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MICROBIAL REMEDIATION OF PETROLEUM SLUDGE OR SPILLAGE ON SOIL ECOSYSTEM (*REVIEW*)

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ABSTRACT

Crude oil, petroleum sludge, refined petroleum products, as well as polycyclic aromatic hydrocarbons are ubiquitous in nature and their ubiquity in various environments makes it possible to bioaccumulate in food chains where they disrupt biochemical and physiological activities of many organisms, thus predisposing to carcinogenesis, mutagenesis with genetic impairments. Soil contamination from oil spills has a limiting factor to soil fertility and hence crop productivity. On this basis, its release into the environment is monitored by Environmental Protection Agency which classified it as one of the environmental pollutants. These deleterious effects have made it mandatory to have a counter measure for the petroleum spillage in the environment. Bioremediation of petroleum hydrocarbon-contaminated environment is a potentially important application of Environmental Biotechnology. Soil ecosystem stressed with discharges from petroleum can be decontaminated by the use of microorganisms which are utilized under some specified conditions to ameliorate the negative effects in a cost-effective and environmentally friendly manner. This recovery process which uses consortia of microorganisms (indigenous or foreign) is termed bioremediation. Bioremed iation is becoming an increasingly an effective, eco-friendly treatment and cost effective in remediating an oil polluted environment. Given that large quantities of organics and inorganics are released into the environments yearly from petroleum, petroleum hydrocarbon still remains the ultimate source of energy and hence a global environmental pollutant.

Key words: Crude Oil, Petroleum hydrocarbons, Environmental biotechnology, Bioremediation

INTRODUCTION

Reports have it that, large quantities of organic and inorganic compounds are released into the environments every year as a result of human activities causing serious environmental problems. Paramount among these problems is oil contamination of soil and water from industrial sources and other activities (Mrayyan and Battikhi, 2005). Petroleum (hydrocarbon) continues to be used as principle source of energy and hence a large global environmental pollutant. Safe disposal of oily sludge has been one of the challenges facing oil refineries during the processing of crude oil. Improper disposal of oily sludge is an environmental threat which leads to environmental pollution, particularly soil and ground water contamination. (Argun *et al*., 2010; Dhote *et al*., 2009). Among the several clean-up techniques available to remove petroleum hydrocarbons from the soil and groundwater, bioremediation processes are gaining ground due to their simplicity, higher efficiency and cost effectiveness when compared to other technologies (Mariano *et al*., 2001). With the stimulated research into the environmental fate and biological effects of spilled oil in the 1970s and to date, many advances have been recorded in the field of Environmental Biotechnology, with respect to crude oil pollution control and clean-up operations. Some of the difficulties include the fact that crude oil and petroleum are very complex mixtures of several thousands of hydrocarbons and other indeterminate structures and additives. The complexity of some ecosystems do not make for easy accessibility to determine the spilled oil condition at the moment of impact and thereafter. Petroleum with its complex mixture of non-aqueous and hydrophobic components makes it toxic, mutagenic and carcinogenic (Mandal *et al*., 2012) and US Environmental Protection Agency made it a priority watch as a global environmental pollutant.

Clean-up measures based on physiochemical treatment techniques such as incineration, thermal desorption, chemical oxidation, are expensive, energy intensive and not sustainable (Liu *et al*., 2010). With respect to their environmental impact which includes damage to the soil structure and toxicity to plants these techniques do not eliminate the problem (Liu *et al*., 2010) but sequester the contaminants. These limitations have been the basis of search for more economically and environmentally friendly approaches to remediate contaminated soil. Biological treatment of organic pollutants is a promising field of research, which gives reliable, simple and cheap technologies over chemical and physical processes. (Liu *et al*., 2010).

