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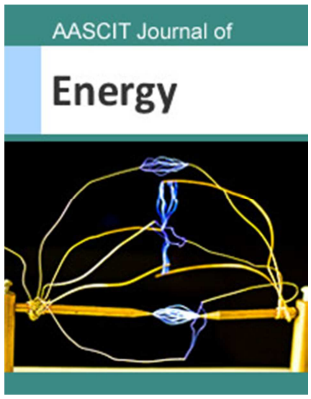

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Status Upgrade of Nigerian Petroleum (Crude Oil) by Fungal Degradation on Clay Surface at Room Temperature

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Abstract
The status upgrade of Nigerian petroleum crude oil by fungal degradation on clay surface was carried out. The substrates and fungi (Saccharomyces cerevisiae) were mixed together in a reactor and monitored for the period 21 days for complete degradation reaction. Finally, the structures of the products were elucidated by the use of GC-MS analysis. Crude oil sample that was not subjection to fungal degradation was analyzed and a total of 20 components were found. These components are a mixture of linear alkanes, iso –alkanes and few amides. The degradation products of Nigerian petroleum on clay surface revealed the presence of 19 components among which 7 are new compounds and were not present in the crude petroleum. The new compounds produced were identified as follows octacosanamide (Mwt: 423) ($C_{28}H_{57}NO$), tetracosane (Mwt: 338) ($C_{24}H_{50}$), 9, 12, 15, 18, 21-cotapenteniene, (Mwt: 412) ($C_{30}H_{52}$), nonacosanamide (Mwt: 437) ($C_{29}H_{59}O$), triacontane (Mwt: 422) ($C_{30}H_{62}$), 9-hexacosenamide, (Mwt: 393) ($C_{26}H_{51}NO$) and 9, 12, 15, 18-nonacosatetraieneamide (Mwt: 429) ($C_{29}H_{51}NO$) respectively. The seven (7) new compounds in the degraded products that were not originally present in the crude oil functionalize the technique of fungal degradation. Petroleum (crude oil) can be degraded and eventually upgraded by the action of fungi to produce valuable chemicals and higher molecular weight hydrocarbons to meet refinery feedstock specifications. These components with increased C/H ratio are of high commercial value.

1. Introduction

Petroleum is one of the most important natural resources. It is formed from decaying plants and dead animals acted upon by high pressure and temperature, and by micro-organisms beneath earth's surface [1]. Petroleum (crude oil) is a major source of energy globally and the deposit is plentiful. [2]. Hence, the importance of petroleum in our technological age cannot be over emphasized. This is because the entire human activities today depend on it. Its scarcity can as a matter of fact pose serious economic challenges to many and even commercial activities can be brought to a halt, and in the same vein, could make life difficult and uncomfortable. Apart from the importance of petroleum as

a fuel, petroleum products obtained from refining have as a matter of fact given rise to a number of industries like the petrochemical and pharmaceutical industries. In recent times, the use of biotechnology in the upgrade of crude oil in petroleum industry has gradually become an emerging technology [3]. This has successfully provided new tool to modify, crude oil and extra-heavy crude oil (EHCO) status and quality. However, standard technologies developed for crude oil upgrading include a process that has to do with carbon rejection, and hydrogen addition as cited in [4]. Enzymatic processes may also provide an alternative to usual methods for crude oil upgrade, due to the advantage of being environmentally friendly and economically less demanding. One of such method is biodegradation, defined as the biologically catalyzed reduction process in the complexity of an organic compounds as cited by Joutey *et al* [5] in [6]. Usually, certain microorganisms exhibits amazing, natural microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds among which are hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), radionuclides and metals [7]. Microbial degradation of crude oil by fungi pre-grown on wood meal was studied and the result showed that application of a carrier-based indigenous microorganism like fungi could be employed to remediate soil contaminated by crude oil [8]. Similarly, [9] carried out a research on Isolation of autochthonous non-white rot fungi with potential for enzymatic upgradingof Venezuelan extra-heavy crude oil and reports, for the first time, the use of o-phenylenediamine dihydrochloride (OPD) as substrate to measure extracellular ligninolytic peroxidases (ELP) in culture broths of filamentous fungi (Fusarium solani HP-1), which is the first formal study of the fungal community associated with the EHCO of the Orinoco Oil Belt.

Fungi are important groups of degrading micro biota. Unlike bacteria; they can grow in low moisture environment and in low pH solutions, which aids their breakdown ability [10]. Yeasts however, are better hydrocarbon degraders than bacteria [11]. Biodegradation of aliphatic hydrocarbons that takes place in petroleum and some petroleum products were investigated, most especially for yeasts. This study found that the n-alkanes are the most widely used group hydrocarbons, with those between the chain length of C10 and C20 being most suitable as substrates for microfungi degradation [12]. However, the biodegradation of n-alkanes with chain lengths up to C24 has also been established [13]. A good number of yeasts could breakdown n-alkanes. The classes of alkane-utilizing yeasts include, *Rhodoturularubra, C. tropicalis*, *Candida lipolytica*, *Aureobasidion* (*Trichosporon*) *pullulans*, among others. All these, have the ability to degrade diesel oil as cited by [5] in [14]. There are several other research that worked on anaerobic biodegradation of organic matter by yeast to produce bioliquids and biogas among which are; Nyong *et al* [15] carried out studies on the production of methylesters from fungal degradation of soya bean oil and mechanism of reaction; the result showed that after the urea adduction of the vegetable oil, methyl esters were the predominant bioliquid produced as opposed to n-alkanes. Ekwenchi and Yaro, [16] conducted a research on extraction of bioliquid and quantitative determination of saturates, aromatics and organic polar from fermented Banana (musa sapintum) leaves and the research revealed that essential organic compounds like saturates, aromatics and polar compounds can be produced and isolated from banana leaves by the means of anaerobic fungal degradation. Most researches on crude oil degradation are on bioremediation, and not on improving the status of crude oil by elongating the carbon length. This research therefore, seeks to employ the use of fungi (*S. cerecvisiae*) to upgrade the petroleum (crude oil) status at room temperature and to propose plausible mechanism for the degradation. The method is envisaged to be very cheap and environmentally friendly.

2. Materials and Methods

The materials used for this research are clay, crude oil, yeast *(Saccharomyces cerevisiae),* chemicals (absolute methanol, n-hexane) which are of analytical grade and some glass digesters.

2.1. Sample Collection and Preparation

In this present work, Nigerian crude oil sample was studied. The crude oil was sourced from ExxonMobil Port-Harcourt, Rivers State. Clay was obtained from Kwi in Ryom LGA of Plateau state and transported in polythene bags to the laboratory where it was crushed into fine particles and sieved using a sieve with mesh size of 3.50mm. The clay was dissolved in water and stirred thoroughly, the mixture was allowed for some time to settle, and the decanted to separate water from the clay. This was repeated three times and dried under the sun. The dried clay was then treated with n-hexane and methanol to remove organic and polar matters that might interfere with the analysis. Finally, the clay was then dried in an oven in a crucible, at a temperature of 105°C for two hours after which it was bottled andkept in the desiccators for onward use.

