease and solids collected at the bottom of the primary settler after screening and grit removal. The secondary or activated sludge is composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment and collected in the secondary settler. A portion of the collected secondary is recycled back to the aeration basins to maintain a sufficient concentration of microorganism. Lipids could yield an unexploited source of cheap and readily available feedstock for biodiesel production[3]. According to complex sources of sewage the lipids in that exist in several forms which would make the extraction not easier. But lipids are generally soluble in organic solvents, so we can use solvents to extract lipids from sludge. Some solvents such as ethanol, ethyl ether, petroleum ether, hexane and methanol have been used for extraction of lipids. In which hexane and methanol are widely used[1]. Transesterification, also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis. This process has been widely used to reduce the viscosity of triglycerides, thereby enhancing the physical properties of renewable fuels to improve engine performance. Thus fatty acid methyl esters obtained by transesterification can be used as an alternative fuel for diesel engines.



3. MATERIALS AND METHDOLOGY

3.1 *CHEMICALS*

Hexane, sodium chloride, Magnesium sulphate monohydrate, sulphuric acid, sodium bicarbonate, anhydrous sodium sulphate and methanol were purchased from RANKEM private limited. Analytical grade chemicals were used in this study.

3.2 *SEWAGE SLUDGE*

There are two types of sludge, primary and secondary sludge obtain from Municipal Waste Water Treatment Plant. Here Secondary Sludge was chosen for the production of biodiesel. It is also called as Activated Sludge. Activated sludge is the solid or semisolid produce during biological treatment of industrial and municipal waste waters.

3.3 *COLLECTION AND HANDLING OF SLUDGE*

Secondary Sewage Sludge was collected from a Municipal Waste Water Treatment Plant located in Koyambedu, Chennai, Tamilnadu. Secondary sludge was collected from the aerobic waste sludge line which fed into the anaerobic digester. The collected sludge were stored icepack after it was immediately delivered to laboratory and stored at 4°C prior to use and maximum storage time 4 days. The sludge samples were taken every 2-3 weeks.

3.4 *SAMPLE PREPARATION*

Upon collection the sludge flocks were allowed to undergo gravity settling. The sludge was dewatered by centrifugation and filtration. Centrifugation was performed with Cooling Centrifuge, C24 REM I at 8000 rpm for five minutes. The sludge was filtered using whatman filter paper. The solids obtained from sludge were removed and dried. The dried sludge was separately taken in another vessel. Then the dried sludge was transferred to mortar and pestle, and then it was grounded well. This process is called as homogenization. Thus, the sample was prepared for lipid extraction [4].

3.5 *EXTRACTION OF LIPIDS*

Secondary sludge was disintegrated under room temperature. 200ml of sludge sample

was placed in 250ml conical flask. The sludge sample was disintegrated at a room temperature for the time of 10 minutes. From that 20g of dried sludge was taken and 0.3ml of fuming Hydrochloric acid was added. Then the sample was dried and 25g of magnesium sulfate monohydrate was added. Then leave it for solidified and stored in desiccators at room temperature for overnight. Next day, the sample was ground under mortar and pestle until the sample was homogeneous as possible. Then 200ml of hexane was added and heated for sometimes and finally lipid was extracted[5].

3.6 *CONFIRMATION OF LIPIDS BY TLC*

The glass slide was cleaned with ethanol and coated with aqueous slurry of silica gel. Heated at 110°C for 1 hour and it was cooled for sometimes and then sprayed the lipid sample over it. After sometimes the sulfuric acid was sprayed and then heated for 10 minutes. Appearance of brown color spots indicates the presence of lipids.

3.7 *TRANSESTERIFICATION*

Transesterification is also called as Alcoholysis, defined as the reaction of fat or oil with an alcohol to form ester and glycerol. A catalyst is usually used to increase the reaction rate and yield. Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the product side. There are three types of transesterification process, they are Acid, Alkali and Enzyme catalyzed reactions. Commonly used alkali catalysts are sodium hydroxide, potassium hydroxide, carbonates and alkoxides. Acid catalysts such as sulfuric acid, sulfonic acid, hydrochloric acid were used. Lipases are used as biocatalysts.The conversion of lipids extracted from sludge to FAME (biodiesel) was carried out through acid catalyzed transesterification method. The lipid sample up to 50mg was placed in a vial and dissolved in 1ml of hexane. After that 2ml of 1% sulfuric acid in methanol was added to the vial. The vial was capped and heated overnight at 50°C[3].

3.8 *RECOVERY OF FAME (BIODIESEL)*

To recover the FAME 5ml of 5% sodium chloride in water was added and the FAME was extracted 2 times with 5 ml of hexane using a vortex to provide efficient mixing in the vial. Then the hexane phase was washed and biodiesel was recovered[3].