Environmental biotechnology for crude oil clean-up

Biotechnology is a set of scientific techniques that utilizes living organisms or parts of oxidation from organisms to make, modify or improve products (which could be plants or animals). It is the development of specific organisms for specific application or purpose and may include the use of novel technologies such as recombinant DNA, cell fusion and other new bioprocesses (Anon, 1991.) It is also a biotechnology that specifically addresses issues in environmental pollution control and remediation (Onwurah, 2000). As a result many disciplines such as biology, agriculture, engineering, health care, economics, mathematics, chemistry, computer science and physics are involved. It is regarded today as fundamentally an engineering application of microbial ecology (Rittman *et al*., 1990) and process design. The engineering aspect of environmental biotechnology involves the design /construction and design of special machines or equipment referred to as reactors or bioreactors. Environmental biotechnology also encompasses quantitative mathematical modeling whereby understanding and control of many inter-related processes become possible. Mathematical modeling technology transcends the boundary of single traditional scientific disciplines and technologies, whereby a logical framework resolves related problems (Ziegler 2005; Onwurah 2002b). They are tools utilized economically for explaining the cost and effectiveness of different options of clean-up technology and control (Onwurah and Alumanah 2005; Ziegler 2005).

Statement of the problem

One of the greatest challenges to humanity today is the endangering of biota as a result of environmental pollution from crude oil. To estimate the biological danger of oil after a spill, knowledge of the harmful effects of the components is necessary. In other to obtain or ascertain the effects of such polluting substance, every living being and life function can be considered a potential biomarker or bio-indicator. Some ecologists, when discussing the adverse effects of pollutants usually consider the accumulation of pollutant residues in the tissues of organisms as adverse. The most ecologically relevant measurements in assessment of ecotoxicity are those that describe changes in ecosystem structure and function (Kelly and Harwell, 1989). However, such measurements are often difficult and time consuming to make, and are sometimes predictive. Blanck *et al.* (1988) postulated that pollutants that do not exert a selection pressure cannot cause any significant biological effects to an ecosystem, since these substances are unable either to restructure communities or to change the genotypic distribution in the populations. A lot of pathological changes and biochemical markers have been identified that signal exposure to pollutants at the cellular and molecular levels (McCarthy and Shugart, 1990).

A biomarker is an organism or part of it, which is used in establishing the possible harmful effects of a pollutant on the environment or the biota. Bio-monitoring or biological monitoring is a promising, reliable means of quantifying the negative effect of an environmental contaminant. In a broad sense, biological markers (biomarkers) are measurement in any biological specimens that will elucidate the relationship between exposure and effect such that adverse effects could be prevented (NRC., 1992). Microorganisms can be used as indicator organism for toxicity assay or in risk assessment. Tests performed with bacteria are considered to be most reproducible, sensitive, simple, economical and rapid (Mathews, 1980). Some examples include the 'rec-assay' which utilizes *Bacillus subtilis* for detecting hydrophobic substances (hydrocarbons) that are toxic to DNA (Matsui, 1989), *Nitrobacter sp*, which is based on the effect of crude oil on oxidation of nitrite to nitrate (Okpokwasili and Odukuma, 1994), and *Azotobacter sp*, used in evaluating the effect of oil spill in aquatic environment (Onwurah, 1998).

Bioremediation has become a major method employed in the restoration of oil polluted environments, and attempts to accelerate the natural hydrocarbon degradation rates by overcoming factors that limit bacterial hydrocarbon degradation activities (Liu *et al*., 2010; Jakson, 1996). 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).

Bioremediation technologies for hydrocarbon contaminated sites

Bioremediation is a technology that exploits the abilities of microorganisms and other natural habitats of the biosphere to improve the environmental quality of all species, including man. The development of innovative bioremediation technology as a functional tool in clean-up of oil polluted environments has depended largely on the basic knowledge of the physiology and ecology of the indigenous microbial community found in such polluted sites. Many advances in biochemistry and molecular biology are now applied in various bioremediation methods (Olson and Tsai, 1992). According to some investigators (Barbee *et al*. 1996; Ritter and Scarborough, 1995), bioremediation does not always result to complete mineralization of organic compounds. Many of these compounds are naturally transformed to metabolites of unknown persistence and toxicity. Therefore some basic steps that may be necessary for a successful bioremediation project will include: compliance analysis, site characterization, and methods of selection / feasibility studies (Bonaventura *et al*., 1995). Compliance analysis requires examination of the polluted site in the light of the governing regulation and the action plan. Examination of the site will lead to its characterization and this is a very challenging and difficult aspect of a bioremediation technique. Knowledge of soil parameters such as cation exchange capacity (CEC), relevant nutrient availability, acidity (soil pH), aeration or oxygen level, hydraulic properties etc are of relevance and this requires the assistance of specialists in these areas. The last stage of any bioremediation project should include bioassay of the treated site. This confirms complete or near complete removal of the PHC pollutant.

