EVALUATION OF CRUDE OIL BIODEGRADATION POTENTIALS OF SOME INDIGENOUS SOIL MICROORGANISMS

ABSTRACT

This study evaluated the crude oil degradation potentials of some indigenous soil microorganisms. The microbial isolates were among those obtained from crude oil contaminated and uncontaminated agricultural soils of Awoye, Orioke-Iwamimo, Igodan-Lisa and Oba-Ile all in Ondo State, Nigeria. The isolates were tested for crude oil degradation potentials by visual turbidity, extent of shredding of overlaid oil and the optical density by spectrophotometry method at the wavelength of 540nm. *Brevundimonas diminuta, Bacillus subtilis, Flavobacterium* species*, Enterobacter* species, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Bacillus megaterium*, *Klebsiella edwardsii*, *Bacillus aryabhattai, Aspergillus flavus*, *Kodamaea ohmeri*, *Cephalosporium* species*, Mucor mucedo, Paecilomyces variotii*, *Candida parapsilopsis* and *Trichoderma* species were among the sixteen bacterial and seven fungal isolates tested. The findings in this study revealed varying optical densities of 0.324-0.647 for bacteria and 0.497 -0.812 for fungi at days 11 and 17 respectively thus suggesting different responses and potentials to breakdown crude oil. The highest degradative ability was shown by *Klebsiella edwardsii* (OD 0.647) followed by *Pseudomonas aeruginosa* (OD 0.575) and *Klebsiella pneumoniae* (OD 0.490). *Paecilomyces variotii* showed the highest degradative ability (OD 0.812) among the fungi. The results also suggest that these microorganisms with high degradative ability may be useful in seeding petroleum hydrocarbon polluted agricultural soils for bioremediation.

KEY WORDS: Degradation potentials, soil microorganisms, agricultural soils, Ondo State, spectrophotometry, optical densities, bioremediation.

INTRODUCTION

Crude oil is a complex mixture of hydrocarbons composed of aliphatics, aromatics and asphaltene fractions along with nitrogen, sulphur and oxygen containing compounds (Jain *et al*., 2005; Odeyemi, 2014). The release of this complex mixture into the environment consequent upon diverse human activities is a world- wide problem of pollution. Environmental pollution problems arising from crude oil spill is of greater dimension in the oil producing region of Nigeria due to negligence, sabotage and vandalisation of well heads and flow lines and the lack of effective regulatory laws.

Crude oil spill, no matter its quantity and size (minor, medium, major or disaster) may cause minor or severe damages to the environment and all forms of life dependent on the environment. The release of crude oil into the environment causes enormous damages to the environment due to the presence of many toxic compounds such as polycyclic aromatic hydrocarbons, benzene and its substituted and cycloalkane rings in relatively high concentration (Agarry *et al*., 2012). Oil spill on land may lead to retardation of vegetation growth and cause soil infertility for a long period of time (Onifade *et al* ., 2007), causing alterations in soil physicochemical and microbiological properties (Ijah and Antai, 2003; Odokuma and Dickson, 2003). The overall effects of crude oil on agricultural land may be due to nutritional imbalances created by the spilled oil (Ijah *et al*., 2008; Chorom *et al*., 2010), causing reduced agricultural yield and consequently adversely affecting the socio-economic lives of the people residing in the affected area due to high unemployment and poverty rates.

The negative impact of crude oil pollution have necessitated the exploration of several physicochemical and biological strategies in order to return contaminated sites to their pre-contamination status(Jain *et al*., 2011; Onuoha *et al*., 2014). Biodegradation as a method of

petroleum hydrocarbon degradation and removal have been adjudged by several researchers as having advantage over the physicochemical techniques for the restoration of polluted sites because it is inexpensive, environmentally friendly and simple (Jain *et al*., 2011; Odeyemi, 2014; Onuoha *et al*., 2014). This method also known as bioremediation which involves the use of microorganisms to remove or reduce the complexity of organic pollutants can occur on its own without human intervention (natural attenuation or intrinsic bioremediation) or can be enhanced through the addition of appropriate microbial nutrients (fertilizers) to increase bioavailability and stimulate the indigenous microflora to enhance pollutant degradation within the polluted site (biostimulation). Bioremediation can also be enhanced via the addition of matched strains to the site or medium to enhance the resident microbes population's ability to breakdown the contaminant(bioaugmentation).

