British Microbiology Research Journal
9(2): 1-6, 2015, Article no.BMRJ.6196
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Bioremediation of Crude Oil Contaminated Soils Using Surfactants and Hydrocarbonoclastic Bacteria

C. A. Etok[1], O. D. Akan[1] and A. A. Adegoke[1*]

[1]Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol, and author ODA wrote the first draft of the manuscript. Authors ODA and AAA managed the analyses of the study. Author ODA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/6196
Editor(s):
(1) Joao Lucio Azevedo, University of São Paulo, Department of Genetics, Brazil.
Reviewers:
(1) Anonymous, Bu-Ali Sina University, Iran.
(2) Anonymous, Poland.
(3) Anonymous, Italy.
(4) Ameh Alewo O, Ahmadu Bello University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1217&id=8&aid=9745

Original Research Article

Received 30th July 2013
Accepted 9th October 2013
Published 12th June 2015

ABSTRACT

A study of the rate of crude oil remediation in soils with the application of surfactants and hydrocarbonoclastic bacterial population was undertaken. Locally sourced particulate surfactants (wood and palm bunch ashes) were compared with synthetic surfactant, Tween 80, and found to be more microbial friendly and efficient in contaminant removal from the soil. The microbial count recorded in biostimulated set up ranged from 2.08 $x10^4$ to 1.43 $x10^7$ cfu/g for wood ash; 2.08 $x10^4$ to 2.22 $x10^7$ cfu/g for palm fruit bunch ash while Tween 80 had a range of 2.08 $x10^4$ to 3.38 $x10^7$ cfu/g. The bioaugmented set up had microbial counts of 8.00 $x10^3$ to 2.50 $x10^8$ cfu/g with wood ash treatment; 9.90 $x10^3$ to 2.50 $x10^8$ cfu/g for palm fruit bunch ash treatment while Tween 80 recorded 1.00 $x10^3$ to 2.50 $x10^8$. The highest reduction of 94.54% was observed in bioaugmented soil treated with palm fruit bunch ash. While the control sample with indigenous population and no surfactant treatment had 36.32% reduction. Biosurfactant aided the utilization of the crude oil by hydrocarbonoclastic bacteria in the soil. Therefore, stakeholders in the oil and gas/petroleum energy sector should encourage the development of cheaper, safe and readily sourced remedial agents.

**Corresponding author: Email: anthonyadegoke@yahoo.co.uk; aadegoke@ufh.ac.za;*

Keywords: Bioremediation; biosurfactant; hydrocarbonoclastic bacteria; wood ash.

1. INTRODUCTION

Soil, or any other environmental component, contaminated with pollutants can cause extensive damage to local systems since the accumulation of pollutants in animals and plant tissues may cause mutations or even death [1]. April, [2] suggested that bioremediation is cost effective amongst other methods for remediating soil contamination while working with filamentous fungi. Being an evolving remedial method, it involves the use of biological agents, like microorganisms, to detoxify or remove pollutants from the environment including the products of petroleum industry [3,4]. Although, microorganisms are found everywhere in the environment, oil degrading organisms are most abundant in areas where there have been petroleum seeps or spillages metabolizing petroleum hydrocarbons as food and energy source [5]. The rate at which microbial cells can convert crude oil contaminants during bioremediation depends on the rate of its uptake and metabolism and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor [6]. These bioavailability problems can be overcome by the use of surfactants [7], which increase the availability of contaminants for microbial degradation. Biosurfactants act by partitioning preferentially at interphases and exhibiting high surface and emulsifying activities [8]. Varied works on locally sourced biosurfactants have been reported with high effectiveness some examples are Essien [9] using wood ash and sawdust, Agbor [10] experimenting with plantain peels and cocoa pod husk etc. The aim of this study was to check the remedy of crude oil soil polluted with microbial and surfactant enhancements.

