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Kinetics study of biologically remediated crude oil polluted soil using a bacteria and fungi

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Abstract: The effectiveness of remediating soils polluted with crude and treated hydrocarbon oil using a fungi - *Aspergillus niger*, bacteria - *Pseudomonas aeruginosa* and the combination of the two were investigated and the first order kinetics were studied. Eight systems of 500g soil sample were polluted with both raw and treated crude oil. Four systems were polluted with 100 ml treated crude oil while other remaining systems with same quantity of raw crude oil. Two systems with raw and treated crude oil were left as control (RCC and TCC). Samples of soil polluted with raw crude oil were amended with *A. niger* (RCA) and *P. aeruginosa* (RCP) respectively, while treated crude samples were also treated with *same* (TCA) and (TCP) only. The last two systems were treated with both *P. aeruginosa and A. niger* (RCAP and TCAP). First order bioremediation kinetics and biostimulant efficiency for these systems were studied by monitoring Total Petroleum Hydrocarbon (TPH). The result obtained at the end of the bioremediation period, revealed that treated crude oil polluted soil remediate faster and better than raw crude oil polluted soil. The highest level of bioremediation occurred in systems amended with both *A. niger* and *P. aeruginosa* which had about 98 % TPH decrease.

Keywords: bioremediation; Ex-situ; *Aspergillus niger; Pseudomonas aeruginosa;* Total Petroleum Hydrocarbon; crude oil; biostimulant efficiency.

1. Introduction

The innovation of the motor engine and its quick appropriation in all vehicle structures broadened the occupation of natural resource. This caused the expansion of the petroleum business. Method and way to recover the large percentage of oil in the depleted producing field remains a major concerns to the oil producing industries today [1-3]. Contamination problems comes as a result of processes required in the recovering of crude oil and converting it into more valuable products. Since these pollution challenges cannot be totally eliminated they still bring several issues to the environment [4,5]. Bioremediation is the clean-up process in which the degrading property of microorganisms (fungi or bacteria) is employed for the degradation or decrease of harmful substances that pollute the environment. Substances in this group include hydrocarbons at various degrees, metals, pesticides and mechanical solvents [6,7]. Of all the technologies and methods researched into in recent years for oil clean up, bioremediation is still the best approach to remediating polluted soil or water by hydrocarbons due to its ability to prevent and inhibit the accumulation of contaminants. It is also a cheap method of remediation [8].

A. niger as a crude oil degrading microorganism is a haploid fungi that is filamentous in nature. It is an important microorganism in the field of biotechnology. *A. niger* has found wide relevance in bio-transformations and management, such as the conversion of waste paper to fermentable sugar, orange peel to bioethanol e.t.c. [1-3]9-11]. The fungi is popularly found in deteriorating or decaying vegetation [12]. Fungi perform a key function in degrading hydrocarbons by releasing some capable enzymes which acts on them. This is probably as a result of their aggressive growth, extensive hyphal growth in soil, and greater biomass production. Fungi has a very high tendency for biodegradation technology [13,14]

P. aeruginosa as hydrocarbon degrading microorganism is a rod-shaped, gram-negative bacterium with an unbelievable nutritional versatility. *Aeruginosa,* as well as many other species of *Pseudomonas*, degrade hydrocarbons and also capable of breaking down toluene. This bacteria degrade toluene which is the simplest form of methylbenzene through the oxidation of methyl group to alcohol, aldehyde and acid, which is then converted to catechol. Because of this characteristic, *P. aeruginosa* can be used in environmental pollution control [15]. It is naturally found in some environments such as soil, sewage, plants, water, humans, animals and hospitals [4]**.**

Bioremediation kinetics seeks to investigate how different experimental conditions can influence the rate of the microbial degradation of the crude oil. With the study of microbial degradation kinetics, it is possible to know and understand the kinetics of soil bioremediation and also to determine the quantity of crude oil left at a particular time [17,18].

[19] reported that pollution caused by crude oil spill happens as a result of land runoff, accidents that occur involving pipelines and oil carrying vessels, oil exploration and production engineering operations, oil shipping activities and improper effluent discharge into the environment. These spills have negative and disastrous economic, environmental, and social effects on the society. As a result, oil spill accidents have created some media interest and have brought many together in a battle regarding the response of the government to oil spill incidents and the actions that are being taken to curb the menace [20]**.** The type of oil that spills, the quantity or severity of the oil spill, the environment in which the spill occurs and the prevailing weather conditions are factors that must be considered before choosing the most effective technique to clean up the spill [21].

Raw crude oil is basically crude coming directly from the depths of the earth through the wellhead. It may or may not have some amount of water and dissolved natural gas within it. Treated crude oil in this case is not necessarily crude oil that has gone through the refining process. Treated crude refers to crude oil that has been passed through an oil production platform. These platforms are located in central locations of major oil producing fields. Raw crude from the different wellheads is channelled to the platform. Here, the crude is stripped off dissolved natural gas, excess water which forms emulsion with the crude and condensates. Raw crude also comes with a high level of sand and this is also removed.
Before the knowledge of bioremediation came into limelight, there had been the existence of some crude oil spill clean-up techniques some of which are in use till this day. According to [5], these remediation techniques are:

1. The physical remediation methods
2. The chemical remediation methods
3. The thermal remediation methods

A lot of microorganisms naturally have the enzymatic or catalytic ability to degrade or simplify petroleum hydrocarbons. Some of the microbes are specific in their action. Alkanes may be degraded by certain types of micro-organisms. Other micro-organisms may degrade only aromatic compounds. It has been experimentally proven that alkane compounds that range from C_{10} to C_{26} are the most easily biodegradable. Very toxic aromatic compounds like benzene, toluene and are also easily biodegraded by microorganisms. More complex structures are very resistant to biodegradation. There are only few microbes that can degrade these compounds and the biodegradation rate is much lower than in pure alkanes [23]. A mixed microbial population may guarantee a higher level of biodegradation. The speed and efficiency of the clean-up of a soil contaminated with petroleum and petroleum products largely depends on the presence crude oil-degrading microorganisms in the soil. Temperature, oxygen, pH, content of nitrogen and phosphorus are factors necessary for microbial population growth. The type of soil in which the process occurs determines and influences the degree and rate of biodegradation [24].

