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OSEI Corporation Summary Nigeria University of Port Harcourt Bioremediation of crude oil impacted soil utilizing Enzyme Amendment Oil Spill Eater II

The University of Nigeria, in conjunction with Shell Oil Company, performed a test on soil which had been contaminated with Shell company oil. The University experiment consisted of fourteen treatment reactor vessels subdivided into Groups EA (OSE II), UA, SD, EUS (OSE II), SLG- GS and SLG and set up in triplicate concentrations. Individual reactor vessels contained 20kg of crude oil impacted soil and 7kg of agricultural soil. Groups EA, UA, SD and EUS were treated with an anionic surfactant sodium dodecyl sulfate (SDS), enzyme additive, oleophilic nutrient (uric acid) and a combination of SDS, enzyme additive, uric acid respectively. Control group SLG did not receive any treatment while Control group SLG-GS received agricultural soil only.

The tests were performed in triplicate, where they mixed OSE II with two different amendments of different nutrient and surfactant amendments. It is unfortunate the experimental design did not include EA (OSE II) alone, since it has been our experience that when you amend OSE II with other ingredients, compounds, mixtures, microbes, it generally upsets the balance of OSE II and the reduction rates for total TPH are slower than if OSE II was used alone.

Some groups have attempted to introduce foreign bacteria or microbes into OSE II. This practice hinders the reduction process because non-native bacteria, when introduced into a foreign ecosystem, face competition from the native bacteria. In general, the native bacteria tend to outcompete the non-native ones, causing a temporary slowdown in the process until the native bacteria become established.

Conclusion

In the present study, the combination of Enzyme additive (OSE II), Oleophilic nutrient and surfactant recorded the best performance in reducing TPH in the impacted soil .

OSE II, despite the amendments showed a huge reduction over the other nutrients and the control, showing that OSE II produces very effective remediation rates whenever utilized through the OSEI Corporation instructions/protocols. This is yet another study verifying the efficacy of OSE II!

Steven Pedigo

CEO OSEI Corporation



RESEARCH PAPER

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Bioremediation of crude oil impacted soil utilizing surfactant, nutrient and enzyme amendments

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Abstract

The present study was designed to investigate possible methods to enhance the rate of biodegradation of crude oil highly impacted soil excavated from a site at K-Dere in Gokana Local Government Area of Rivers State, Nigeria. The experiment consisted of fourteen treatment reactor vessels subdivided into Groups EA, UA, SD, EUS, SLG-GS and SLG and set up in triplicate concentrations. Individual reactor vessels contained 20kg of crude oil impacted soil and 7kg of agricultural soil. Groups EA, UA, SD and EUS were treated with an anionic surfactant sodium dodecyl sulfate (SDS), enzyme additive, oleophilic nutrient (uric acid) and a combination of SDS, enzyme additive, uric acid respectively. Control group SLG did not receive any treatment while Control group SLG-GS received agricultural soil only. TPH reduction in the impacted soil varied between 4.0 and 45.0% within five weeks as well as between 11.0 and 64.0% after nine weeks of applying the treatments respectively. The group that received a combination of SDS, enzyme additive and uric acid showed the highest reduction in TPH by the end of the ninth week. Total Organic Carbon (TOC) reduction ranged from 4.0 and 30.0% between day 0 and week 5 as well as from 0.0 and 50.0% between weeks 5 and 9 respectively in the test groups. The pH of the degrading impacted soil fluctuated between 6.12 and 9.49 during the same period. When compared with the 0.0% TPH reduction in the Control group SLG, the added treatments evidently increased the rate of bioremediation of the impacted soil.

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Introduction

Petroleum industry effluents, oily sludge and oil spills cause a serious threat to the environment as their constituents are toxic, mutagenic and carcinogenic (Mandal et al., 2012). Traditionally, petroleum hydrocarbon contaminated soils have been dealt with by excavation and disposal to landfill. However, as landfills have become scarcer and more cost prohibitive, this method has become less feasible. Various physicochemical treatment techniques have been developed to clean up contaminated soil such as incineration, thermal desorption, chemical oxidation, immobilization and solvent extraction (Liu et al., 2010). In general, such treatments are more expensive, energy intensive and not sustainable with respect to their environmental impacts which include damage to soil structure and toxicity issues associated with chemical additives (Alamri, 2009). Many of these techniques simply dilute or sequester the contaminants or transfer them from one environmental compartment to another and therefore do not eliminate the problem (Semple, 2001). These limitations have been the basis of search for more economical and environmentally sound approaches to remediate contaminated soils.