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing

The soil samples were collected following the protocol of Okop, [11] with modifications. Five to ten (5-10) centimeters deep topsoil was collected with shovel into clean bucket from a site at the Pharmacy farm and transported to the Microbiology Postgraduate laboratory, University of Uyo Town campus. Bonny light crude oil was sourced from Elf Petroleum, Nigeria. The wood ash was obtained from charcoal residue. The wood lump was chopped and ashed in the oven at 100°C. The char residue was grounded, sieved and stored in airtight containers at room temperature. The commercial surfactant Tween® 80 (Ariaria market in Aba, Abia State, Nigeria) was also considered.

The soil was divided into six portions and all received 5% crude oil contamination. The six portions (each weighing 1000 g) were then separated into two, out which half was used for biostimulation with surfactants only while the other half was used for bioaugmentation with surfactants inclusive. The surfactants were then added at 2.5% concentration. Soils with and without crude oil contamination served as controls set ups. They were contained in perforated wood boxes and kept outside the laboratory.

Throughout the monitoring period there was constant tilling and moistening of the soil samples for aeration, optimum microbial growth and even distribution of contaminant for increased microbial-contaminant contact using a scapular and sterile water. For each soil sample 30ml of sterile water was used for moistening every three days.

2.1.1 Physicochemical properties of samples

The physicochemical properties of the soil was measured using standard methods to check if the soil was fit for microbial growth and capable of supporting bioremediation in the soil. The Walkely and Black methods for organic carbon as reported by Osuji et al. [12]; the Bowman [13] method for available phosphorus; the Kjeldhal method for soil total nitrogen as reported by [14]. Also the surfactants were also analysed for their chemical component, starting with their emulsification index using the Batista [15] method, nitrogen, organic matter, pH and phosphorus.

2.1.2 Preparation of isolates used for bioaugmentation

Organisms were isolated from previously contaminated soil at 5% contamination (this was to simulate a minor spill and ensure microbial survival and adaptation) using Mineral salt agar (MSA) and re-incubated into fresh nutrient agar

plates. Their morphology and pigmentation were done visually, while cell shape and Gram stain were determined. Other biochemical tests were carried out as described in the Bergey's manual of determinative bacteriology. These cultured organisms were later introduced to a set of sterilized soil. The isolates identified were of the *Pseudomonas, Corynebacterium, Bacillus* and *Alicaligene* genera.

2.1.3 Microbial counts

Soil sample (1 gram) was gotten every two weeks to check for Total Heterotrophic Count in the soils. The soil samples were diluted in sterile water using ten-fold dilution and pour plated with nutrient agar in petri dishes. The set up was incubated for 24 hours, the bacterial density was reported as mean of triplicate determination and recorded as cfu/g of soil.

2.1.4 Total residual hydrocarbon content

The remediation is usually calculated by subtracting the residual amount from the initial amount. One gramme of the contaminated soil sample was mixed in 10 ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using Whatman No 1 filter paper and the filtrate diluted by taking 1ml of the extract into 50 ml hexane. The absorbance of the solution was read at 460 nm with Mamotte 701 Spectrophotometer using *n*-hexane as blank [16].

3. RESULTS AND DISCUSSION

The experimental soil was slightly acidic at pH of 5.3, while the surfactants had pH of 10.8, 7.8 and 4.9 for palm fruit bunch ash, wood ash and Tween 80 respectively. Kamalu and Isirimah [17] suggested that soils samples in the Niger Delta region are usually slightly acidic. Udoetok [18] and Udosen [19] reported similar results for the palm fruit bunch ash and wood ash respectively. The biosurfactants' addition to the soil aided to increase the pH of the soil toward alkalinity, thereby, enabling optimal microbial growth and crude oil degradation [19]. The acidity of Tween 80 reflected in the extension of the microbial adaptation period with initial lowered counts in the soil mixtures [20]. From Table 1, Tween 80 surfactant also had the lowest chemical content while palm fruit bunch ash had good nutrients levels followed by wood ash. The high chemical content of the biosurfactants (wood and palm fruit bunch ashes) showed they could be used to fortify less fertile soils [18,19] and contained nutrients for the growth of crude oil degrading organisms [4].

