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Fungal Degradation of Soybean Oil (Glycine Max Oil) on the Surface of Clay at Room Temperature

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Abstract

The fungal degradation of glycine max oil on the surface of clay at room temperature was carried out. The influence of clay, nutritive additive and concentration on the degradation process was monitored by Gas Chromatography–Mass Spectrometry analysis of the products. 0.4g of glycine max oil, 0.16g yeast, and 3.6g of treated clay and 25 ml of distilled water were mixed together to form slurry in an airtight glass digester and allowed to stand for 21 days at room temperature. It was established that the clay provided a useful surface for the effective degradation of the triglyceride molecule as evident in the type of products obtained. The addition of urea at concentration of 0.1 to 0.2M increases the yield of the bio liquid. Also, an increased in the quantity of the substrate leads to a corresponding increase in the yield of the bio liquid. It was also inferred that the process followed a first order kinetics. The GC-MS analysis of the products indicates wide distribution of compounds.

Keywords: Fungal Degradation; Triglycerides; Clay surface; Gas Chromatography; Mass Spectrophotometry; Glycine max oil.

Introduction

Rising energy consumption, depletion of fossil fuels and increased environmental concern (ozone depletion, global warming, greenhouse warming) have shifted the focus of energy generation towards biofuel use. These factors have caused an increased in research on alternative renewable and eco-friendly fuel sources. Biodiesel has shown its ability to meet the energy demands of the world in transportation, agriculture, commercial and industrial sectors of the economy (Akoh *et al*., 2007; Topal, 2009). The annual world consumption of diesel is approximately 934 million tonnes of which Canada and United States consume 2.14 and 19.07, respectively (Fuels Institute, 2014). As a green, renewable and potentially unlimited, biodiesel has recently come out as the superlative alternative fuel which can be used in compression ignition engines with minor or no modification (Xu and Wu, 2003). The use of biofuel in place of conventional fuels would slow the progression of global warming by reducing sulphur and carbon monoxide and hydrocarbon emissions (EPA, 2016). Because of economic benefits and more power output, biodiesel is often blended with diesel fuel in ratio of 2, 5 and 20%. The higher the ratio of biodiesel to diesel, the lower emission of carbon monoxide. Using a mixture containing 20% biodiesel reduces the carbon dioxide net emission by 15.67 while using pure biodiesel makes the net emission of carbon dioxide zero (Fukuda *et al*., 2001, Vasudevan and Briggs, 2008).

Biodiesel is a mixture of fatty acid methyl ester which is produced from renewable resources (Srivatava and Prasad, 2000). Because of the high viscosity and low volatility of vegetable oils, its direct use in diesel engine can cause high carbon deposits, scuffing of engine liner,

injection noozle failure, gum formation, lubricating oil thickening, high colour and pour points (Fukuda *et al.*, 2001, Murugesan *et al*., 2009).

Biodiesel can be produced from vegetable oil (Triglycerides) through three methods; pyrolysis which involves chemically reducing triglycerides molecules to fatty acid alkyl esters by the application of extreme heat in the absence of air (Wiggers, *et al* 2009, Donovan, 1998 and Pollard, 2005). Microemulsion which involve the use of solvents to physically reduce the viscosity of the feedstock, it is thermodynamically stable dispersion of oil in water and water in oil and uses surfactants as the principal agent to enable water and oil to mix (Ma and Hanna, 1998; Renganathan *et al*., 2005). Transesterification, which involves the reaction of triglycerides with a low molecular weight alcohol in the presence of a catalyst to produce a mixture of monoalkyl esters of fatty acid and glycerol, (Akoh *et al*., 2007 and Robles *et al*., 2009). In the present research, the Pyrolysis method is adopted in which fungal degradation of soybean oil (Glycine max oil) on clay surface was carried out to assess the potential of the substrate for bio liquid generation, influence of clay surface, nutritive additives and concentration of substrate on bio liquid produced.

Table 1: The physical and chemical properties of soybean oil

Parameter	Value
Colour	Golden yellow
Density	0.987 Kg/m^3
Refractive index	1.469
Viscosity	50 mPa.s
Solubility in water	insoluble
Flash point	280^oC
Acid value	0.6 mgKOH/g
Free fatty acid	0.3 mgKOH/g
Saponification value	189 mgKOH/g
Iodine value	123mg I_2/100g
Peroxide value	10 meqv/Kg

Materials and Methods

Sample collection: Freshly prepared soybean oil was obtained from Grand Cereals and Oil Mills Limited, Jos in a polyethylene theraphthalate container and kept in the laboratory at room temperature. Urea adduction of the sample was carried out to remove any secondary metabolite that might be present. The clay was obtained from a clay deposit in Kwi, Riyom Local government area of Plateau state. The clay was treated with n-hexane to remove organic matter that might interfere with the analysis.