Microorganisms, namely heterotrophic bacteria and fungi have evolved a tremendous ability to metabolize simple and complex hydrocarbon contaminants (King et al., 1998). By harnessing their metabolic ability, it is possible to remediate contaminated environments, a technique referred to as bioremediation (Riser-Roberts, 1998). This represents a viable alternative to physicochemical remediation technologies as it enhances a natural process, resulting in the complete or partial biotransformation of organic contaminants into cell biomass and stable innocuous end products such as carbon dioxide and water (Semple, 2001; Liu et al., 2010). Bioremediation, through natural attenuation (intrinsic bioremediation) and enhanced bioremediation, promises possible approaches for destruction of contaminants in soils. Using natural processes involving microbial growth and enzymatic production, bioremediation can convert target

contaminants ultimately to non-toxic end products (Wilson and Jones, 1993; Abramowicz, 1995; Liu and Jones, 1995).

Petroleum hydrocarbons are biodegradable in geological time, although some in the human context can be particularly recalcitrant. The slow rate of natural bioremediation is generally caused by a number of rate-limiting factors including those imposed by the contaminant (biodegradability, bioavailability and concentration) (Mann et al., 1995) and soil environmental factors (nutrients, oxygen, moisture, pH and temperature) which affect the growth and activity of microorganisms. However, high molecular weight contaminants such as polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs), persist in petroleum contaminated soils, biodegrading only slowly while strongly partitioning to the soil and bio-accumulating up the food chain, ultimately reaching humans (Santos et al., 2011).

There are two main techniques to enhance soil bioremediation efficiency. Biostimulation refers to the adjustment of soil inorganic nutrients and/or organic nutrient substrates to stimulate the activity of contaminant degrading indigenous microorganisms (Scullion, 2006). A useful approach to overcome the problem of water-soluble nutrients being rapidly washed out is to utilize oleophilic organic nutrients (Ladousse and Tramier, 1991). The rationale for this strategy is that oil biodegradation occurs mainly at the oil-water interface; since oleophilic fertilizers are able to adhere to oil and provide nutrients at the oil-water interface; enhanced biodegradation should result without the need to increase nutrient concentrations in the bulk pore water. Another developing bioremediation technique is the use of surface active agents (surfactants) to increase the bioavailability and therefore biodegradation of recalcitrant compounds (Mohan et al., 2006).

Collectively, microorganisms have a great metabolic diversity which allows their ubiquity. Because of their ubiquitous nature, the biotechnological potential of

microorganisms is virtually endless with many possible applications. One of these applications is the utilization of enzymes generated by microorganisms in petroleum bioremediation approaches (Madigan et al., 2010). According to US EPA (2012), *Bioremediation agents* include enzyme additives that are deliberately introduced into an oil discharge and that will significantly increase the rate of biodegradation to mitigate the effects of the discharge.

The present study is therefore, designed to evaluate the use of anionic surfactant sodium dodecyl sulfate (SDS), lipophilic nutrient (uric acid) and enzyme additive to enhance the rate of biodegradation of crude oil impacted soil with extremely high pollution level and recalcitrance of contaminants for biodegradation. Data gathered in this study would assist in the development of a new technology that might be helpful to solve remediation problems in a cost effective manner.

Materials and methods

Crude oil impacted soil

Crude oil impacted soil excavated from a contaminated site at K-Dere in Gokana Local Government Area of Rivers State, Nigeria was used for this study. The waste was sealed in polyethylene materials which preserved its integrity. The contaminated soil samples were observed to be black and laden with highly weathered crude. The waste was thoroughly mixed to ensure uniform distribution of crude oil and other contaminants.

Agricultural soil sample

Agricultural soil (sand – 70%, clay – 9%, silt – 21%, Total Organic Carbon – 3.77g/kg, pH 7.9, and Total Hydrocarbon Content – 122mg/kg) used for bulking was collected from a site with no history of crude oil impact located at Eleme, Rivers State, Nigeria. The soil sample was sieved using a 1.7mm sieve.

