Kathmandu University
Journal of Science, Engineering and Technology

Biodegradation of components of mixture of hydrocarbons by a defined pure and consortium of selected hydrocarbon degraders

Ogirima Olawole Olanipekun* , Atoke Olaide Ogunbayo , and Rahaman Adisa Bello

Department of Chemical & Petroleum Engineering, University of Lagos, Nigeria

Abstract

The Biodegradation of a mixture of aliphatic and polyaromatic hydrocarbons as multicomponent substrates in a liquid medium by pure and mixed cultures of two bacteria, *Providential rettgeri* and *Streptococcus salivarius*, two mould, *Trichoderma harzianum* and *Aspergillus flavipes,* and one yeast, *Candida famata* was investigated in this study. The microbes were isolated from petroleum hydrocarbon contaminated soil in the Niger Delta Nigeria. The fate of the various components in the substrate was monitored individually. Each component was found to decrease during the degradation period. The degrading ability of the consortium was further studied by quantifying the growth of the culture using cumulative Carbon (vi) oxide produced and optical density method. The rate of degradation of each hydrocarbon was monitored, and growth was observed correspondingly to the degradation of the substrates. At the end of 5 days, 86.54, 81.85 and 81.71 percentage of 2- methylnaphthalene, 1- methylnaphthalene and 1,3- dimethylnaphthalene respectively were degraded while 59.65 and 73.61 percentage of hexadecane and heneicosane respectively were degraded. Further degradation was obtained after 11 days resulting in 2.36, 3.30, 5.90, 6.13 and 6.35 percentage of 2-methylnaphthalene, 1- methylnaphthalene, 1,3- dimethylnaphthalene, hexadecane and heneicosane respectively. Kinetic parameters such as the maximum substrate consumption rates of 0.507, 0.194. 0.798, 1.490 and 0.731 g/g/hr and the affinities of 20.70, 6.31, 50.60, 601.0 and 358.0 were obtained for 2- methylnaphthalene, 1- methylnaphthalene, 1,3- dimethylnaphthalene, hexadecane and heneicosane respectively. This result showed the prospect of the defined consortium for bioremediation of multicomponent substrates.

Keywords: Biodegradation; Multicomponent; Substrate; Consortium; Aliphatic; Polyaromatic

1. Introduction

Among various factors that affect the design of bioremediation processes are the nature of substrates and microbial compositions in the environment. Inadequate understanding of these factors can lead to a setback in the success of the remediation process particularly when the contaminants are in mixtures such as in crude oil and some of its fractions. Besides that, the degradation of one can be inhibited by other in the mixture and different conditions may be required to degrade different compounds within the mixture. One of such conditions is the availability of members of the consortium of microorganisms with adequate abilities to degrade the various components.

Contamination of the environment with crude oil results in co-contamination of the environment with mixture of hydrocarbons, leading to a general situation which can be described as a case of multiple cells competing for multiple substrates. Crude oil is a natural, heterogeneous mixture of hydrocarbons with potentially large chemical components, ranging from alkanes with different chain lengths through cycloalkanes, mono-aromatic to polycyclic aromatic compounds [1]. This is an important issue in biodegradation and bioremediation studies since there is need to understand the biodegradability of the hydrocarbon components of the mixture.

Individual microorganisms metabolise only limited range of hydrocarbon substrates [2,3], so biodegradation of crude oil requires mixtures of different microorganisms (consortia) to degrade a wider range of hydrocarbons [2,4]. Therefore, development of treatment strategies for crude oil-contaminated environment (soil) requires consideration of individuality of the component hydrocarbons. But many, if not most of the existing literature have not adequately described the fate of the substrate, especially, crude oil and its derivatives, which are a mixture of hydrocarbons, and hence a multi-components substrate.

The hydrocarbon compositions of crude oil vary widely in their physicochemical properties such as their viscosities, solubility, and capacities to be absorbed, as well as varying in their biodegradability and toxicity [5]. When there is crude oil pollution in the soil, the low molecular weight fractions such as C5 - C9; n alkanes and benzene, toluene, ethyl-benzene and Xylene (BTEX) normally volatilize to the atmosphere, the C10 - C16 n alkanes and monoaromatic hydrocarbons are transportable in the soil and biodegradable and are mainly removed by indigenous bacteria. But the high molecular weight components such as alkanes with carbon number higher than twenty, polycyclic aromatics hydrocarbons (PAHs) such as naphthalene, anthracene, phenanthrene, chrysene and so on. and their alkylated derivatives have proved difficult to biodegrade in the soil [6,7,8].

The aim of this study is to investigate the biodegrading capacity of pure and different defined consortium of microorganisms obtained from indigenous isolates from Niger Delta, Nigeria to degrade multicomponent substrate formulated by mixture of higher molecular weight and polycyclic aromatic hydrocarbons selected from the components of Nigeria crude oil.