2.2. Experimental Procedures

The experiment was carried out in two stages:

2.2.1. Process Fungal Degradation of Crude Oil on Clay Surface

Clay (3.6g), was weighed and placed in to a glass digester, 0.4g of crude oil was then weighed and added to the clay which gave a total of 4g of the substrate. Then about 10ml of n-hexane was measured and poured in to the digester and swirled in order to spread the crude oil evenly on the surface of the clay. The n-hexane was evaporated off in a fume cupboard before the introduction of fungus. About 0.0162g of yeast, was weighed into a beaker, 25ml of distilled water was measured and put in to the beaker containing yeast and stirred, the mixture was then poured and mixed together to

form a slurry in an air-tight glass digester and allowed to stand at room temperature for 21 days which is the optimum condition for biogas production as reported by Ekwenchi *et al* [17] and [18].

During this degradation period, the digester was agitated twice daily. After the degradation process is completed, 25ml of hexane was added to the slurry and properly agitated and allowed to stand after which it was decant off into a beaker and then the remaining slurry was filtered into the same beaker using a filter paper and funnel. The filtrate was then transferred into a separating funnel where the two layers (hexane and water) were allowed to separate. The two layers were collected separately into 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 GCMS analysis.

2.2.2. GC/MS Analysis of the Products of Fungal Degradation and Crude Oil Sample (Control Sample)

The GC/MS analysis of the product of fungal degradation and crude oil were obtained using model QP2010 GC/MS machine at NARICT Zaria. 0.1mg/ml of the sample was taken for analysis, out of which 1micro liter of the sample was injected into the GC inlet with injection part temperature at 200°C. The carrier gas flow rate was 1ml/min with the initial temperature of the oven set at 50°C. The temperature of the oven was programmed to increase at a rate of 8°C/min to a final temperature of 280°C, after which the GC chamber was set at this constant temperature for 8 minutes. After this, the Gas chromatogram and the Mass spectrum of the samples were obtained.

3. Results and Discussion

The experimental results (weights) obtained from the products of fungal degradation of crude oil at room temperature using clay as a surface, and GCMS analysis of raw crude oil sample (control sample) are shown below.

Table 1. *Weight of both Hexane and Water Fraction of the Fungal Degradation Process In The Presence of Clay as a Surface.*

Expt. Setup	N-Hexane Fraction (g)	Water Fraction (g)
1	0.157	0.0045
2	0.165	0.0061
3	0.152	0.0053

3.1. Gravimetric Analysis of Products of Fungal Degradation of Crude oil on Clay Surface

Table 1: presents the weights of both n-hexane and water soluble fractions of the products of fungal degradation process. The n-hexane fractions have higher weights compared to their corresponding water soluble fractions. It can be observed from this result therefore, that the fungus was able to degrade the petroleum (crude oil). This was confirmed when the two weights for n-hexane fraction and water soluble fraction of the products of degradation were compared with the weights of the original starting material 0.4g of petroleum (crude oil), when the two weights are combined and totaled, it was found to be almost half the original weight used. This is however, evident enough to show that degradation and possibly recombination had taken place producing upgraded products of petroleum (crude oil) with the other half which could not be accounted for as biogas that were produced simultaneously during the enzyme reactions. The comparison between the GC-MS results obtained from the original petroleum crude oil (control) and the product of the fungal degradation gives a clear evidence of upgrade. The Gas Chromatogram of petroleum (crude oil) sample is shown in Figure 1 with several peaks, followed by the mass spectra of the corresponding match from the library of the suggested compounds as presented in figure 2 and 3

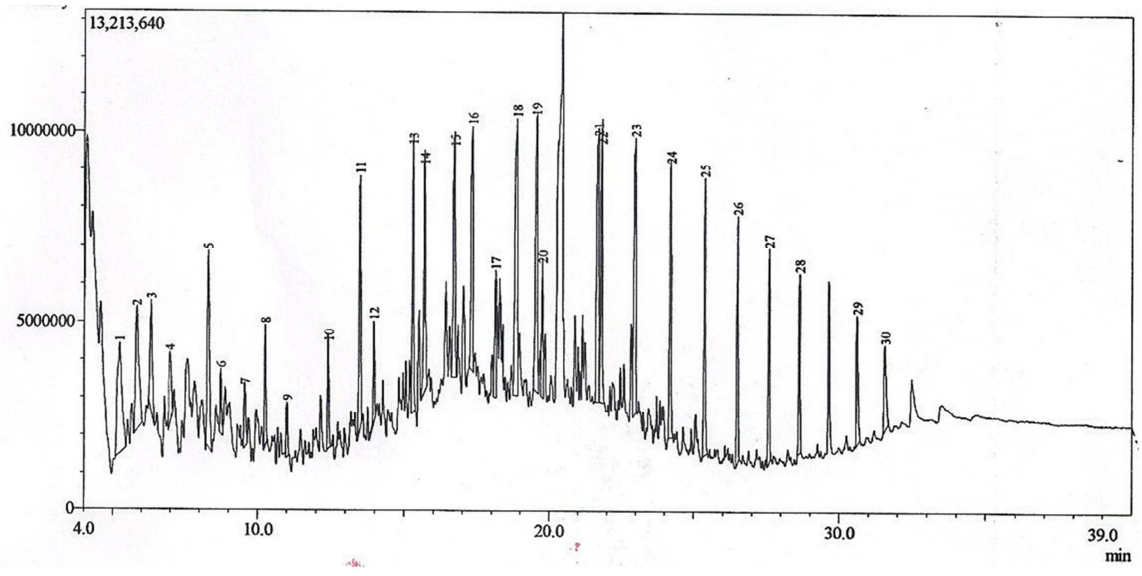


Figure 1. *Gas Chromatogram of petroleum (crude oil) sample.*

Mass Spectra of Suggested Compounds of Petroleum (Crude Oil) Sample

The mass spectra of the corresponding match from the library of the suggested compounds.

