Pollution, 5(2): 269-277, Spring 2019
DOI: 10.22059/poll.2018.264288.505
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Degradation of Hydrocarbons and Lignin-like compounds by *Alcaligenes* sp. strain 3k isolated from Ilorin

Adetitun, D. O.,[1,2] *Fathepure, B.,[1] Hugh, H.,[1] Kolawole O. M.[2] and Olayemi, A. B.[2]

1. Department of Microbiology and Molecular Genetics Oklahoma State University, Stillwater, OK, USA
2. Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria

Received: 24.08.2018 Accepted: 14.01.2019

ABSTRACT: The primary goal of this study was to isolate hydrocarbon-degrading organisms and assess their ability to bioremediate petroleum-contaminated soil and water. Nigeria is one of the major oil producing countries and petroleum contamination is widespread in agricultural soil. *Alcaligenes* sp. strain 3k was isolated from a kerosene-polluted soil in Ilorin, Nigeria. We also assessed its ability to degrade plant lignin, as lignin is a complex aromatic heteropolymer commonly found in soil and aquifer environment. Strain 3k was originally grown on mineral salts medium with kerosene as a sole energy and carbon source. The capacity of the isolate to degrade both aromatic, aliphatic hydrocarbons and lignin-like compounds was tested. Among the tested compounds, the organism utilized kerosene, hexadecane, cyclohexane, phenol and benzoate as the sole sources of carbon. In addition, strain 3k also degraded various lignocellulose compounds as the sole source of carbon. However, hexane, benzene, toluene, ethylbenzene and xylene were not metabolized. Our study demonstrates that soil organisms like *Alcaligenes* could play important role in the reclamation of petroleum-contaminated soil and water. Utilization capacity of lignin as the sole carbon source suggest that these organisms can survive on plant detritus and also have the ability to degrade hydrocarbons upon accidental or deliberate contamination of agricultural soil and water.

Keywords: *Alcaligenes* sp; hydrocarbons; lignin-like; aromatics; heteropolymer.

INTRODUCTION

Nigeria has Africa's largest reserves of oil and gas resources, mostly in the Niger Delta and on the continental shelf of the country. Oil extraction in the Niger Delta has been going on since the 1950s and Nigeria exports a significant amount of oil every day. Oil spills have occurred repeatedly for decades in the Niger Delta and large parts of the land and wetlands are intermittently and chronically affected by oil spills that destroy crops and aquaculture through the contamination. There is also rampant mismanagement of the land resources. These have significant negative consequences on crop yield and land productivity, which further impoverish the already poor farmers in these areas. Cost of farm produce also increases due to shortage of supply. With the increasing soil infertility because of the destruction of soil microorganisms and dwindling agricultural productivity, farmers have been forced to abandon their land to seek non-existent alternative means of livelihood.

* Correspondence author, Email: babu.fathepure@okstate.edu

Kerosene is a colourless flammable hydrocarbon liquid obtained from the fractional distillation of petroleum at 150°C and 275°C (Gouda *et al*., 2008). Despite the several usefulness of kerosene, it also constitutes a major environmental concern worldwide (Saratale *et al*., 2007). Kerosene is not considered a carcinogen but repeated exposure of animals to kerosene has been linked to skin cancer (Chilcott, 2006). In addition to aesthetic problem and economic damage caused by kerosene spills, crops and animals in both land and water are affected negatively (Ikpeme *et al*., 2007). Microorganisms with the ability to degrade oil are ubiquitously distributed in soil and marine environments (Cao *et al*. 2009; Harayama *et al.* 2004; Head *et al*. 2006; Jyothi *et al*. 2012; Marques-Rocha *et al*. 2000). Degradation of Jet fuel by Gram-negative bacteria isolated from kerosene-polluted soil had been reported previously (Adetitun *et al*., 2018).

The degradation rates of hydrocarbons are affected by several chemical, bio - physical parameters such as pH, temperature, nutrient and quantity of hydrocarbons (Santhini *et al*. 2009). Alkanes vary in chain length from one in methane to greater than 48 carbon atoms. They are the major components of petroleum and petroleum products and make up about 20-50% of crude oil (So & Young, 1999). Several microorganisms (Rojo, 2009) can efficiently degrade most alkanes. Aerobic degradation of alkanes begins with oxidation of one of the methyl groups (either terminal or subterminal) to produce the corresponding primary alcohol by the enzyme alkane hydroxylases. The alcohol is finally converted to a fatty acid (van Beilen *et al*. 2003; Ji *et al.* 2013).