*Corresponding author. Email: oolanipekun@unilag.edu.ng

2. Methodology

2.1. Substrate preparation

The substrate was composed of hexadecane, heneicosane, 1-methylnaphthalene, 2-methylnaphthalene and 1, 3-dimethylnaphthalene which are confirmed component of crude oil based on analysis as provided in Olanipekun thesis [11]. The mixture was prepared by dissolving 21.49, 18.48, 14.58, 6.88 and 18.56 mg/l of hexadecane, heneicosane, 1-methylnaphthalene, 2-methylnaphthalene and 1, 3-dimethylnaphthalene based on their percentage composition in Nigerian crude oil, respectively in acetone because of the insolubility of components in water, and this was then dissolved in the mineral salts medium pre-prepared, to serve as spiking solution to inoculate the experimental vessels.

2.2. Preparation of bacterial and fungal inocula

Bacteria (*Providential rettgeri* and *Streptococcus salivarius*) and fungi (two Mould, *Trichoderma harzianum* and *Aspergillus flavipes* and a yeast, *Candida famata*) were transferred in sterile environment to separate flasks for inoculum preparation. Bacterial inocula were grown at 25 ± 2 °C and 175 rpm in Nutrient broth, (NB) (500 ml in 1-liter flasks) for 48 hrs. The Cells were harvested by centrifugation, washed twice with sterile BSM, and suspended in an appropriate volume of basal salt medium, (BSM) whose composition in g/l was NaCl, 10.00; $MgSO_4.7H_2O$, 0.42; KCl, 0.29; KH_2PO_4, 0.83; $Na_2HPO_4.H_2O$, 1.25 and $NaNO_3$, 0.42 in distilled water (pH, 7.2).

Fungal and yeast inocula were prepared by growing isolates on PDA plates at 30 °C for 7 days. Spores were harvested into 25 ml of YPD broth, and 10 ml of the suspension was used to inoculate 250 ml of YPD. After 48 h at 25 ± 2 °C and 175 rpm, the mycelial pellets were collected by filtration through Whitman no. 1 paper under vacuum and washed thrice with sterile BSM, and then suspended in appropriate volume of BSM.

The cell suspensions of the microbes were adjusted with BSM to obtain ~1.00 OD (600_{OD}) [9] and used as inocula in all the experiment in line with the works of Chang et al. and Sebiomo et al. [8, 10].

2.3. Experimental set-up design for pure and various mixed cultures

A 2^5 full factorial experimental design was performed with five factors (microbes) and two levels (positive or negative) in sacrificial tubes, to determine the abilities of the individual microbe and various possible consortium of microbes to degrade the mixed hydrocarbons substrate. The test tubes were prepared in duplicates for the various sampling points containing 4 ml broth of 1 % (v/v) substrate whose composition stated in section 2.1. 10 % (v/v) inoculum of different variants, (Table 1) and 89 % culture media (BSM, composition as section 2.2). The controls were also setup to verify if there are losses due to volatilization, abiotic factors and, if there will be growth of the microbes in pure and mixed culture in the BSM without the substrate. The experiment was carried out for 7 days at 25 ± 2 °C continuously agitated in a shaker with the sampling at 0, 1, 2, 4, 8, 12, 24 hours, 3, 5 and 7 days.

2.4. Bioremediation experiment

Bioremediation experiment using the variant 30 whose composition was *Providencia rettgeri*, *Streptococcus salivarius*, *Trichoderma harzianum*, and *Aspergillus flavipes* that has shown higher degradation than other variants after 8 hours and also, because it has wider representation of microorganisms over variant 19 [11], was carried out in sterile 250 ml conical flasks. The experiment was divided into two parts. The first part was carried out in conical flasks which were sealed with non-absorbent cotton wool containing 100 ml Broth of the following composition: 1 ml of the target compound whose composition was stated in section 2. 10 ml of inoculum (the consortium) and 89 ml of culture media. The second part was exactly the same as the first except that it was hermetically sealed with an outlet for sampling and to trap CO_2 generated in another flask containing NaOH solution. All the conical flasks in the setups were covered to avoid access to light, and with the exemption of the controls were put in a rotary shaker 125 rpm and maintained at 25 ± 2 °C. The samples were periodically withdrawn using chemical resistance liquid sampling pipette for aqueous broth of the first and second setups at 0, 24, 72, 120, 168, 216 and 264 hours for analysis.

2.5. Microbial growth analysis

The rate of growth of consortium was followed using dry weight and optical density techniques simultaneously. Samples were taken periodically during incubations and rapidly cooled and stored at 4 °C if necessary. The growth was assessed by optical density using a MDS Analytical Technologies UV-VIS spectrophotometer and quartz cuvettes with a 1-cm path length at wavelength of 540 nm as adopted by Vipulanandan et al. [12], 22.5 °C and displaced with softmax pro on monitor.

