

## **Bioremediation and Biodegradation of Hydrocarbon Contaminated Soils: A Review**

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**Abstract:** *Bioremediation is environment friendly process that utilised a range of communities of microorganisms in combination with series of techniques to decontaminate polluted sites. This has however remained in pilot scale and pot experiment studies and as far as the application of these techniques in field realities are concerned, there is need for compilation of research on the subject. This review found that the application of BS and BA techniques should be done only after a pilot study confirmed the feasibility of using either techniques or both. Otherwise, the remediation may incur unnecessary and avoidable cost due to the use of these techniques while they are not important. Selection of suitable and cost-effective amendments and more efficient hydrocarbon degraders or a consortium of hydrocarbon degraders are very critical to the success of biological remediation of hydrocarbon contaminated sites. Biostimulation using organic substances such the poultry manure, cow dung, biochar and food waste are more effective in optimising the process of bioremediation. Aerobic degradation process is the most viable technique for field application of bioremediation of soils.*

**Key words:** *Bioremediation, Biostimulation (BS), Bioaugmentation (BA) and Decontamination.*

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### **I. Introduction**

Bioremediation is the act of reduction, removal, transformation or degradation of pollutants or contaminants to less harmful substances through biological means. Bioremediation technology has been accepted as a cost-effective and environmental friendly means of clean-up of contaminated lands (Calvo et al., 2009; Lynch and Moffat, 2005; Yang et al., 2009). The use of biological process to degrade contaminating substances was initially developed to treat contamination of petroleum hydrocarbons (Juwarkar et al., 2010). Today, there are more than 70 known genera of oil-degrading microorganisms each capable of breaking down a specific group of molecules. They include bacteria such as *Achromobacter*, *Acinetobacter*, *Bacillus* and fungus like *Allescheria*, *Aspergillus*, *Candida* and many others which are widely distributed in the soil (Joo et al., 2008; Rufino et al., 2013; Zanaroli et al., 2010). These are either found naturally in the contaminated soils mostly due to situation of chronic contamination and may require only biostimulation to encourage active growth of degraders as the case with Exxon Valdez oil clean up (Lindstrom et al., 1991). Others may need additional BA to enrich the overall capabilities of the degrading community of microorganisms as have been reported successful in several studies (Zhang et al., 2010; Joo et al., 2008; Xu and Lu, 2010). The aim of this work is to review and bring together a range of recent researches in the area of bioremediation. The literature is reviewed under a number of subheadings which includes the roles of natural attenuation, biostimulation and bioaugmentation in bioremediation, hydrocarbon degrading microorganisms, roles of O<sub>2</sub> supply and other factors on bioremediation, selection of amendment options and conclusion

### **Roles of Natural Attenuation (NA), Biostimulation (BS) and Bioaugmentation (BA) in Bioremediation**

Hydrocarbon-degrading microorganisms commonly exist in the soil but may not be enough to remove contaminants. It may be useful sometimes to inoculate the polluted sites with effective hydrocarbon-degrading microbial consortia to support the existing microbes (Chowdhury et al., 2012; Joo et al., 2008; Sagarkar et al., 2014). In some cases these microbial communities require the necessary conditions such as the addition of fertilisers and other forms of nutrient sources referred as BS (Juwarkar et al., 2010).

Several researches have demonstrated the roles of BS, BA and controlled NA in bioremediation of oil contaminated soils (Andreoli et al., 2015; Bento et al., 2005; Chuluun et al., 2014; Colla et al., 2013; Fan et al., 2014; Lindstrom et al., 1991; Mancera-López et al., 2008; Mroziak and Piotrowska-Seget, 2010; Smith et al., 2015; Tyagi et al., 2011). Multiple Seeding with indigenous microbial strain isolated from oil-contaminated soil, under BS was investigated by Lebkowska et al. (2011). The experiment involved the use of 3 biopiles in field condition which were inoculated with microorganisms at different frequency. The results showed that multiple inoculation improved bioremediation by 50% compared to control with no inoculation and 30% higher than treatments with single inoculation. The efficiency was about 80-93% in samples with double concentration of contaminants at 28-90 days after treatments. This result was supported by several laboratory findings showing

about 60-80% removal of hydrocarbon and a significant difference with the controls (Beskoski et al., 2011; Gomez and Sartaj, 2014; Lin et al., 2010; Nwaichi et al., 2011; Teccari et al., 2012; Thangarajan et al. 2011). Coversely, Chagas-spinelli et al. (2012) and Pontes et al. (2013) reported no significant differences between NA, BS and BA+BS processes. The results show about 80-97% removal in NA, BS and BS+BA. Recent studies confirmed this argument through experiments in bioreactors (Alavi et al., 2014), and biopiles (Mair et al., 2013). Furthermore, Ikhajiagbe et al. (2014) studied a monitored NA where by developing a soil seed bank. The study reported higher efficiencies in the degradation of oil from the polluted site.