Experimental design

The pilot scale experiment was performed under a roof cover. The experiment was carried out using

constructed wooden boxes which served as Reactor Vessels (RV). Individual compartments were lined with High Density Polyethylene (HDPE) liners. The degradation experiments was performed using a set of fourteen (14) Reactor Vessels grouped as RV1, RV2, RV3, RV4, RV5 and RV6 representing four different operating conditions. Two Reactor Vessels served as controls. Leachate generated was captured and recycled into the Reactor Vessels.

Twenty kilograms (20kg) of crude oil impacted soil was weighed into each of the 14 reactor vessels. The experimental groups are detailed in Table I. The Experimental Groups EA, UA, SD, EUS, SLG-GS and SLG were set up in triplicate concentrations. Table II shows a detailed definition of the various subgroups. 7kg of sieved agricultural soil was added to each of the reactors in turn. This mix ratio (i.e. impacted soil: agricultural soil = 3:1) was uniform for all reactor vessels. The mixture was thoroughly mixed and allowed to settle for seven days so that microbial activity could ensue before the application of the various enhancements respectively. The experiments lasted for 63 days. Mixing and watering were repeated every three days.

Determination of Extractable Total Petroleum Hydrocarbon (ETPH)

Extractable Total Petroleum Hydrocarbon was determined using the USEPA 8015 method described by US EPA (1996).

Determination of Total Organic Carbon (TOC)

Total Organic Carbon was determined using the wet oxidation method described by Williams (1969) and Oceanography International Corp. (1970).

Determination of pH

pH of experimental samples was determined by the ASTM D4972 method described by ASTM (1995).

Statistical analysis

Results of all the studies are expressed as mean± standard deviation. Statistical analysis was carried out using analysis of variance (ANOVA). Data

between groups were analyzed using SPSS®: Version 16.0. $P < 0.05$ versus respective initial value was taken as significant.

Results

The various groups involved in the study are defined as in Table I.

Table I. Groups in the study

Reactor Vessels	Group Name	Enhancement received
RV1	EA	Enzyme Additive + agricultural soil
RV2	UA	Uric acid + agricultural soil
RV3	SD	Sodium Dodecyl Surfate + agricultural soil
RV4	EUS	Mixture of Enzyme additive, Uric acid & Sodium Dodecyl Surfate (SDS) + agricultural soil
RV5	SLG-GS	Agricultural Soil only
RV 6	SLG	None

Table II. Subgroups in the study

GROUP NAME	SUBGROUPS		
	1	2	3
EA	20kg impacted soil + 7kg Garden soil + 0.5L Enzyme additive in 1L river water	20kg impacted soil + 7kg Garden soil + 0.8L Enzyme additive in 1L river water	20kg impacted soil + 7kg Garden soil + 1L Enzyme additive in 1L river water
UA	20kg impacted soil + 7kg Garden soil + 40g Uric Acid in 200ml water (10%w/v)	20kg impacted soil + 7kg Garden soil + 60g Uric Acid in 200ml water (15%w/v)	20kg impacted soil + 7kg Garden soil + 80g Uric Acid in 200ml water (20%w/v)
SD	20kg impacted soil + 7kg Garden soil + 200g SDS in 5L water (4%w/v)	20kg impacted soil + 7kg Garden soil + 400g SDS in 5L water (8%w/v)	20kg impacted soil + 7kg Garden soil + 600g SDS in 5L water (12%w/v)
EUS	20kg impacted soil + 7kg Garden soil + 0.5L Enzyme Additive in 1L river water + [40g Uric acid + 200g SDS] in 5L water	20kg impacted soil + 7kg Garden soil + 0.8L Enzyme Additive in 1L river water + [60g Uric acid + 400g SDS] in 5L water	20kg impacted soil + 7kg Garden soil + 1L Enzyme Additive in 1L river water + [80g Uric acid + 200g SDS] in 5L water
SED	20kg impacted soil only	-	-
SED-GS	20kg impacted soil + 7kg Garden soil	-	-

The results of the effects of the various amendments on Total Petroleum Hydrocarbon are shown in Table III. There were significant ($p < 0.05$) decreases in Total Petroleum Hydrocarbon in the groups treated with SDS, Enzyme Additive and Uric acid as well as a combination of SDS, Enzyme Additive and Uric acid at weeks 5 and 9. The Control group that received no form of amendment (SLG) and the group that was

bulked with agricultural soil only (SLG-GS) showed non-significant changes in Total Petroleum Hydrocarbon at the 5th and 9th weeks respectively. Figure 1 shows Percent reduction in Total Petroleum Hydrocarbon in Experimental and control groups. The results showing Total Organic Carbon in Experimental and Control groups are presented in Table IV. Significant ($p < 0.05$) changes in Total

Organic Carbon were noticed in all the treatment groups. The control groups showed non-significant ($p > 0.05$) decreases in TOC. Percent reduction in

Total Organic Carbon in Experimental and Control groups is presented in Figure 2. Table V shows the pH levels in the treatment and control groups.