The microbial biodegradation of crude oil and other aliphatic and aromatic hydrocarbons can be carried out by both autochthonous and allocthonous species that brings about biotransformation, reducing the complex mixture of harmful materials to simple nutrients in soil or aquatic ecosystem (Burland and Edward, 1999). Salam *et al*., (2011) reported that authochtonous microorganisms in the polluted environment has been widely accepted as a formidable approach for biodegradation and bioremediation owing to the high degree of success recorded by researchers. Ikuesan, (2015) also reported that intrinsic soil microorganisms were responsible for 53.08-58.74% crude oil removal by natural attenuation in soils polluted with 5% of crude oil. When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial population are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating hydrocarbon (Ijah and Abioye, 2003). Bioremediation especially by indigenous microbial population is a very

attractive strategy of returning hydrocarbon polluted site to its pristine state. The process relies upon diverse microbial enzymatic activities to degrade and utilize different hydrocarbons pollutants from the environment as a source carbon and energy (Das and Chandran, 2011; Nduka *et al*., 2012). The ability to degrade petroleum hydrocarbon substrate is exhibited by a wide variety of bacteria genera which are widely distributed in oil polluted as well as pristine soils (Bogan *et al*., 2003; Hamamura *et al*., 2006; Cappello *et al*., 2007; Van Beilen and Funhoff, 2007; Chikere *et al*., 2009). Several general of fungi have also been implicated in crude oil biodegradation (Odeyemi, 2014)

The ubiquitous nature of microorganisms and their ease of isolation from crude oil polluted environment confirm that they play important role in crude oil degradation (Onifade, 2007). The success of bioremediation technologies applied to hydrocarbon polluted environments highly depends on the biodegrading capabilities of native microbial populations or exogenous microorganisms used as inoculants. The communities which are exposed to hydrocarbons become adapted exhibiting selective genetic changes (Al- Wasify and Hamed, 2014; Odeyemi, 2014). Although, crude oil degrading microorganisms are ubiquitously distributed in soil and water environments, they may not be present in sufficient number to warrant effective removal of hydrocarbon pollutant, hence, it may be necessary to bioaugment the degradation process with highly efficient strain for effective crude oil removal(Joo *et al*., 2008). Therefore, the overall objective of this research is to evaluate the crude oil biodegradation potentials of some intrinsic soil microorganisms from crude oil contaminated and uncontaminated agricultural soil samples that may be useful in seeding polluted sites in bioremediation.

MATERIALS AND METHODS

Sample Collection

The microbial isolates used in this study soil were collected from the stock obtained by Ikuesan, (2015) from crude oil contaminated soils of Awoye (5^o 59' 0'' N, 4^o 55' 0''E) and Orioke-Iwamimo (6^o11' 0''N, 4^o 41 '0'' E) and the uncontaminated soil samples of Igodan- Lisa (6^o 27' 0''N, 4^o 47'0''E) and Oba-Ile (7^o 16' 0''N, 5^o 15' 0''E), all in Ondo State, Nigeria. Bacteria and fungi isolates used for this study were *Brevundimonas diminuta* (1A), *Pseudomonas aeruginosa* (1B), *Klebsiella pneumoniae* (1B2), *Bacillus subtilis* (1C), *Enterobacter* species (1E), *Klebsiella pneumoniae* (2A), *Pseudomonas aeruginosa* (2B), *Bacillus aryabhattai* (2C), *Klebsiella pneumoniae* (2E), *Pseudomonas aeruginosa* (2G), *Klebsiella edwardsii* (3A), *Bacillus subtilis* (3C), *Alcaligenes faecalis* (3C2), *Bacillus megaterium* (4A), *Klebsiella edwardsii*(4C), *Klebsiella pneumoniae* (4F), *Cephalosporium* species, *Aspergillus flavus*, *Candida parapsilopsis*, *Kodamaea ohmeri*, *Paecilomyces variotii*, *Trichoderma* species and *Mucor mucedo*