For dry weight determinations, the methods used by Baneerjee et al. and Chang et al. [8,13] were adopted. The samples were first centrifuged for 10 min at 5000 rpm, and then the dry weight of the clear supernatant was measured in order to subtract the dry weight of medium constituents from the first measurement.

2.6. Analytical methods

The Chemical analysis was for the estimation of the concentration of the components of the mixed substrate.

The hydrocarbons mixture concentration in hexane extract were analysed on Agilent 7890 gas chromatograph coupled with an Agilent 7000B triple quadrupole (QqQ) mass spectrometer, MS fitted with an electron Ionization, EI source and a collision cell. The MS was operated in the scan mode to obtain spectral data for identification of individual components and monitoring selected ions with SIM mode for quantification of the target compounds.

The column used was Agilent DB-5MS column with dimension of length 30 m by diameter 0.25 mm by film 0.25 um. The chromatograph conditions were as follow, carrier gas helium (0.8 mL/min), Injection mode was pulse splitless, injection and detector temperatures were 290 and 320 °C respectively and the oven temperature program was 50 °C (0.8 min hold), 40 °C/min to 250 °C (2 min hold); 30 °C/min to 325 °C (1 min hold) and total run time of 11 min was used.

The quantification was carried out on the GC-MS in SIM mode rapid response factors, RRFs for each compound determined during the instrument calibration. The ions monitored were 141 for 1-methylnaphthalene, 2-methylnaphthalene and 1, 3-dimethylnaphthalene, 57 for hexadecane, and heneicosane. The RRF for each target compound was calculated from the authentic standards. The hydrocarbons were quantified by employing Agilent Technologies Masshunter Ms quantification software.

3. Results and discussion

3.1. Biodegradation of the component of the model substrate by pure and mixed cultures of the selected microorganisms

The microbes in pure and various combination (variants) as shown earlier in Table 1, have shown the abilities to degrade substantially the components of the substrate, though at different degrees as depicted in the Fig. 1-3.

Table 1: Experimental variants of the member of the formulated consortium to determine their abilities in pure and mixed cultures on the mixture of the hydrocarbons.

Variant	Compositions
C1	BSM without any microbes and without Model Substrate
C2	BSM + Model Substrate
1	BSM + Model Substrate + bacterium I
2	BSM + Model Substrate + bacterium II
3	BSM + Model Substrate + bacterium I &II
4	BSM + Model Substrate + mould I
5	BSM + Model Substrate + mould II
6	BSM + Model Substrate + yeast
7	BSM + Model Substrate + mould I & II
8	BSM + Model Substrate + mould I & yeast
9	BSM + Model Substrate + mould II & yeast
10	BSM + Model Substrate + mould I + II + yeast
11	BSM + Model Substrate + bacterium I + mould I
12	BSM + Model Substrate + bacterium I + mould II
13	BSM + Model Substrate + bacterium I + yeast
14	BSM + Model Substrate + bacteria II + mould I
15	BSM + Model Substrate + bacteria II + mould II
16	BSM + Model Substrate + bacteria II + yeast
17	BSM + Model Substrate + bacteria I & II + mould I
18	BSM + Model Substrate + bacteria I & II + mould II
19	BSM + Model Substrate + bacteria I & II + yeast
20	BSM + Model Substrate + bacteria I + mould I & II
21	BSM + Model Substrate + bacteria II + mould I & II
22	BSM + Model Substrate + bacteria I + mould I + yeast
23	BSM + Model Substrate + bacteria I + mould II + yeast
24	BSM + Model Substrate + bacteria II + mould I +yeast
25	BSM + Model Substrate + bacteria II + mould II + yeast
26	BSM + Model Substrate + bacteria I + mould I & II + yeast
27	BSM + Model Substrate + bacteria II + mould I & II + yeast
28	BSM + Model Substrate + bacteria I & II + mould I & II
29	BSM + Model Substrate + bacteria I & II + mould I + yeast
30	BSM + Model Substrate + bacteria I & II + mould II + yeast
31	BSM + Model Substrate + bacteria I & II + mould I & II + yeast

Key: Bacteria I- *Providencia rettgeri*, Bacteria II- *Streptococcus salivarius*,
mould I- *Trichoderma harzianum*, Mould II- *Aspergillus flavipes* and Yeast-*Candida famata*. BSM-Basal salts media, Model substrate- mixture of hexadecane,heneicosane, 1-Methylnaphthalene, 2-Methylnaphthalene, and 1,3-Dimethylnapthalene.

