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RESEARCH ARTICLE

METHYL ESTER (BIODIESEL) PRODUCTION FROM MICRO ALGAE AND LINSEED MIXING OIL

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Abstract

This paper discusses a detailed study on the production of methyl ester using a mixture consist of Schizochytrium micro algae oil and linseed oil in different volume percentage say 15:85ml through two-step trans-esterification process. The effects of molar ratio of methanol to oil (3:1-9:1), wt% of catalyst (0.5-2wt%), reaction temperature (40-70°C), and reaction time (30-120 min) were studied on the percentage yield of oil extracted. The methyl esters with best yield (90.99%) and quality was produced in case of a mixture consist of Schizochytrium micro algae oil and linseed oil (Mixed methyl ester) (MME), at 6:1 mole ratio, 1.5wt% of catalyst (NaOH), Temperature 60^0C and reaction time 120 min. It was noted that increase or decrease the concentration of (NaOH) or methanol ratio than the optimal values, the reaction either did not fully occur or lead to soap formation, that will be causes reduced the yield of methyl ester.The quality of the methyl ester produced was analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). The study also includes examination of physical and chemical properties such as pH value, viscosity, density, flash point, fire point and acid values on the produced methyl ester as well as on the conventional diesel for comparison. The study revealed that the properties of the methyl esters were very close to the conventional diesel.

Introduction:-

Scarcity of fossil fuel and adverse impact of emission from petroleum diesel on environment has created a need for alternative fuel being renewable and having similar characteristics to that of fossil fuel. Biodiesel is one of the renewable alternative fuels having characteristics similar to diesel. Biodiesel, with the advantage of being a potential renewable source of energy, is non-toxic, eco-friendly (leading to lower CO_2, CO, SO_2 emissions compared to petroleum diesel), easy to store and transport and has better lubricity properties [1]. Biodiesel is superior to conventional petroleum-based diesel in terms of its sulphur and aromatic content, and flash point. However, the high cost of biodiesel in compared to petroleum-based diesel, is a major barrier to its commercialization. It costs approximately 1.5 times higher than petroleum-based diesel depending on sources of feedstock oils [2]. It is reported that approximately 70-95% of the total biodiesel production cost arises from the cost of raw materials (e.g. virgin vegetable oil) [3].

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Introduction Biodiesel is defined as a fuel composed of alkyl esters of long-chain fatty acids, mainly present in vegetable oils as triglycerides. Therefore, vegetable oils are normally used as the main reactant for biodiesel preparation via a transesterification process with short-chain alcohols. Transesterification is considered to be the best choice as the physical characteristics of fatty acid and esters are very close to those of petroleum-based diesel and the process is relatively simple. Besides, it also tends to lower the viscosity of the biodiesel and produces glycerol as a by-product which has commercial value as well. The transesterification is a chemical process involving a number of consecutive reversible reactions between a triglyceride (fat/oil) and an alcohol that form esters and glycerol [4]. The triglyceride is converted stepwise to diglyceride, monoglyceride, and finally glycerol whereby 1 mol of alkyl esters is removed in each step. The formation of alkyl esters from monoglycerides is believed to be the step that determines the reaction rate since monoglycerides are the most stable intermediate compound [5]. A catalyst is usually used to enhance the reaction rate and biodiesel yield. Since the reaction is reversible, excess alcohol is required to shift the equilibrium to the product side. Among the alcohols that can be used in the transesterification reaction are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most often as methanol is inexpensive and ethanol is derived from agricultural products and is renewable and environment-friendly.

The Energy security has become a serious global issue and a lot of research is being carried out to look for economically viable and environment-friendly alternatives. The only solution that appears to meet futuristic needs is the use of renewable energy. The prospects of producing carbon-neutral biofuels from microalgae appear because of their unique features such as suitability of growing in open ponds required for production of a commodity product, high CO_2-sequestering capability, and ability to grow in wastewater/seawater/brackish water and high-lipid productivity.

Growing bio-diesel could be a sustainable process, using the energy of the sun and waste carbondioxide to produce useful lipids that can be processed into bio-diesel fuel. This could be a useful natural solar panel transforming sunlight into the chemical energy of oil. Our reliance on fossil fuels has caused carbon dioxide enrichment of the atmosphere, and is the primary contributor to the generally-accepted phenomenon called global warming. Because using coal produces even greater CO_2 emissions than oil, the depletion of oil will be unlikely to improve this pattern of CO_2 enrichment. In order to realize a stable energy alternative that will meet world demand while mitigating climate change, it is necessary to develop renewable clean fuels [6]. In the conventional trans-esterification process, linseed oil, micro algae oil methanol and KOH in various concentrations were refluxed together in a 500 ml round bottom flask equipped with magnetic stirrer and water condenser. After the complete conversion of the oil, the reaction was stopped and the mixture was allowed to stand for phase separation: the ester mixture formed the upper layer and glycerine formed the lower layer. The residual catalyst and unreacted alcohol were distributed between the two phases. After phase separation, using a separating funnel, the ester mixture was dried over anhydrous sodium sulfate. This study focuses on the production of Methyl ester (biodiesel) from a mixture of third and second generation feed stocks (mixture of microalgae oil and linseed oil) via transesterification process investigating the effects of process parameters, for example (i) molar ratio of feedstock to methanol (ii) catalyst concentrations (iii) reaction temperature and (iv) reaction period on the yield of methyl ester.