Using bacteria and fungi to degrade hydrocarbons has proven to be the most environmentally friendly oil spill clean-up method known [25,26]. Microorganisms provides potential for biodegradation technology processes [6]. Figure 1 shows the schematic diagram for the general overview of Bioremediation process.

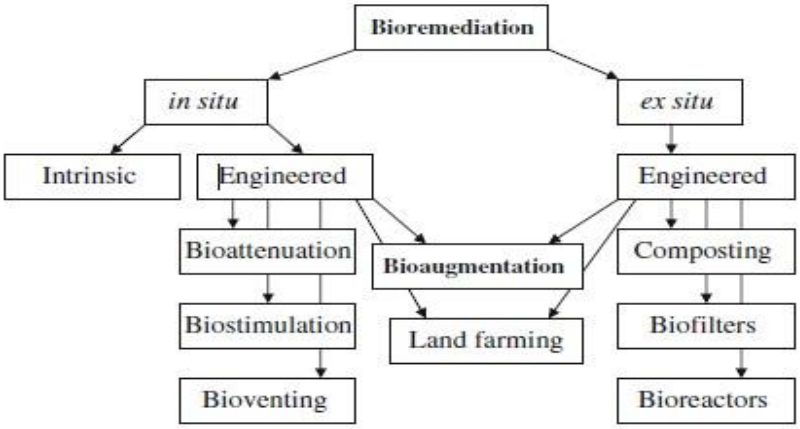

Fig. 1 Bioremediation Overview [28]

2. Materials and method

2.1. Materials

Soil samples used was obtained from Covenant University Farms, Canaan land Ota while the Crude oil samples were from Chevron Nigeria Limited, Delta state Nigeria.

2.1.1. Preparation of Microorganisms

The two microorganism used were sourced from Microbiology Department, Covenant University, Ogun State, Nigeria. [29-36] method were used for the preparation and inoculation.

2.2. Soil Treatment

The soil used sample was collected from the top layer of about 10 to 20cm below the surface. Sample was air dried to get rid of the excess moisture. The crude oil spill/pollution was artificially simulated in the laboratory. Before the pollution, 1kg of the soil was collected and analysed for all the parameters that were used to monitor the level of bioremediation of the polluted soil. To simulate an ideal pollution for this study, 8 transparent buckets were filled with 500g of the loamy soil. Each soil filled bucket was referred to as a system. These buckets were then randomly perforated in different areas to create needle like holes through which the soil particles would not pass through but air could pass through. This was done to ensure proper aeration of each system. Four out of the eight systems were polluted with 40g raw crude to achieve 8% w/w pollution. Four other systems were polluted with 40g treated crude. The *A. niger and the P. aeruginosa* were respectively inoculated into the eight systems. The systems inoculated with microorganisms were replenished with nutrients once every three days to ensure the continuous survival. Samples were withdrawn from each system at intervals of 5 days or sometimes 10 days for various tests to be run.

2.3. Sample preparation

All the samples except the two control samples were inoculated. For all samples polluted with crude to be treated with *A. niger* only, (RCA and TCA), 200 ml sterile Yeast Broth was inoculated with 4 ml of the *A. niger* suspension and this was then used to dose the respective soil systems. The same procedure was repeated with the soil systems treated with *P. aeruginosa* (RCP and TCP). In this case, 4 ml of the *P. aeruginosa* suspension was used to inoculate 200 ml of Mueller Hinton Broth. The procedure was a little

different for systems treated with both *A. niger* and *P. aeruginosa*. In this case, for the systems polluted with crude (RCAP and TCAP), 100 ml of Mueller Hinton Broth and 100 ml of Yeast Broth were mixed in a sterile beaker and inoculated with 2 ml of *A. niger* suspension and 2 ml of *P. aeruginosa* suspension and used to dose the respective systems. Samples were taken from all systems for testing before inoculation and the inoculated systems were left for five days before the next batch of samples were withdrawn for testing.

2.4. Total Hydrocarbon Content Determination [THC]

Total Hydrocarbon Content was determined using Jenway 6405 UV/VIS Spectrophotometer to read off the absorbance of the samples. 1g of soil sample from each system was dissolved in 10ml of hexane and shaken with a magnetic stirrer which was used to ensure the proper mixing and extraction of crude in hexane for 30 minutes. 1ml of this extract was measured and made up to 10ml with n-hexane and its absorbance was determined using spectrophotometer at 420nm wavelength for crude oil. The wave length was chosen after screening of several dilutions of crude oil in the spectrophotometer. Soil samples from each of the systems were tested by the same way mentioned above at 0 days, 5 days, 10 days, 15 days, 20 days and 30 days. Hexane chart was used for the conversion of absorbance to concentration. The standard curve calibration was plotted by obtaining the absorbance of different concentrations of crude oil in hexane in mg/ml converted to mg/kg of soil. To determine the TPH at any point, equation 1 was used [36].

$$\frac{TPHo - TPHt}{TPHo} * 100 \qquad [1]$$

Where:
TPH_o = Total Petroleum Hydrocarbon at day 0 [=] mg/kg
TPH_t = Total Petroleum Hydrocarbon at any day t [=] mg/kg

3. Results and discussion

3.1. Total Hydrocarbon Content analysis

Figures 2 – 9 shows plots for the Total Petroleum Hydrocarbon [TPH] and the natural logarithm for each system over the course of the first 30 days.