Within the 8 hours of the degradation experiment, the PAHs components were reduced by about 80 % the pure variants 1, 2, and 3, which are the bacteria in pure and mixed cultures. The alkane components, hexadecane and heneicosane were respectively degraded by about 65 % and 40 %, 80 % and 45%, 78 and 18%, and 55% and 32%, and 37% and 32% in culture variants 1, 2, 4, 5, and 6 respectively. Other variants showed different degrees of disappearance of the alkanes but variant 30 demonstrated the best degradation ability because it reduced all the PAHs and hexadecane to less than 20% and heneicosane to less than 40% after 8 hours of incubation.

After 72 hours (3 days) of incubation, there were further significant disappearances of the various components of the substrates. All the PAHs were reduced to between 10 - 20% in the variants even less than 10% in variants 1, 2, 3, and 20. The rates of the disappearances of the PAHs were also slow in variants 5 and 6 when compared to other pure culture variants such as 1 and 2. The variants 15 and 31 showed the degradation of PAH components of more than 90% and above. And about 80% in variants 7 and 14. Also, the alkane components especially the hexadecane was reduced to 40% and below in all the variants. The most reduction of heneicosane was in variants 23, 28, 29 and 30 where there was about 80 % reduction.

At 120 hours (5 days) of the experiment, there were further degradation (disappearance) of all the components (Fig. 3). The variants 15, 16, 20 and 23, reduced the heneicosane to 20 % or less. The bacteria in pure cultures, that is variants 1 and 2, showed higher abilities to degrade all the components of the mixture more than the mould and the yeast (variants 4, 5 and 6). Variant 1 reduced the PAHs, 1-MN, 2-MN and 1, 3-DMN to less than 10 %, the hexadecane to less 20 % and heneicosane to less than 40 %. Variant 2 (bacteria II) also reduced PAHs to less than 10 %, hexadecane and heneicosane to less than 30 %.

The mould in pure cultures that is variants 4 and 5 (fungus I & II respectively) reduced the PAHs to less than 10 %, while variant 4 reduced hexadecane and heneicosane to less than 30 % and 50 % respectively, variant 5 reduced heneicosane to about 60 % and hexadecane to about 30 %. Yeast pure culture, variant 6 showed higher degree of removal than the fungi by reducing the hexadecane and heneicosane to less than 40 %.

The mixed cultures showed varying abilities in the removal of the components and this may be due to the relationship exhibited by the presence of any of the microbes. For instance, variant 3, that is mixed culture of bacteria I and II reduced hexadecane to about 30 % while variant I and variant II reduced it to less than 20 % and 30 % respectively. Also, variants 1 and 2 reduced heneicosane to about 40 % and 35 % respectively but the mixed culture could only reduce it to less than 60 %.