Materials and Methods:-

Collection of Micro Algae:

Microalgae was purchased from ETA, Kolathur, Chennai. The species of micro algae chosen was Schizochytrium sp.

Lipid Extraction:

Soxhlet method is used to extract the lipid from micro algae. The organic solvents n-hexane was used. A mass of 20gm of dry microalgae was extracted with 150 mL of solvent. This type of extraction is based on the evaporation, condensation and percolation of the solvent through the microalgae during 4 hours [7] .After that, solvent was removed and lipids recovered by water bathing process. Figure 1 shows the soxhlet apparatus for lipid extraction. The total lipid content of Schizochytrium microalgae was obtained to be 0.1015 g/g biomass.

Figure 1:- Soxhlet Apparatus.

Mixing of Microalgae Oil and Linseed Oil:

Microalgae oil and linseed oil are mixed thoroughly in different ratio (15:85) and checked the viscosity using redwood viscometer and density by density meter. The mixing ratio, observed value of viscosity and density are presented in the Table-1.

Table 1:- Viscosity of oil at Different Mixing Ratio.

Mixing Ratio	**Viscosity (at 50^0C)**	**Density(kg/m^3)**
Micro algae oil	9.18 mm^2 /s	930
Linseed oil	18.94 mm^2 /s	929
15ml micro algae oil +85ml linseed oil	9.05 mm^2 /s	925

Pre-esterification Process:

The mixed oil has high free fatty acids (FFA) and requires pre-treatment. In pre-esterification process the mixed oil is react with 20% v/v methanol and 0.5% v/v acid catalyst at 60^0C. The reactions were carried out using a 500ml round bottom flask equipped with a reflux condenser, and magnetic stirrer. The experiments were performed at methanol temperature reflux. The round bottom flask was filled with algae oil, linseed oil, acid catalyst and methanol and heated under constant agitation speed of 1500 rpm for 1.5 hours. The pre- esterification was carried out to reduce algae oil acidity from 29% to less than 1%. After completion of the reaction, the mixture was filtrate to remove catalyst and the excess of methanol was recovered by washing. Add sodium sulfate for remove the water content of the oil. The acid value was determined by titration method.

Acidity (%) = $\frac{282*v*n}{1000*m}$ x100

Where v = volume (ml) of NaOH solution;
n= normality,
m=sample weight

Trans-esterification Process:

In trans-esterification process measured 0.5 gram of NaOH. Measured quickly since the catalyst absorbs water from the atmosphere rapidly and this water can interfere with the trans-esterification reaction. Then, mix the NaOH with 20 ml of methanol in a sturdy, heat proof glass bottle with a narrow neck to prevent splashing. Constantly mix or stir the solution to quickly dissipate the heat given off by the reaction. The mixing process takes about 15 minutes, pour

100ml of mixed oil in the container, and heat the container to about 50°C. The trans-esterification reactions were carried out using a 500 ml round bottom flask equipped with a reflux condenser, and magnetic stirrer. The experiments were performed at methanol temperature reflux. The round bottom flask was filled with algae oil, linseed oil, NaOH and methanol and heated under constant agitation speed of 1500 rpm for 1.5 hours. Keep the temperature below 60°C since methanol will boil at 65°C and will be lost. Then allow the mixture to settle overnight. The system should be closed to the atmosphere to prevent loss of methanol during the reaction. The reaction will take about 12 hours to complete. Figure 2 shows the trans-esterification reaction.

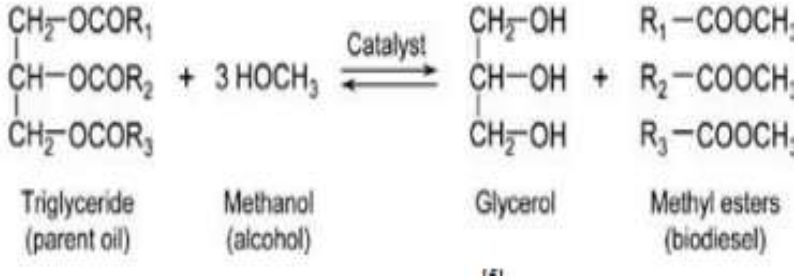


Figure 2:- Trans-esterification Reaction [10].