Microbiological analysis of samples

(i) Purity of microbial isolates and crude oil degradation

The isolates were confirmed for purity by repeated streaking on molten nutrient agar (NA) and malt extract agar (MEA) respectively for bacteria and fungi. The identities of the isolates were also confirmed using colonial characteristics, microscopic and standards biochemical tests using Holt *et al*, (1994) as reference for bacteria and Onions *et al*, (1981) and Barnett and Hunter, (1983) were used for the identification of fungi. Isolates were also confirmed to be crude oil degraders by cultivation on Mineral Salt Medium (MSM). The NA plates for bacteria were incubated at 35^{o}C for 48 hours while MEA plates (for fungi) after gelling were incubated at 28±2^{o}C for 7 days. The MSM used was Bushnell-Hass broth incorporated with 1.5% agar (for

bacteria), 1.2% agar (for fungi). Crude oil (2%) sterilized using 0.45µm Millipore filter served as carbon source. The MS-oil medium for crude oil degrading bacteria and crude oil degrading fungi were then incubated at 28°C±2°C respectively for 14 and 21 days.

(ii)Evaluation of crude oil degradation capabilities of microbial isolates: Microbial growth by utilization of crude oil as sole source of carbon and energy was determined by measurement of optical density (OD) using the spectrophotometry method. The crude oil degradative ability of Sixteen bacteria species belonging to six genera and seven genera of fungi were investigated using the modified method of Omotayo *et al*., (2012). The mineral salt medium (Bushnell-Hass) used for this experiment was dispensed into culture tubes in 49.5ml amount and sterilized by autoclaving at 121°C for 15 minutes. These tubes were allowed to cool and 0.5ml crude oil (sterilized using 0.45µm Millipore filter) which served as source of carbon and energy was added to make a final volume of 50ml. Each isolate (0.5ml) in nutrient broth was subsequently inoculated in separate tube containing the liquid medium. Culture tubes were agitated daily to provide oxygen required by the aerobes for crude oil utilization. Triplicate samples were incubated at 28°C ± 2°C for 15 and 21 days respectively for bacteria and fungi. The optical density (OD) of growth in culture tubes were also taken at a wavelength of 540nm (Ekpenyong and Antai, 2007) at 2 days interval and observed for turbidity and shredding, scored as high (+++), moderate (++), low growth (+) for turbidity and high (H), moderate (M) and low (L) for the degree of shredding or breakdown of overlaid oil. Control liquid culture containing the liquid MS medium and crude oil but without organism was also prepared to standardize the spectrophotometer. The most efficient microbial isolates were identified base on measurement of optical density and extent of breakdown of overlaid oil.

Statistical analysis

Data obtained were analyzed by one way Analysis of Variance (ANOVA) using SPSS version 18.0 (2010) while the mean were compared by Duncan's Multiple Range Test (DMRT) at 95% confidence level values. Differences were considered significant at $P \leq 0.05$.

RESULTS

Crude oil degradation potentials of microbial isolates.