Presented in Table 2 is the microbial counts for the control, biostimulated and bioaugmented soil samples for the 90 days. There was observable difference in the microbial counts from the two processes of biostimulation and bioaugmention. While microbial numbers in bioaugmented soils reduced drastically from their initial counts [21], the ones in the biostimulated portions increased steadily [22,23]. The bioaugmented isolates reduced in numbers due to adaptation to the new environment and contaminant stress, while the biostimulated indigenous organisms only took time to produce the enzymes needed for degradation of crude oil. The counts from the soil portions over the 90 day period followed the trend observed by Bahrampour and Moghanlo [24], Essien and Udosen [25]; Atlas [22] with low initial counts before acclimatization, then increased microbial number during heightened degradation. These numbers rescinded with lowering contaminant levels, increased waste metabolites and probable toxic degradation by-products [26,27].

The residual hydrocarbon content of each contaminated soil sample revealed that biodegradation took place to different extents. Table 3 revealed that the bioaugmented samples had higher crude oil content reductions compared to the reductions observed in biostimulated soils. The highest reduction was observed in bioaugmented soil treated with palm bunch ash (94.54%), while the lowest reduction was seen in contaminated soil without surfactant treatment. The reduction results obtained in treated soils with 'trained' consortia population were higher than those obtained from soils with indigenous population. This result is in contrast to the observations of Demque [28] who used acclimatized indigenous bacterial populations to treat diesel contaminated soils.

Table 1. Physicochemical properties of soil and surfactant samples

Parameters	Wood ash	Palm fruit bunch ash	Tween 80	Soil sample
pH	7.8	10.9	4.9	5.3
Emulsification index	25.00	20.40	83.33	ND
Total carbon	82.40	80.00	32.72	1.17
Total nitrogen	0.002	97.80	0.22	0.105
Phosphorus	8.12	47.50	6.15	22.10

Table 2. Microbial counts (cfu/g) in remedied soil samples over 90 days

Biostimulation			Bioaugmentation			Control samples	
Wood ash	Palm ash	Tween 80	Wood ash	Palm ash	Tween 80	Contaminated soil	Soil alone
$2.08 \times 10^4 \pm 0.02$	$2.08 \times 10^4 \pm 0.02$	$2.08 \times 10^4 \pm 0.02$	$2.50 \times 10^8 \pm 0.10$	$2.50 \times 10^8 \pm 0.10$	$2.50 \times 10^8 \pm 0.10$	$2.08 \times 10^4 \pm 0.02$	$2.08 \times 10^4 \pm 0.02$
$7.27 \times 10^4 \pm 0.77$	$2.47 \times 10^5 \pm 0.36$	$1.20 \times 10^5 \pm 0.05$	$1.01 \times 10^5 \pm 0.02$	$2.15 \times 10^4 \pm 0.05$	$3.85 \times 10^4 \pm 0.22$	$1.88 \times 10^5 \pm 0.25$	$2.08 \times 10^5 \pm 0.02$
$1.89 \times 10^6 \pm 0.11$	$1.99 \times 10^6 \pm 0.12$	$1.44 \times 10^6 \pm 0.12$	$1.70 \times 10^5 \pm 0.10$	$1.15 \times 10^5 \pm 0.09$	$8.50 \times 10^4 \pm 0.60$	$2.16 \times 10^6 \pm 0.12$	$2.30 \times 10^6 \pm 0.15$
$1.43 \times 10^7 \pm 0.25$	$2.22 \times 10^7 \pm 0.02$	$3.38 \times 10^7 \pm 0.12$	$5.00 \times 10^4 \pm 0.13$	$1.25 \times 10^5 \pm 0.05$	$5.50 \times 10^4 \pm 0.25$	$2.66 \times 10^7 \pm 0.16$	$2.84 \times 10^7 \pm 0.14$
$2.13 \times 10^7 \pm 0.06$	$1.83 \times 10^7 \pm 0.22$	$2.00 \times 10^7 \pm 0.10$	$1.95 \times 10^4 \pm 0.10$	$1.35 \times 10^5 \pm 0.15$	$2.45 \times 10^4 \pm 0.25$	$2.20 \times 10^7 \pm 0.17$	$2.20 \times 10^7 \pm 0.07$
$2.77 \times 10^6 \pm 0.07$	$3.00 \times 10^6 \pm 0.36$	$2.90 \times 10^6 \pm 0.12$	$1.21 \times 10^4 \pm 0.03$	$1.33 \times 10^4 \pm 0.18$	$1.45 \times 10^4 \pm 0.09$	$6.00 \times 10^6 \pm 0.90$	$3.30 \times 10^6 \pm 0.08$
$2.17 \times 10^5 \pm 0.07$	$5.00 \times 10^5 \pm 0.45$	$1.40 \times 10^5 \pm 0.10$	$8.00 \times 10^3 \pm 0.30$	$9.90 \times 10^3 \pm 0.11$	$1.00 \times 10^4 \pm 0.00$	$4.00 \times 10^5 \pm 0.40$	$2.10 \times 10^5 \pm 0.09$