Method*:* The method of Ekwenchi *et al*., 1989 was used for the fungal degradation of the glycine max oil. About 3.6 g of clay, 0.16 g of yeast, 0.4 g of treated soybean oil and 25 ml of distilled water were mixed together to form slurry in an air tight glass digester and allowed to stand at room temperature for 21 days. During the process, the digester was agitated twice daily. After the 21 days , 25 ml of n-hexane was added to the slurry properly agitated and allowed to stand

after which it was decant off into a beaker and the remaining slurry filtered into the beaker using a filter paper and funnel. The filtrate was then transferred into a separating funnel where the two layers (water and hexane) were allowed to separate. The two layers (portion) were collected separately into pre-weighed beakers. The water fraction was dried by evaporating on a water bath to constant weight while the hexane fraction was allowed to dry in a fume cupboard and the weight of the extracted fractions were obtained and kept for Gas Chromatography-Mass Spectrometry analysis.

The procedure was repeated by addition of nutritional additives (0.01 and 0.02 M solution of urea) to the process, doubling of the reaction parameters and carrying out the fungal degradation without a surface (absence of clay surface). The Gas Chromatography and Mass Spectrometry analysis of the extract was done using QP 2010 GC/MS machine. 0.1 mg/ml of the extract was taken for analysis; 1µl was injected into the GC inlet with the injection port temperature of 200^{o}C. The carrier gas flow rate was 1 ml/minute with the initial temperature of the oven at 50^{o}C. The oven temperature was programmed to increase at a rate of 8^{o}C/min to a final temperature of 280^{o}C after which the GC chamber was maintained for 8 minutes. The results of the samples were obtained as mass spectrum.

Results and Discussion

Tables 2 and 3 show that the fungus was able to degrade the oil in the presence of the clay compared to when there was no clay. This is evident when the weights of the product from both extracts were compared to the original weight of the starting material (0.4g) for that with and without clay as a surface. The combined weight when clay was used as a surface is observed to be half the original weight which indicated that degradation occurred producing bio liquid with the other half representing the biogas that was produced concurrently with the bio liquid (equations 4 and 5). It is also observed that the weight without clay was almost the same as the original weight of the starting material (Table 3) which went further to show that there was little reaction process that occurred, indicating lesser biogas production. According to Khandelwa & Mahdi (1986), Biogas production from organic substrate is a microbial process that involves the combined action of four groups of bacteria in four stages. The first stage is the degradation of higher molecular weight substrate like starch, cellulose, fats, proteins, oils etc presence in organic materials into low molecular weight compounds like fructose, glucose etc that are able to pass through bacterial membrane by a group of hydrolytic bacteria. Similarly, Isaacs, (1984) reported that, polymers are transformed into monomers by enzymatic hydrolysis as follows;

Table 2: Weight of hexane and water extract (fraction) of the fungal degradation process in the presence of clay surface

Determination	n-hexane fraction (g)	Water fraction (g)	Cumulative(g)	Difference(g)
1.	0.090	0.105	0.195	0.205
2.	0.106	0.120	0.226	0.174
3.	0.092	0.118	0.210	0.190
4.	0.095	0.119	0.214	0.186
5.	0.090	0.116	0.206	0.194
∑	0.473	0.578	1.051	0.949
∑/n	0.095	0.116	0.210	0.190

Table 3: Weight of hexane and water extract (fraction) of the fungal degradation process in the absence of clay surface

Determination	n-hexane fraction (g)	Water fraction (g)	Cumulative(g)	Difference(g)
1.	0.223	0.136	0.359	0.041
2.	0.221	0.139	0.360	0.040
3.	0.225	0.134	0.359	0.041
4.	0.220	0.139	0.359	0.041
5.	0.226	0.138	0.364	0.036
∑	1.115	0.686	1.801	0.199
∑/n	0.223	0.137	0.360	0.040

$n(C_6H_{12}O_5)(s) + nH_2O \rightarrow nC_6H_{12}O_6$ ……..(1)

The product of the first stage is converted into organic acids and by-products like CO_2, H_2O, H_2, NH_3 etc by a group of bacteria known as acetogens, which are collectively called 'acid formers'

$n(C_6H_{12}O_6)(aq) \rightarrow 3nCH_3COOH\ (aq)$ ……..(2)

The third stage is the conversion of hydrogen and simple carbon compounds produced in the second stage into ethanoic acid by a group of bacteria called homoacetogens.