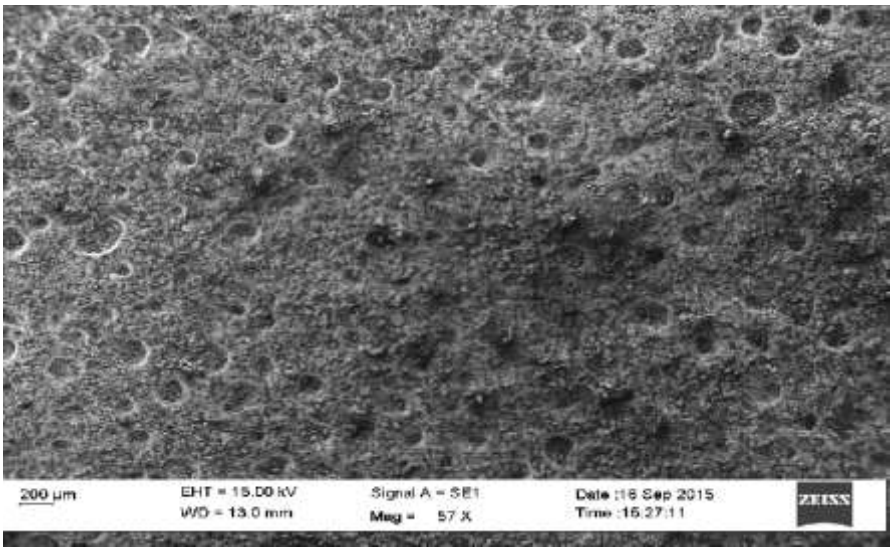
Separation:
As soon as the reaction is completed, pour the mixture from the round bottom flask into a separating funnel for settling and screw on the lid tightly. Allow the mixture to settle 12-24 hours. After settling, there will be two phases in the bottle with a clear interface. Dark colored glycerol byproduct will collect at the bottom, with crude methyl ester on top. The methyl ester varies in color depending on the oil used. Carefully remove the bottom layer. Be sure to not inadvertently mix up the glycerol layer with the methyl ester.

Crude Methyl ester Washing and Filtering:
The crude biodiesel still contains contaminants such as soaps, excess methanol, residual catalyst, and glycerol. It can be purified by washing with warm water to remove residual catalyst or soaps. The methyl ester was washed by 5% water until it was become clean. The washing procedure is effective because the residues are more readily dissolved in water. The ester mixture was dried over anhydrous sodium sulfate [9]. In filtration process, it is filtered with the use of a filter paper.

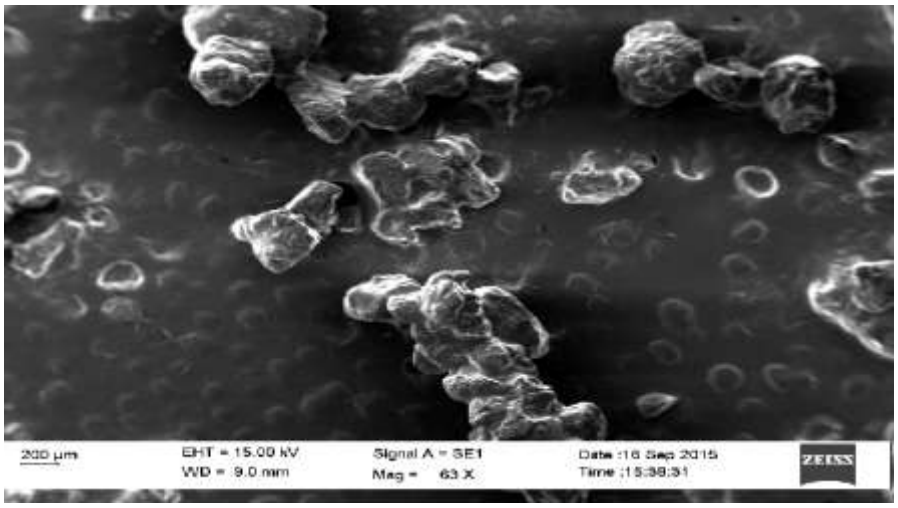
Results and Discussion:-

In this study A mixture consist of Schizochytrium micro algae oil and linseed oil in different volume percentage say 15:85 ml through two-step trans-esterification process. Characterization of biodiesel using GC-MS, FTIR techniques and elemental analysis. This chapter also provides different physico-chemical properties of fuel produced.

SEM analysis:
Figure 3(a) shows the SEM image before reaction. It resembled honey comb, porous in structure and possessed active sites. This indicated the catalyst has more surface area for reaction. Figure 3(b) shows the SEM image of the catalyst after reaction. It had fewer voids because of the shear change during transesterification. The number of pores in the catalyst after reaction was found to be reduced, it can be attributed to the fact that the active sites were utilized for transesterification reaction.



(a)



(b)

Figure 3:- SEM images of (a) Before and (b) After reaction respectively.