Table 3. Total residual hydrocarbon content (mg/kg)

Surfactants	Biostimulation			Bioaugmentation			Control
	Wood	Palm	Tween	Wood	Palm	Tween	
Percentage reduction	87.10	73.10	89.76	90.91	94.54	90.28	36.32

4. CONCLUSION

Microbial growth and hydrocarbon reduction results from this study showed a lot can be achieved with the application of cheap and readily sourced agricultural/industrial by-products like the wood and palm fruit bunch ashes in bioremediation of crude oil contaminated soils. With the 2015 projection of 15.63 x10^6 m^3 day^{-1} world petroleum consumption by the United States Energy Information Administration [29] high rate of oil spills are inevitable. Speedy and efficient removal of these contaminants from polluted environment would curtail the negative impact spilled crude oil or its products could bring to fore, on human, plant and environmental health. Taking into account the environmental friendliness of these locally sourced surfactants, on the one hand and the re-use of substances considered 'waste' to ensure a cleaner environment, locally sourced biosurfactants have high prospects in the oil and gas sector. The addition of surfactant and use of 'trained' bacterial cells showed high effectiveness in contaminated soil remediation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alvarez PJJ, Vogel TM. Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Applied and Environmental Microbiology. 1991;57(10):2981–2985.
2. April TM, Foght JM, Currah RS. Hydrocarbon degrading filamentous fungi isolated from flare pit soils in Northern and Western Canada. Canadian Journal of Microbiology. 2000;46(1):38–49.
3. Okoh AI. Biodegradation alternative in the clean-up of petroleum hydrocarbon pollutants. Biotechnology and Molecular Biology Review. 2006;1(2):38-50.
4. Vidali M. Bioremediation. An Overview. Pure Applied Chemistry. 2001;73(7):1163-1172.
5. Seymour RJ, Geyer RA. Fates and effects of oil spills. Annual Reviews of Energy and Environment. 1992;17:261-283.
6. Boopathy R, Manning J. A laboratory study of the bioremediation of 2,4,6-trinitrotoluene-contaminated soil using aerobic anaerobic soil slurry reactor. Water Environmental Research, 1998;70:80-86.
7. Boopathy R, Manning J. Surfactant-enhanced bioremediation of soil contaminated with 2,4,6-trinitrotoluene in soil slurry reactors. Water Environmental Research. 1999;71:119-124.
8. Martienssen M, Reichel O, Schirmer M. Use of surfactants to improve the biological degradability of petroleum hydrocarbons. Chemie Ingenieur Technik. 2003;75(11): 1749-1755.
9. Essien JP, Ubom RM, Udosen A. Bioremedation of petroleum contaminated soil: Effect on the population dynamics and capacities of hydrocarbonclastic bacteria. Journal Biology and Applied Chemistry. 1995;43:23-27.
10. Agbor RB, Ekpo IA, Udofia UU, Okpako EC, Ekanem EB. Potentials of cocoa pod husk and plantain peels in the degradation of total petroluem hydrocarbon content of crude oil polluted soil. Archives of Applied Science Research. 2012;4(3):1372-1375.
11. Okop JI, Okorie FS, Obadimu CO. Quantitative evaluation of spatial distribution and penetration of liquid hydrocarbons in petroleum spilled soil. Global Advanced Research Journal of Environmental Science and Toxicology. 2012;1(6):152-161.
12. Osuji LC, Nwoye I. An appraisal of the impact of petroleum hydrocrbons on soil fertility: the Owaza experience. African Journal of Agricultural Resources. 2007; 2(B):318-324.
13. Bowman RAA. Rapid method to determine total phosphorus in soils. Soil Science Society of American Journal. 1988;52: 1301-1304.
14. Bremner JM. Determination of nitrogen in soil by the Kjeldahl method. Journal of Agricultural science. 1960;55(1):11-33.
15. Batista SB, Mounteer AH, Amorim FR, Totola MR. Isolation and characterization of Biosurfactant/Bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresource Technology. 2006;97: 868–875.
16. Valcarcel M. Principles of analytical chemistry, New York: Springer-Verlag Berlin Heidelberg; 2000.
17. Kamalu OJ, Isirimah NO. In: Essien JP, Udosen ED. (2000). Distribution of actinomycetes in oil contaminated ultisols of the Niger Delta (Nigeria). Journal of