Peak 12, R. Time: 13.958

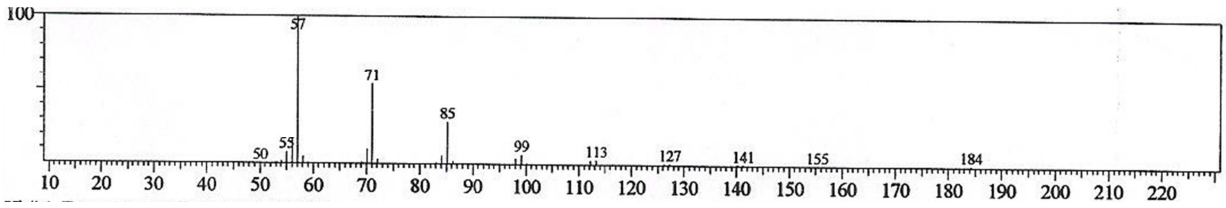


Figure 2. *Mass spectra of unknown compound from crude oil.*

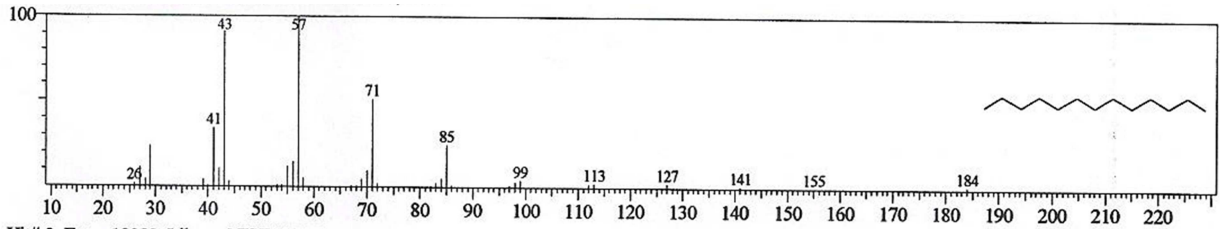
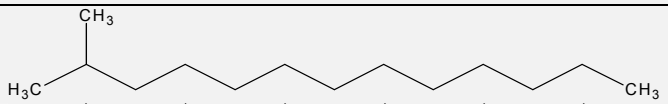
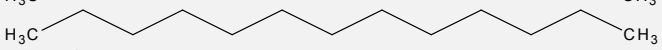
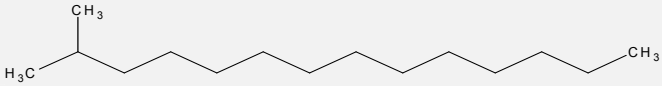
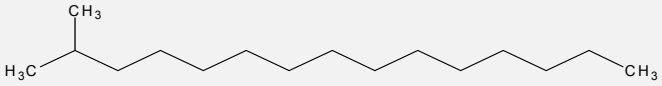
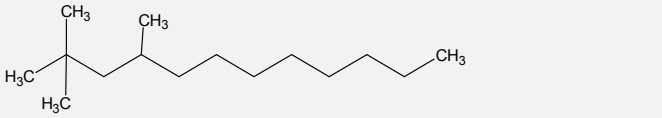
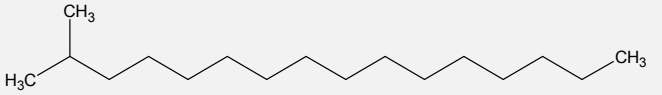
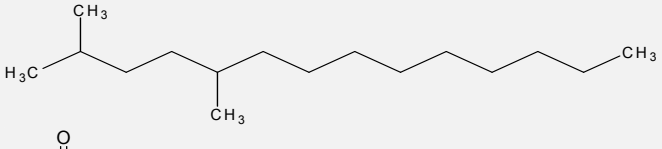
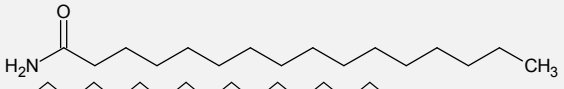
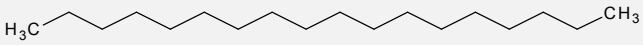
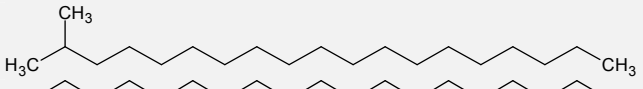
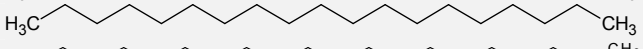
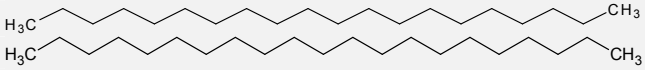
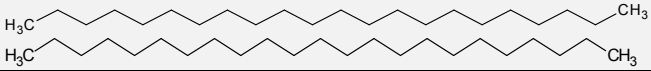
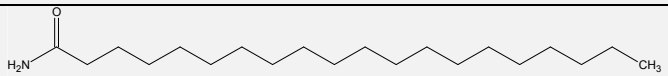
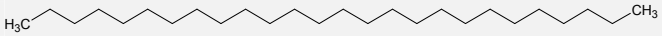
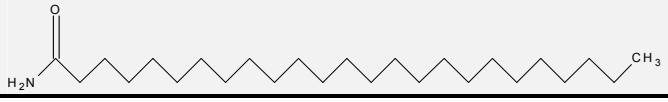


Figure 3. *Mass Spectra of known compound from NIST library (standard) Tridecane ($C_{13}H_{28}$).*

Table 2. *Identified reaction products from petroleum (crude oil) sample.*

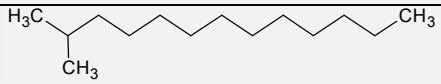
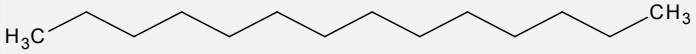
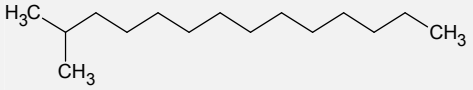
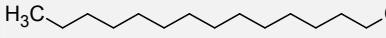
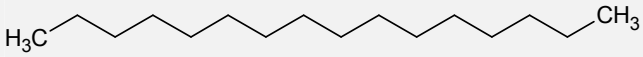
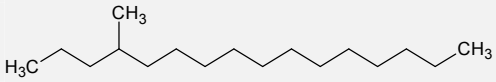
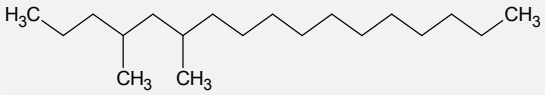
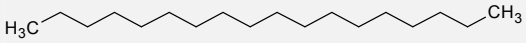
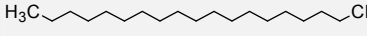
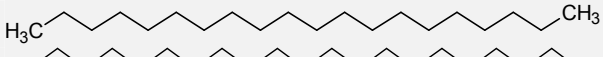
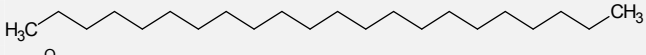
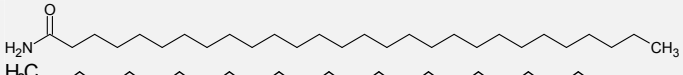
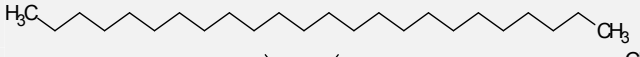
T. Line	R. T	M. W	Molecular formula	Name	Structure
11	13.483	198	$C_{14}H_{30}$	2-methyltridecane	
12	13.958	184	$C_{13}H_{28}$	Tridecane	
13	15.292	212	$C_{15}H_{32}$	2-methyltetradecane	
14	15.700	206	Unidentified		
15	16.733	226	$C_{16}H_{34}$	2-methylpentadecane	
16	17.333	212	$C_{15}H_{32}$	2,2,4-trimethyldodecane	
17	18.125	240	$C_{17}H_{36}$	2-methylhexadecane	
18	18.875	226	$C_{16}H_{34}$	2,5-dimethyltetradecane	
19	19.550	255	$C_{16}H_{33}ON$	Hexadecanamide	
20	19.742	240	$C_{17}H_{36}$	n-heptadecane	
21	21.675	254	$C_{18}H_{38}$	n-octadecane	
22	21.808	282	$C_{20}H_{42}$	2-methylnonadecane	
23	22.975	268	$C_{19}H_{40}$	n-nonadecane	
24	24.192	282	$C_{20}H_{42}$	n-eicosane	
25	25.367	296	$C_{21}H_{44}$	n-heneicosane	
26	26.500	310	$C_{22}H_{46}$	n-docosane	
27	27.583	324	$C_{23}H_{48}$	n-tricosane	