Benzoate has been widely used as a prototype compound for the study of the bacterial catabolism of aromatic compounds (Gibson & Harwood, 2002; Carmona *et al*. 2009). The monoaromatic hydrocarbons such as benzene, ethylbenzene, toluene and *o*-, *m*-, and *p*-xylene (BTEX) are constituents of petroleum and its products such as gasoline and diesel fuel. (Dean, 1985). BTEX compounds are toxic and carcinogenic and difficult to degrade, especially under anaerobic conditions (Phelps & Young, 1999).

Lignin is a complex heteropolymer comprised of a network of aromatic phenypropanoid units linked by C–C and C–O–C binding (Brown & Chang 2014; Ralph *et al.* 2004). Microorganisms have evolved the capacity to degrade lignin to gain access to plant polysaccharides to meet their metabolic needs. The exploitation of this capacity offers a natural means for preparing plant biomass for biofuels production (Brown & Chang 2014; Bugg & Rahmanpour, 2015; Mosier *et al*. 2005). It is known that many fungi and bacteria produce lignin degrading enzymes such as peroxidases and laccases (Bugg *et al*. 2011a, b; Kumar *et al*. 2009; Martinez, *et al*. 2009) that not only depolymerize or degrade lignin, but also degrade petroleum hydrocarbons (Falade *et al*. 2017; Marco-Urrea *et al.* 2012). Lignin is a major component of soil organic matter (Datta *et al*. 2017) that can support soil microbial community including hydrocarbon-degrading organisms. This is important as these organisms can quickly degrade hydrocarbons upon sudden contamination of soil and water by petroleum compounds. In addition, these organisms can play important role in carbon cycling and delignification of plant biomass for improved biofuel production (Brown & Chang 2014; Bugg & Rahmanpour, 2015; Mosier et al. 2005). Puentes-Téllez & Salles (2018) reported that cellulose and hemicellulose degradation were either negatively or not affected by functional diversity whereas functional diversity positively correlated with the breakdown of lignin.

The present work aims at characterization of *Alcaligenes* sp. strain 3k and assessing its degradation of hydrocarbons and lignin-like compounds in vitro with the hope that the

same results obtained can be replicated on non-experimental samples.

MATERIALS AND METHODS

Hexadecane, hexane, cyclohexane, benzoate, phenol, benzene, toluene, ethylbenzene and xylene used in this study were obtained from Sigma-Aldrich (St Louis, MO, USA). Also, lignocellulose such as vanillic acid, vanillin, veratric acid, methylvanillin, syringic acid, cinnamic acid, anisoin (4,4-dimethoxybenzoin), o-benzylvanillin (4-benzyloxy-3-methoxy-benzaldehyde), alkali lignin, and cellobiose were purchased from Sigma-Aldrich. Xylan (birch wood) was purchased from Fluka. Most of the reagents used in this study were of reagent grades.

Kerosene-metabolizing microorganisms were briefly enriched in mineral salts medium (Dalvi *et al*. 2016) containing kerosene as the carbon source from alfisol loam artificially contaminated with kerosene at the University Of Ilorin, Nigeria. Pure cultures were isolated by 10-x dilution of the enrichment culture and spreading 0.1-ml aliquots of the diluted culture onto agar plates. Plates were incubated at 30 ^{0}C and well-isolated colonies were re-streaked three more times on new plates prior to transferring into 250-ml conical flasks containing 100 ml of mineral salts medium (MSM) with 1 ml of kerosene. The isolate was identified as a species of the genera *Alcaligenes* closely related (99 % similarity) to *Alcaligenes faecalis* using PCR. Genomic DNA was isolated using a commercially available kit (FastDNA spin kit for soil, MP Biomedicals, Santa Ana, CA). 16S rRNA-genes were amplified with 27F and 1492R primers as described before (Spear *et al*., 2005). Amplified PCR products were cleaned using ExoSAP-IT and sequenced at the DNA core facility, Oklahoma State University, Stillwater, OK. Amplified 16S rRNA-gene sequence was analyzed using the National Center for Biotechnology Information (NCBI) database using BLASTn.

Degradation of hexane, hexadecane, cyclohexane by strain 3k was carried out in flasks containing 50 ml of MSM supplemented with 24.7 mM hexane (65 μL neat hexane), 11 mM hexadecane (65 μL neat hexadecane), or cyclohexane 30.05 mM (65 μL neat cyclohexane) as the sole source of carbon. Bottles were inoculated with roughly 10^4 to 10^5 cells/ml and incubated at 30^0 C static in the dark. Degradation of hydrocarbons was monitored by determining the growth of the organism. Culture sample (1 mL) was withdrawn periodically from each flask and plated on to LB plates. Colony forming units (CFU) was determined.