One major drawback of Lebkowska's work is the differences in porosity of the samples used which could affect aeration and nutrient transfer in the soil. Nwaichi et al. (2011) overlooked the importance of interactions between microorganisms in poultry manure and those in the rhizosphere. An interesting finding in Gomez and Sartaj (2014) was that the biopiles were able to operate under cold condition. Several factors could lead to similar results in all samples used by Chagas-spinelli et al. (2012). But the most likely cause is colonisation of the samples in BS and BA by fungi and suppressing hydrocarbon degrading bacteria. The similar pattern assumed by the degradation suggests that degrading bacteria must have been responsible for contaminant removal in NA. This shows that the experiment should have been adequately planned and conducted to be more dependable. Pontes's article would be more useful if the experiment was set up to simulate ground aquifers because sediments are bounded by permeable partitions in 3 dimensional directions which dictate draining after tidal actions. Only vertical drain was simulated that practically seems least important.

The assertions of Nwaichi et al. (2011) that bioavailability of hydrocarbon in the soil increases with increasing contaminant concentration has been found to improve the efficiency of degradation processes was clearly shown in Lebkowska et al. (2011). This further reveals that the outcomes reported by Lin et al. (2010) may likely be affected by these effects. Moreover, recent evidence suggests that Lag phase of microbial growth cycle might have been delayed in samples with higher contaminant concentration (Juwarkar et al., 2010). It is evident from the above literature that the findings of Lebkowska et al. (2011) are more viable and dependable. It attempted to bridge the gap of pot experiments and complexities of field application in the use of bioremediation to clean-up oil from soils. More so, the developed technology has been patented and applied in Poland on a field scale to treat over 150,000 tons of soil. Nevertheless, the viability of NA is realisable but its limitations outweighed its merits as observed by Genovese et al. (2008) and Liu et al. (2014), and attributed it to lack of nutrients and adverse environmental conditions.

Toluene bioremediation was conducted by Afrouzossadat et al. (2013) in which toluene degrading bacterium was isolated from wastewater using basal salt media. The bacterium was found to be more effective under aerobic condition and has effectively removed toluene by up to 77.5%. Under anaerobic condition, the same bacterium removed toluene up to 45% and both controls have shown no change in toluene concentrations. These outcomes have been supported by a research on bioventing procedure to remediate petroleum in contaminated sandy soil. Amin et al. (2014) reported even higher efficiency of up to 99.5% degradation after 96 hours of injection of air at a stable flow rate of 250mL/min. On the contrary, Li et al. (2013) investigated the application BA to decontaminate clay textured soil (36.32% sand, 21.27% silt and 42.41% clay). Removal efficiency for toluene is reported to be up to 80% with no significant difference between all the treatments and the control.

The research by Afrouzossadat has highlighted the capability of a bacterium to degrade toluene. However, higher effectiveness could be attained if community of organisms were used because different bacteria act on different part during metabolic process. The higher efficiency obtained by Amin's process could be explained by the diversity of microbial population isolated from a site contaminated with oil for over 100 years. The study however overlooked the fact that, if the vessels were made from semi permeable material which would better reflect the unconfined nature of the vadose zone and could affect the result. There are no explicit mentions of the Li's experimental procedure, which appeared confusing. It is also difficult to specify whether BA or NA were applied throughout the entire process.

### **Hydrocarbon Degrading Microorganisms**

Previous and recent researches have conducted microbial isolations from a variety of media and were used in the bioremediation processes (Liu et al., 2014; Zhang et al., 2010; Suja et al., 2014; Rufino et al., 2013). A study by Jung et al. (2014) investigates the use of red clay and processed red clay as biostimulant to decontaminate diesel from soils. It was clearly demonstrated that bioremediation was enhanced by red clay and processed red clay by some 4.9 and 6.7. It was also observed that that there were consistent microbial growths alongside diesel bioremediation. Both types of red clay were found to increase the population of *Acinetobacteroleivorans* DRI, *Pseudomonas putida* and *Cupriavidus*. Jung et al concluded that biostimulating capabilities of red clay and processed red clay were able to support microbial growth without apparent selection for specific bacterial species. A study that investigated the capabilities of fungi to degrade hydrocarbons was carried out by Al-Jawhari (2014) where fungi such *A. niger*, *A. fumigatus*, *F. solani* and *P. funiculosum*

degraded of up to 75% of the contaminants after 21 days. Vinas et al. (2005) reported an insignificant bioremediation in all treatments using biostimulation and NA. The results indicated that *Sphingomonas* and *Azospirillum* as the dominant species during the early stage of the remediation. At the later stage, *Sphingomona*, *Bacteroidete*, became dominant in the un-amended treatments. While In the amended treatments *Xathomona*, *Alcaligenes* and *Achromobacter* and *Sphingomonas* were dominant. The results also showed 72 to 79% and 83 to 87% decontamination of TPH and PAH respectively.