Influence of Catalyst weight on MME yield:

The effect of catalyst weight on the transesterification of study A mixture consist of Schizochytrium micro algae oil and linseed oil in different volume percentage say 15:85 ml was investigated with its concentration varying from 0.5, 1.0, 1.5 and 2.0 to 1.75 wt.% (based on the weight of raw oil), while maintaining methanol to oil molar ratio, 7:1 , 90 min reaction time, 60^0 C and stirring speed of 150 rpm.For 1.5 wt. % of catalyst shows 89% biodiesel yield.Whereas2.0 wt% catalyst shows biodiesel yield 78% decreases due to its higher concentration of addition catalyst gives negative effect of biodiesel production.This is because the addition of excess alkaline catalysts caused more triglycerides participation in the saponification reaction, resulting in increased production of soap and reduction of the esters yield. So, any increase in concentration of catalyst beyond the neutralization limit results in decrease in biodiesel conversion. The yields of mixing algae oil and linseed oil methyl ester (MME) at different catalyst weight were shown in Figure 4.

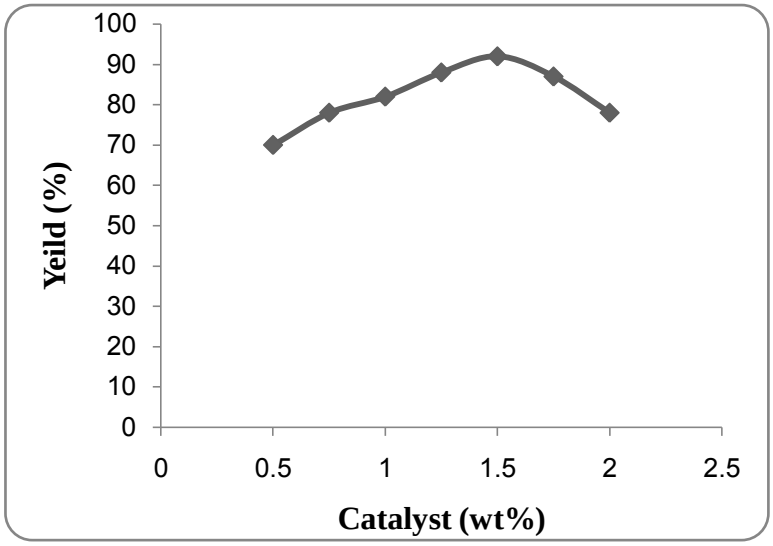


Figure 4:- Effect of catalyst weight.

Influence of Temperature on MME yield:

In order to study the effect of reaction temperature on FAME formation, the experiments were conducted at temperature ranging from 40 to 70 ºC at 5 ºC intervals. The effect of reaction temperature was shown in Figure 5.Experimental results showed that the transesterification reaction could proceed within the temperature range studied but the reaction time to complete the reaction varied significantly with reaction temperature. The maximum conversion efficiency (90%) during transesterification was obtained at 60°C. With the temperature increased above optimum, the product yield started to decrease with respect to all the oil samples used in the study, the reason for this is that higher temperature accelerates the side saponification reaction of triglycerides and although a reflux condenser was used in the experimental set up to avoid methanol losses when the reaction temperature approaches or exceeds the boiling point of methanol (65ºC), the methanol molecules would vaporise and form a large number of bubbles that then inhibits the reaction

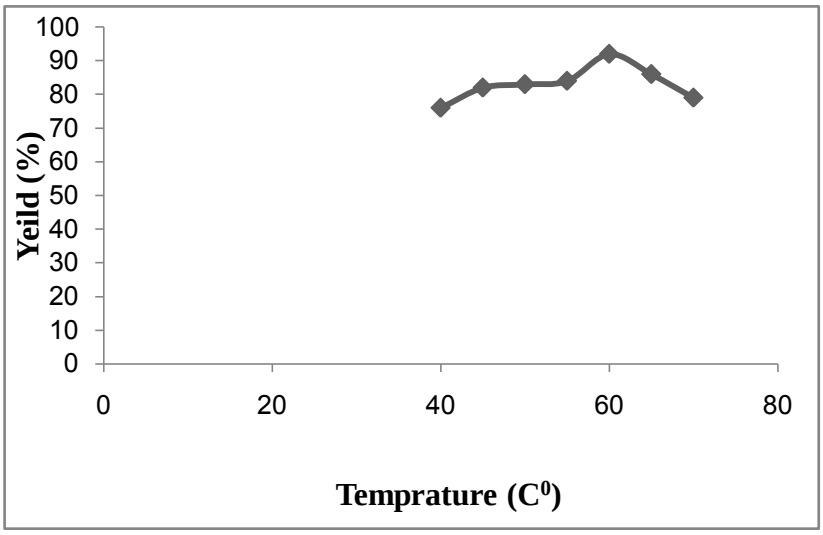


Figure 5:- Effect of temperature.

Influence of Reaction Time on MME yield:

The effect of reaction period on MME yield was investigated by carrying out the transesterification reaction over a period of 45 to 135 min, while other parameters remained constant (catalyst concentration of 1.5% weight of oil, reaction temperature of 60 ^{0}C and stirring speed of 150 rpm). The yields of biodiesel at different reaction periods are

presented in Figure 6. The maximum Mixed Methyl Ester (MME) yields (90.0%, was obtained at the reaction period of 120 min. It was also observed that the yield increased significantly from 45 to 120 min which indicated that most of the transesterification occurred during 120 min. This could be associated with the molecular structure of the oil that contains saturated fatty acids. These fatty acids have higher activation energy thus require longer period of heating to react. Therefore, the yield of biodiesel increased significantly at 120 min reaching maximum value as the activation energy was achieved after which the yield declined. It was believed that during 2nd and 3rd hour the process reached equilibrium.

