PROGRESS IN THE MICROBIAL REMEDIATION STRATEGIES FOR OIL CONTAMINATED SITES: A REVIEW

Vijay A.K.B. Gundi*
Department of Biotechnology, Vikrama Simhapuri University, Nellore - 524 320, Andhra Pradesh, India
*Corresponding Author Email: gundi.vijay@gmail.com

ABSTRACT
The natural crude oil community is a valuable resource for bioremediation of pollutant environments. The hydrocarbonoclastic bacteria naturally present can adapt to different aquatic sources, and when exposed to a hydrocarbon source can promptly commence degradation. Bioremediation, a process that use microorganisms to degrade oils that cause serious environmental and human problems. Bioremediation processes typically involve microbes which act either singly or consortium to complete the degradation process. Both in situ (in place) and ex situ (removal and treatment in another place) remediation approaches are used. The versatility of microbes to degrade a variety of oils makes bioremediation a technology that can be applied in different environmental conditions. Though it can be inexpensive and in situ approaches can reduce disruptive engineering practices, bioremediation is still not a common practice. There are many advantages to be gained from a quick clean-up of an oil spill, some of which relate not to the marine ecosystem, but to other concerns. These include economic impacts from lost us of shorelines for recreation, legal liabilities and settlement of claims, and aesthetic considerations. Besides, rapid oil disappearance may lead seashores and beaches safer for local wildlife and minimized the movement of undegraded oil from the beaches into the water column.

KEY WORDS
Bioremediation, Pollutant, Hydrocarbonoclastic bacteria.

INTRODUCTION
Oil contamination is one of the most facing environmental problematic issues today. Since from industrialization of the world began over two centuries ago the petrochemical industry occupies major place and petrochemical oils became major energy sources of the world and daily life as the part of globalization. Eventually accidental leakages and spillages of the oils during the exploration and shifting have been occurred regularly. Though it was not a major environmental problematic issue in the starting days, now a day it became a major pollution causes and dangerous threat to the marine life. It was estimated that the amount of natural crude oil leakage in the ocean was ranging from 200,000 tons with a lower limit to 2,000,000 tons per annum with an upper limit (14). Releasing of aromatic and aliphatic petroleum derived hydrocarbons in to environment showing great adverse effects on soil and see water. These compounds may accumulate in the animals through food chains and even up to humans. Long exposure to the petrochemical oils may cause liver problems, kidney diseases and possible bone
marrow damages (12). Hence it needs to see the clean-up processes of oil contaminated areas. The physicochemical techniques were developed to remove oil contaminations which including incineration, base-catalyzed dechlorination, UV oxidation, fixation and solvent extraction, but these methods include disadvantages like cost and intermediate metabolites. This can be resolved by the addition of microorganisms instead of chemicals to degrade different organic compounds. The degradation of hydrocarbons using microorganisms is a promising method to remove the contaminants from the environment and the process is called as biodegradation or bioremediation. And it was identified that Bacteria, fungi and yeast are the main organisms to degrade the petrochemical oils and hydrocarbons (21). Moreover bacteria has been considered as more predominant agents for hydrocarbon degradation among the three microorganisms (6).

Table 1. Different methods used to remove petrochemical oils from contaminated sites (adopted from (23))

<table>
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<tr>
<th>Method</th>
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<td>Physical methods</td>
<td>Booms</td>
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<td>Sediment relocation or Surface washing</td>
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<td>Chemical methods</td>
<td>Dispersants</td>
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The success of the biodegradation of oils is based on the metabolic capability of microorganisms to degrade the contaminants and the conditions which are established to enhance the biodegradation rates and the physicochemical properties of the oil and oil contaminated area (5). Success is also based on the previous environment or the nativity of the organism where it previously grown or the contaminated site from which the organism is isolated. Because if the organism has previous exposure to the hydrocarbons, it shows selective enrichment and genetic changes may takes place (22). The adopted microbial communities has shown quick response with in the short period of time and higher biodegradation capacity when compared with the exogenously added microorganisms which has no previous exposure to the contaminated sites (1).

STRATEGIES OF THE MICROBIAL REMEDIATION

The awareness of harmfulness of the petrochemical oils to the human welfare and to the environment has turned the attention of research to develop various remediation methods which are mainly classified as physicochemical methods. Many of these conventional methods are tuff to perform and expensive because of transporting of the contaminated materials for...
treatment and cost of excavation. Hence the biological treatment methods of contaminated sites came in to existence (3).

1. Natural Attenuation

Natural attenuation is also called as intrinsic bioremediation method, this is the basic bioremediation process where the native or indigenous microorganisms are used to treat the contaminated sites (13). The process involves aerobic or anaerobic degradation of toxic compounds and different kind of detoxification strategies which actually microorganisms shows includes dilution, sorption, volatilization decay, biological or chemical stabilization, and transformation of contaminants. In this method no genetically modified organisms are used. The process involves not only biological phenomena, the detoxification of hydrocarbons through physical methods includes advection, dispersion, dilution, diffusion, volatilization, desorption and chemical mechanisms like ion exchange, complexation, biotic or abiotic transformation also takes place. Perhaps the process is good if there is no time limit at low concentration of contaminated sites and the organisms with suitable catabolic activity are not available on the polluted sites is a limiting factor of this process (13). If the clean-up is not enough to remove the contaminants well scientists will monitor the conditions of the process to get satisfactory remediation, this alteration in the attenuation is called monitored natural attenuation (17).