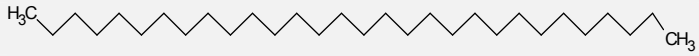
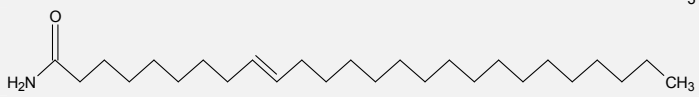
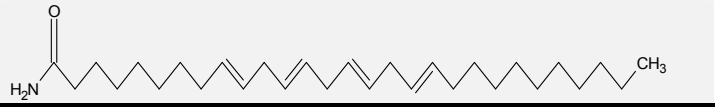
T. Line	R. T	M. W	Molecular formula	Name	Structure
28	28.633	309	$C_{20}H_{29}NO$	Cosanamide	
29	30.608	366	$C_{26}H_{54}$	n-hexacosane	
30	31.550	381	$C_{25}H_{51}NO$	Pentacosanamide	

3.2. Products of Analysis of Crude Oil (Control) Sample

Table 2: presents the results of GC. MS of petroleum crude oil. The result revealed the presence of 20 components as presented in the Gas Chromatogram Figure 4. These are 2-methyltridecane, tridecane, 2-methyltetradecane, 2-methylpentadecane, 2, 2, 4-trimethyldodecane, 2-methylhexadecane, 2, 5-dimethyltetradecane, hexadecanamide, n-heptadecane, n-octadecane, 2-methylnonadecane, n-nonadecane, n-eicosane, n-heneicosane, n-docosane, n-tricosane, cosanamide, n-hexacosane, and pentacosanamide. Followed by mass spectra of the corresponding match from the library of the suggested compound as shown in figure 5 and 6 respectively. The result is composed of a mixture of straight chain alkanes, iso-alkanes, and amides. These compounds have molecular weights (Mwt.) that ranges from 198, ($C_{14}H_{30}$) to 381 ($C_{25}H_{51}NO$) as the highest. Looking at their molecular weights (Mwt.) and their corresponding retention time (RT.), some hydrocarbons for example $C_{15}H_{32}$, reading from target line 13 having Mwt. of 212, with RT of 15.292 and the other with the same molecular weight 212, target line 16, with the RT 17.333 appeared twice. The next are hydrocarbon $C_{16}H_{34}$, target line 15 and line 18 with Mwt. of 226 eluted at different RT of 16.733 and 18.875 and finally, target line 22 and 24 having the same Mwt. (282) $C_{20}H_{42}$ elutes at different RT of 21.808 and 24.192 respectively. The variation in their RT meanwhile, having the same molecular weights is a clear indication that there are isomers in the mixture. In the original petroleum crude oil (control sample) result, the highest carbon chain length is C_{23}. However, amides with carbon chain length of C_{16}, C_{20}, and C_{25} were also present.

Table 3. *Identified reaction products from fungal degradation of petroleum crude oil on clay surface, n-hexane fraction.*

T.Line	R.T	M. W	Molecular Formula	Name	Structure
11	15.267	212	$C_{15}H_{32}$	2-methyltetradecane	
12	15.658	198	$C_{14}H_{30}$	n-tetradecane	
13	16.675	226	$C_{16}H_{34}$	2-methylpentadecane	
14	17.282	212	$C_{15}H_{32}$	pentadecane	
15	18.833	226	$C_{16}H_{34}$	hexadecane	
16	19.525	254	$C_{18}H_{38}$	4-methylheptadecane	
17	20.383	268	$C_{19}H_{40}$	4,6-dimethylheptadecane	
18	21.658	254	$C_{18}H_{38}$	Octadecane	
19	22.958	268	$C_{19}H_{40}$	Nonadecane	
20	24.192	282	$C_{20}H_{42}$	Eicosane	
21	25.367	296	$C_{21}H_{44}$	n-heneicosane	
22	26.500	310	$C_{22}H_{46}$	n-docosane	
23	27.592	423	$C_{28}H_{57}ON$	octacasane amide	
24	28,633	338	$C_{24}H_{50}$	n-tetracosane	
25	29.642	412	$C_{30}H_{52}$	9, 12, 15, 18, 21-cotapenteniene	

26	30.825	437	$C_{29}H_{59}ON$	nonacosanamide	
27	32.850	422	$C_{30}H_{62}$	n-triacontane	
28	34.950	393	$C_{26}H_{51}ON$	9-hexacosenamide	
29	34.317	429	$C_{29}H_{51}ON$	9, 12, 15, 18-nonacosatetraieneamide	