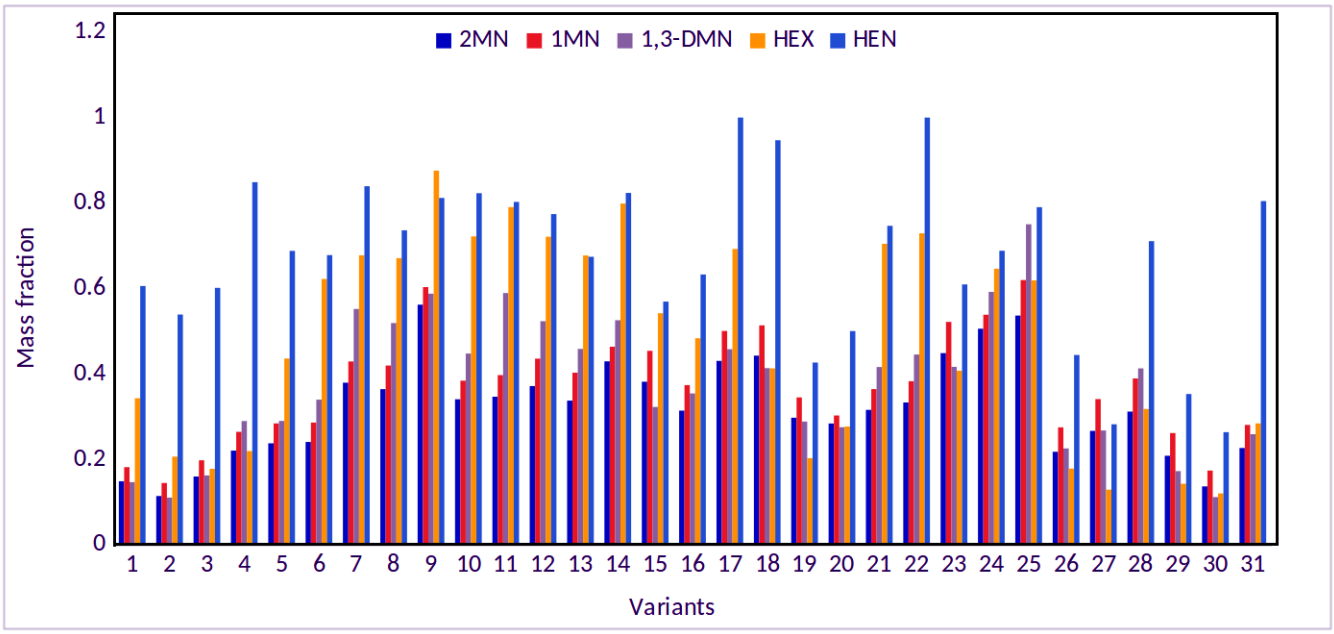


Figure 1: Degradation rate of the various components of the model substrate after 8 hours of biodegradation.

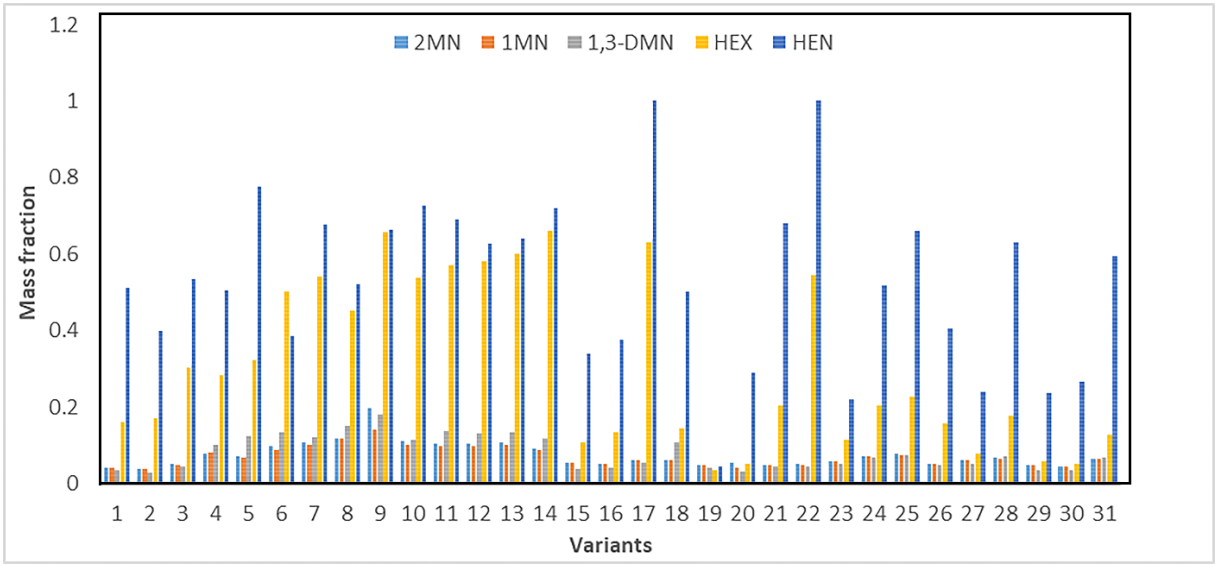


Figure 2: Degradation rate of the various components of the model substrate after 72 hours of biodegradation.