3.9 *ANALYSIS OF BIODIESEL*

Biodiesel produced can be analyzed by Gas chromatography- Mass spectrometry. In GC, the mixture is separated mainly by the boiling point and the structure *of* the individual compounds. To carry out a GC analysis, the sample is usually dissolved in low concentrations in an organic solvent and then injected into the gas chromatograph. In some cases, a sample needs to be derivatized with a specific reagent in order to obtain a useful gas chromatogram. This is the case for biodiesel. Glycerol and the mono- and diacylglycerols contain free hydroxyl groups, causing these materials not to perform well in GC. Derivatization improves their performance considerably. Derivatization can provide better resolution between compounds with similar properties.In contrast to chromatographic methods, spectroscopic methods analyze the intact sample at the same time, i.e., impurities contribute to the results and must be taken into consideration. However, when coupling spectroscopic methods to chromatographic methods, uncertainties remaining in the use of the latter are eliminated. The reason is that the (pure) compounds have obtained unique spectra. This information is related directly to the structure of the compound. Note that chromatographic methods only detect if a compound is eluting, not its identity or structure. The identity or structure needs to be established through the use of standards as far as possible. Thus, the spectroscopic method of detection in combination with a chromatographic method yields more detailed information aboutbiodiesel analysis[6].

4. RESULTS AND DISCUSSION

Figure1 ***DRIED SLUDGE***

Figure 2 ***EXTRACTION OF LIPIDS***

Dried sludge was shown in fig (1).The lipids were extracted from the sewage sludge by using solvents like hexane, methanol, etc., from the experimental results it can be seen that the amount of extracted lipid from the sludge sample depends on the solvent used for a extraction. Hexane was used for the extraction process .The extraction process consist of many cycles, 20 gram of sludge was taken and 200ml of hexane was added and lipid was extracted. After the completion of cycles for extraction then the remaining sludge was removed and the solvent containing lipid was alone taken into another vessel. Then the solvent containing lipid mixture was heated for sometimes for the evaporation of solvent and lipid was obtained. Once the lipid was extracted (fig 2) then it should be confirmed. For the confirmation of lipids Thin Layer Chromatography was performed. (fig 3).

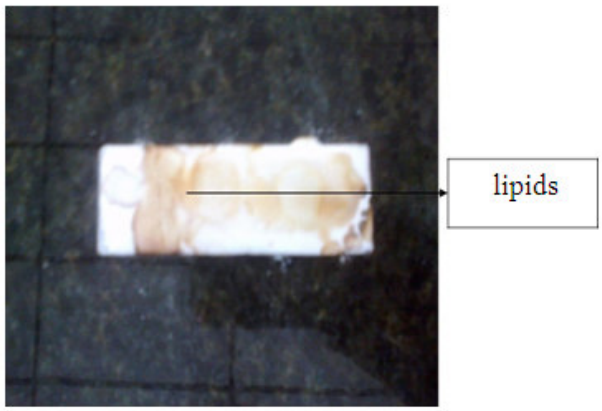


Figure 3 ***CONFIRMATION OF LIPIDS***

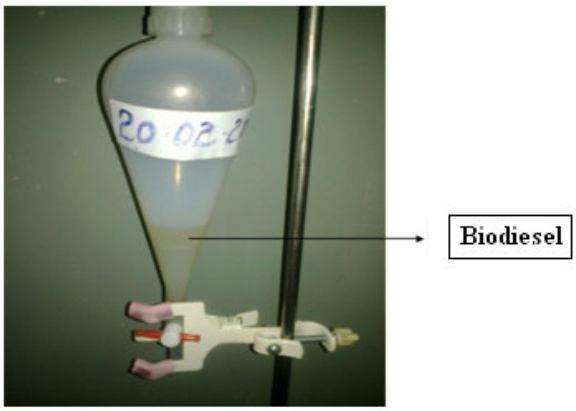


Figure 4 ***RECOVERY OF BIODIESEL***

The lipids extracted from the sludge were converted to Fatty Acid Methyl Esters (Biodiesel). This process was carried out through acid catalyzed transesterification process, where the lipids were added with an alcohol, solvent and then heated and Fatty Acid Methyl Esters (Biodiesel) was produced.(fig 4).The FAME produced by transesterification was analyzed by using an Agilent Gas Chromatograph JEOL - JMS gc mat ell with a flame ionization detector. The separation was achieved in a HP-MS capillary column with Helium as carrier gas and injector temperature of 220°C. The injection volume of sample was 1µl and the oven temperature was 50- 250°C at a rate of10°C/min.(fig5).