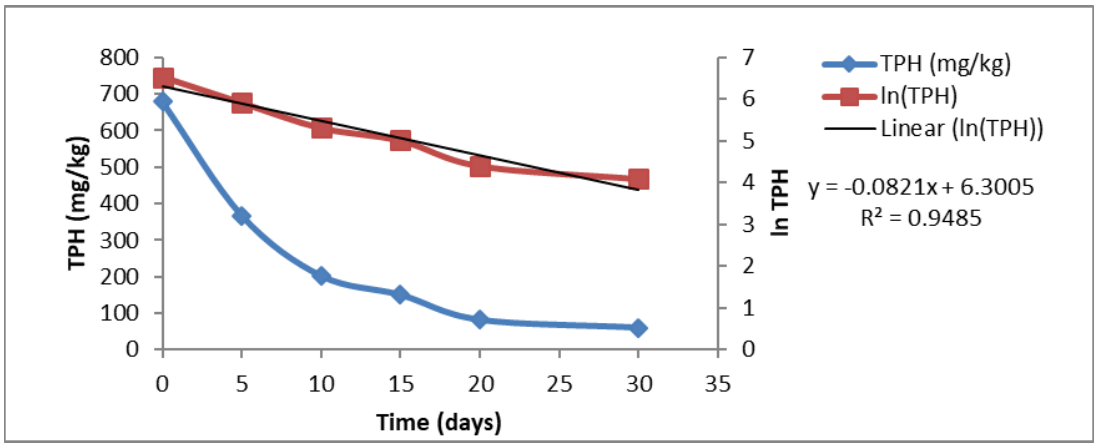

Fig. 2 TPH and LN (TPH) for RCA

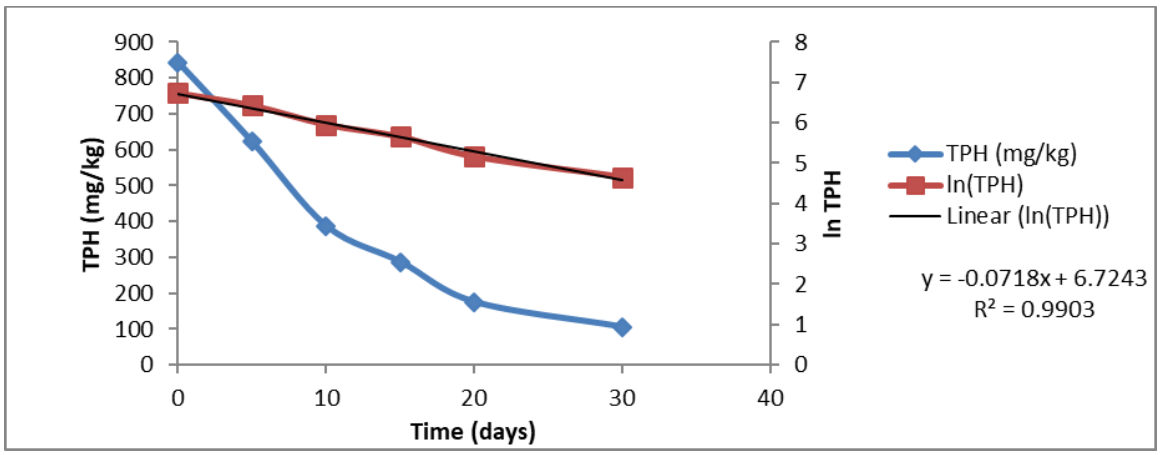

Fig. 3 TPH and LN (TPH) for TCA

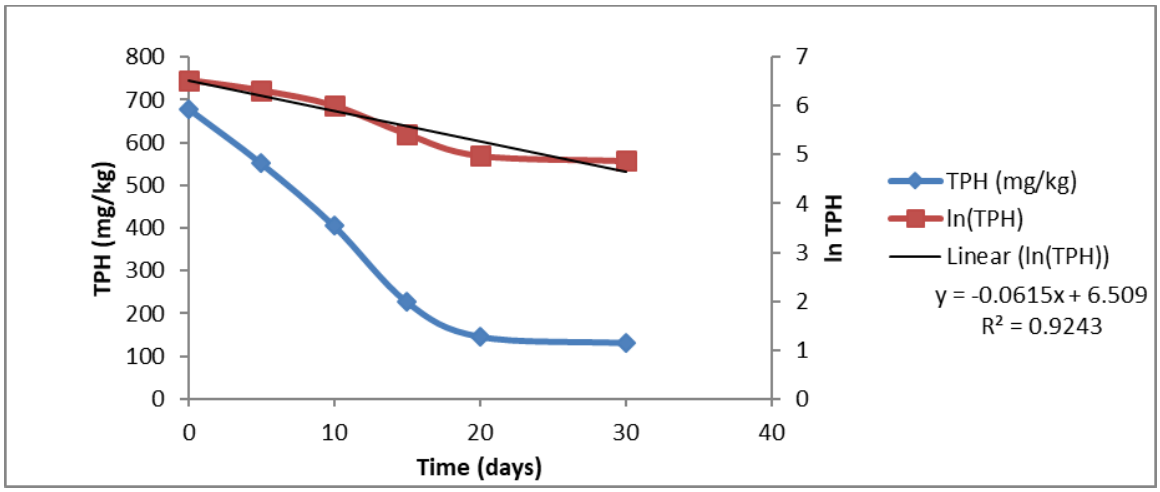

Fig. 4 TPH and LN (TPH) for RCP

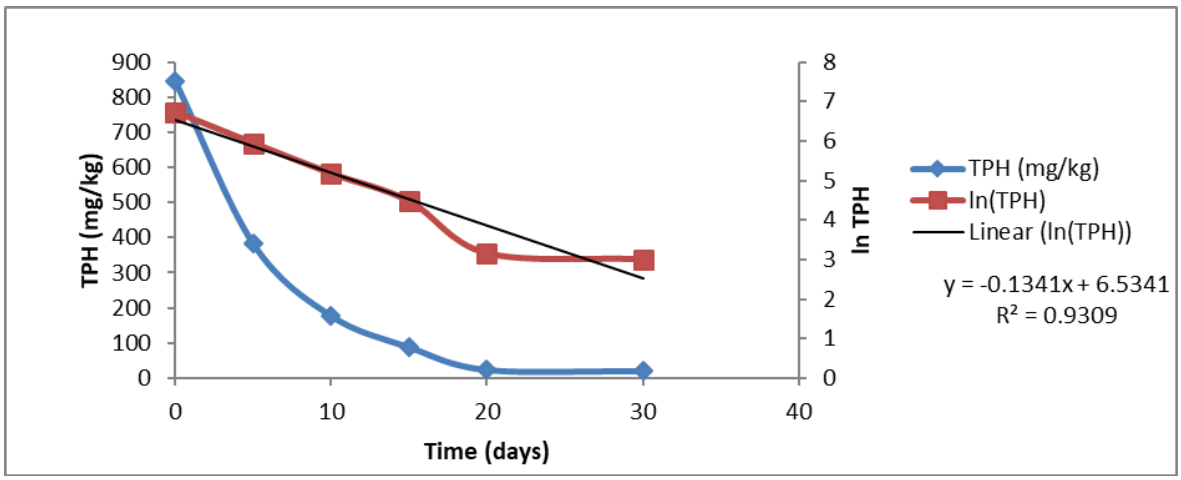

Fig. 5 TPH and LN (TPH) for TCP

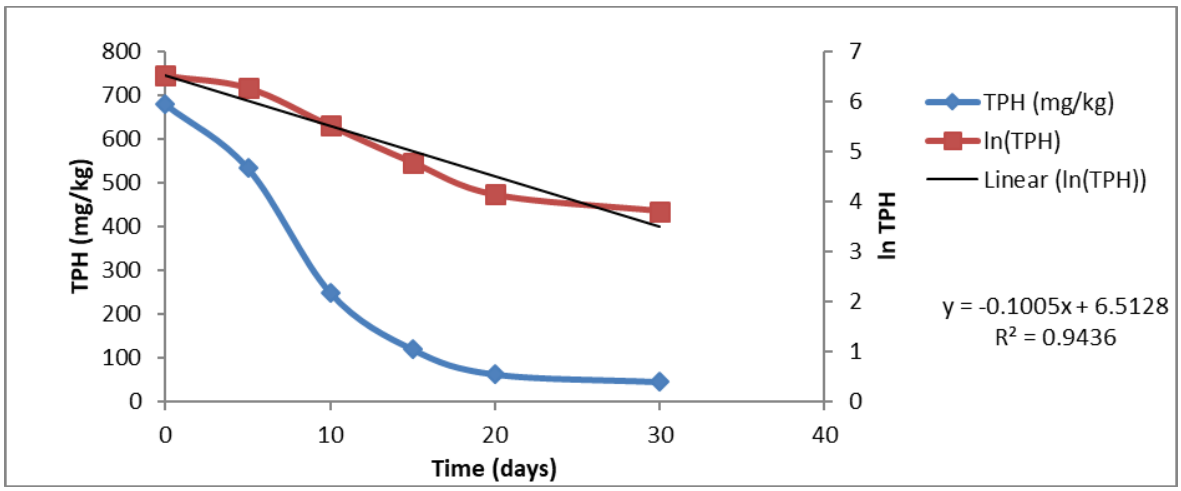

Fig. 6 TPH and LN (TPH) for RCAP

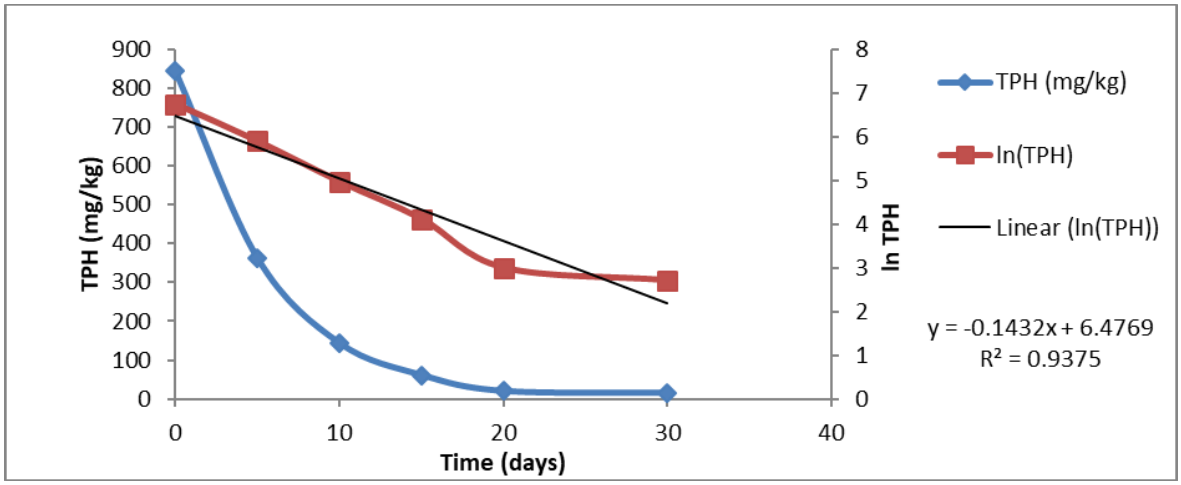

Fig. 7 TPH and LN (TPH) for TCAP

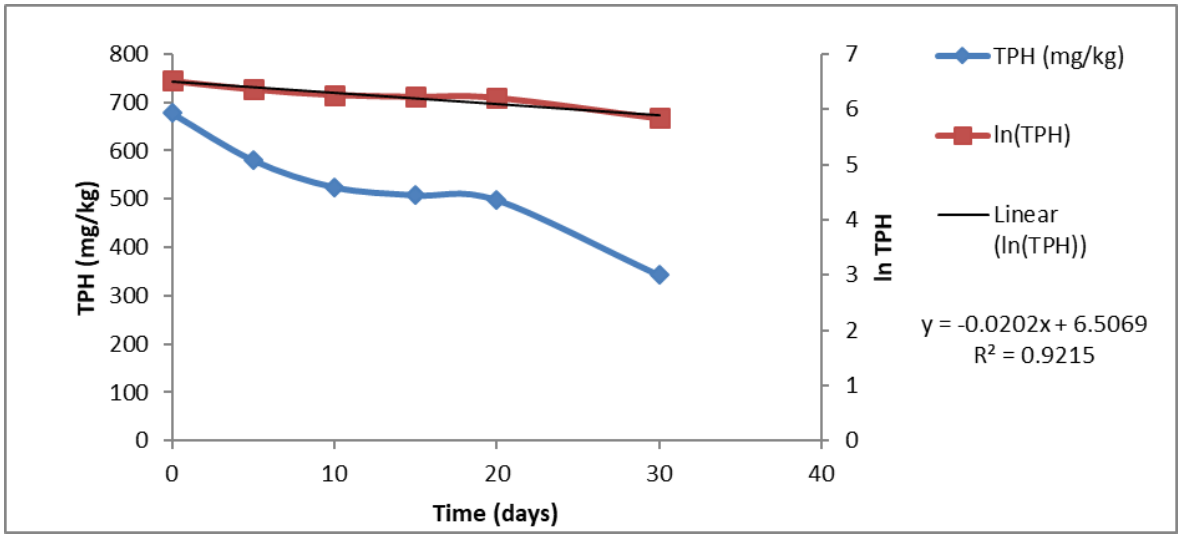

Fig. 8 TPH and LN (TPH) for RCC

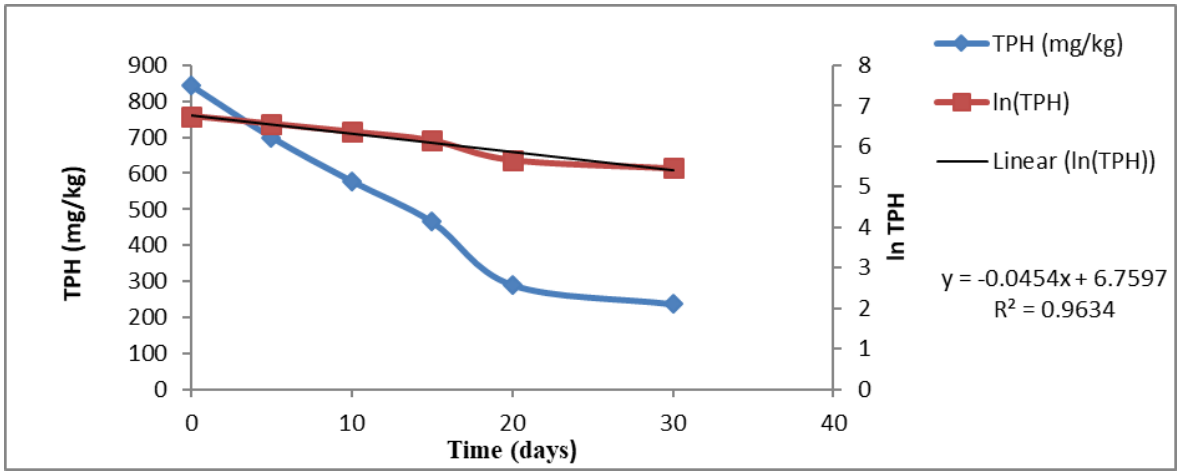

Fig. 9 TPH and LN (TPH) for TCC

3.2. First order bioremediation kinetics