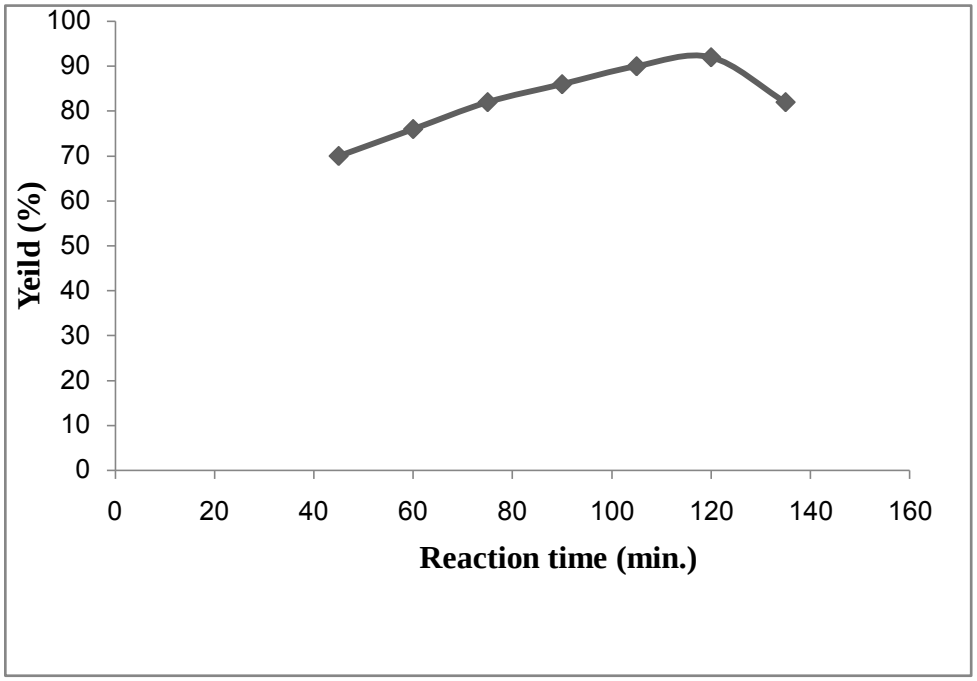


Figure 6:- Effect of reaction time.

Influence of Molar Ratio on MME yield:

The methanol/oil molar ratio is considered to be one of the most important factors affecting the yield of Methyl ester. Although the required stoichiometric ratio is 3:1 the transesterificationprocess is usually carried out with an extraamount of alcohol in order to shift the equilibrium to theexpected product, methyl ester side. It is reported that thetransesterification is insufficient at the ratios of methanol/oilbelow 5:1. The effect of variation of methanol addition onthe biodiesel yield was analyzed as presented in Figure 7.The yield of biodiesel was found to be highest atthe molar ratio of 6:1 resulting in 90.99% [11]. However, the yield of MME declined beyond the molar ratio (methanol to oil) of 6:1. The decrease of yield at molar ratios of 7:1, 8:1 and 9:1could be attributed to the fact that excess methanol deactivated the catalyst, hence reducing its effectiveness. Upon transesterification, produced glycerin was settled at the bottom layer, while the upper layer was of methyl ester. Therefore, the excess methanol also tended to blur the separation border between glycerin and methyl ester, making it difficult to extract the methyl ester. Based on this result, the molar ratio of 6:1was considered to be an optimum condition and thus this ratio was maintained throughout the experimentations to investigate the effects of other process parameters.

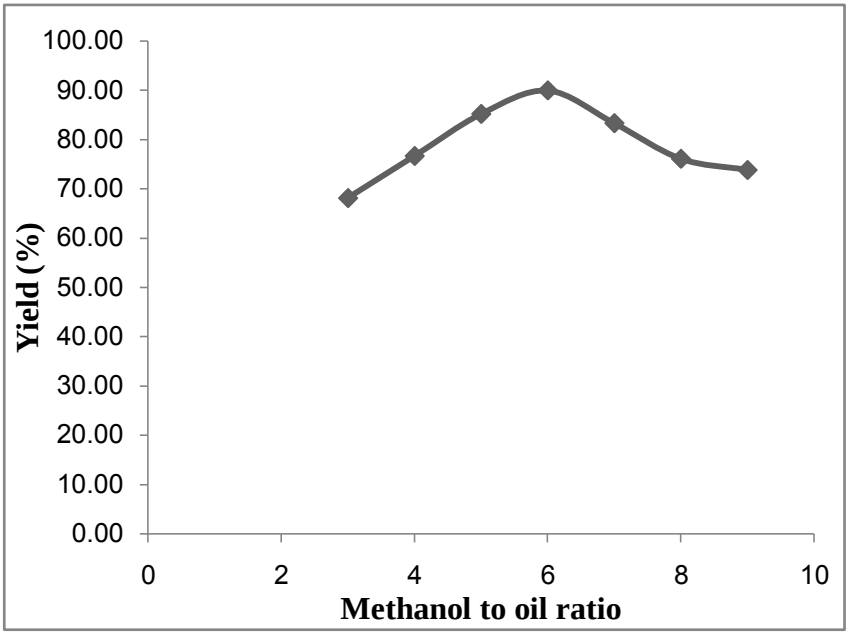


Figure 7: Effect of Molar ratio

FFA Analysis of MME using GC-MS:

The fatty acid composition of the oils seems to have an important role in the performance of the methyl ester in diesel engines. Based on the fatty acid composition and many other parameters the biodiesel specifications will be mandatory to limit the oxidative stability, as it may be a crucial parameter for injection pump performances. The stability of the fuel is a quality parameter established by the ASTM American Society for Testing and Materials, being its evaluation and control necessary. Vegetable oils are natural products consisting of ester mixtures derived from glycerol (triglyceride), whose chains of fatty acid contain about 14 to 20 carbon atoms with different degrees of unsaturation. The trans esterification reaction consists in the conversion of the triglyceride molecules by means of the action of short chain alcohol, like, methanol, ethanol into the corresponding fatty acid esters

The prepared biodiesel of (mixing algae oil and linseed oil methyl ester) was analyzed by GC-MS system to determine the composition of fatty acids. The methyl ester is mainly formed by transesterification of saturated and monounsaturated fatty acids while the remaining polyunsaturated and some bulk saturated fatty acid are responsible for high viscosity. The higher level of unsaturated fatty acid reduces fuel quality, because of its easy oxidation. The GC-MS report of methyl esters are shown below in Figure 8. The measured values of fatty acids present in the CSME are in Table 2.

Table 2:- GC-MS Analysis for the Mixed Methyl ester (MME) (A mixture consist of Schizochytrium micro algae oil and linseed oil).

Fatty Acid	Systematic Name	Structure	Wt%
Myristic	Methyl tetradecanoate	C14:0	0.61
Palmitic Acid	Hexadecenoic acid	C16:0	20.78
Palmitoleic acid	9-Hexadecenoic acid	C16:1	0.41
Margaric acid	Heptadecanoic acid	C17:0	0.10
Stearic acid	Octadecanoic acid	C18:0	42.01
Vaccenic acid	6-Octadecenoic acid	C18:1	1.79
Linolelaidic acid	Methyl 10-trans,12-cis- octadecadienoic acid	C18:2	29.76
Arachidic acid	Eicosanoic acid	C20:0	1.63
Gondoic acid	cis-11-Eicosenoic acid	C20:1	1.17
Behenic acid	Docosanoic acid	C22:0	0.72
Erucic acid	13-Docosenoic acid	C22:1	0.22