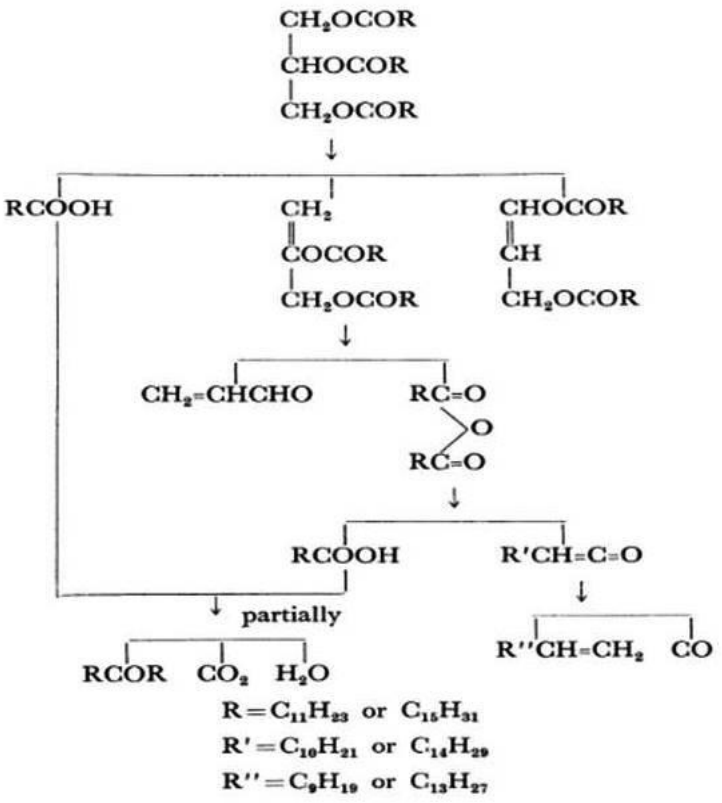
$4H_2(g) + 2CO_2(g) \rightarrow CH_3COOH(aq) + 2H_2O(l)$ ……(3)

The fourth stage is the conversion of ethanoic acid and some compounds like CO_2 and H_2 into methane (CH_4) by a group of bacteria known as methanogens.

$CH_3COOH(aq) \rightarrow CO_2(g) + CH_4(g)$ ……(4)

$CO_2(g) + 4H_2(g) \rightarrow CH_4(g) + 2H_2O(l)$ ……(5)

In the present study, the mechanism can be summarized as follows:



R may be higher than 9, 10 and 11
Scheme 1: Proposed mechanism of pyrolysis of triglyceride (Wiggers *et al*, 2009)

The aforementioned confirmed that in heterogeneous catalysis, there must be proper contact between the substrate and catalyst for degradation to occur. The clay, which was ground to fine particles / powder sizes, provided a large surface area for proper contact between the oil and the fungus (yeast) for effective degradation process to occur.

As shown on Table 4, when the concentration of the reactants was doubled, there was a corresponding increase in the quantity of bio liquid produced. This implies that the fungal degradation of soybean oil on clay surface follows a first order reaction rate. As observed by Ekwenchi and Yaro (2012), the use of urea at certain concentration range enhances the yield of bio liquid as shown on Table 5. Addition of urea shows a marked increased in the quantity of bio liquid generated compared to when there was no addition of urea to the mixture. This could be attributed to the fact that urea is a nutritive additive which enhances the action of the yeast a plant based enzyme.

Table 4: Weight of hexane and water extract (fraction) of the fungal degradation process on clay surface and doubling of reactants

Determination	n-hexane fraction (g)	Water fraction (g)	Cumulative (g)	Difference (g)
1.	0.210	0.202	0.412	0.388
2.	0.221	0.209	0.430	0.370
3.	0.224	0.208	0.432	0.368
4.	0.222	0.211	0.433	0.367
5.	0.225	0.213	0.438	0.362
∑	1.102	1.043	2.145	1.855
∑/n	0.220	0.209	0.429	0.371

Table 5: Weight of hexane and water extract (fraction) of the fungal degradation process on clay surface on addition of urea

Determination	n-hexane fraction (g)	Water fraction (g)	Cumulative (g)	Difference (g)
1.	0.169	0.129	0.298	0.102
2.	0.173	0.131	0.304	0.096
3.	0.171	0.129	0.300	0.100
4.	0.170	0.133	0.303	0.097
5.	0.169	0.130	0.299	0.101
∑	0.852	0.652	1.504	0.496
∑/n	0.170	0.130	0.301	0.099