Degradation of benzoate and phenol was carried out in 50 mL tubes containing 25 ml of MSM with 2 mM sodium benzoate or phenol as the sole carbon source. Tubes were inoculated with 10^3 cells/ml of strain 3k and incubated static at 30^0 C in the dark. Un-inoculated control tubes with benzoate or phenol were set up similarly. Culture sample (1 mL) was withdrawn from each tube and the concentration of benzoate and phenol was determined by UV absorption at 223 nm and 269 nm, respectively using a spectrophotometer. The spectrophotometer was blanked with distilled water.

Utilization of BTEX by the isolate was carried out in 110 mL of serum bottles containing 50 mL of MSM with 28 μ mole benzene (2.5 μL neat), 23.85 μ mole toluene (2.5 μL neat), 20.4 μ mole ethylbenzene (2.5 μL neat) and 20.35 μ mole xylene (2.5 μL neat). Bottles were capped with Teflon-coated septa and aluminum crimps. For each compound, three live and two autoclaved control bottles were setup. Headspace samples (100 μL) was withdrawn periodically and analyzed using a gas chromatograph using the methods described elsewhere (Nicholson & Fathepure, 2004).

Utilization of lignin monomers and dimers as well plant polysaccharides (xylan

and cellulose) was carried out in 250 ml flasks containing 100 ml of MSM and 0.02% of anisoin, 0.02% benzylvanillin, 0.02% methylvanillin, 0.02% syringic acid, 0.02% cinnamic acid, 0.02% vanillic acid, veratric acid, 0.02% veratryl alcohol, 0.02% alkali lignin, 0.02% xylan, and 0.02% cellobiose as the sole sources of carbon. Starter culture was prepared by transferring strain 3k colonies to a flask containing 100 ml of 1/5 strength LB broth and grown for 24 hours at 30^{o}C. Aliquots of 0.1 ml of appropriately diluted (10^{6}-fold dilution) culture were used as the inoculum. Flasks were incubated static in the dark at 30 ^{o}C. Culture (1 ml) was withdrawn periodically from each flask and degradation of lignin compounds was determined by plating the appropriately diluted culture onto LB plates and colony-forming unit (CFU) was determined.

RESULTS AND DISCUSSION

One of the major environmental problems today is the contamination of soil and water by pollutants from petrochemical industries. Bioremediation is the promising technology for the remediation of contaminated sites since it is cost-effective and will lead to complete destruction of pollutants. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. The primary goal of this study was to determine natural bioremediation potential of soil organisms. We intentionally contaminated garden soil with kerosene and attempted to isolate kerosene degrading organisms. Among the isolates, we chose *Alcaligenes* sp strain 3k for further study as this organism grew well on kerosene. Figure 1 shows the growth of strain 3k on hexadecane. The bacterium grew well on hexadecane as the sole carbon and energy source. Growth of the organisms increased from initial 67, 000 cfu to 30,500,000,000 cfu in 24 days suggesting an increase in population by almost 6-log unit. These data show that strain 3k grows well on hexadecane. This is important because hexadecane is present in the aliphatic fraction of crude oil and is one of the major components of diesel (Chénier *et al*., 2003). In addition, alkanes, especially longer-chain alkanes like hexadecane are non-polar molecules with very low chemical activity and water solubility, (Rojo 2009) hence; their degradation by microbes constitutes an important aspect of reclamation of polluted soil for agricultural purposes. Strain 3k showed growth on cyclohexane but not as much as it did on hexadecane (Figure 2). Interestingly, strain 3k did not utilize hexane as the carbon source (Table 1). These findings correlate with that of Zampolli *et al*. (2014), where *Rhodococcus opacus* R7 was unable to degrade hexane and octane. It was however able to degrade decane, dodecane, hexadecane, eicosane, tetracosane and hexatriacontane.