Table III. Total Petroleum Hydrocarbon in Experimental and Control groups

		ETPH (mg/kg)			
GROUPS		Day 0	Week 1	Week 5	Week 9
SD	SD 1		143848 ± 101	137632 ± 86*	93719 ± 106*
	SD 2		149766 ± 149	101704 ± 222*	82782 ± 138*
	SD 3		105370 ± 151	88037 ± 129*	78016 ± 151*
UA	UA 1		86091 ± 288	68409 ± 156*	60727 ± 117*
	UA 2		128128 ± 303	118340 ± 202*	84319 ± 184*
	UA 3		123186 ± 140	94416 ± 303*	67339 ± 109*
EA	EA 1		143276 ± 109	122457 ± 127*	79372 ± 109*
	EA 2		139767 ± 247	124049 ± 183*	97478 ± 130*
	EA 3		208695 ± 132	115421 ± 183*	103873 ± 131*
EUS	EUS 1		183872 ± 202	175387 ± 321*	62495 ± 95*
	EUS 2		131206 ± 232	99653 ± 169*	42890 ± 59*
	EUS 3		161390 ± 102	116265 ± 200*	34954 ± 52*
CONTROLS	SLG	133078 ± 101	133013 ± 92	132936 ± 106	132865 ± 109
	SLG-GS	113116 ± 73	112848 ± 113	112098 ± 190	111979 ± 98

Values are Mean ± SD. Values with asterisks are significantly different from Day 0/Week 1 values for control & experimental groups respectively

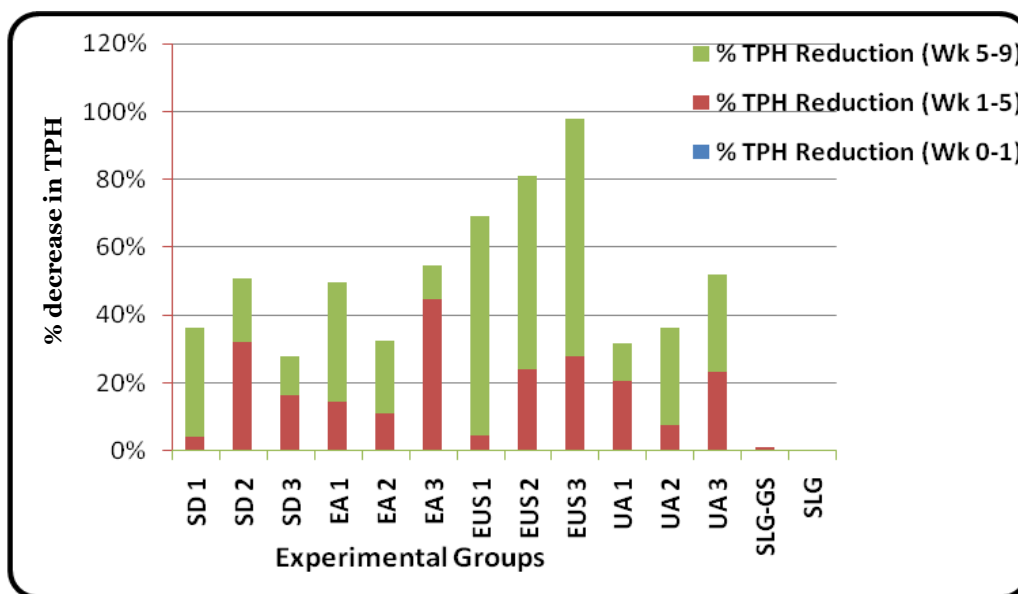


Figure 1. Percent reduction in Total Petroleum Hydrocarbon in Experimental and Control Groups