Tables 1(a- b) and 3 respectively show the crude oil degradative abilities of the 16 bacterial and 7 fungal isolates on the basis of their optical densities at 2 days interval until day 15 and 21 for bacteria and fungi respectively. Tables 2 and 4 show the optical density, turbidity and extent of shredding of crude oil at the peak of degradation. The isolates exhibited varying degrees of crude oil utilization and growth, showing extensive shredding i.e breakdown of over laid oil in tubes. The results revealed that apart from *Bacillus subtilis* (1C) and *B. subtilis* (3C) which exhibited highest potentials at day 13, all the bacterial isolates showed highest ability to utilize crude oil at day 11(tables 1a - b) while all the fungi except *Aspergillus flavus* were best at day 17 of growth (table 2). *Klebsiella edwardsii* and *Paecilomyces variotii* exhibited the highest activity respectively among the bacteria and fungi tested for crude oil biodegradation. Assessment of degradation potentials by measurement of optical density (OD) values at 540nm revealed OD values of 0.647 and 0.575 for *Klebsiella edwardsii* and *Pseudomonas aeruginosa* respectively among the bacteria. The optical density for fungi was 0.812 > 0.763 > 0.742 for *Paecilomyces variotii, Kodamaea ohmeri* and *Cephalosporium* species respectively among the fungi. *Paecilomyces variotii* showed leading potential to degrade crude oil from day 5 – 19 of growth.

Table 1a: Optical Density (OD) at 540nm of bacterial isolates from Awoye and Orioke-Iwamimo crude oil contaminated soil samples (Day 1-15)

ORGANISM	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
Brevundimonas diminuta 1A	0.0003 ± 0.0006^{a}	0.0093 ± 0.0006^{ab}	0.0250 ± 0.0036^{a}	0.0927 ± 0.0012^{a}	0.2286 ± 0.0042^{b}	0.3620 ± 0.0020^{c}	0.3070 ± 0.0045^{c}	0.2686 ± 0.0041^{d}
Pseudomonas aeruginosa 1B	0.0010 ± 0.0010^{ab}	0.0130 ± 0.0026^{c}	0.0393 ± 0.002^{b}	0.1400 ± 0.0035^{d}	0.3477 ± 0.0052^{d}	0.4293 ± 0.0023^{e}	0.3893 ± 0.0031^{e}	0.3233 ± 0.0030^{e}
Klebsiella pneumoniae 1B2	0.0000 ± 0.0000^{a}	0.0083 ± 0.0006^{a}	0.0360 ± 0.0020^{b}	0.1093 ± 0.0006^{c}	0.2313 ± 0.0031^{b}	0.3856 ± 0.0045^{d}	0.2870 ± 0.0043^{b}	0.2340 ± 0.0010^{c}
Bacillus subtilis 1C	0.0023 ± 0.0012^{b}	0.0106 ± 0.0006^{ab}	0.0587 ± 0.0042^{c}	0.1507 ± 0.0042^{e}	0.3673 ± 0.0050^{e}	0.3656 ± 0.0040^{c}	0.4706 ± 0.0023^{g}	0.4136 ± 0.0035^{g}
Enterobacter sp. *1E*	0.0003 ± 0.0006^{a}	0.0097 ± 0.0015^{ab}	0.0360 ± 0.0036^{b}	0.1030 ± 0.0036^{b}	0.2106 ± 0.0031^{a}	0.3873 ± 0.0041^{d}	0.3290 ± 0.0010^{d}	0.2720 ± 0.0020^{d}
Klebsiella pneumoniae 2A	0.0010 ± 0.0010^{ab}	0.0157 ± 0.0015^{d}	0.0680 ± 0.0040^{d}	0.1627 ± 0.0012^{f}	0.2920 ± 0.0072^{c}	0.4900 ± 0.0036^{f}	0.3960 ± 0.0040^{f}	0.3313 ± 0.0035^{f}
Pseudomonas aeruginosa 2B	0.0010 ± 0.0010^{ab}	0.0093 ± 0.0006^{ab}	0.0407 ± 0.0023^{b}	0.1063 ± 0.0012^{bc}	0.2100 ± 0.0060^{a}	0.3520 ± 0.0040^{b}	0.2890 ± 0.0020^{b}	0.2126 ± 0.0037^{b}
Bacillus aryabhattai 2C	0.0000 ± 0.0000^{a}	0.0110 ± 0.0000^{bc}	0.0363 ± 0.0040^{b}	0.0940 ± 0.0020^{a}	0.2183 ± 0.0040^{a}	0.3243 ± 0.0494^{a}	0.2450 ± 0.0043^{a}	0.1943 ± 0.0025^{a}