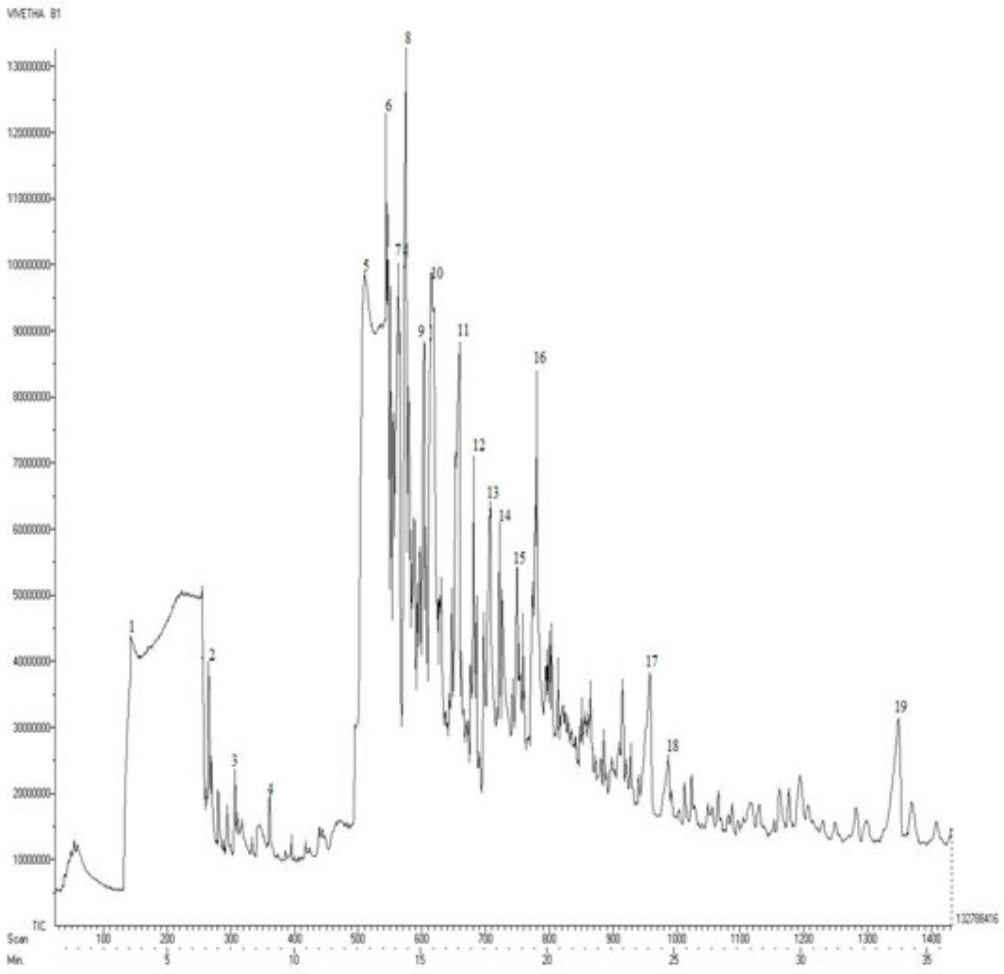


Figure 5
GC-MS CHROMATOGRAM FOR BIODIESEL

The compounds identified from the peak of chromatogram are 1,6-pentalenedione,hexahydro-6a-(2-propynyl), pentetic acid, 8,11,14-Eicosatrienoic acid, hexadecanoic acid- octadecyl ester, hexadecane,1,1-bis (dodecyloxy), 9,12,15- octadecatrienoic acid- 2,3- dihydroxy propyl ester, stigma stanol, hexadecanoic acid- 1,1- dimethylethyl ester, cyclopentane aceticacid-3-oxo-2-(2-pentynyl)-methyl ester, 9-octadecenoic acid- 2,3- dihydroxy propyl ester, 1,2-cyclo heptanediol, 6-hydroxy-8- methoxy octanoic acid- methyl ester, nonanoic acid – 9-chloro-9-oxomethyl ester, pterin-6- carboxylic acid, 5,8,11-heptadecatrien-1-ol, cyclopropane butanoic acid-methyl ester. The peak area values are calculated and given in table no, 1.