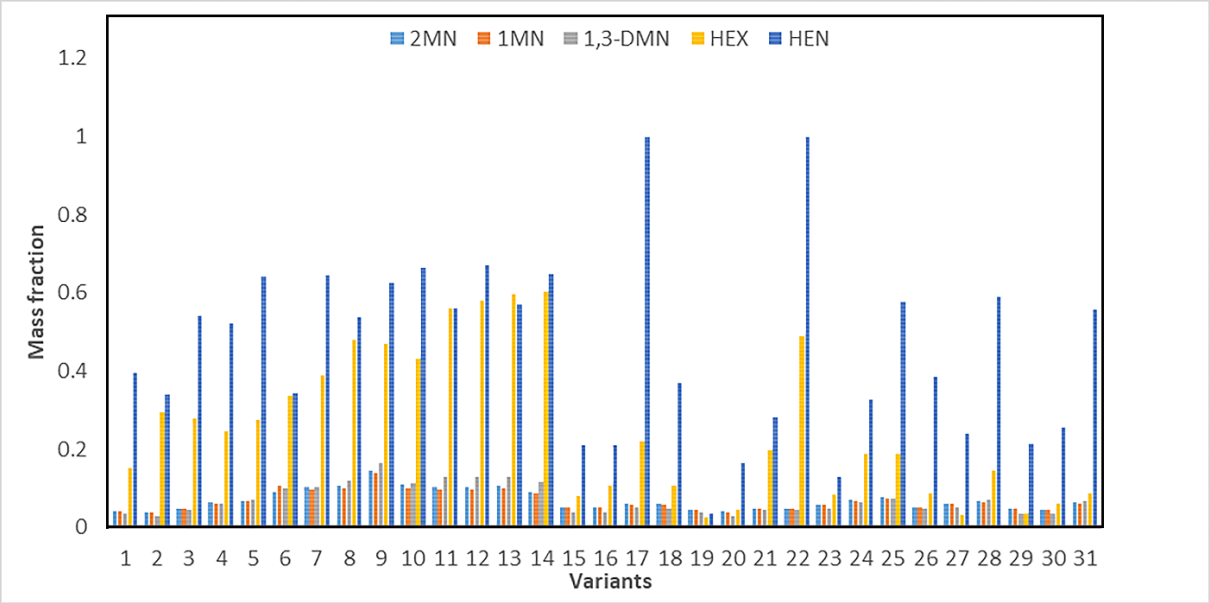


Figure 3: Degradation rate of the various components of the model substrate after 120 hours of biodegradation.

Similarly, while the mixed culture of the mould (variant 7) degraded the alkanes, hexadecane and heneicosane to 40 % and 60 % respectively, the pure culture of the moulds I & II (variants 4 and 5) degraded the same components to about 25 - 50 %, and 20 - 60 % respectively.

It is generally true that mixed cultures may be required to remove mixture of hydrocarbons, not all mixed cultures are effective as it has been observed. It is believed that the type of interactions existing between various microbial population as well as the products of the metabolic pathways may have to be taken into consideration.

The presence of bacterium II and/or mould II in any variants were observed to be significant to disappearance of many of the components of the substrate, for example in variants 9, 12, 14, 15, 16, 17, 18, 28 and 31.

3.2. Microbial growth and biodegradation of model substrate

The consortium has demonstrated the potential to degrade the substrate though in different degrees and at different rates for various components (Fig. 4 a,b). The result showed that at the earlier stage of degradation, most of the heneicosane was consumed more than the other hydrocarbons in the model substrate. It was observed that after 12 hours of the experiment about 53.50 % heneicosane had been degraded as against 18.34, 15.63, 34.99 and 32.38 % of 2MN, 1MN, 1, 3-DMN and HX respectively.

After 5 days (120 hours) most of the polyaromatic hydrocarbons had been significantly degraded: 86.54 % 2MN, 81.85 % 1MN and 81.71% 1, 3-DMN while only 59.65 and 73.61 % of HX and HN respectively were degraded. This is an indication that the interaction in the consortium developed an appropriate combination of enzymes that helped in the rapid degradation of the PAHs. After 11 days, the degradation rate showed that the percentages of 2MN, 1MN, 1, 3-DMN, HX and HN remaining were 2.36, 3.30, 5.90, 6.13 and 6.35 respectively.

It was also observed that the rate of degradation of polyaromatic hydrocarbons was affected by the position and degree of alkylation, as the rate of degradation of 2-MN, 1-MN and 1,3-DMN increased in that order at any time during the experiment. Most of the 2-MN disappeared rapidly more than 1-MN while 1, 3-DMN degraded more slowly than either of the two. This rapid degradation of 2-MN was

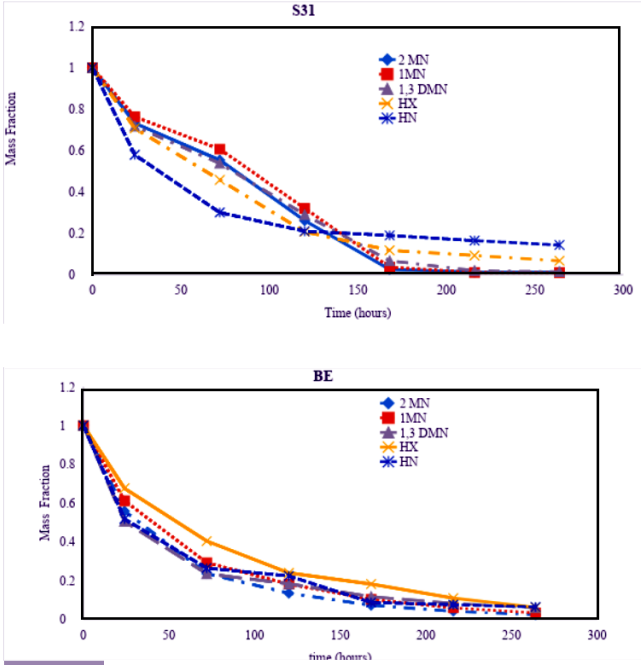
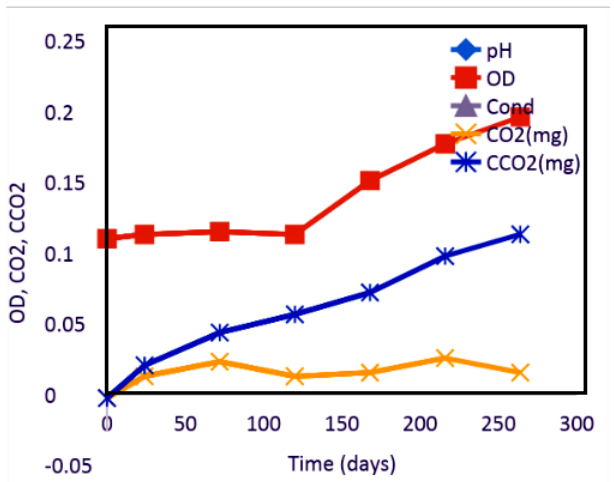