The first order Bioremediation kinetics for this experiment was computed using the data obtained from the Total Petroleum Hydrocarbon computation. The kinetics was used to averagely compute the half-life of degradation of crude in each system. The computation was done using the model postulated by [17, 37]. The plots above (figures 2-9) shows the decreasing trend in the TPH of each system and also show how the first order kinetics was computed. The slope of each of the LN (TPH) curves gives the first order bioremediation rate constant for each system. It is this rate constant that is used to compute the half-life. The first order plots are made by plotting LN (TPH) against time (t). This is based on the computation in equations 2-5.

$$TPH_F = TPH_{ie^{-kt}} \quad [2]$$

$$\frac{TPHf}{TPHi} = e^{-kt} \quad [3]$$

$$\ln\frac{TPHf}{TPHi} = -kt \quad [4]$$

$\ln(TPH_f) = -kt + \ln(TPH_i)$ [5]

Where

TPH_i = Total Petroleum Hydrocarbon at day 0 [=] mg/kg

TPH_f = Total Petroleum Hydrocarbon at any day t [=] mg/kg

k = biodegradation rate constant [=] day^{-1}

The values of the half-life of each system were obtained from the biodegradation rate constant k using equation 6

$$\text{Half-life, } t_{1/2} = \frac{0.693}{k} \quad [6]$$

Table 1 shows the result obtained from the kinetics analysis.