3.3. Products Analysis of Fungal Degradation of Petroleum (Crude Oil) on Clay Surface

The GC.MS results of the identified products of fungal degradation presented in Table 3, showed a clear disparity with that of the original petroleum (crude oil) that was not acted upon by fungus which served as control. This results revealed a total of 19 components and are; 2-methyltetradecane, n-tetradecane, 2-methylpentadecane, pentadecane, hexadecane, 4-methylheptadecane, 4,6-dimethylheptadecane, Octadecane, Nonadecane, Eicosane, n-heneicosane, n-docosane, octacasane amide, n-tetracosane, 9, 12, 15, 18, 21-cotapenteniene, nonacosanamide, n-triacontane, 9-hexacosenamide, and 9, 12, 15, 18-nonacosatetraieneamide. The products formed are more of linear alkanes than the iso-alkanes; hence fungal activity on the crude oil yielded higher molecular weights alkanes which ranges from molecular weight of 212 ($C_{15}H_{32}$) to 429 ($C_{29}H_{51}NO$), with hydrocarbon chain length of C_{30}as the highest. The result further revealed the existence of isomers found in (target line) T.L 11 and 14, with Mwt. 212 ($C_{15}H_{32}$) which elutes at different retention time (R.T) 15.267 and 17.282. Similarly, target line 13, and 15, having the Mwt. 226 ($C_{16}H_{34}$) eluted at R.T. 16.675, and 18.833 respectively. The research therefore, found that fungus was able to degrade carbon-carbon chain, recombine and eventually elongates the carbon chain. The identification of seven (7) new compounds in the degradation of the crude oil that were originally absent in the crude oil sample functionalizes the technique of fungal degradation. These components with increased C/H ratio are of high commercial value. The compounds produced have the following molecular weights; 423 ($C_{28}H_{57}NO$) octacosanamide, 338 ($C_{24}H_{50}$) tetracosane, 412 ($C_{30}H_{52}$) 9, 12, 15, 18, 21-cotapenteniene, 437 ($C_{29}H_{59}O$) nonacosanamide, 422 ($C_{30}H_{62}$) triacontane, 393 ($C_{26}H_{51}NO$) 9-hexacosenamide and, 429 ($C_{29}H_{51}NO$) 9, 12, 15, 18-nonacosatetraieneamide respectively. Hence, it can be seen that, these new products formed were as a result of fungi activity. This is evident to say that the fungus has upgraded the status of the original petroleum (crude oil) sample by elongating the carbon-carbon chain length.

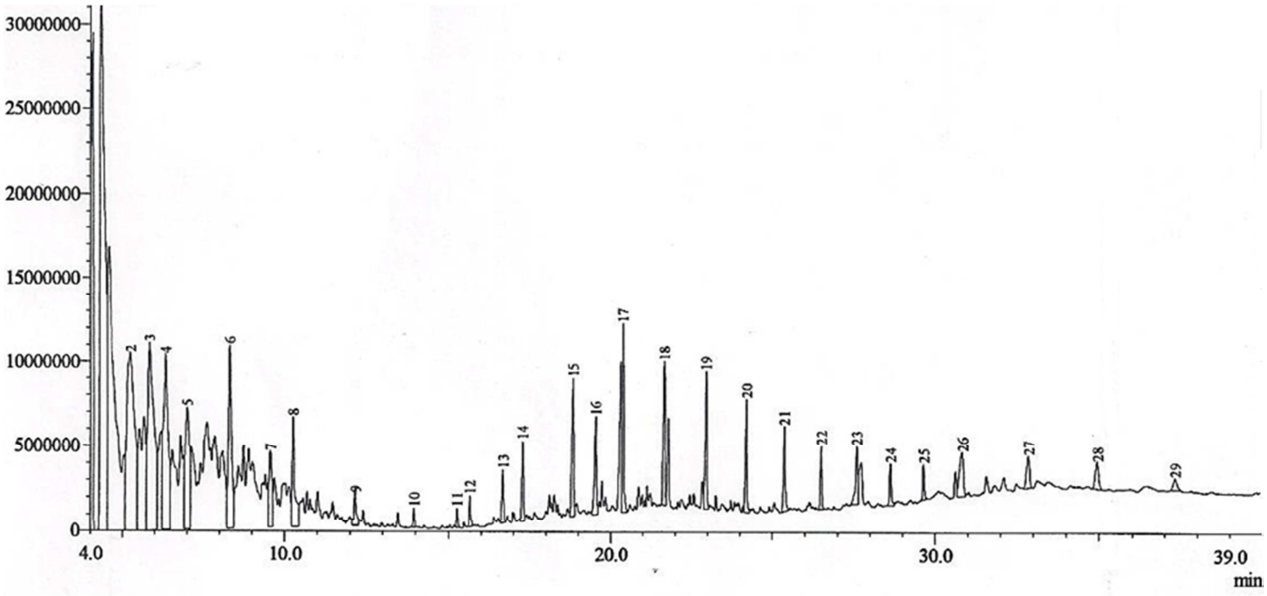


Figure 4. *Gas Chromatogram of the n-hexane fraction of the products of fungal degradation of Petroleum crude oil on Clay surface.*

Mass Spectra of Suggested Compounds of Upgraded Products

The mass spectra of the corresponding match from the library of the suggested compounds.

Peak 14, R. Time: 17.283

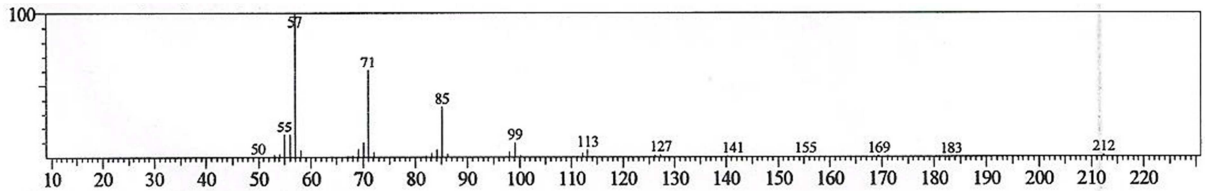


Figure 5. *Mass spectra of unknown compound from Fungal degraded crude oil.*

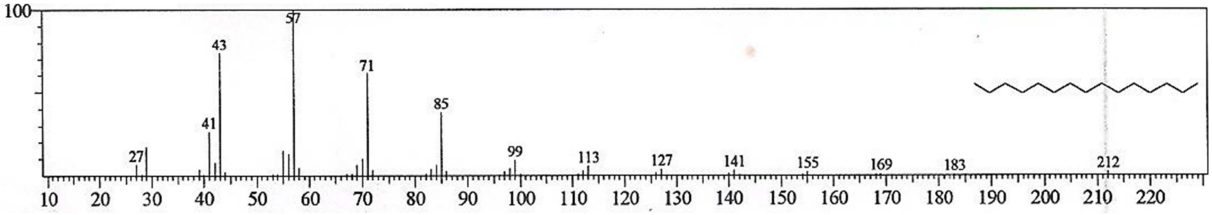
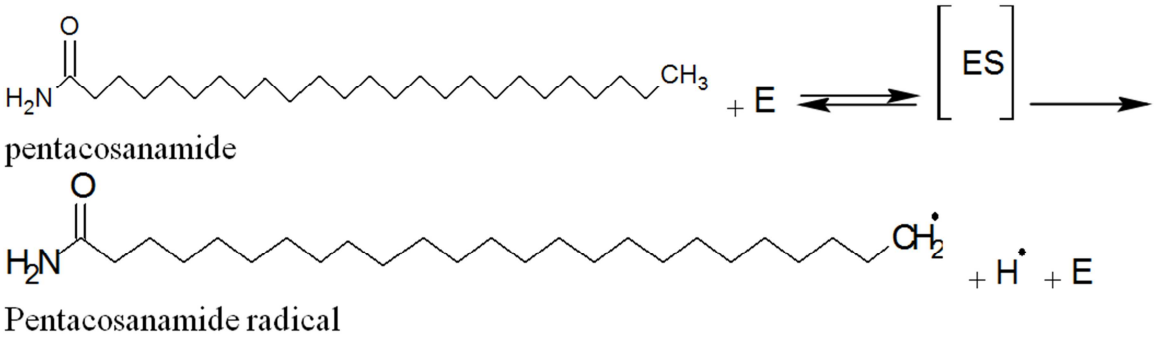