Legend: isolates 1A, 1B, 1B2, 1C and 1E were obtained from Awoye soil
isolates 2A, 2B and 2C were obtained from Orioke Iwamimo soil

Table 1b: Optical Density (OD) at 540nm of bacterial isolates from Igodan -Lisa and Oba-Ile soil samples (Day 1-15)

ORGANISM	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
*Klebsiella pneumoniae 2E**	0.0000 ± 0.0000^{a}	0.0083 ± 0.0005^{a}	0.0313 ± 0.0011^{a}	0.1183 ± 0.0005^{ab}	0.2526 ± 0.0047^{b}	0.3920 ± 0.0040^{c}	0.2570 ± 0.0030^{b}	0.2156 ± 0.0020^{b}
*Pseudomonas aeruginosa 2G **	0.0020 ± 0.0000^{cd}	0.0146 ± 0.0015^{b}	0.0750 ± 0.0030^{f}	0.1813 ± 0.0030^{f}	0.3786 ± 0.0061^{e}	0.5750 ± 0.0060^{f}	0.4876 ± 0.0005^{g}	0.4073 ± 0.0030^{e}
Klebsiella edwardsii 3A	0.0023 ± 0.0011^{d}	0.0166 ± 0.0025^{bc}	0.0593 ± 0.0023^{d}	0.1910 ± 0.0036^{g}	0.4086 ± 0.0020^{f}	0.6466 ± 0.0050^{g}	0.4696 ± 0.0015^{f}	0.4203 ± 0.0011^{f}
Bacillus subtilis 3C	0.0000 ± 0.0000^{a}	0.0183 ± 0.0028^{c}	0.0513 ± 0.0025^{c}	0.1370 ± 0.0030^{d}	0.2796 ± 0.0077^{d}	0.4263 ± 0.0047^{d}	0.5010 ± 0.0036^{h}	0.4280 ± 0.0036^{g}
Alcaligenes faecalis 3C2	0.0013 ± 0.0005^{bc}	0.0103 ± 0.0015^{a}	0.0413 ± 0.0030^{b}	0.1213 ± 0.0041^{b}	0.2386 ± 0.0041^{a}	0.3940 ± 0.0026^{c}	0.3633 ± 0.0041^{e}	0.3360 ± 0.0036^{d}
Bacillus megaterium 4A	0.0000 ± 0.0000^{a}	0.0100 ± 0.0026^{a}	0.0343 ± 0.0015^{a}	0.1133 ± 0.0040^{a}	0.2743 ± 0.0040^{d}	0.4353 ± 0.0035^{e}	0.3180 ± 0.0036^{d}	0.2616 ± 0.0040^{c}
Klebsiella edwardsii 4C	0.0000 ± 0.0000^{a}	0.0083 ± 0.0015^{a}	0.0313 ± 0.0030^{a}	0.1303 ± 0.0015^{c}	0.2383 ± 0.0066^{a}	0.3780 ± 0.0052^{b}	0.2836 ± 0.0030^{c}	0.2126 ± 0.0015^{b}
Klebsiella pneumoniae 4F	0.0010 ± 0.0000^{b}	0.0140 ± 0.0010^{b}	0.0670 ± 0.0030^{e}	0.1456 ± 0.0023^{e}	0.2623 ± 0.0015^{c}	0.3626 ± 0.0011^{a}	0.2463 ± 0.0020^{a}	0.1816 ± 0.0025^{a}