Although, the study by Vinas et al. (2005) has demonstrated the application of adequate bioremediation monitoring techniques, and appeared well implemented with substantiated findings, the processes involved were very complex and difficult to comprehend. Furthermore, all the studies reviewed under this subheading were not able to adequately report on the interaction between the microorganisms in the soils and the soil physical properties such as porosity, particle size distribution, soil structure and permeability. However, strong relationship between these soil properties and the type of microorganisms was reported by Wolf et al. (2013).

Precisely 15 bacteria were prepared into consortia of microorganisms during an investigation by Malik and Ahmed (2014). These microorganisms were incubated at 37 °C and hydrocarbon was used as the only carbon and energy source. The bioremediation efficiency of the microbial community was assessed with 2% contaminant concentration in a shake flask transformation in mineral salt medium for 24 days. Total removal of aliphatic and aromatics was 94.64% and 93.75% at the end of the experiment. The result also indicated that the biotic depletion of alkanes were 90.96% for tridecane (C<sub>13</sub>), 77.95% for pentadecane (C<sub>15</sub>), 74.1% for octadecane (C<sub>18</sub>), and 56 to 69% for other alkanes after 24 days of incubation. This study has demonstrated the ability of bacteria as a viable degrading agent but the authors failed to indicate the names of the microorganisms used probably due to commercial confidentiality reasons as mentioned in Rowe et al. (2008). In a trial on bacterial diversity along petroleum contamination gradient, about 42 to 89 % of all the population of microorganisms observed in contaminated crude oil contaminated soil were of phylum Proteobacteria. Though, Betaproteobacteria, Gammaproteobacteria, Bacilli, and Actinobacteria were also present (Abed et al., 2015). This indicates that the former is more likely to be more effective degraders than the latter particularly in arid areas. Betaproteobacteria, Gammaproteobacteria, Bacilli, and Actinobacteria are by this evidence potential degraders (Jung et al., 2014). *Pseudomonas xanthomarina* was very effective in highly contaminated sites compared to *Pseudomonas* sp and *Arthrobacter nitroguajacolicus* during a study on the treatment of crude oil tank bottom sludge in Libya (Mansur et al., 2015).

### **Roles of O<sub>2</sub> Supply and other Factors on Bioremediation**

Oxygen (O<sub>2</sub>) is the most widely used electron receptor in aerobic bioremediation to help the degradation of pollutants (Dmytreko, 2007). Organic and inorganic pollutants are broken-down faster under aerobic condition because it enhances the performance of majority of the microorganisms (Khorasanizadeh, 2014; Levi et al., 2014; Sanscartier et al., 2011). Although Merlin Christy et al. (2014) argued that anaerobic process is more cost-effective, it is however only realistic in bioreactors and not as effective in field application which is the contemporary challenge in bioremediation. A recent study by Walworth et al. (2013) attempted to develop optimal O<sub>2</sub> level for bioremediation. This was done by investigating the efficacy of different O<sub>2</sub> levels terminal electron acceptor depletion and hydrocarbon degradation in an oil-contaminated soil. The result shows that degradation declined with increased consumption of O<sub>2</sub> during the entire processes. Moreover, O<sub>2</sub> level above or below 10.4% tend to impede degradation of hydrocarbons. A recent study Sihag and Pathak (2014) reported similar percentage but further reported a range 10 to 40% as optimum hydrocarbon oxygen requirements.

A research article by Mori et al. (2013) assesses whether a cost effective procedure can be developed using biostimulation in soils with high volume of macropores. The results indicated higher bioremediation in the unsaturated condition credited to non-clogging due to macropores and presence of air. Micropores might have reduced the toxic effects of contaminant concentration on the degrading microorganisms (Gogoi et al., 2003). Although, the depletion of contaminant is obvious, it is difficult to actually attribute it to biodegradation. Moreover, the article gave no account of hydrocarbon removal from the concentration of 5000 mg/kg. It could be argued that the removal of hydrocarbons resulted more from leaching than bioremediation. Furthermore, macropores should have been substituted by particle size manipulation to support adequate ventilation and moisture retention. Kim and Crawley (2013) observed substantial diversity of bacteria in clay soils which was higher on the outer than the inner parts of the samples. It was further observed that consistent growth and colony of a particular taxon was observed with coarse silt, coarse and fine sand. This is likely due to the presence of O<sub>2</sub> that allows for a rapid metabolism and eventual growth in diversity and population on the outer parts of the surface (Liu et al., 2014). This could also be an explanation to the result obtained by Mori.