During full-scale bioremediation technologies of oil polluted ecosystems, many rate-limiting factors are known (Atlas, 1991; Prince, 1992), and they include the presence of other toxic compounds other than oil pollutant, as well as the aerobic status and nutrient availability (particularly nitrogen and phosphorus), temperature and pH. Other factors are moisture content or water availability, biodiversity of hydrocarbonoclastic and co-metabolizing microbes at the site. The adsorptive capacity of the hydrocarbons to the soil and sediment, and rate of mixing and mass transfer are also important factors.

In terrestrial ecosystem, spilled oil adsorbs to the soil particles, forming a cohesive toxic mixture that is deleterious to the indigenous microbial communities. These events on soil characteristics led to reduction or increase in the bioavailability of petroleum hydrocarbons, the inherent toxicity and hence biodegradability. These factors are responsible for the long delays in the mineralization of the petroleum hydrocarbons (PHC) by the indigenous or adapted microbial populations. Effective metabolism of (crude or spent) oil requires adequate oxygen supply as electron acceptor. Under low oxygen tension as in the mangrove ecosystem, the use of alternate electron acceptors as biologically active absorbent (Gregorio, 1996) to fix the oil and effect medium term biodegradation is desired. It should be noted that the extent of oil impact on the soil equally depends on the concentration spilled, ease of dissociation from the soil matrix, particle size of the soil, porosity, or permeability. To facilitate bioremediation requires methods that can dissociate the PHC and create conditions for mass transfer process (Onwurah, 2000).

Bioremediation of oil polluted environment may require some engineering process, so as to facilitate recovery efforts. Engineering may include construction of booms, trenches, and barriers for contaminant containment, boreholes, bio-cells and using engineered microbial systems. Enhancement of the bioavailability of the PHC can be achieved by physically processing the oil-polluted soil or sediment by excavation and mixing. The above processes maximize aeration and surface area for microbial activity. Some specific bioremediation processes that may require engineering application include; *in situ* bioremediation, land farming, adapted bacterial cultures, genetically engineered microorganisms (GEMs) etc.

***In-situ* bioremediation**

In this remediation, the soil is not excavated but treated as it is. This method is cost effective judging from the fact that excavation is expensive and also the cost of transport to treatment sites. Selection of the best treatment method depends on the nature and quantity of the contaminants, treatment costs, the soil type and the environmental conditions on the site, among other things (US. DOD, 1994; Khan *et al*., 2004). It is needful to carry out a thorough investigation of the properties of the soil before selecting the best treatment method(s). This investigation will enhance *in-situ* remediation because performance of the remediation process is more difficult to monitor and control during treatment than traditional *ex-situ* remediation processes (Morgan and Watkinson, 1992).

In- situ remediation is a good option on many occasions judging from its cost effective nature. Excavating the soil may be too expensive or even impossible especially, if the area to be treated is very large or the contaminated zone lies deep in soil. It is usually costly to excavate the whole mass and transport it for remediation. If the contaminated site has buildings or is otherwise in active use, excavation is not an option (Carberry and Wik, 2001). However, challenges exist with *in-situ* treatment. How to achieve uniform remediation throughout the treatment area is a major challenge with all *in-situ* treatment methods. The heterogeneous nature of the contaminated soils usually makes uniform delivery of chemicals to the whole area very difficult (Federal Remediation Technologies Roundtable, 2000). *In-situ* biological treatment technologies include bioventing, land treatment, phytoremediation and natural attenuation.