Table IV. Total Organic Carbon in Experimental and Control groups

		TOC (g/kg)			
GROUPS		Day 0	Week 1	Week 5	Week 9
SD	SD 1		186 ± 11	178 ± 9	163 ± 7
	SD 2		210 ± 9	147 ± 7*	127 ± 16*
	SD 3		225 ± 13	188 ± 11	174 ± 20*
UA	UA 1		233 ± 7	195 ± 19	188 ± 18*
	UA 2		267 ± 9	242 ± 12	159 ± 16*
	UA 3		203 ± 12	180 ± 7	149 ± 9*
EA	EA 1		286 ± 28.5	235 ± 7	225 ± 19*
	EA 2		328 ± 7	229 ± 24*	229 ± 14*
	EA 3		280 ± 10	277 ± 20	208 ± 21*
EUS	EUS 1		266 ± 11	200 ± 7*	185 ± 19*
	EUS 2		211 ± 14	185 ± 8	145 ± 11*
	EUS 3		219 ± 21	206 ± 12	102 ± 13*
CONTROLS	SLG	203 ± 12	198 ± 14	195 ± 12	191 ± 17
	SLG-GS	256 ± 8	251 ± 21	232 ± 17	229 ± 16

Values are Mean ± SD. Values with asterisks are significantly different from Day 0/Week 1 values for control & experimental groups respectively

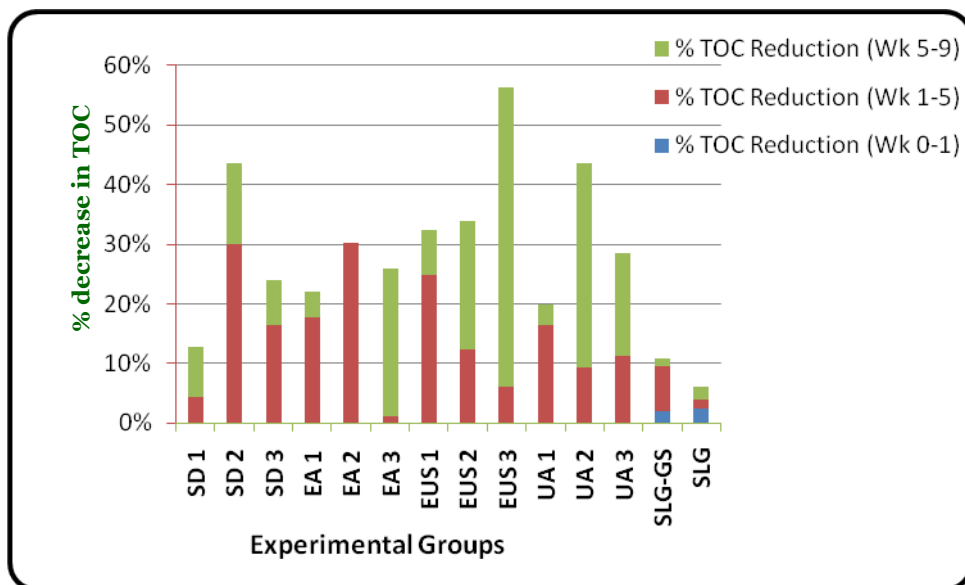


Figure 2. Percent reduction in Total Organic Carbon in Experimental and Control groups

Table V. pH in Experimental and Control groups

		pH			
GROUPS		Day 0	Week 1	Week 5	Week 9
SD	SD 1		6.14	6.80	8.13
	SD 2		8.02	6.31	6.59
	SD 3		7.99	8.66	7.39
UA	UA 1		6.12	9.66	9.12
	UA 2		8.35	7.34	8.04
	UA 3		7.24	7.65	8.05
EA	EA 1		6.92	6.45	7.03
	EA 2		6.55	6.62	6.75
	EA 3		6.17	6.25	6.48
EUS	EUS 1		6.09	7.43	6.83
	EUS 2		9.49	8.1	6.35
	EUS 3		6.48	6.72	6.57
CONTROLS	SLG	6.50	7.46	7.8	7.15
	SLG-GS	7.10	7.33	8.79	7.33