TABLE 1
PEAK AREA VALUES

Peak no	Retention time	Area
1	3.58	178235384
2	6.65	44448224
3	7.67	17177096
4	9.03	22358872
5	12.8	442252052
6	13.65	351989248
7	14.13	204805328
8	14.43	557156784
9	15.13	176755824
10	15.42	432297008
11	16.55	381230656
12	17.1	251341768
13	17.75	263569128
14	18.13	103454736
15	18.83	149076680
16	19.58	327965368
17	24.05	133945624
18	24.78	68292872
19	33.9	113444608

TABLE 2
FORMULA, MOLECULAR WEIGHT, AND MELTING POINTOF FATTY ACIDS AND THEIR METHYL ESTERS

Fatty acid methyl ester	Formula	Acronym	Molecular weight	Melting point (°c)
Palmitic acid Methyl Palmitate	C16H32O2 C17H34O2	C16:0	256.428 270.457	63-64 30.5
Stearic acid Methyl Stearate	C18H36O2 C19H38O2	C18:0	284.484 298.511	70 39
Oleic acid Methyl oleate	C18H34O2 C19H36O2	C18:1	282.465 296.495	16 -20
Linoleic acid Methyl Linoleate	C18H32O2 C19H34O2	C18:2	280.450 294.479	-5 -35
Linolenic acid Methyl Linolenate	C18H30O2 C19H32O2	C18:3	278.434 292.463	-11 -52/-57

The compounds present in biodiesel and their characteristics, molecular weight, common name, melting point etc., the peak area values were also given for the respective peak with the retention time. The respective compound name for the retention time was also given.(Table 2). It was discussed from the above experimental results that the amount of extracted lipids depends on the solvent used to extract lipids from the sewage sludge. Comparison of sludge fatty acid profiles by various extraction methods with the profile of a standard soybean sample shows that all sewage sludge samples have a much larger concentration of saturated fatty acids. Although the higher levels of saturated fatty acids may present a problem in cold weather owing to gelling, the higher saturated content will produce a better burning biodiesel[7]. The amounts of lipids extracted from the secondary sewage sludge sample for first time were significantly higher than that those of the lipid extracted from sample for second and third time. The results showed that Municipal Secondary Sludge produced during waste water treatment is a potential source of suitable lipids for biodiesel production. The Gas Chromatography – Mass Spectrometry analysis of produced biodiesel indicated a similarity between the fatty acids- esters composition. It was observed that majority of fatty acids- esters found in sludge are same as hexadecanoic acid, octanoic acid- methyl ester, Eicosatrienoic acid etc., From the results of Gas Chromatography- Mass Spectrometry the peaks were obtained from that the area was calculated **.**Fatty acids composition of secondary sludge was compared with common biodiesel feedstock such as vegetable oil (soybean) and animal fat (lard). From that it is observed that majority of fatty acids in sludge are same as fatty acids of vegetable oil and animal fat such as palmitic acid, oleic acid, linoleic acid. However the notable difference is in observed is the high

amount of linoleic acid present in biodiesel of vegetable oil. Several reviews on the production, analysis, and use of biodiesel in internal combustion engines are available in the literature and the reader is encouraged to read them for a more comprehensive view of the subject[2, 8, 9,10,11,12,13,14,15]. The sewage sludge will improved oxidation stability of biodiesel than vegetable oil. Some of the compounds detected but not identified or not quantified were presented as total. The results indicated that secondary sewage sludge is a potential candidate as raw material for the production of biodiesel.

5. SUMMARY AND CONCLUSION

Biodiesel is a fuel compressed of alkyl esters of long chain fatty acids. Now the world is witnessing an unprecedented increase in interest and demand for biodiesel and other fuels derived from renewable biomass due to rising petroleum fuel costs, increasing concern for the environmental impact of emissions from combustion of fossil fuels. However pure vegetables oils are expensive as a result use of municipal sewage sludge gaining traction around the world. There are numerous challenges faced by biodiesel production and they are determining how best to collect the sludge and treat them for maximum lipid extraction and another one is transesterification, choice and amount of solvent used. Based on this the amount of biodiesel obtained can be calculated. The secondary sewage sludge is used for biodiesel production through acid- catalyzed transesterification reaction. The Acid-catalyzed Transesterification was chosen to reduce soap formation and glycerol content. Finally the biodiesel was recovered and analyzed by Gas Chromatography- Mass Spectrometry (GC-MS). From the analytical report of GC-MS for biodiesel the compounds like 6-hydroxy-8- methoxy octanoic acid-methyl ester, 9,12,15- octadecatrienoic acid-2,3- dihydroxy propyl ester [Z,Z,Z], cyclopentane aceticacid-3-oxo-2-(2-pentynyl)-methyl ester, cyclopropane butanoic acid-methyl ester, hexadecanoic acid- octadecyl ester, hexadecanoic acid- 1,1-dimethylethyl ester were present which shows the sample produced is biodiesel. From this it is concluded that Municipal Sewage Sludge is better and cheap feedstock for biodiesel. The biodiesel can be used most effectively as a supplement to other energy forms particularly in mining and marine areas where lower pollution level is important.

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