Genetically engineered microganisms (GEM)

Apart from using adapted organisms, genetically engineered microorganisms are in use. In a bid to improve the microbial degradative potentials, Novel oil degrading "super bugs" has been engineered by inoculating into them plasmids from bacterial species with different biodegradative capabilities (Chakrabarty, 1982). The advent of high-throughput methods for DNA sequencing and analysis of genomes as well as modeling of microbial processes have revolutionized environmental biotechnology (Lovely, 2003). Table 1.0 shows some novel microbial systems that have been applied in PHC degradation. Some of these cultures have been developed as proprietary products through selection of genetically able (not engineered) microorganisms from mixed cultures that are found in a natural contaminated environment as opposed to the genetically engineered strains of microorganism.

Table 1.0: Successfully used microbial system or strains in bioremediation of oil polluted environment

Microbial system or strain	Remediation mechanism	Reference
Pseudomonas aeruginosa (UG2)	Production of biosurfactant that emulsifies crude oil	Scheibenbogen *et al*., 1994
Pseudomonas sp/ Azotobacter vinlandii consortium	Optimization of nitrogen fixation for crude oil metabolism and co-metabolism	Onwurah, 1999b Onwurah and Nwuke, 2004
DBCRS (IBS blend of hydrocarbon-degrading microbes)	Adapted microbial consortium that degrades many components of crude oil	Adams and Jackson, 1996

Genetically modified or engineered microorganisms have greater potential in bioremediation of oily waste in a polluted environment because they have been tailored to perform such a function. There is however a great concern that the use of GEMs may adversely affect biodiversity. Supposedly, engineered microbe specifically designed to mineralize spilled crude oil may wreck havoc on stored fuel supplies and even to the extent of depleting crude oil reserves or deposits if not controlled. Owing to the great uncertainty and regulation, the prospects of bioremediation with GEMs are yet to be realized.

Microbiology of remediation

Environments contaminated with petroleum derivatives such as hydrocarbons from spent oil remain impacted for a very long period unless remedied. In every oil-polluted environment, microorganisms are found clustered in greater numbers in the oil/water interphase of a hydrocarbon layer (Berwick, 1994). Thus hydrocarbons increase the abundance of hydrocarbon degrading microorganisms, but on the other hand induce a limitation in microbial diversity (Ebulue *et al*., 2017). A well articulated bioremediation programmes will definitely reduce the time of decontamination. Microbial degradation of petroleum and its derivatives is initiated by the production of biosurfactant molecules by some species of microorganisms (Scheibenbogen *et al*., 1994). These organisms solubilize the petroleum hydrocarbons (PHCs) by increasing their surface contact with the microorganisms in the environment. The biosurfactant molecules first encapsulate a small quantity of the oil which is an emulsification process. The oil is then transported across the bacterial cell wall/membrane. Once the oil is internalized, its degradation occurs through α- or β- oxidation metabolism. The degradation or decomposition of these compounds leads to production of carbon dioxide and water (mineralization).

Biochemistry of remediation

Three general pathways of microbial metabolism are known, and these are aerobic, anaerobic respiration, and fermentation. For each of these pathways, enzymes are responsible for catalyzing the transformation of the contaminants (substrates) to harmless products. The degradation of the internalized substrate will depend on the following: the binding of the substrate (chemical) to the enzyme and conformational changes at the active site of the enzyme. In most enzymes, chemically induced conformational changes play a very important role. When the suitable conformational changes in the enzyme have taken place, then enzymatic reaction will commence. Aliphatic compounds are first oxidized to alcohol, followed by dehydrogenation to an aldehyde and then to carboxylic acid, which is finally transformed into acetic acid. In contrast, two hydroxyl groups are added to aromatic molecules via a dioxygenase in order to saturate a pair of adjacent carbons. Oxygen may also be incorporated into products of microbial metabolism through the action of oxidase enzymes. The transformation facilitates enzymatic cleavage of the delocalized annulus to yield β-keto acids or short chain acids. Both acetic and the adipic acids are then assimilated into the microbial tricarboxylic acid (TCA) cycle for production of energy in the form of adenosine triphosphate (ATP).

CONCLUSION

In this review, crude oil being the major source of energy has increased the risks of accidental spills and hence environmental threat. Environmental biotechnology for oil spill clean-up is a multidisciplinary approach aimed at recovery of polluted ecosystems. Bioremediation of ecosystem stressed with hydrocarbon utilizes consortia of microorganisms to restore the biochemical equilibrium of the soil.

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