Figure 6. *Mass spectra ofknown compound from NIST library (standard) Pentadecane ($C_{15}H_{32}$).*

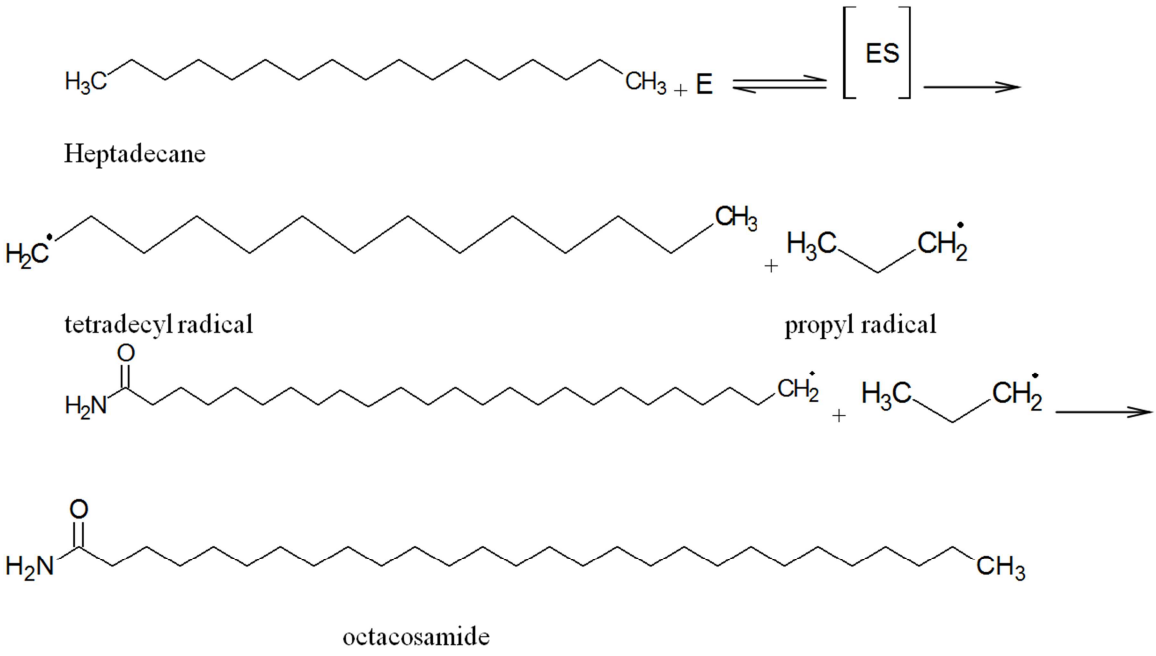
3.4. Proposed Mechanism for the Products Formation of Fungal Degradation

Below are the suggested mechanisms for all the new products resulted from fungal degradation of petroleum (crude oil).

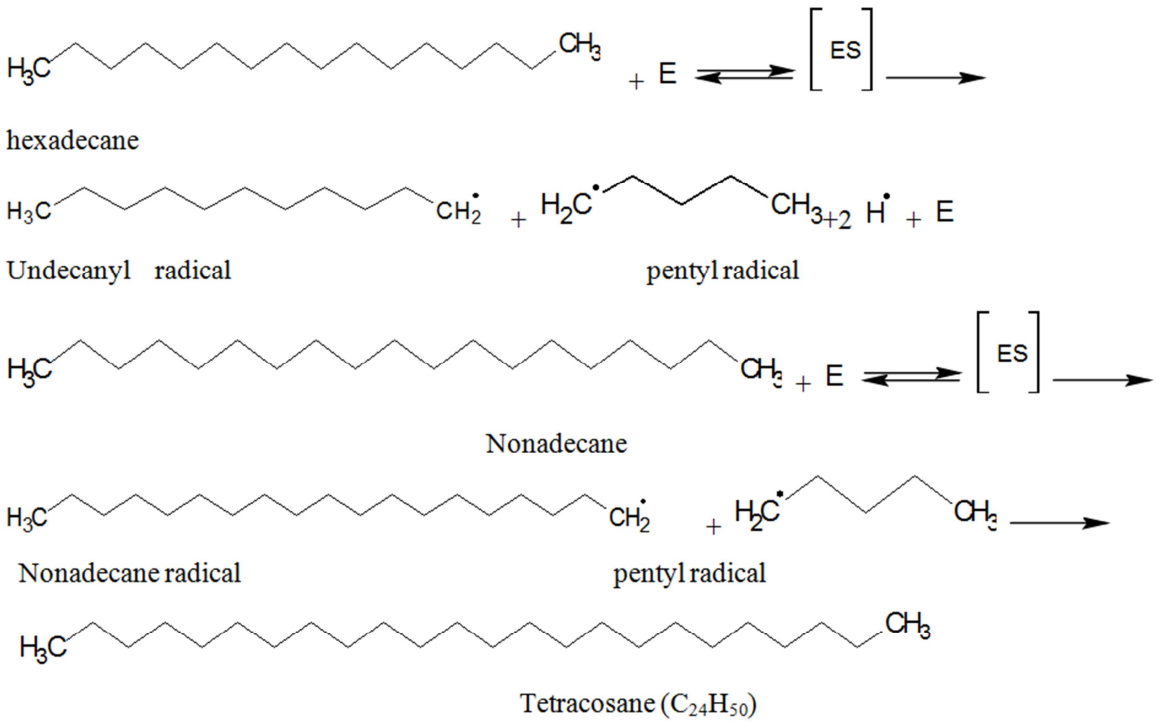
A. The formation of octacosamide ($C_{28}H_{57}NO$)



The enzyme then utilizes and cleaves heptadecane in the reactor and produced propyl radical and tetradecyl radical, which recombined with pentacosamide radical to form the product.