Figure 3 depicts the utilization of phenol as the sole carbon source by strain 3k. The organism degraded almost 80% of the initially added phenol within 7 days. Many researchers had previously reported Biodegradation of phenol. Bai *et al*. (2007) reported that Alcaligenes faecalis could utilize 100 mg L^{-1} phenol in just 10 hours as the sole source of carbon. Jiang *et al*. (2007) isolated *A. faecalis* from acclimated activated sludge and it was shown to degrade high concentration of phenol (1600 mg L^{-1}) in 76 h. Agarry *et al*. (2008) reported that *Pseudomonas aeruginosa* NCIB 950 and *Pseudomonas fluorescens* NCIB 3756 were able to degrade phenol. Chakraborty *et al*. (2010) had reported that one of three local bacteria detected in coke processing water was able to degrade phenol. Zhenghui *et al*. (2016) reported the biodegradation of phenol by *Acinetobacter calcoaceticus*. They reported that the strain removed 91.6% of the initial 800 mgL^{-1} phenol within 2 days. Environmental fate of phenol is important as this compound is toxic, recalcitrant, bioaccumulate in organisms and negatively affects aquatic biota (Annachatre & Gheewala, 1996).

Strain 3k degraded > 90% of the added benzoate within 2 days of incubation (Figure 4). Benzoate has been widely used as a model compound for the study of the bacterial catabolism of aromatic compounds. Benzoate (or benzoylCoA) is the most common intermediate in anaerobic metabolism of aromatic compounds and recently, benzoate has been reported to be an intermediate of anaerobic benzene biodegradation (Caldwell & Suflita, 2000). *Pseudomonas* and *A. eutrophus* have been reported to degrade benzoate *via* the *ortho* cleavage pathway (Ampe *et al*. 1997; Feist & Hegeman, 1969) but other studies have shown the possibility of both *meta* and *ortho* pathways (Nakazawa & Yokota, 1973). In some organisms, benzoate is reportedly converted to catechol using chromosomally encoded enzymes. Catechol is then further degraded to trichloroacetic acid cycle intermediates by an ortho ring cleavage pathway (Harwood & Parales, 1996).

Strain 3k rapidly utilized lignin monomers and dimers as well as sugar polymers (Table 2). The ability to utilize lignocellulosic compounds is important for overall carbon cycling in soil (Datta *et al*. 2017) and for delignificaiton of plant biomass for rapid saccharification of plant polysaccharides for efficient fermentation of sugars to biofuels and other chemicals (Brown & Chang 2014; Mosier *et al*. 2005). The observation that strain 3k with the ability to degrade aromatic compounds is also able to degrade lignin provides a possible link between aromatic degradation and lignin degradation, which is logical, given that lignin is the ultimate source for much of the aromatic material found in soil.

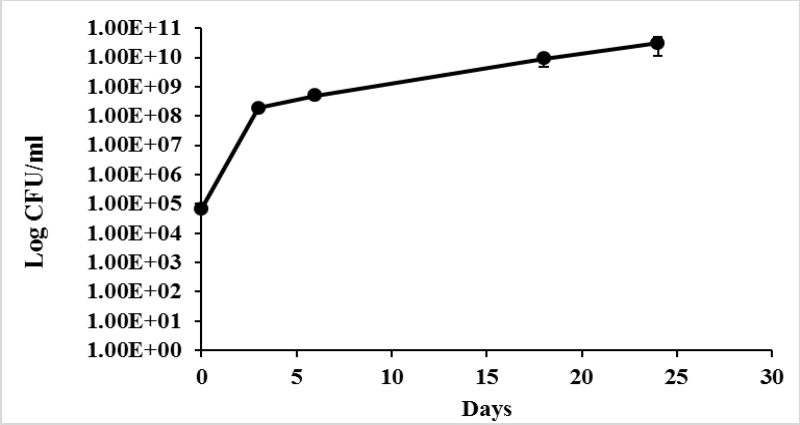

Fig. 1. Degradation of hexadecane by *Alcaligenes* sp. strain 3k. Data are the mean of triplicate plate counts, and bars indicate ± standard deviation.

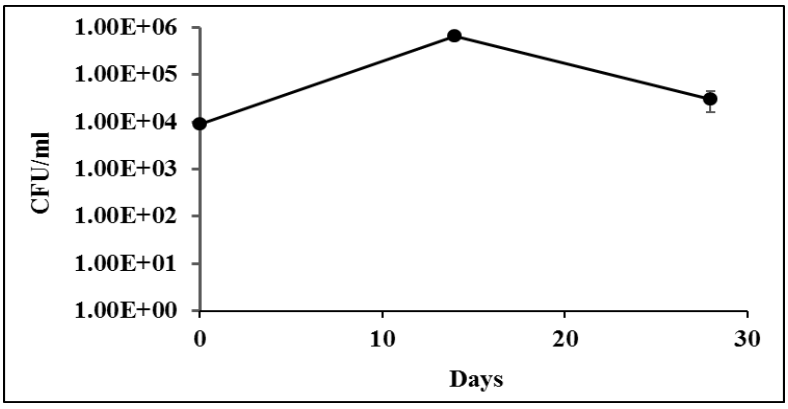

Fig. 2. Degradation of cyclohexane by *Alcaligenes* sp. strain 3k. Data are the mean of triplicate plate counts, and bars indicate ± standard deviation.