Table 1. Rate Constant and Half life

Sample	k (day^{-1})	Half-life (day)
RCA	0.0821	8.443
RCP	0.0797	8.697
RCAP	0.1005	6.897
RCC	0.0202	34.314
TCA	0.0718	9.654
TCP	0.1341	5.168
TCAP	0.1432	4.840
TCC	0.0454	15.267

Key:

TCC Treated Crude Control sample
RCC Raw Crude Control sample
RCA Raw Crude Treated with *A. niger*
TCA Treated Crude Treated with *A. niger*
RCP Raw Crude Treated with *P. aeruginosa*
TCP Treated Crude Treated with *P. aeruginosa*
RCAP Raw Crude Treated with *A. niger* and *P. aeruginosa*
TCAP Treated Crude Treated with *A. niger* and *P. aeruginosa*

The Biostimulant Efficiency (BE) analysis was done to determine the efficiency of each biostimulant used to remediate the soil samples in each system. An average of the biostimulant efficiency for each biostimulant in the respective soil samples was done to concisely determine which of the biostimulant was most efficient. The BE is calculated from the TPH data as follows:

% BE = % TPH loss of soil remediated with biostimulant - % TPH loss of corresponding control sample remediated with no biostimulant.

From the table 1 to determine the BE of *A. niger,* all samples remediated with this biostimulant and their respective control samples are taken into consideration.

% BE of *Aspergillus niger* in RCA = % Loss in TPH of RCA - % Loss in TPH of RCC

This is done for all other system remediated with *Aspergillus niger* and the average % BE is found. The same procedure is repeated for the other biostimulants (*Pseudomonas aeruginosa* alone and *Aspergillus niger* combined with *Pseudomonas aeruginosa*).

Table 2 shows the average % BE for each biostimulant used for this research.

Table 2. Biostimulant Efficiency Summary

Soil Samples	% Loss in THC	Biostimulant Efficiency (%)
RCC	49.51	0
TCC	71.94	0
RCA	91.15	41.65
RCP	80.59	31.08
RCAP	93.37	43.86
TCA	87.55	15.61
TCP	97.63	25.69
TCAP	98.22	26.28

Table 3. Average Biostimulant Efficiency

Biostimulant	Average % BE
Aspergillus niger	28.63
Pseudomonas aeruginosa	28.39
Aspergillus niger + *Pseudomonas aeruginosa*	35.07

3.3. Total Petroleum Hydrocarbon [TPH] and Bioremediation Kinetics

The results show that all eight systems experienced a level of reduction in the TPH content over the space of 30 days. Most of the systems experienced a significant level of remediation within the first fifteen days. The least hydrocarbon content decrease was observed to be in the control systems (figures 8 and 9). Between the two control systems polluted with raw and treated crude, the system polluted with treated crude experienced a higher level of remediation when compared to the control system polluted with raw crude. At the end of 30 days, the hydrocarbon content in the control system RCC decreased from 678.33mg/kg to 342.5mg/kg which is about 49.50% hydrocarbon removal. The rate constant of biodegradation was obtained to be 0.0202/day as shown in figure 8. The half-life for this system is 34.3 days.

The control system TCC (figure 9) had its hydrocarbon content decrease from 843.3 mg/kg to 236.6 mg/kg an equivalent of 71.9% petroleum hydrocarbon removal, a rate constant of 0.0454/day and half-life of 15.26 days. For the samples remediated with microorganisms, general and precise inferences can be made. It was observed that on the average that figures 6 and 7, the systems remediated with *A. niger* and *P. aeruginosa* (RCAP and TCAP) experienced the best level of remediation having an average remediation of the two samples of 95.793%. This was closely followed by the soil samples remediated with *P. aeruginosa* alone (RCP and TCP) which had an average remediation of the two samples of 90% (figures 4 and 5).

Figures 2 and 3 showed the least remediation performance of 87% in the average of the two samples amended with *A. niger* alone (RCA and TCA).

A more critical analysis of the data showed that *A. niger* alone remediated the systems amended with raw crude (figure 1) better than the systems amended with treated crude (figure 2). This was observed from the percentage crude oil removal of each of these systems. At the end of 30 days, RCA experienced a percentage crude oil removal of 91.2% as opposed to its direct counterpart TCA that had a hydrocarbon removal of 87.5% as shown in figures 2 and 3.

The results in figures 4 and 5 also showed that *P. aeruginosa* alone remediated the systems polluted with treated crude better than the systems polluted with raw crude. This was observed from the percentage crude oil removal of each of these systems. RCP experienced a percentage crude oil removal of 80.6% as opposed to its direct counterpart TCP that had a hydrocarbon removal of 98%.

Figures 6 and 7 showed the systems remediated with both *P. aeruginosa* and *A. niger* approximately remediated at 93.3% and 98.2% for RCAP and TCAP are respectively.

The analysis of the first order bioremediation kinetics for each system showed that the system TCAP had the smallest half-life of approximately 4.8 days as shown in table 1. RCA had a half-life of 8.4 days and RCP 8.7 days. The sample with the greatest half-life was observed to be RCC (34.3 days). It can be inferred from this that on the average effective bioremediation occurred fastest in samples remediated with *Aspergillus niger* and *Pseudomonas aeruginosa.* The control samples remediated the slowest.