Legend ; isolates 3A, 3C and 3C2 were obtained from Igodan – Lisa soil

isolates 4A, 4C and 4F were obtained from Oba - Ile soil

*isolates were from Orioke Iwamimo crude oil contaminated sample

Table 2: Growth and extent of crude oil utilization by the test bacterial isolates at the Peak (day 11)

Organisms	Turbidity	Extent of Shredding	Optical density
Brevundimonas diminuta 1A	+	L	0.3620 ± 0.0020^{c}
Pseudomonas aeruginosa1B	++	H	0.4293 ± 0.0023^{e}
Klebsiella pneumoniae 1B2	+	L	0.3856 ± 0.0045^{d}
Bacillus subtilis 1C	++	H	* 0.3656 ± 0.0040^{c}
Enterobacter specie*.1E*	++	M	0.3873 ± 0.0041^{d}
Klebsiella pneumoniae2A	+++	H	0.4900 ± 0.0036^{f}
Pseudomonas aeruginosa 2B	++	M	0.3520 ± 0.0040^{b}
Bacillus aryabhattai 2C	+	L	0.3243 ± 0.0043^{a}
Klebsiella pneumoniae 2E	++	M	0.3920 ± 0.0040^{c}
Pseudomonas aeruginosa 2G	+++	H	0.5750 ± 0.0060^{f}
Klebsiella edwardsii 3A	+++	H	0.6466 ± 0.0050^{g}
Bacillus subtilis 3C	++	H	* 0.4263 ± 0.0047^{d}
Alcaligenes faecalis $3C_2$	++	M	0.3940 ± 0.0026^{c}
Bacillus megaterium 4A	++	H	0.4353 ± 0.0035^{e}
Klebsiella edwardsii 4C	++	M	0.3780 ± 0.0052^{b}
Klebsiella pneumoniae 4F	++	M	0.3626 ± 0.0011^{a}

Legend: Isolates 1A,1B,1B2 1C and 1E were obtained from Awoye soil
Isolates 2A,2B, 2C, 2E and 2G were obtained from Orioke Iwamimo soil
Isolates 3A,3C,3C2 were obtained from Igodan - Lisa soil
Isolates 4A,4C and 4F were obtained from Oba- Ile soil
*values obtained at day thirteen of growth