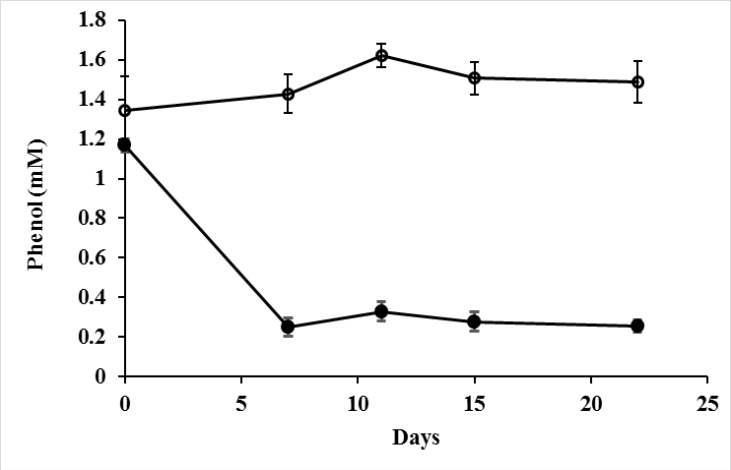

Fig. 3. Degradation of phenol by *Alcaligenes* sp. strain 3k. Data are the mean of triplicate tubes, and bars indicate ± standard deviation (Control = o).

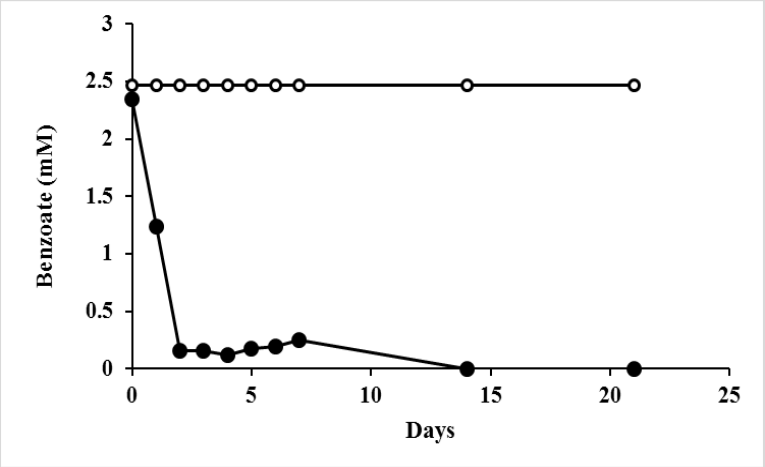

Fig. 4. Degradation of benzoate by *Alcaligenes* sp. strain 3k. Data are the mean of triplicate tubes, and bars indicate ± standard deviation (Control = o).

Table 1. Degradation of aliphatic and aromatic hydrocarbons by *Alcaligenes* sp. strain 3k [1]

Compound	Degradation
Kerosene	++
Benzene	-
Toluene	-
Ethylbenzene	-
Xylenes	-
Phenol	++
Benzoate	++
Cyclohexane	+
Hexane	-
Hexadecane	++

1. *Alcaligenes* sp. strain 3k was gown in MSM supplemented with a petroleum compound as the sole source of carbon. ++ Best growth, + poor growth, and - no growth.

Table 2. Degradation of lignocellulose compounds by *Alcaligenes* sp. strain 3k[1]

[1]Lignocellulose compound	2 weeks	4 weeks
Vanillic acid	3.99E+05	7.00E+07
Veratric acid	6.00E+04	1.01E+07
Methylvanillin	2.00E+05	2.00E+05
Syringic	1.55E+05	2.00E+05
Veratryl alcohol	3.86E+05	5.10E+06
Cinnamic acid	5.70E+04	5.00E+07
Anisoin	2.00E+06	4.00E+05
O-Benzylvanillin	9.61E+05	1.01E+07
Alkali lignin	2.50E+05	2.00E+05
Cellobiose	5.00E+05	5.45E+06
Xylan	5.00E+05	1.15E+07

1. All flasks were inoculated initially 8.62E+04 ± 1.03E+04.
2. Each flask was amended with 0.02% lignocellulosic compound as the sole source of carbon. Flaks were incubated at 30 0C in the dark.