For the results of the Biostimulant Efficiency, it shows on table 3 that on the average, a combination of *Aspergillus niger* and *Pseudomonas aeruginosa* had the highest efficiency of approximately 35%.

4. Conclusion

Raw crude oil soil samples amended with *A. niger* alone shows higher level of remediation than treated samples of polluted soil. Hence, for raw crude oil polluted soil/site, fungi (*A. niger*) would be a good biostimulant compared to bacteria (*P. aeruginosa*). The result obtained from the biostimulant efficiency (BE) and percentage crude oil removal revealed that the combination of both *A. niger* and *P. aeruginosa* can also serve as an effective biostimulant compared to using either *P. aeruginosa* or *A. niger* alone. Using *P. aeruginosa* alone as a biostimulant only shows effectiveness in the remediation of soil samples polluted with treated crude oil and not with raw crude oil. Therefore, for an oil spill site/soil polluted with treated crude oil from a production platform or even the flow line, *P. aeruginosa* (bacteria) is a better choice of biostimulant compared to *A. niger* (fungi).

The result revealed that treated samples of crude oil polluted soil remediate faster and to a larger extent than raw samples of crude oil polluted soil, probably because of the removal of some other heavier hydrocarbons. This research confirmed the previous work carry out on either in-situ or ex-situ bioremediation of crude oil polluted water or soil by different microorganisms.

Conflicts of interest: The authors declare no conflict of interest.

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References

[1] Ojewumi, M.E., Emetere, M.E., Babatunde, D.E., Okeniyi, J.O. (2017). In Situ Bioremediation of Crude Petroleum Oil Polluted Soil Using Mathematical Experimentation. *International Journal of Chemical Engineering*, Volume 2017, Article ID 5184760.

[2] Ojewumi, M.E., Obielue, B.I., Emetere, M.E., Awolu, O.O., Ojewumi, E.O. (2018). Alkaline Pre-Treatment and Enzymatic Hydrolysis of Waste Papers to Fermentable Sugar. *Journal of Ecological Engineering*, 19(1), 211–217. https://doi. org/10.12911/22998993/79404.

[3] Ojewumi, M.E., Okeniyi, J.O, Ejemen, V.A., Okeniyi, E.T., Ikotun, J.O., Popoola, A.P. (2018). Bioremediation: Data on *Pseudomonas aeruginosa* Effects on the Bioremediation of Crude Oil Polluted Soil. *Data in Brief*, 19, 101-113. https://doi.org/10.1016/j.dib.2018.04.102

[4] Pala, M., Carvalho, D. D., Pinto, D., & J.C. Sant Anna Jr. (2006). A suitable model to describe bioremediation of a petroleum-contaminated soil. *Journal of international Bioderadation*, 254-260.

[5] Ojewumi, M.E., Oyeyemi, K.G., Emetere, M.E., Okeniyi, J.O. (2018). Data on the rheological behavior of cassava starch paste using different models. *Data in Brief,* Volume 19, 2163-2177. DOI: https://doi.org/10.1016/j.dib.2018.06.112.

[6] Korda, A., Santas, P., Tenente, A., & Santas, R. (1997). Petroleum Hydrocarbon Bioremediation: sampling and analytical techniques, in situ treatments and commercial microorganisms currently used. *Journal of Applied Microbiology and Biotechnology*, 677-686.

[7] Ojewumi, M.E., Anenih, V.A., Taiwo, S.O., Adekeye, B.T., Awolu, O.O., Ojewumi, E.O. (2018). A Bioremediation Study of Raw and Treated Crude Petroleum Oil Polluted Soil with *Aspergillus niger* and *Pseudomonas aeruginosa*. *Journal of Ecological Engineering*, 19(2), 226–235. https://doi.org/10.12911/22998993/83564.

[8] El-Nawawy, I., Abdal, E.-B. M. and Khalafawi, M. (1992). Biodegradation of oily sludge in Kuwait soil. *World Journal of Microbiology and Biotechnology*, 618-620.

[9] Ojewumi M.E., M.E. Emetere, C.V. Amaefule, B.M. Durodola and O.D. Adeniyi (2019), Bioconversion of Orange Peel Waste by *Escherichia coli* and *Saccharomyces cerevisiae* to Ethanol. *International Journal of Pharmaceutical Sciences and Research*, 10(3), 1246-1252. DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(3).1000-07.

[10] Ojewumi M.E., I.J. Akwayo, O.S. Taiwo, O.M. Obanla, A.A. Ayoola, E.O. Ojewumi, and E.A. Oyeniyi (2018), Bio-Conversion of Sweet Potato Peel Waste to BioEthanol Using *Saccharomyces cerevisiae, International Journal of Pharmaceutical and Phytopharmacological Research,* 8(3), 46-54, 2018.

[11] Ojewumi M.E., E.O. Kolawole, D.T. Oyekunle, S.O. Taiwo, A.O. Adeyemi (2019), Bioconversion of Waste Foolscap and Newspaper to Fermentable Sugar. *Journal of Ecological Engineering*, 20(4), 35-41.

[12] Schuster, E., Dunn-Coleman, N., Frisvad, J., & Van Dijck, P. (2002). On the safety of *Aspergillus niger* – a review. *Applied Microbiology and Biotechnology,* 59, 426-435.

[13] Kenneth, E. (1995). Mechanisms of polycyclic aromatic hydrocarbon degra-dation by ligninolytic fung. *Environmental Health Perspective*, 41-43.

[14] Ojewumi, M.E., Omoleye, J.A., Adesola, A.A., Alagbe, E. E. (2018). Evaluation of fermentation rate for the production of a protein based African seed condiment. *PREPRINT*. doi:10.20944/preprints201806.0359.v1.