Other factors affecting bioremediation include bioavailability, pH, porosity, permeability, contaminant concentration and toxicity, temperature and mineral nutrients (Khorasanizadeh, 2014; Sihag and Pathak, 2014).

### **Selection of Amendment Options for Bioremediation**

Lee et al. (2008) in agreement with Llado et al. (2013) clearly demonstrates that high microbial population and nutrients in compost made it exhibited the highest decontamination compared to inorganic nutrients. Generally, degradation was about some 9-31% higher than observed in the control. Several studies supported this outcome (Nwaichi et al., 2011; Taccari et al., 2012; Gomez and Sartai, 2014). Llado et al. (2013) further argued that the use of lignocellulosic substrates was found to promote the highest degradation after 60 days of treatment. Removal efficiencies of 71-73% TPH and 59-87% PAHs degradation were achieved. Similarly, an amendment with cow dung yielded higher removal efficiency of about 80% and the dung was found to contain reasonable population of hydrocarbon degraders (Adams et al., 2014). Nevertheless, the importance of inorganic fertiliser was reported by Amenaghawon et al. (2013) where higher bioremediation efficiencies were observed. Recent studies have confirmed these findings but however reported slightly lower efficiencies (Soleimani et al., 2013; Suja et al., 2014). Nwaichi et al. (2011) obtained a removal efficiency of 80% with poultry manure in a phytoremediation using *Centrosema pubescens* Benth.

The results obtained for biomass outweighed that of inorganic fertilisers in terms of time and removal efficiency. However, lack of information on methods for TPH monitoring and aeration, and bio-monitoring used can only reflect a process and not the actual degradation. These coupled with inadequate data on control weakens the argument of Llado et al. (2013). The major limitation of Amenaghawon's article is that the samples were not analysed prior to the treatments. The samples used in the experiment could contain high organic matter which could be useful in un-amended soils (Liu et al., 2014).

The use of biochar produced from rice straw in bioremediation of hydrocarbons has been studied by Qin et al. (2013). It improves removal by 16-23% and the highest removal was observed in samples supplied with biochar at day 80. A similar result was obtained by Joo et al. (2008) in which composting containing food waste and diesel contaminated soil subjected to bioremediation was investigated. Treatment has clearly shown 36% decontamination difference with the control. Kuran et al. (2014) utilises humates and zeolite as organic and abiotic amendments in combination with BA in the bioremediation of hydrocarbons in agricultural soils. The contaminant was successfully reduced by 60% in all the treatments. Both humates and zeolite resulted in insignificant impacts on degradation rate but have affected significantly the amount of phospholipids fatty acid (PFA). It has improved the soil microenvironment and homogenisation. Nevertheless, a study by Szulc et al. (2014) has shown insignificant impact of using the organic BS on bioremediation of petroleum contaminated soil.

The study by Qin shows the feasibility of biochar as an amendment option but a longer period of treatment makes it less viable compared to the work of Joo et al. (2008) where decontamination was achieved after 13 days of treatment. Nevertheless, both studies have limitations to the cost of producing energy for heat used in biochar production and bioreactor which could be expensive. Joo et al. (2008) provides a cheap alternative means of sourcing biostimulant but keeping the process at 65 °C is another thing to be considered. The study by Kuran has clearly demonstrated the ability of zeolite and humates to improve the condition of the soil and have shown a comprehensive application of monitoring and analytical techniques. It is however difficult to accede with the author's conclusion that BA was the most important treatment. The comparison between controls and the treatments could be a better way to assess whether BA is the most significant treatment than comparing the total PFA and microbial activities. A disadvantage of using zeolite is its antimicrobial property and the possibility of contaminants being adsorbed to its wider porosity (Andrejkovičová et al., 2012), which cannot be analysed but could be attributed to biotic depletion of the contaminants. However, it has the merit of providing wider surface area for microbial contact with the contaminants (First et al., 2011).

## **II. Conclusion**

Bioremediation of petroleum in soils has extensively been studied and the literature has shown that various techniques have been utilised both in laboratories and pilot scales to provide enough data for enhanced understanding of the process. Bioaugmentation and/or BS are particularly useful in improving degradation rate and efficiency depending on site requirements (Genovese et al., 2008). Oxygen is the most widely used electron receptor in bioremediation and is influenced by soil porosity which is a factor of soil type and texture. These have been found to affect the process of bioremediation.

This review has shown that the selection of amendment option and microorganisms is critical to the success of any BS application to optimise the process. Amendments of organic nature have yielded more positive results, but inorganic fertilisers are also useful. The cost and availability of these amendments have to be evaluated to ensure that the process is still cost effective. Zeolite has been used to optimise bioremediation but has been found to have an antimicrobial characteristic which could impede the process.

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