CONCLUSION

This study shows that strain 3k is capable of degrading both aliphatic and aromatic compounds and not BTEX compounds. Previous studies, however, have shown *Alcaligenes* ability to degrade BTEX compounds (Płaza *et al*. 2007; Yeom & Yoo, 2002) as well compounds present in produced water (Okoro & Amund, 2010; Igwo-Ezikpe *et al.* 2009). The reason that strain 3k lacks the ability to degrade BTEX compounds is not known. Overall, *Alcaligenes* have the ability to degrade common toxic pollutants found in the environment, thus can play important role in the reclamation of contaminated agricultural soil and aquatic environments. *Alcaligenes* sp. strain 3k is expected to degrade hydrocarbons and lignin-like compounds on the field just as it has been demonstrated in this laboratory scale experiment.

ACKNOWLEDGEMENT

The authors appreciate the US government for the Fulbright award and the Association of African Universities (AAU) for the small grant for thesis and dissertations granted to David Olugbenga Adetitun in 2015.

REFERENCES

Adetitun, D.O., Akinmayowa, O.V., Atolani, O. and Olayemi, A.B. (2018): Biodegradation of jet fuel by three Gram negative bacilli isolated from kerosene contaminated soil. Pollution, 4(2), 291-303.

Agarry, S. E., Durojaiye, A. O., Yusuf, R. O. and Aremu, M. O. (2008). Biodegradation of phenol in refinery wastewater by pure cultures of *Pseudomonas aeruginosa* NCIB 950 and *Pseudomonas fluorescence* NCIB 3756. International Journal of Environment and Pollution, 32(1), 3-11.

Ampe, F., Uribelarrea, J., Aragao, G. M. F. and Lindley, N. D. (1997). Benzoate Degradation via the ortho Pathway in *Alcaligenes eutrophus* is perturbed by Succinate. Applied and Environmental Microbiology, 63(7), 2765–2770.

Annachatre, A.P., Gheewala, S.H. (1996) Biodegradation of chlorinated phenolic compounds. Biotechnology Advances, 14, 35–56.

Bai, J., Wen, J., Li, H. and Jiang, Y. (2007). Kinetic modeling of growth and biodegradation of Phenol and m-cresol using Alcaligenes faecalis. Process Biochemistry, 42, 510-517.

Brown, M. E. and Chang, M. C. (2014). Exploring Bacterial Lignin Degradation. Current Opinion in Chemical Biology, 19, 1–7.

Bugg, T. D. and Rahmanpour, R. (2015). Enzymatic Conversion of Lignin into Renewable Chemicals. Current Opinion in Chemical Biology, 29, 10–17.

Bugg, T.D., Ahmad, M., Hardiman, E.M. and Rahmanpour, R. (2011)a. Pathways for degradation of lignin in bacteria and fungi. Natural Product Reports, *28*, 883-1896.

Bugg, T. D., Ahmad, M., Hardiman, E. M. and Singh, R. (2011)b. The Emerging Role for Bacteria in Lignin Degradation and Bio-product Formation. Current Opinion in Biotechnology, 22, 394–400.

Caldwell, M. E. and Suflita, J. M. (2000). Detection of Phenol and Benzoate as Intermediates of Anaerobic Benzene Biodegradation under Different Terminal Electron-Accepting Conditions. Environmental Science and Technology, 34, 1216–1220.

Chakraborty, S., Bhattacharya, T., Patel, T. N. and Tiwari, K. K. (2010). Biodegradation of Phenol by Native Microorganisms Isolated from Coke Processing Wastewater. Journal of Environmental Biology, 31(3), 293-6.

Chénier, M, R., Beaumier, D., Roy, R., Driscoll, B.T., Lawrence, J.R., Greer, C.W. (2003).

Impact of seasonal variations and nutrient inputs on nitrogen cycling and degradation of hexadecane by replicated river biofilms. Applied Environmental Microbiology, 69, 5170– 5177.

Cao, B., Nagarajan, K. and Loh, K.C. (2009). Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Applied Microbiology and Biotechnology, 85, 207-228.