ORGANISM	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15	DAY 17	DAY 19	DAY 21
Cephalosporium sp.(FI)	0.0036 ± 0.00058^{bc}	0.0143 ± 0.0015^{bc}	0.0900 ± 0.0017^{g}	0.1750 ± 0.0030^{e}	0.2783 ± 0.0037^{g}	0.3913 ± 0.0040^{h}	0.4896 ± 0.0025^{I}	0.5933 ± 0.0012^{g}	0.7416 ± 0.0005^{h}	0.7096 ± 0.0021^{J}	0.6113 ± 0.0031^{J}
Aspergillus flavus (F2A)	0.0010 ± 0.0010^{a}	0.0140 ± 0.0020^{abc}	0.0643 ± 0.0025^{c}	0.1250 ± 0.0020^{b}	0.2300 ± 0.0030^{d}	0.3003 ± 0.0021^{c}	0.3950 ± 0.0034^{c}	0.4650 ± 0.0036^{c}	0.5206 ± 0.0015^{c}	0.5210 ± 0.000^{c}	0.4473 ± 0.0025^{d}
Candida parapsilopsis(F4A)	0.0007 ± 0.0012^{a}	0.0100 ± 0.0017^{a}	0.0503 ± 0.0032^{b}	0.1076 ± 0.0006^{a}	0.1867 ± 0.0030^{a}	0.2786 ± 0.0058^{b}	0.3703 ± 0.0021^{b}	0.4320 ± 0.0017^{b}	0.4970 ± 0.0055^{b}	0.4399 ± 0.0021^{b}	0.3643 ± 0.0025^{b}
Kodamaea ohmeri (F2B)	0.0043 ± 0.0012^{bc}	0.0156 ± 0.0015^{bcd}	0.0990 ± 0.0026^{h}	0.2123 ± 0.0025^{g}	0.3060 ± 0.0020^{I}	0.4210 ± 0.0000^{I}	0.5470 ± 0.0017^{J}	0.6456 ± 0.0032^{h}	0.7633 ± 0.0040^{I}	0.6956 ± 0.0047^{I}	0.6183 ± 0.0035^{K}
Paecilomyces variotii (F4B)	0.0010 ± 0.000^{a}	0.0150 ± 0.0030^{bc}	0.1240 ± 0.0020^{ij}	0.2450 ± 0.0030^{h}	0.3706 ± 0.0081^{J}	0.4646 ± 0.0035^{J}	0.6113 ± 0.0012^{K}	0.7096 ± 0.0025^{J}	0.8120 ± 0.0030^{J}	0.7476 ± 0.0006^{K}	0.5716 ± 0.0015^{I}
Trichoderma sp (F3A).	0.0013 ± 0.0012^{a}	0.0123 ± 0.0006^{abc}	0.0696 ± 0.0025^{d}	0.1346 ± 0.0023^{c}	0.2216 ± 0.0025^{c}	0.3286 ± 0.0015^{e}	0.4240 ± 0.0017^{e}	0.5430 ± 0.0017^{e}	0.6366 ± 0.0035^{e}	0.5733 ± 0.0023^{e}	0.4793 ± 0.0045^{f}
Mucor mucedo (F3B)	0.0043 ± 0.0019^{bc}	0.0126 ± 0.0012^{abc}	0.0736 ± 0.0006^{e}	0.1696 ± 0.0425^{d}	0.2600 ± 0.0020^{f}	0.3326 ± 0.0031^{e}	0.4310 ± 0.0036^{f}	0.5440 ± 0.0024^{e}	0.6460 ± 0.1106^{d}	0.5950 ± 0.0026^{f}	0.4700 ± 0.0020^{e}

Table 3: Optical Density (OD) at 540nm of fungal isolates from crude oil contaminated and uncontaminated soil samples (Day 1-21)

Legend: isolate F1; fungal isolate from Awoye
isolate F2A and F2B; fungal isolates from Orioke Iwamimo
isolate F3A and F3B; fungal isolates from Igodan- Lisa
isolate F4A and F4B; fungal isolates from Oba- Ile

Table 4: Growth and extent of crude oil utilization by the test fungal isolates at the Peak (day 17)

Organism	Turbidity	Extent of shredding	Optical density
Cephalosporium specie	+++	H	0.7416±0.0005[b]
Aspergillus flavus	++	H	*0.5210±0.0015[c]
Candida parapsilopsis	++	M	0.4970±0.0055[b]
Kodamea ohmeri	+++	H	0.7633±0.0040[I]
Paecilomyces variotii	+++	H	0.8120±0.0030[J]
Trichoderma specie	++	H	0.6366±0.0035[e]
Mucor mucedo	++	H	0.6460±0.1106[d]

Legend: isolate F1; fungal isolate from Awoye
isolate F2A and F2B; fungal isolates from Orioke Iwamimo
isolate F3A and F3B; fungal isolates from Igodan- Lisa
isolate F4A and F4B; fungal isolates from Oba- Ile
*value obtained at day 19 of growth

The highest degradative ability was exhibited by *Klebsiella edwardsii* at OD of 0.647 followed by *Pseudomonas aeruginosa* isolated from Orioke-Iwamimo sample at OD of 0.575.