Figure 4: Biotic loss due to consortium of the components of the substrate in (a) cotton wool sealed flask (S31) and (b) hermetically sealed flask (BE).



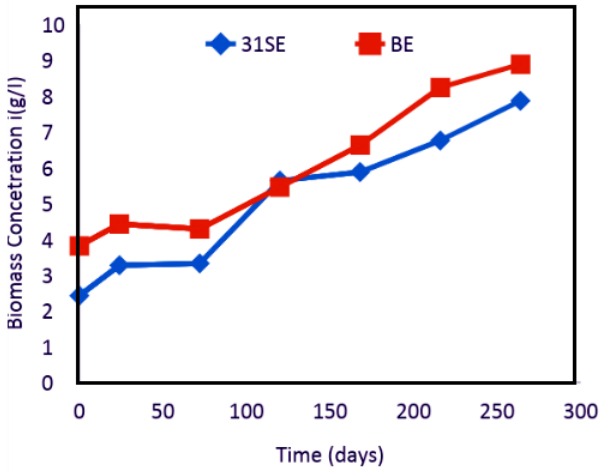


Figure 5: The growth characteristic of the microbes on the model substrate in the (a) hermetical sealed flask and (b) Dry biomass concentration of the consortium in wool sealed and hermetically sealed flasks.

observed also in the work of Lblonde et al. [14]. Therefore, suggesting that high degree of alkylation reduces the rate of degradation.

This can be attributed to the ability of the consortium to grow on the substrate as shown by increase in the biomass concentration (Fig. 5 a,b) as the various components decreased. Fig. 5a depicts the three indicators used in monitoring the growth of the consortium and their correlation. That is, as the biomass concentration increased the cumulative CO_2 increased, so also did the optical density.

The growth pattern observed during the study is expected as the individual microbe that made up the consortium would have grown at different rates under different interactions resulting in pattern growth similar to serial growth. The consortium's biomass concentration increased as the weight fraction (concentration) of each component of the substrate decreased, an indication that they are not only homologous but supported the growth of the consortium.

3.3. The growth kinetics of the consortium on components of the substrate

Adopting the Lawrence and Macarty kinetic model, the parameters evaluated are the saturation constant, H_a, and maximium specific substrate consumption rate, q^{max}, for individual component relative to the presence of the others depicted in Table 2. This gives an insight into the ability of the consortium to degrade various components of the substrate.

Table 2: Kinetic parameters for the degradation of the model substrate by the consortium.

EXPT	Parameter	2-MN	1-MN	1,3-DMN	HEX	HEN
BE	H_a (g/l)	107.0	43.8	295.0	602.0	231.0
	q^{max} (g/g/hr)	1.13	0.564	1.44	3.67	1.17
S31	H_a(g/l)	20.7	6.31	50.6	601.0	358.0
	q^{max} (g/g/hr)	0.507	0.194	0.798	1.49	0.731

Key: BE-hermetically sealed flask, S31E-cotton wool sealed flask, 2-MN- 2- methylnaphthalene, 1-MN- 1-methylnaphthalene, 1,3-DMN- 1,3-dimethylnaphthalene, HEX-hexadecane and HEN-heneicosane.

The value of H_a gives indication that though there were competitions the consortium showed more affinity for 1-Methylnapthalene than for any other component in the substrate. The affinity decreased from 1-methylnaphthalene through 2-methylnaphthalene to 1,3-dimethylnaphthalene. This can be explained by the fact that the position of alkylation and the degree of alkylation affected the affinity for the polyaromatic compounds. This finding supports the previous observations that alkyl substituents are more resistant to biodegradation and the position of the alkylation slow down the transformation rate. [14,15, 16].

In the alkane end, the consortium showed more affinity for heneicosane than the hexadecane with the value of the saturation constant of 231.0 and 602.0 g/l respectively for heneicosane and hexadecane. This is an indication that heneicosane seemed to be more recalcitrant to the consortium than the hexadecane.