Carmona, M., Zamarro, M. T., Blázquez, B., Durante-Rodríguez, G., Juárez, J. F., Valderrama, J. A., Barragán, M. J., García, J. L. and Díaz, E. (2009). Anaerobic Catabolism of Aromatic Compounds. A Genetic and Genomic View. Microbiology and Molecular Biology Reviews, 73, 71–133.

Chilcott, R. P. (2006). Compendium of Chemical Hazards: Kerosene (Fuel Oil). Health Protection Agency. Didcot, Oxfordshire, OX11 0RQ, United Kingdom. 36P.

Dalvi, S., Youssef, N. H. and Fathepure, B. Z. (2016). Microbial Community Structure Analysis of a Benzoate-Degrading Halophilic Archaeal Enrichment. Extremophiles, 20, 311–321.

Dean, B. J. (1985). Recent Findings on the Genetic Toxicology of Benzene, Toluene, Xylenes and Phenols. Mutation Research, 145, 153–181.

Datta, R., Kelkar, A., Baraniya, D., Molaei, A., Moulick, A., Meena, R.S. and Formanek, P. (2017). Enzymatic degradation of lignin in soil: a review. Sustainability, 9, 1163.

Falade, A.O., Nwodo, U.U., Iweriebor, B.C., Green, E., Mabinya, L.V. and Okoh, A.I., (2017). Lignin peroxidase functionalities and prospective applications. Microbiology Open, 6(1); p.e00394.

Feist, C. F. and Hegeman, G. D. (1969). Phenol and Benzoate Metabolism by *Pseudomonas putida*: Regulation of Tangential Pathways. Journal of Bacteriology, 100, 869– 877.

Gibson, J. and Harwood, C. S. (2002). Metabolic Diversity in Aromatic Compound Utilization by Anaerobic Microbes. Annual Review of Microbiology, 56, 345–369.

Gleixner, G.; Czimczik, C.J.; Kramer, C.; Lühker, B.; Schmidt, M.W.I. (2001). Plant compounds and their turnover and stabilization as soil organic matter. Global Biogeochemical Cycles in the Climate System, 201–215.

Gouda, M. K, Omar, S. H, Nour-Eldin, H. M. and Chekroud, Z. A. (2008). Bioremediation of Kerosene II: A Case Study in Contaminated Clay (Laboratory and Field: Scale Microcosms). World Journal of Microbiology and Biotechnology, 24, 1451-1460.

Harayama, S., Kasai, Y. and Hara, A. (2004). Microbial Communities in Oil-Contaminated Seawater. Current Opinion in Biotechnology, 15, 205–214.

Harwood, C. S. and Parales, R. E. (1996). The β-ketoadipate Pathway and the Biology of Self-Identity. Annual Review of Microbiology, 50, 553–590.

Head, I. M., Jones, D. M. and Roling, W. F. (2006). Marine Microorganisms make a Meal of Oil. Nature Reviews Microbiology, 4, 173–182.

Igwo-Ezikpe, M. N., Gbenle, O. G., Ilori, M. O., Okpuzor, J and Osuntoki, A. A. (2009).

Evaluation of *Alcaligenes faecalis* Degradation of Chrysene and Diesel Oil with Concomitant Production of Biosurfactant. Research Journal of Environmental Toxicology, 3, 159-169.

Ikpeme, E. M., Nfongeh, J. F. and Etim, L. (2007). Comparative Bioremediation Enhancement Procedures on Kerosene Polluted ultisol from Niger Delta Region, Southern Nigeria. Research Journal of Microbiology, 2 (11), 856-860.

Jiang, Y., Wen, J., Bai, J., Jia, X. and Hu, Z. (2007). Biodegradation of phenol at high initial concentration by *Alcaligenes faecalis*. Journal of Hazardous Materials, 147, 672- 676.

Ji, Y., Mao, G., Wang, Y. and Bartlam, M. (2013). Structural Insights into Diversity and n-alkane Biodegradation Mechanisms of Alkane Hydroxylases. Frontiers in Microbiology, 4, 1–13.

Jyothi, K., Babu, S. K., Nancy Clara, K. and Kashyap, A. (2012). Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization. Helix, 2, 105-111.

Kubicek, C.P. The Plant Biomass (2012). In Fungi and Lignocellulosic Biomass; Kubicek, C.P., Ed.; Wiley-Blackwell: Oxford, UK, 2012; pp. 1–28

Marco-Urrea, E. and Reddy, C. A. (2012). Degradation of chloro-organic pollutant by white-rotfungi. In S. N. Singh (Ed.), Microbial degradation of xenobiotics (pp. 31–66). Berlin: Springer.