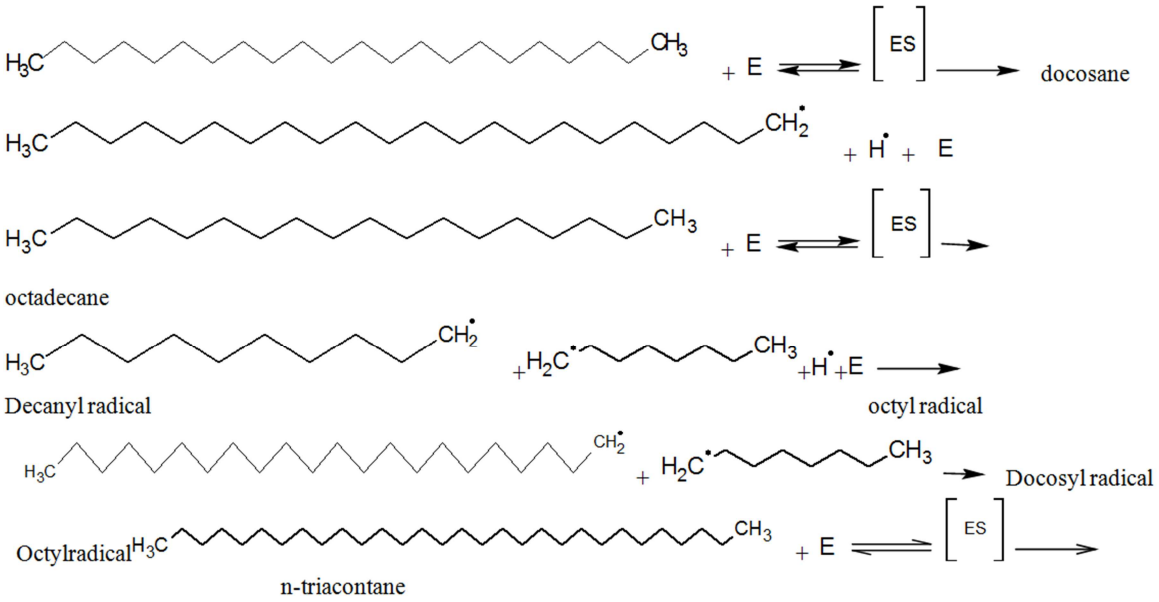


B. Formation of Tetracosane ($C_{24}H_{50}$)

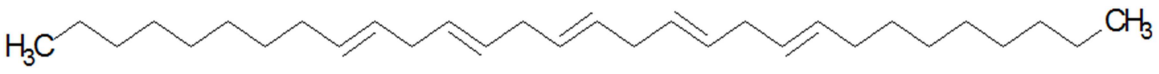


The above enzyme catalysis begins with hexadecane present in the crude oil. The fungus cleaves this compound and produced lowermolecular weights products; undecanyl and pentyl radical. The fungi then abstracts hydrogen atom from nonadecane, this radical thenrecombined with pentyl radical and gave tetracosane.

C. Formation of 9, 12, 15, 18, 21-cotapenteniene ($C_{30}H_{52}$)

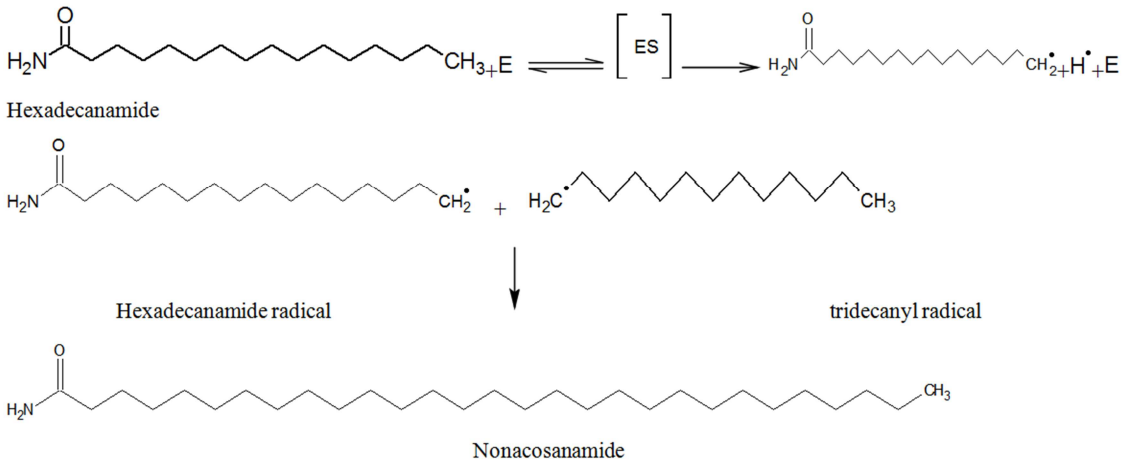


In the above reaction, hydrogen abstraction occurs facilitated by the fungi in the reactor, this leads to the formation of double bonds on carbon 9, followed by 12, 15, 18 and 21 to give the final product 9, 12, 15, 18, 21-cotapenteniene

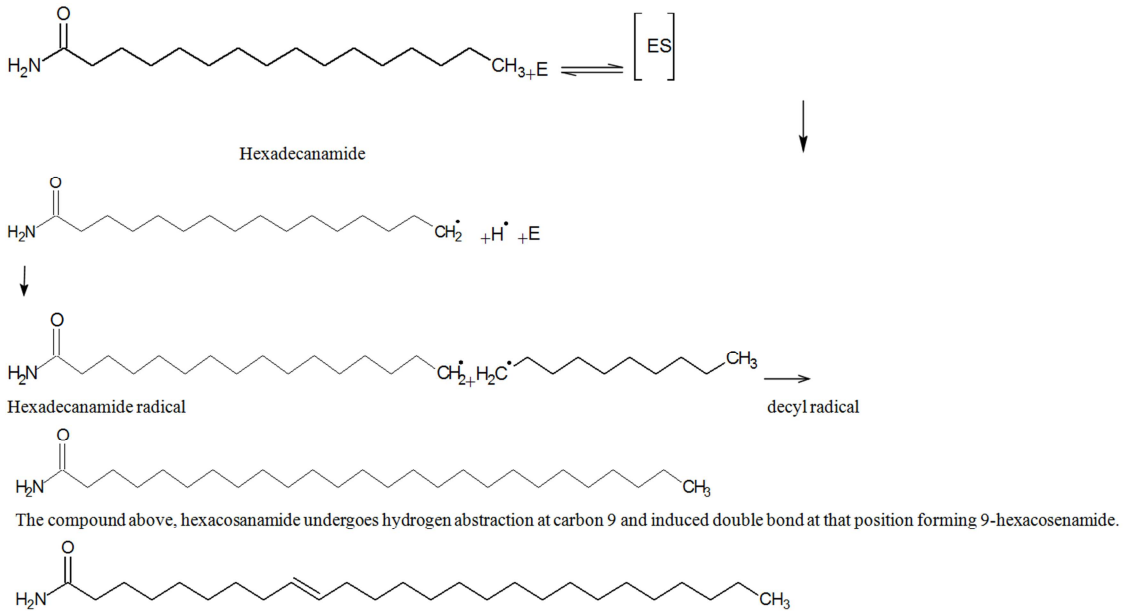


9,12,15,18,21-cotapenteniene

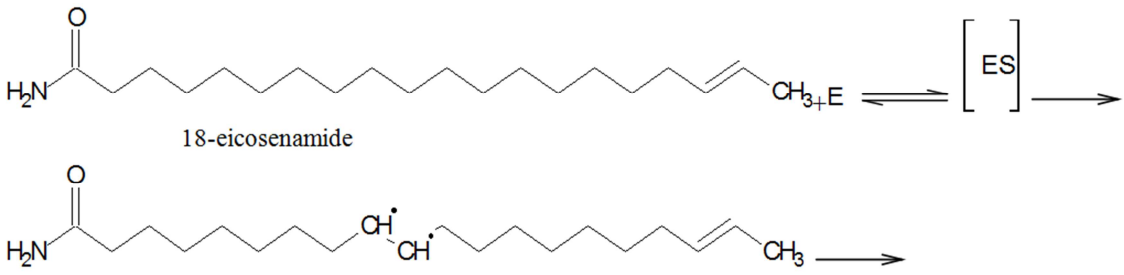
D. Formation of nonacosanamide ($C_{29}H_{59}NO$)