Lignoceric acid	Tetracosanoic acid	C24:0	0.80

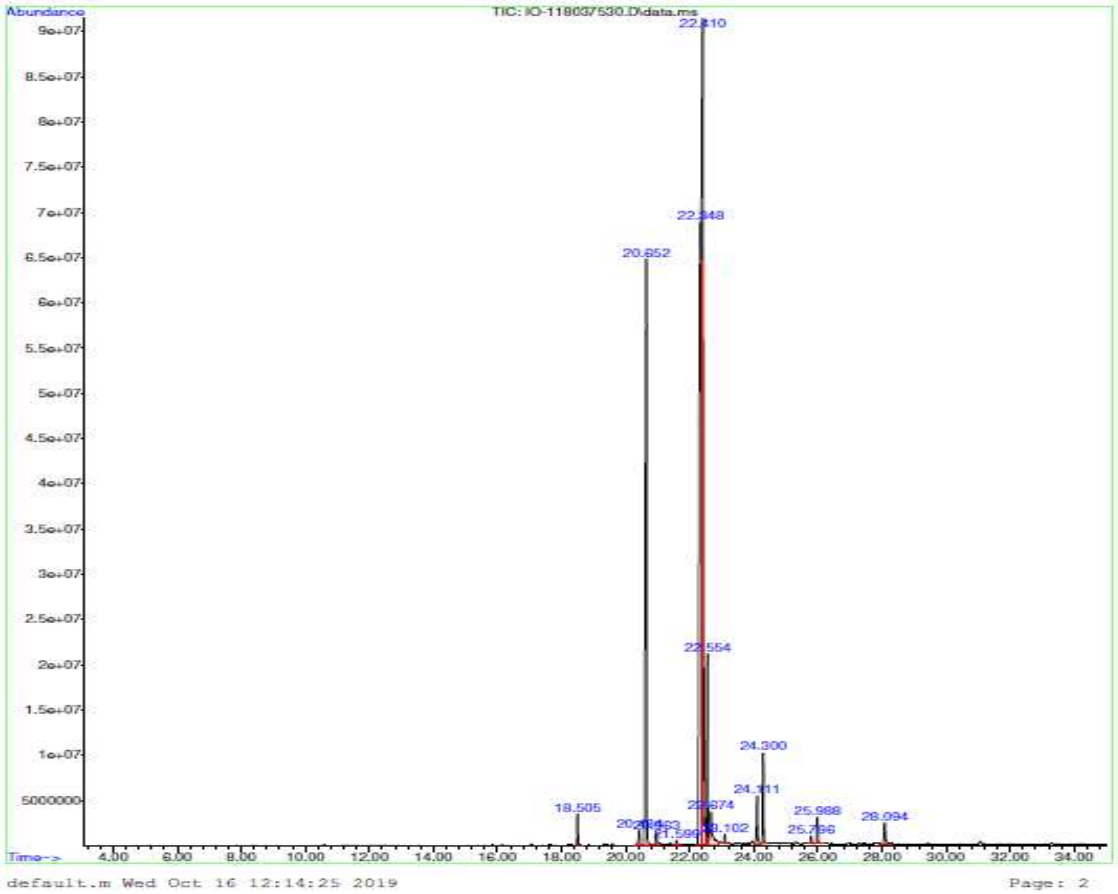


Figure 8:- GC-MS Analysis for the MME.

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis:

Infra-red spectrum of mixing algae oil and linseed oil biodiesel originated from Perkin Elmer Spectrum 1FTIR spectrometer. The instrument consists of mercury vapour and globar lamp as source interferometer chamber (with KBr and mylar beam splitter), sampler and detector The spectrum, covers the range of 450- 4000cm^{-1} wave length. The mixed methyl ester (biodiesel) that obtained after the trans esterification process is sent for FT-IR analysis and the results obtained in Figure 9, the FTIR is used to determine the functional groups such as alcohol, alkane, alkynes, alkenes and other such groups present in the sample. Figure 9 and Table 3 shows spectra and FTIR studies of mixing algae oil and linseed oil methyl ester.