[15] Johnson, G., & Olsen, R. (1997). Multiple Pathways for Toluene Degradation in Burkholderia sp. Strain JS150. Applied and Environmental Microbiology, 4047-4052.

[16] Lederberg J.e.a.P.S.E.V.S.D., 2000. p. 876-891, *Pseudomonas.* Encyclopedia of Microbiology: p. 876-891, 2000.

[17] Agarry, S. E. (2013). Application of carbon-nitrogen supplementation from plant and animal sources in in-situ soil bioremediation of diesel oil experimental analysis and kinetic modelling. *Journal of Environment and Earth Science, 3*(7), 1-13.

[18] Agarry, S. E., Aremu, M. O., & Aworanti, O. A. (2013). Kinetic modelling and half-life study on enhanced soil bioremediation of bonny light crude oil amended with crop and animal-derived organic wastes. *Journal of Petroleum & Environmental Biotechnology*, 1-11.

[19] Lucas, Z., & Macgregor, C. (2006). Characterization and source of oil contamination on the beaches and seabird corpses, Sable Island, Nova Scotia, 1996-2005. *Marine Pollution Bulletin : the international journal for marine environmentalists, scientists, engineers, administrators, politicians and lawyers, 52*(7), 778-789.

[20] Broekema, W. (2015). Crisis-induced learning and issue politicization in the EU: the Braer, Sea Empress, Erika, and Prestige oil spill disasters. (A. B. Lodge, Ed.) *Public Administration*, 778-789.

[21] Choi, H.-M., & Cloud, R. M. (1992). Natural Sorbents in Oil Spill Cleanup. *Environmental Science and Technology*, 772-776.

[22] Larson H., *Responding to oil spill disasters: The regulations that govern their response.* ASTM International 2010.

[23] Van Hamme, J., Singh, A., & Owen , W. P. (2003). *Microbiology and Molecular Biology Review*, 503-549.

[24] Rittmann, B. E., & McCarty, P. L. (2001). *Enviromental Biotechnology:Principles and Applications.* New York: McGraw-Hill.

[25] Facundo, J., Vanessa, H., & Teresa, M. (2001). Biodegradation of diesel oil in soil by a microbial consortium. *Water, Air and Soil Pollution*, 313-320.

[26] Robert, M., & Stephen, J. a. (2003). Biodegradation of fuel oil under laboratory and arctic marine conditions. *Spill Science and Technology Bulletin*, 297-302.

[27] Kenneth E.H., Mechanisms of polycyclic aromatic hydrocarbon degra-dation by ligninolytic fung*. Environmental Health Perspective*: p. 41-43, 1995.

[28] Boopathy, R. (2000). Factors limiting bioremediation technology. *Bioresource Technology*, 63-67.

[29] Ojewumi, M.E, Omoleye J.A., Ajayi, A.A. (2016) Optimum fermentation temperature for the protein yield of *Parkia biglobosa* seeds (Iyere). Proceeding of the 3rd International conference on African Development Issues CUICAD, 2016; 584-587. ISSN:2449-075X.

[30] Ojewumi, M. E., Omoleye, J.A., Ajayi, A.A. (2016). The Effect of Different Starter Cultures on the Protein Content in Fermented African Locust bean (*Parkia biglobosa*) Seeds. *International Journal of Research & Technology*. 5(04), 249 – 255. ISSN: 2278-0181, IJERTV5IS040435. http://www.ijert.org.

[31] Ojewumi, M. E., Omoleye, A.J., Ajayi, A.A., (2016). The Study of the Effect of Moisture Content on the Biochemical Deterioration of Stored Fermented *Parkia Biglobosa* Seeds. *Open Journal of Engineering Research and Technology.* 1 (1), 14 – 22. www.ojert.com.

[32] Ojewumi, M.E., Omoleye, J.A., Emetere, M.E., Ayoola, A.A., Obanla, O.M., Babatunde, D.E., Ogunbiyi, A.T., Awolu, O.O., Ojewumi, E.O. (2018). Effect of various temperatures on the nutritional compositions of fermented African locust bean (*Parkia biglobosa*) seed. *International Journal of Food Science and Nutrition*, 3(1), 117-122.

[33] Ojewumi, M.E., Omoleye, J.A., Ayoola, A.A., Ajayi, A.A., Adekeye, B.T., Alaba, A.O. (2018). Effects of Salting and Drying on the Deterioration Rate of Fermented Parkia biglobosa Seed. *Journal of Nutritional Health & Food Engineering*, 8(1), 1-5.

[34] Ojewumi, M.E., Omoleye, J.A., Nyingifa, A.S. (2018). Biological and chemical changes during the aerobic and anaerobic fermentation of African locust bean. *International Journal of Chemistry Studies*, 2(2), 25-30.

[35] Ojewumi, M.E., Omoleye, J.A., Ajayi, A.A. (2017). Optimization of Fermentation Conditions for the Production of Protein Composition in *Parkia biglobosa* Seeds using Response Surface Methodology. *International Journal of Applied Engineering Research,* 12(22), 12852-12859.

[36] Ojewumi M.E. and V. A. Ejemen (2018).The Ex-Situ Bioremediation Kinetics of Raw and Treated Crude Oil Polluted Soil Using *Aspergillus niger* and *Pseudomonas aeruginosa.* doi:10.20944/preprints201807.0202.v1202

[37] Mohajeri, L., Aziz, H., Isa, M., Zahed, M., & Mohajeri, S. (2010). Ex-situ Bioremediation of Crude Oil in Soil, a Comparative Kinetic Analysis. *Bulletin of Environmental Contamination and Toxicology, 85*(1), 54-58.