DISCUSSION AND CONCLUSION

In this study, crude oil degradation potentials of Sixteen (16) bacteria species and Seven (7) fungal isolates indigenous to crude oil contaminated and uncontaminated soil were evaluated by measurement of optical density, visual turbidity and extent of breakdown of overlaid oil in tubes. The results revealed that different genera and types of microorganisms are involved in the degradation of crude oil. Many of these isolates were among those identified by several researchers like Ijah and Abioye (2003), Ajayi *et al*., (2008), Das and Chandran (2011),

Omotayo *et al*., (2012). The findings in this study revealed that these microbes exhibited different responses and potentials to breakdown crude oil and utilize as source of carbon and energy. The report of this study is in agreement with the report of Nduka *et al*., (2012) that microorganisms have enzyme systems that degrade and utilize different hydrocarbons as source of carbon and energy. This variation in crude oil utilization is reflected in the differences in optical densities. The differences in optical density is suggestive that the pattern of microbial growth and crude oil utilization differ from organism to organism. The different degrees of response of the tested microbes to crude oil degradation could be attributed to the inherent genetic abilities in utilizing hydrocarbon as substrates. This differences in response implies that different microorganisms have different rates of crude oil utilization. The assertion is in line with the report of Nwaogu *et al*.,(2008) that microorganisms have different rates at which they utilize and degrade hydrocarbons in the soil or water. Magid *et al*., (2008) and Onuoha *et al*., (2014) suggested that the differences in the rate of hydrocarbon degradation may be due to natural ability of the microbes in hydrocarbon utilization. The bacterial isolates exhibited their highest degradative abilities at days 11 and 13 while the fungal isolates showed highest ability based on optical density at days 17 and 19 of growth respectively, suggesting that crude oil utilization by fungi was initially slower than bacteria. The progressive increase in optical density of the isolates and the turbidity in culture tubes are suggestive of microbial growth and accumulation of microbial biomass which result from degradation and utilization of crude oil as source of carbon and energy. Highest degradative ability was shown by *Klebsiella edwardsii* (OD 0.647) followed by *Pseudomonas aeruginosa* (OD 0.575) and *Klebsiella pneumoniae*(OD 0.4900). *Paecilomyces variotii* showed the highest degradative ability among the fungi. The higher utilization of crude oil by these microbes as sole source of carbon and energy may be attributed to the presence of

efficient hydrocarbon degradative enzymes system and the presence of catabolic genes involved in petroleum hydrocarbon degradation in the microorganisms (Kyung-Hwa *et al*., 2006; Magid *et al*., 2008; Abioye *et al*., 2010). Atlas and Bartha (1981); Al-Wasify and Hamed (2014) ascribed that these microorganisms are taken as evidence that they are the most active degraders in the environment. The results also suggest that these microorganisms with high degradative ability may be useful in seeding oil polluted soils for bioremediation.

The optical densities of 0.647 and 0.575 demonstrated by *Klebsiella edwardsii* and *Pseudomonas aeruginosa* among bacteria and 0.812, 0.763 and 0.74 respectively by *Paecilomyces variotii*, *Kodamaea ohmeri and Cephlosporium* spp. among fungi suggests that these organisms have better potentials for crude oil degradation. The result of this study also revealed that *Pseudomonas aeruginosa* (1B), *Bacillus subtilis* (1C and 3C) and *B. megaterium*(4A), *A. Flavus, Trichoderma* species, and *Mucor mucedo* which showed moderate growth (table 2 and 4) also exhibited high shredding of overlaid oil. This implies that the ability of microorganisms to produce turbidity does not necessarily suggest efficient hydrocarbon degradation potentials.

Recommendation

The efficiency of indigenous microbial population in crude oil degradation differ. Therefore, these microbes with extensive breakdown of oil in tubes can be applied singly or as a consortium for the degradation of crude oil. It is also recommended that these isolates be tested for hydrocarbon specificities since crude oil is a complex mixture of hydrocarbons. Finally, future research should study the effects of varying concentration of crude oil on the growth of these

271 microbes since the quantity and size of crude oil spill to be degraded vary from minor to medium
272 to disaster.

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