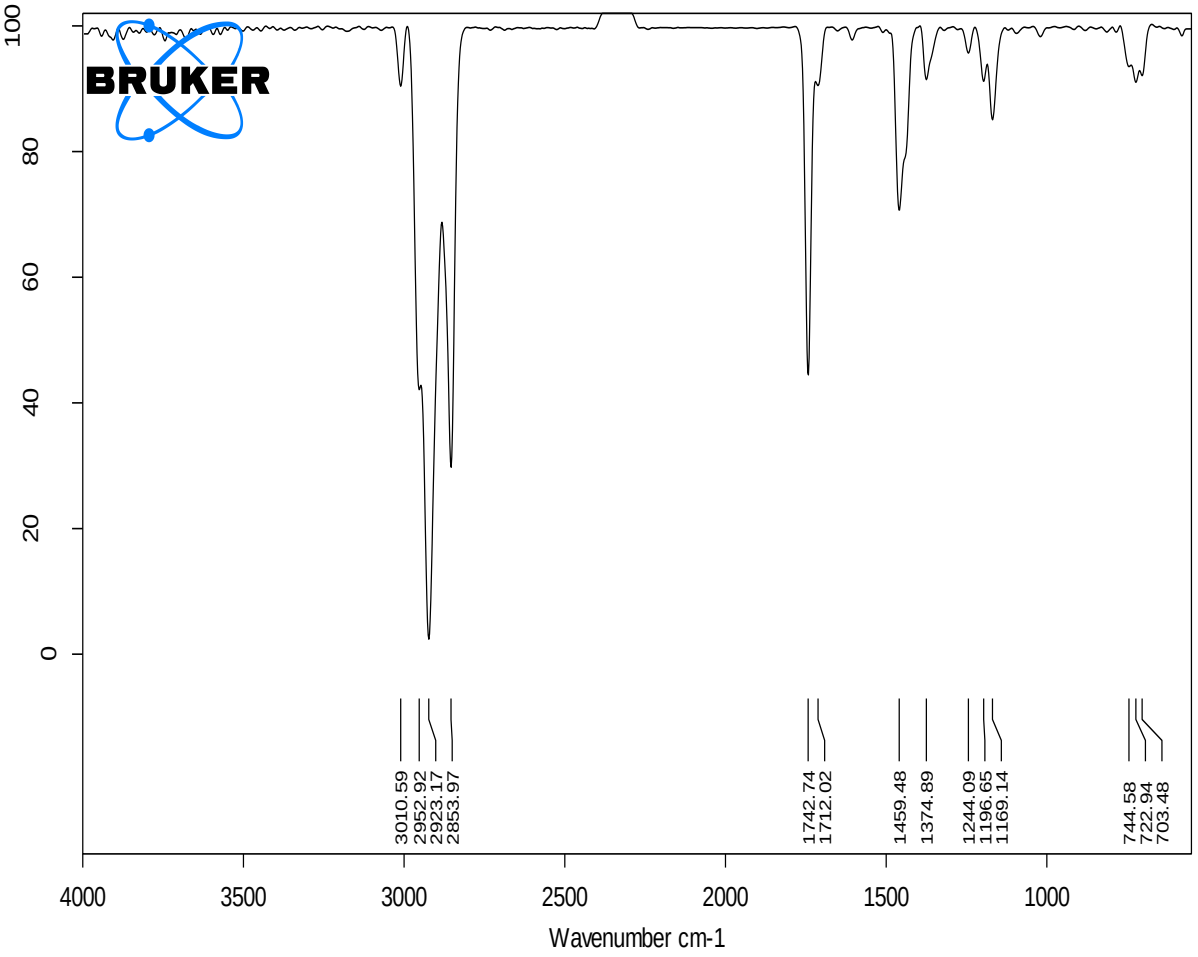


Figure 9:- FTIR Studies of micro algae and linseed oil mixed methyl ester (MME).

S.NO.	Peaks (cm^{-1})	Bond	Compound Type	Mode	Transmittance (%)	Concentration
1	3008.45	C-H, =C-H	aromatics, alkenes	Stretch	58	Strong, medium
2	2942.48	O-H/ C-H	Carboxylic acid/alkanes	Stretch	17	Medium
3	2870.77	O-H/ C-H	Carboxylic acid/alkanes	Stretch	32	Medium
4	2832.07	O-H/ C-H	Carboxylic acid/alkanes	Stretch	52	Medium
5	1739.51	C=O	carboxylic acids/ carbonyls/ esters	Stretch	12	Strong
6	1437.53	C-C/ C-H	aromatics/ Alkanes	stretch/ Bend	74	Medium
7	1364.15	C-H	Alkanes	Rock	78	Medium

8	1243.47	C-N	Aliphatic Amines	Stretch	85	Medium
9	1194.70	C-N	Aliphatic Amines	Stretch	70	Medium
10	1169.14	C-N	Aliphatic Amines	Stretch	68	Medium
1 1	1093.24	C-O	Alcohols/ carboxylic acid	Stretch	83	Strong
12	723.11	C-H	Alkanes	Rock	74	Medium

Table 3:- Studies of micro algae and linseed oil mixed methyl ester (MME).

Elemental analysis:

The methyl ester consists of three basic elements namely:1- Carbon.2- Hydrogen.3- Significant amount of Oxygen. The increase of O_2 in methyl ester is related to the reduction of C and H causes the lower calorific value of biodiesel that produced, as compared to that of diesel. A mixture consist of Schizochytrium micro algae oil and linseed oil methyl ester (MME) contains 70% carbon and 11.43% hydrogen and 9.46% oxygen. All the elements reached ASTM standards which is suitable for environment (Table 4). Table 5 shows that the properties of MME and conventional diesel. The study shows that the properties of the MME are very close to the conventional diesel.

Table 4:- Elemental composition and C/H ratio Comparison of MME with ASTM biodiesel standards.

Element (wt%)	**Petro Diesel**	**(Algae and linseed) MME**	**ASTM**
Carbon(C)	**86.25**	**70.96**	**77**
Hydrogen(H)	12.5	**11.43**	**12**
Nitrogen(N)	**0**	**1.31**	**----**
Sulphur(S)	**0.25**	**0.15**	**0.05**
Oxygen(O)	**1**	**9.46**	**11**
C/H Ratio	**6.9**	**6.20**	**----**

Table 5:- Properties of MME and Conventional Diesel.

Parameter	**MME (15 ml +85 ml) (Microalgae oil + Linseed oil)**	**Diesel**
Density (kg/m^3)	850	859
Viscosity (mm^2/s) at 50^0C	2.6	4.2
Acid value mg/KOH gm	0.33	-
Flash point (K)	399	341
Fire point (K)	403	351
pH	7	7

Conclusion:-

From the above study, the following conclusions can be deduced: ¬ The algae oil and readily available linseed oil were tested individually for their properties. The combination of both the algae oil and the sunflower oil may be used as resource to obtain biodiesel. In this way micro algae and sunflower oil can be used as renewable energy. The best conditions of operation for maximum yield (90.99%) are: Molar ratio alcohol to oil: 6:1; Catalyst concentration: 1.5 wt% ; Reaction temperature: 60 °C and Reaction time: 120 min. The mixing of algae oil and linseed oil methyl ester (MME) was analyzed by GC-MS system to determine the composition of fatty acids. From FTIR analysis it is observed that the bonds such as C-H, C-O, O-H and C-N are abundant in the (mixing of algae oil and linseed oil methyl ester), and the modes of the bonds are stretch, bending and rocking.

The experimental result shows that the trans-esterification is a promising area of research for the production of biodiesel in large scale. The study also revealed that the properties of the methyl ester (bio-diesel) are very close to the conventional diesel. ¬ Micro algae and other biodiesel feedstock shall be cultivated exclusively for the purpose of biodiesel production, so that the cost of the oils from these feedstock used for other purposes do not change.

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