4. Conclusion

The microbes in their pure and variants mixed cultures showed proficient and excellent degradation capacities for a mixture of hexadecane, heneicosane, 1-methylnaphthalene, 2-methylnaphthalene and 1, 3-dimethylnaphthalene. The result obtained indicated that though there were degradations of the components of the substrate, it can be deduced that the interactions of the microorganisms affected the degradation rates. The degradation of the substrate which is a mixture of hydrocarbons and the kinetic parameters evaluated showed that the substrate supported the growth of the defined consortium *Providencia rettgeri*, *Streptococcus salivarius*, *Trichoderma harzianum*, and *Aspergillus flavipes*. The mixed cultures that is the defined consortium shew the abilities to degrade the components of the substrate though in different degree and have different affinities for each component. The defined consortium demonstrated ability for the mixed substrate in the aqueous medium with different affinities for individual components shows its efficiency for bioremediation of environment polluted with multicomponent like petroleum products.

Acknowledgment

This research was supported by PATOFUL through the Research grant and we also appreciate Prof. Joel Hayworth of Civil Engineering Department, Auburn University, Auburn, USA for providing a Laboratory and useful materials to carry out the research.

References

[1] McGenity T J, Folwell B D, McKew B A & Sanni G O, Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquatic Biosystems*, *8*(10) (2012) 1-19.

[2] Al-Saleh E, Drobiov H & Obuekwe C, Predominant culturable crude oil-degrading bacteria in the coast of Kuwait. *International Biodeterioration and Biodegradation*, *63*(4), (2009) 400-406.

[3] Al-Wasify R S & Hamed S R, "Bacterial biodegradation of crude oil using local isolates," International Journal of Bacteriology, 2014, Article ID 863272, 8.

[4] Bordenave S, Goñi-Urriza M S, Caumette P & Duran R, Effects of heavy fuel oil on the bacterial community structure of a pristine microbial mat. *Applied and Environmental Microbiology*, *73*(19) (2007) 6089–6097.

[5] Liang Y, Zhang X, Dai D & Li G, Porous biocarrier-enhanced biodegradation of crude oil contaminated soil. *International Biodeterioration & Biodegradation*, *63* (2009) 80-87.

[6] Dutta T K & Harayama S, Analysis of longside-chain alkylaromatics in crude oil for evaluation of their fate in the environment. Environmental Science and Technology, 35(1) (2001) 102-107.

[7] Oberoi A S, Philip L & Bhallamudi S M, Biodegradation of various aromatic compounds by enriched bacterial cultures: part A-monocyclic and polycyclic aromatic hydrocarbons. Applied Biochemistry and Biotechnology, 176(7) (2015) 1870-1888.

[8] Chang L K, Ibrahim D & Omar I C, A laboratory scale bioremediation of Tapis crude oil contaminated soil by bioaugmentation of *Acinetobacter baumannii*, T30C, *African Journal of Microbiology Research*, *5*(18) (2011) 2609-2615.

[9] Obuekwe C O & Al-Zarban S S, Bioremediation of crude oil pollution in the Kuwaiti desert: the role of adherent microorganisms, *Environment International*, *24*(8) (1998) 823–834.

[10] Sebiomo A, Awosanya, A O & Awofodu A D, Utilization of crude oil and gasoline by ten bacterial and five fungal isolates. *Journal of Microbiology and antibacterials*, *3*(3) (2011) 55-63.

[11] Olanipekun O O, *Bioremediation potential of microorganisms in selected hydrocarbon contaminated soils of the Niger-Delta*, *Nigeria*, Ph. D. Thesis, University of Lagos, 2017.

[12] Vipulanandan C, Wang S & Krishnan S, *Bioremediation of phenol*. In: Wise, D L & Trantolo D J (Eds), Remediation of hazardous waste contaminated soils. Marcel Dekker Inc. New York, 1994.

[13] Banerjee C, Ghosh S, Sen G, Mishra S, Shukla P & Bandopadhyay R, Study of algal biomass harvesting through cationic cassia gum, a natural plant-based biopolymer.

[14] Leblond J D, Schultz T W & Sayler G S, Observations on the preferential biodegradation of selected components of polyaromatic hydrocarbon mixtures. *Chemosphere*, *42* (2001) 333-343.

[15] Cheng X, Hou D, Mao R & Xu C, Severe biodegradation of polycyclic aromatic hydrocarbons in reservoired crude oils from the Miaoxi Depression, Bohai Bay Basin. *Fuel*, 211 (2018) 859-867.

[16] Sihag S, Pathak H & Jaroli D P, Factors Affecting the rate of biodegradation of polyaromatic hydrocarbons. *International Journal of Pure and Applied Bioscience,* *2*(3) (2014) 185-202.