Environmental Sciences. 1992;12(3):296–302.
18. Udoetok IA. Characterization of ash made from oil palm empty fruit bunches (oefb). International Journal of Environmental Sciences. 2012;3(1):518-524.
19. Udosen ED, Essien JP, Ubom RM. Bioamendment of petroleum contaminated utisol: effect on oil content, heavy metals and pH of tropical soil. Journal of Environmental Sciences. 2001;13(1):92-98.
20. Nkereuwen ME, Edem ID, Fagbola O. Bioremediation of oil- polluted soils with organomineral fertilizer (OMF) and Mexican sunflower (*Tithonia diversifolia*). Nigerian Journal Agriculture, Food and Environment. 2010;6(1-2):13-20.
21. Pritchard PH. Use of inoculation in bioremediation. Current Opinion in Biotechnology. 1992;3:232-243.
22. Atlas RM. Microbial hydrocarbon degradation, bioremediation of oil spills. Journal of Chemical Technology and Biotechnology. 1991;52:149-156.
23. Leahy JG, Colwell RR. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews. 1990;54(3):305–315.
24. Essien JP, Ubom RM, Udosen A. Bioremediation of petroleum contaminated soil: Effect on the population dynamics and capacities of hydrocarbondastic bacteria. Journal Biology and Applied Chemistry. 1995;43:23-27.
25. Bahrampour T, Moghanlo VS. Evaluation of soil biological activity after soil contaminating by crude oil. International Journal of Agriculture: Research and Review. 2012;2(6):671-679.
26. Narula N, Vasudeva M. Terrestrial environment. Environmental Microbiology. Haryana Agricultural University. 2006;1-28.
27. van Veen JA, van Overbeek LS, van Elsas JD. Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews. 1997;61:121-135.
28. Demque D, Biggar K, Heroux J. Land treatment of diesel contaminated sand. Canadian Geotechnical Journal. 1997;34: 421-431.
29. EIA, Energy kid's page. Coal: A Fossil Fuel. Energy Information Administration, USA; 2006.
Available:http://www.eia.doe.gov/kids/energy.cfm?page=coal_home-basics

© 2015 Akan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1217&id=8&aid=9745