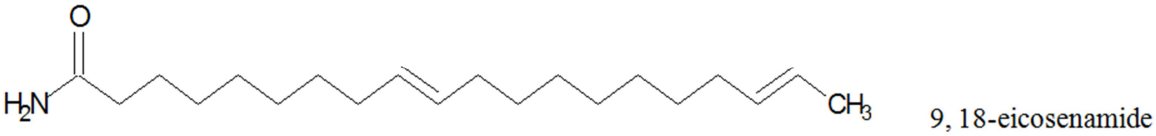
E. Formation of 9-hexacosenamide ($C_{26}H_{51}NO$)



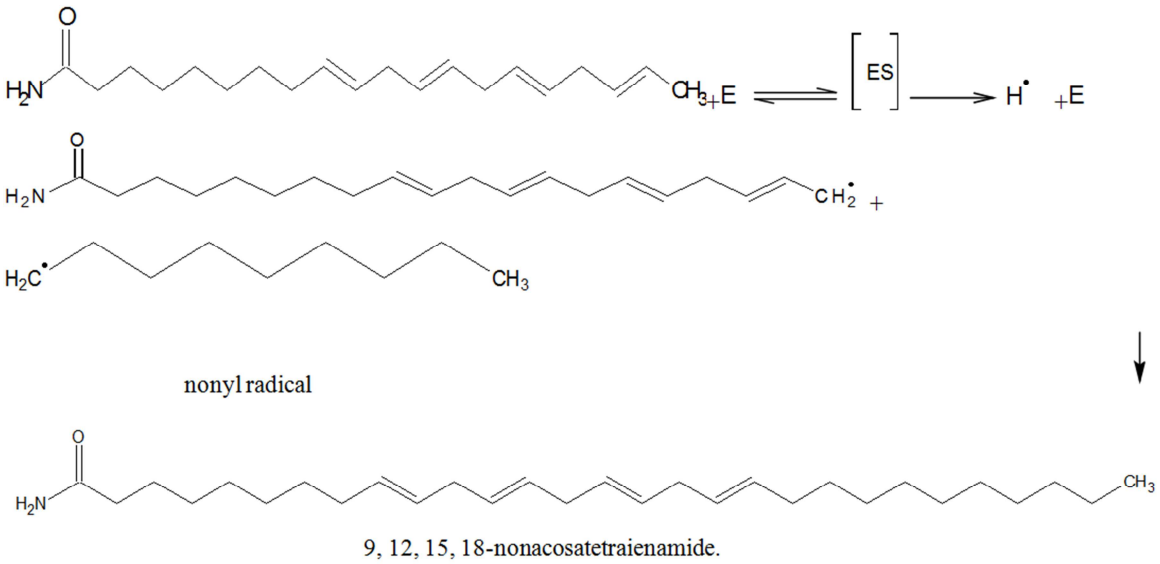
F. Formation of 9, 12, 15, 18-nonacosatetraienamide ($C_{29}H_{51}NO$)



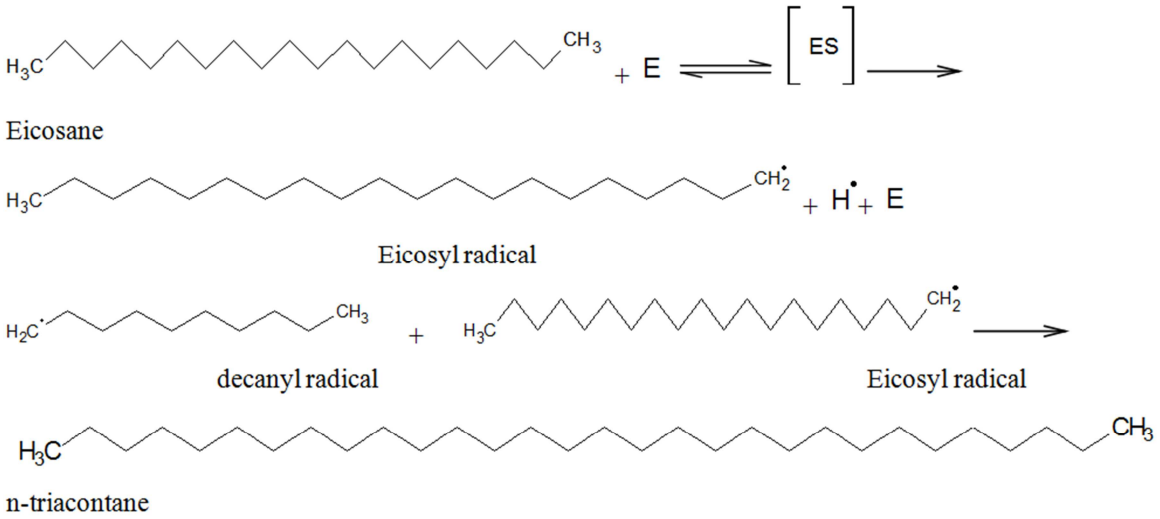
In the above reaction, hydrogen abstraction occurred at C9 and C10 which eventually induced double bond, hence converting 18-eicosenamide, to 9, 18-eicosenamide



The 9, 18-eicosaienamide undergoes further hydrogen abstraction reaction by the enzyme as shown above on C_{12}, and C_{15} toform 9, 12, 15, 18-eicoseienamide, which then recombined with nonyl radical to produce the final product 9, 12, 15, 18-nonacosatetraienamide



G. Formation of n-triacontane ($C_{30}H_{62}$)



3.5. Conclusion

The researcher has succinctly studied the status upgrade of Nigerian petroleum by fungi degradation on clay surface at room temperature and the mechanism of degradation. The results revealed that petroleum (crude oil) can be degraded, recombined and eventually upgraded by the action of fungi to produce higher molecular weights hydrocarbons and valuable chemicals so that when cracked would eventually meet refinery feedstock specification.

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