On Table 6, it can be seen that the breakdown of the triglyceride molecule, the main component of the oil; esters, n-alkanes, aldehydes, acids and alcohols were obtained in both the water and n-hexane fractions. The large number of alkanes (C_{13}-C_{17}) obtained in the process is attributed to the effect of the clay which serves as a surface and enhanced effective catalytic radical formation, breakdown, recombination and decarboxylation of the triglyceride molecule and only few esters and acids were obtained. This is in contrast to the products obtained for the non-clay surface degradation where there were no n-alkanes present as shown on Table 7. This further confirms the positive effect of a suitable surface needed by the enzymes for more effective degradation. The surface provided a useful surface for proper and effective breakdown of the triglycerides. It can also be inferred that n-alkanes from C_1-C_{12} were produced, but absent in the result shown due to the volatile nature of the gases and liquids. Such volatile gases and liquids may have been lost during evaporation of the fractions. Table 7 also indicates the formation of higher molecular weight hydrocarbons compared to the clay surface reaction where lower molecular weight hydrocarbons were produced. This can be attributed to a lack of surface area and proper medium to provide enough contact between the enzymes and the oil leading to lower degradation. This is also evident in previous results shown in Table 3 where the combined weight of both fractions of the products were almost the same as the original substrate (0.4 g) indicating

there was little degradation owing to lack of surface (clay).

Table 6: Identified reaction products from fungal degradation of soybean oil on clay surface

S/N	R.T	M.W	M. F	Name
1.	5.608	102	$C_5H_{20}O_2$	1-methylelthylateisopropyl acetate
2.	5.925	114	$C_7H_{14}O$	2,2 dimethylpentanal
3.	12.125	154	$C_{13}H_{28}$	Tridecane
4.	13.808	184	$C_{14}H_{30}$	Tetradecane
5.	15.530	198	$C_{15}H_{32}$	Pentadecane
6.	16.917	212	$C_{16}H_{32}$	Hexadecane
7.	18.342	226	$C_{17}H_{34}$	Heptadecene
8.	19.642	240	$C_{18}H_{36}$	9-Octadecene
9.	22.175	252	$C_{19}H_{38}$	9-Nonadecene
10.	24.317	266	$C_{20}H_{40}$	9-Eicosene
11.	24.442	280	$C_{16}H_{32}O_2$	Hexadecanoic acid
12.	24.483	298	$C_{20}H_{40}O$	9-Eicosenal
13.	26.608	312	$C_{21}H_{42}O_2$	9-Heneiasenal
14.	27.692	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester
15.	27.692	282	$C_{18}H_{34}O_2$	Oleic acid(9-octadecenoi acid)
16.	28,442	352	$C_{25}H_{52}$	pentacosane

Table 7: Identified reaction products from fungal degradation of soybean oil on absence of clay surface.

S/N. R.T	M.W	M.F	Name
1.	23.217 110	C_8H_{14}	1,4-Octadiene
2.	23.425 256	$C_{16}H_{32}O_2$	Hexadecanoic acid
3.	25.608 280	$C_{19}H_{38}O$	Nonadecenal
4.	28.733 298	$C_{20}H_{38}O$	9-Eicosenal
5.	29.51 390	$C_{24}H_{38}$	1,2-benzenedicarboxylic acid,dioctylester
6.	23.408 111	$C_7H_{13}N$	1,4-Heptadienamie
7.	24.467 116	$C_7H_{16}O$	1-hydroxyheptane
8.	24.650 112	C_6H_8O	1,4-Hexanoic acid
9.	25.650 264	$C_{17}H_{28}O$	1,4-Deptadecenoic acid
10.	27.967 114	$C_6H_{10}O_2$	Hexanoic acid
11.	28.733 129	$C_6H_{15}ON$	Heptanamide
12.	32.900 347	$C_{23}H_{41}O$	Tricosatrienamin

Conclusion

Based on the finding made in this work, it is established that a suitable surface such as clay used in this fungal degradation plays an important role in the products obtained in fungal degradation of triglyceride molecules. It creates a large surface area as well as enhances proper contact between the enzyme and the substrate to achieve a better degradation process. There were reasonable evidences that the degradation process was a surface reaction because the degradation process without the clay as a surface was very negligible. The results also showed extensive surface reaction which gave first order kinetics as shown when the quantity of the

substrate was doubled. Addition of additives such as urea at certain concentration increases the yield of the bio liquid and biogas. The fungal degradation process in the presence of a suitable surface is a useful process for the production of chemical feed stocks. Fungal degradation process, which is an enzymatic process, is a clean and environmentally friendly technique for biodiesel production.

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