Marques-Rocha, F. J., Hernandez-Rodrigues, V. and Lamela, M. A. T. (2000). Biodegradation of Diesel Oil by Microbial Consortium. Water, Soil and Air Pollution, 128, 313-20.

Martinez, A.T., Ruiz-Duenas, F.J., Martínez, M.J., del Rio, J.C. and Gutierrez, A. (2009). Enzymatic delignification of plant cell wall: from nature to mill. Current Opinion in Biotechnology, 20, 348-357.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M. and Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource technology, 96, 673-686.

Nakazawa, T. and Yokota, T. (1973). Benzoate Metabolism in *Pseudomonas putida* (arvilla) mt- 2, Demonstration of Two Benzoate Pathways. Journal of Microbiology, 115; 262–267.

Nicholson, C. A. and Fathepure, B. Z. (2004). Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. Applied Environmental Microbiology, 70, 1222- 1225.

Okoro, C. C. and Amund, O. O. (2010). Biodegradation of Produce Water Hydrocarbons by Pure Cultures of *Alcaligenes* sp. Journal of American Science, 6, 107-112

Pepi, M., Minacci, A., Cello, F. D., Baldi, F. and Fani, R. (2003). Long Term Analysis of Diesel Fuel Consumption in a Co-Culture of *Acinetobacter venetianus*, *Pseudomonas putida* and *Alcaligenes faecalis.* Antonie van Leeuwenhoek, 89, 3-9.

Phelps, C. D. and Young, L. Y. (1999). Anaerobic Biodegradation of BTEX and Gasoline in Various Aquatic Sediments. Biodegradation, 10, 15–25.

Płaza, G.A., Wypych, J., Berry, C. and Brigmon, R.L. (2007). Utilization of monocyclic aromatic hydrocarbons individually and in mixture by bacteria isolated from petroleum-contaminated soil. World Journal of Microbiology and Biotechnology, 23, 533-542.

Puentes-Téllez, P.E and Salles, J.F. (2018). Construction of effective minimal active microbial consortia for lignocellulose degradation. Microbial Ecology, 76, 419–429.

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P.F., Marita, J.M., Hatfield, R.D., Ralph, S.A., Christensen, J.H. and Boerjan, W. (2004). Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. Phytochemistry Reviews, 3, 29-60.

Rojo, F. (2009). Degradation of Alkanes by Bacteria. Environmental Microbiology, 11, 2477–2490.i_

Santhini, K., Myla, J., Sajani, S. and Usharani, G. (2009). Screening of *Micrococcus* sp from Oil Contaminated Soil with Reference to Bioremediation. Botany Research International, 2(4), 248-252.

Saratale, G. D., Bhosale, S. K., Kalme, S. D. and Govindwar, S. P. (2007). Biodegradation of Kerosene in *Aspergillus ochraceus* (NCIM-1146). Journal of Basic Microbiology, 47, 400-405.

Schnitzer, M. and Monreal, C.M. (2011). Quo vadis soil organic matter research? A biological link to the chemistry of humification. In Advances in agronomy, 113, 143-217.

So, C. M. and Young, L. Y. (1999). Isolation and Characterization of a Sulfate-Reducing Bacterium that Anaerobically Degrades Alkanes. Applied Environmental Microbiology, 65, 2969–2976.

van Beilen, J. B., Li, Z., Duetz, W. A., Smits, T. H. M. and Witholt, B. (2003). Diversity of Alkane Hydroxylase System in the Environment. Oil and Gas Science and Technology, 58(4), 427-440.

Yeom, S.H. and Yoo, Y.J. (2002). Analysis of microbial adaptation at enzyme level for enhancing biodegradation rate of BTX. Korean Journal of Chemical Engineering, 19, 780-782.

Zampolli, J., Collina, E., Lasagni, M. and Di Gennaro, P. (2014). Biodegradation of Variable-Chain-Length *n*-Alkanes in *Rhodococcus opacus* R7 and the Involvement of an Alkane Hydroxylase System in the Metabolism. *AMB Express*, 4(73), 1-9.

Zhenghui, L., Wenyu, X., Dehao, L., Yang, P., Zesheng, L. and Shusi, L. (2016). Biodegradation of Phenol by Bacteria Strain *Acinetobacter calcoaceticus* PA Isolated from Phenolic Wastewater. International Journal of Environmental Research and Public Health,13(300), 1-8.