Discussion

TPH reductions in the impacted soil were 4.0 to 45.0% and 11.0 to 64.0% within five and nine weeks of application of various treatments respectively. Overall, a considerable decrease in TPH concentration occurred in most treated groups compared to the unamended control after nine weeks of bioremediation. TPH reduction was significantly ($p < 0.05$) enhanced and was best in the presence of a combination of Enzyme additive, oleophilic nutrient and a chemical surfactant (28% reduction at week 5 and 70% reduction at week 9 in the group that received the highest ratios of these amendments – EUS-3). At week 9, experimental group SD-3 treated with 12%w/v SDS recorded the least reduction in TPH (16% at week 5 and 11% at week 9). Possible reasons for this could be bacteria-surfactants interactions and toxicity of the surfactant, at that concentration, against hydrocarbon degrading bacteria (Liu et al., 2001). The Control group SLG which was mixed and watered regularly as with other experimental groups, could not significantly ($p > 0.05$) reduce TPH (0%) all through the experimental period. Similarly, the

second control group SLG-GS reduced TPH by a limited amount (i.e. 1% at week 5 but 0% at week 9) within the same period of bio-augmentation despite being bulked with agricultural soil, watered and aerated by mixing. This result seems similar to an earlier report by Ayotamuno et al. (2007) on bio-remediation of sludge containing hydrocarbons, where the indigenous micro-organisms in a reactor system were able to reduce the Total Hydrocarbon (THC) by a limited amount (i.e. 6 to 12.8%) within the six-week period of bio-augmentation. This indicates that in achieving significant reduction in TPH, the various treatments in this study had significant advantages over just having the indigenous microbes alone or increasing their number by bulking with agricultural soil.

The changes in Total Organic Carbon (TOC) during the bioremediation process are shown in Fig. 2. The total organic carbon decreased with time in all the amended groups but initially at a slow rate in the 4%w/v SDS treated group till week 5 and then decreased further at a faster rate. This finding seemed

similar to an earlier finding by Dave et al. (2011) that total organic carbon decreased with time, initially at a slow rate till day 24 and then decreased at a faster rate during effective soil washing process for the removal of the hydrocarbons from the contaminated soil. For the EUS group (amended with high ratios of a mixture of Enzyme additive, Uric acid and SDS) which recorded the best TPH reduction, an initial reduction of 6% in Total Organic Carbon from 219 to 206 g/kg was observed after the first five weeks of bioremediation. A further reduction of 30% in Total Organic Carbon from 206 to 102 g/kg was observed between weeks 5 and 9 of bioremediation.

In the present study, there were variations in pH in the various reactor vessels. A number of groups showed slight increases in pH at week five and a further increase at week 9 while some other groups recorded slight decreases at week 9. Overall, pH in the experimental groups varied between 6.45 to 9.66 at week 5 and 6.35 to 9.12 at week 9 respectively. Majority of soil microorganisms thrive best in the pH range of 6 to 8 and adjustment of pH could double the rates of biodegradation (Leahy and Colwell, 1990; Dragun, 1998). This goes to show that the pH of 9.66 and 9.12 observed in the group UA-1 that received oleophilic uric acid might be responsible for the low level of TPH reduction recorded for this group. Interestingly, the group EUS-3 (amended with a combination of Enzyme additive, Uric acid and SDS) which recorded the highest reduction in TPH as well as TOC showed pH in the range that would support the growth of crude oil bio-degraders. It was also observed that control groups SLG and SLG-GS had pH within the range of 6.50 to 8.79 but could not significantly reduce TPH. This corroborates the fact that the rate of hydrocarbon degradation in soils is affected by a myriad of factors and not just pH (Santos et al, 2011).

Conclusion

In the present study, the combination of Enzyme additive, Oleophilic nutrient and surfactant recorded the best performance in reducing TPH in the impacted soil. The complexity of contaminated soils

requiring treatment and the imposition of strict regulatory requirements for the allowable types and levels of contaminants present in soils often precludes the use of only one treatment technique to decontaminate soils. The solution will often require the use of several treatment processes in a "treatment train". Such treatment train could be repeated to ensure that the level of contaminants meet regulatory requirements.

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References

- Abramowicz DA**, 1995. Aerobic and anaerobic PCB degradation in the environment. *Environ. Health Perspective* 103, Supplement 5: 97-99.
- Alamri SA**, 2009. Use of microbiological and chemical methods for assessment of enhanced hydrocarbon bioremediation, *Journal of Biological Sciences* 9: 37-43.
- American Society of Testing and Materials (ASTM)**, 1995. Annual Book of ASTM Standards, Designated D4972 – 95a: Standard Test Method for pH of Soils.
- Ayotamuno MJ, Okparanma RN, Ogaji SOT and Probert SD**, 2007. Bioremediation of a sludge containing hydrocarbons. *J. Appl. Energy* 84(9): 936 – 943.
- Dave D, Alharathi R, Ghaly A**. 2011. Sequential Remediation Processes for Effective Removal of Oil from Contaminated Soils. *American Journal of Environmental Science* 7 (5): 477-491.
- Dragun J**. 1998. *The Soil Chemistry of Hazardous Materials*. 2nd Edition. Amherst Scientific Publishers, Amherst, MA.