2. Bio-augmentation or Bio-stimulation

Bio stimulation is another process used to enhance the transformation of toxic compounds to nontoxic products by the addition of nutrients, trace materials minerals and electron acceptors along with the indigenous microorganisms (16). Whereas the bio augmentation involves the genetically altered microbes or wiled type microorganisms to improve the degradation of toxic compounds. Though it is satisfactory method when compared with natural attenuation it has some limitations. The concentration of the inoculated organism decreased with in short time after addition to the contaminated sites. This might be because of temperature variations, water content, nutrition depletion and pH variations occurred in the contaminated sites. Moreover the indigenous organisms compete with the added microorganism by showing antagonistic reactions like antibiotic production and protozoa, bacteriophages and fungal preying towards the added microorganism (10). This can be overcome by addition of co-inoculation of consortium of microorganisms with different degradation capacities (19). Furthermore the combination of both bio augmentation and bio stimulation could be help full to increase the degradation rate (7).

3. Phytoremediation

Phytoremediation is another strategy for the remediation hydrocarbons. This method involves the usage of plants and plant associated microorganism’s for clean-up of the contaminated sites (20). Phytoremediation is useful for cleaning up of the large scale contaminated sites as it has the potential to degrade the vast range of toxic compounds. Research towards this phytoremediation in the early years explored valuable insights about the degradation ability (3). The phytoremediation can successively degrade many organic contaminants like TCE (trichloroethylene), herbicides like atrazine, explosive compounds like TNT (trinitrotoluene), PHC (Petroleum hydrocarbons) , chemicals like benzene, toluene ethyl benzene and xylene (BTEX), mono aromatic hydrocarbons and poly aromatic hydrocarbons, fuel additives like methyl tertiary butyl ether (MTBE), and polychlorinated biphenyls (PCBs), but some experiments proved that the phytoremediation fail some extent to remove the high concentration of contaminants from the sites due to the inhibition of plant growth.
by the pollutants and oxidative stress, this limits the rate of bioremediation (11).

4. Rhizoremediation

Though phytoremediation is an excellent process, but it has limitations to remove contaminants from sites. However, this problem can be achieved by the together with rhizodegradation and this can be described as a beneficial interaction between plants and rhizobacteria. Bacteria that live on and surroundings of roots of plants have shown the ability to degrade toxic compounds in contaminated sites, potentiality of rhizobacteria can be helpful for improvement of phytoremediation. A wide variety of plant species have been employed in degradation of hydrocarbons was reported in literature (3). The plants which are belongs to species of *Populus* (poplar) and *Salix* have been used successfully which actually introduce oxygen into deeper soil layers through special root systems (23). A huge diversity of hydrocarbon degrading bacteria is present in the rhizosphere of the contaminated sites the organisms belong to the genus of *Rhodococcus*, *Luteibacter* and *Williamsia* are predominantly used (15). Chemicals like flavonoids and some other compounds secreted by plant roots are enhance the growth and activation rhizobacteria and microbes of rhizosphere in turn promotes plant health by enhancing the root growth, uptake of minerals and water and inhibits the other pathogenic soil microorganisms (13). The organisms either release oxidoreductase enzymes along with plant roots and catalyse polymerization of toxic compounds in the soil and on the root surfaces or colonize in the roots of the plants and promote the plant growth which actually called plant growth promoting bacteria (PGPB). Though it is a potential method certain environmental factors influencing the technique and selection of suitable microbial consortium each with different degradation capacities is a limiting factor of this method (3).

5. Bio-surfactant Producing Bacterial Consortium

The degradation of hydrocarbons by the microorganism is because of the production of potential derivative enzymes. But some other kinds of microbes have the ability to produce bio surfactants. Bio surfactants are the heterogeneous chemical compounds with amphipathic in nature. They are surface active compounds produced by different type’s bacterial species. Bio surfactants enhances the biodegradation by reducing the surface tensions of hydrocarbons and ability to digest the micelle of hydrocarbons in aqueous solution mixtures (2). Using single kind of organism for the degradation may not give satisfactory results, because the individual organism can metabolise only a limited range of hydrocarbons. Therefore, inoculation combination of organisms with bio surfactant producing non-biosurfactant producing organisms can be cindered as an interesting strategy. 91% of hydrocarbon degradation in 6 weeks was achieved by a combination *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* with the crude bio surfactant production was reported (4), recently a study has been conducted to test the degradation abilities of different consortium of microorganism. Among the 14 consortia, *Bacillus pumilus* KS2 and *Bacillus cereus* R2 were considered as the best consortium with 68.12% of the hydrocarbon degradation. And the best consortium was selected based on the degradation of total petroleum hydrocarbons (TPH).
Figure 1. Degradation TPH up to 6 weeks by the best microbial consortium (8).

CONCLUSIONS

There are many advantages to be gained from a quick clean-up of an oil spill from the water environments. However, strategies of bioremediation being followed at present are not enough for clean-up of universal organic contaminants. During the bioremediation process, growth and survival of microorganisms is affected by environmental factors like temperature, composition of the contaminant, type of medium and nutrient availability. These factors affect the application of bioremediation as a process of clean-up. Similarly, petroleum hydrocarbons greatly vary in their susceptibility to metabolic breakdown by bacteria. This can limit the scope and effectiveness of bioremediation.

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*Corresponding Author: gundi.vijay@gmail.com*