- King RB, Long GM, Sheldon JK.** 1998. Practical Environmental Bioremediation: The Field Guide, CRC Press/Lewis Publishers, Boca Raton.
- Ladousse A, Tramier B.** 1991. Results of 12 years of research in spilled oil bioremediation: Inipol EAP 22, Proceedings of 1991 Oil Spill Conference. American Petroleum Institute, Washington, DC, pp577-581.
- Leahy JH, Colwell R.** 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews **54** (3): 305-315.
- Liu SM, Jones WJ.** 1995. Biotransformation of dichloromatic compounds in nonadapted and adapted freshwater sediment slurries. Appl. Microbiol. Biotechnol. **43**: 725- 732.
- Liu Y, Zhu L, Shen X.** 2001. Polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air of Hangzhou, China. Environ. Sci. Technol. **35**: 840-844.
- Liu W, Luo Y, Teng Y, Li Z, Ma, LQ.** 2010. Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes, Environmental Geochemistry and Health **10**: 23-29.
- Madigan MT, Martinko JM, Dunlap PV, Clark DP.** 2010. Brock Biology of Microorganisms, 12th edition Benjamin Cummings.
- Mandal AJ, Sarma, PM, Singh B, Jeyaseelan, CP, Channashettar VA, Lal B, Datta J.** 2012. Bioremediation: An environment friendly sustainable biotechnological solution for remediation of petroleum hydrocarbon contaminated waste. ARPN Journal of Science and Technology VOL. 2, Special Issue, ICESR.
- Mann V, Klein J, Pfeifer F, Sinder C, Nitsche V, Hempel DC.** 1995. Bioreaktorverfahren zur Reinigung feinkörniger, mit PAK kontaminierter Böden. (Bioreactor technique for purifying fine-grained soils contaminated with PAHs) TerraTech **(1/95)**: 69-72.
- Mishra S, Jyot J. al.** 2001. In situ bioremediation potential of an oily sludge degrading bacterial consortium. Current Microbiology, **43**: 328.
- Mohan SV, Kisa T, Ohkuma T, Kanaly RA, Shimizu Y.** 2006. Bioremediation technologies for treatment of PAH-contaminated soil and strategies to enhance process efficiency, Reviews in Environmental Science and Biotechnology **5**: 347-374.
- Mrayyan B, Battikhi MN,** 2005. Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge. Journal of Hazardous Materials **120**: 127–134.
- Oceanography International Corp.** 1970. The Total Carbon System operating manual. College Station, Texas.
- Riser-Roberts E.** 1998. Remediation of Petroleum Contaminated Soils: Biological, Physical and Chemical Processes, CRC Press, Boca Raton.
- Santos HF, Carmo FL, Paes JE, Rosado AS, Peixoto RS.** 2011. Bioremediation of mangroves impacted by petroleum. *Water, Air, and Soil Pollution*. **216**(1-4): 329–350.
- Scullion J.** 2006. Remediating polluted soils, Naturwissenschaften **93**: 51-65.
- Semple KT, Reid BJ, Fermor TR.** 2001. Impact of composting strategies on the treatment of soils contaminated with organic pollutants, Environmental Pollution **112**: 269-283.
- U.S. EPA,** 2012. NCP Product Schedule. <http://www.epa.gov/oilspill> & <http://www.epa.gov/oem/content/ncp/products/oseater.htm>
- United States Environmental Protection Agency (US EPA).** 1994. Assessment and remediation of Contaminant Sediments Program, remediation guidance document. Great Lakes National Program Office. EPA/905/R-94/003.

United States Environmental Protection Agency (US EPA). 1996. Method 8015B. Nonhalogenated organics using GC/FID.: US EPA, Washington.

Wilson SC, Jones KC. 1993. Bioremediation of soil contaminated with aromatic hydrocarbons (PAHs): A review. *Environmental Pollution*. **80**: 229-249.

Williams PM. 1969. The determination of dissolved organic carbon in sea water: A comparison of two methods. *Limnol. Oceanogr.* **14**: 297.