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Review

Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies

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The irrepressible quest for a cheap source of energy to meet the extensive global industrialization demand has expanded the frontiers of petroleum hydrocarbon exploration. These exploration activities amongst others often result in pollution of the environment, thus creating serious imbalance in the biotic and abiotic regimes of the ecosystem. Several remediation alternatives have been in use for the restoration of petroleum hydrocarbon polluted systems. In this paper, we present an overview of bioremediation alternative vis-à-vis other cleanup methods and its adaptations in various polluted systems.

Key words: Crude oil, pollution, environment, bioremediation.

INTRODUCTION

Accidental and deliberate crude oil spills have been, and still continue to be, a significant source of environmental pollution, and poses a serious environmental problem, due to the possibility of air, water and soil contamination (Trindade et al., 2005). For example, approx. 6×10^7 barrels of oil was spread over 2×10^7 m^3 soil and 320 oil lakes were created across the desert during the first Gulf War in Kuwait (Al-Saleh and Obuekwe, 2005). The processes leading to the eventual removal of hydrocarbon pollutants from the environment has been extensively documented and involves the trio of physical, chemical and biological alternatives. However, bioremediation which is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition, is an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to CO_2 and H_2O (da Cunha, 1996). It also provides highly efficient and environmentally safe cleanup tools (Margesin, 2000). This technology accelerates the naturally occurring biodegradation under optimized conditions such as oxygen supply, temperature, pH, the presence or addition of suitable microbial population (bioaugmentation) and nutrients (biostimulation), water content and mixing (Trindade et al., 2005). In this paper, we present an overview of bioremediation alternative vis-à-vis other cleanup methods, and its adaptations in various polluted systems.

CLEANUP TECHNOLOGIES – BIOREMEDIATION VERSUS OTHER METHODS

For the past decades, the method of choice for ground water cleanup, for example, involves the pump-and-treat systems. These systems consist of a series of wells used to pump water to the surface and the surface treatment facility used to clean up the extracted water. This method is used to control contaminant migration, and if recovery wells are located in the heart of the plume, it can easily remove contaminant mass. However, since many common contaminants become trapped in the subsurface, complete flushing out may require the pumping of extremely large volumes of water over very long period of time. Because it treats contaminants in place instead of requiring their extraction, *in situ* bioremediation takes

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care of these shortcomings in a cleanup process. Consequently, bioremediation is likely to yield faster results, take a few to several years compared to a few to several decades for the pump-and-treat technology (Testa and Winegardner, 1991).

The microbiological decontamination of oil-polluted soils has been assessed to be an efficient, economic and versatile alternative to physiochemical treatment (Bartha, 1986) even though the rate of hydrocarbon biodegradetion in soils is affected by other physiochemical and biological parameters. While capital and annual operating cost may be higher for bioremediation, its shorter operating time should compensate in a reduction of total cost. Other factors that may contribute to cost reduction in bioremediation compared to pump-an-treat method include reduced time required for site monitoring, reporting and management, as well as reduced need for maintenance, labour, and supplies (National Research Council, 1993). Furthermore, the surface treatment methods that are part of pump-and-treat systems typically use air stripping and/or carbon treatment to remove contaminants from the water. The process is mainly that of transferring the contaminant to another medium (the air or the land) instead of destroying it. Bioremediation on the other hand, can completely destroy contaminants, converting them to carbon dioxide, water, and new cell mass, or at least convert them to non-toxic products some of which may even be useful to the ecosystem.

For cleanup of contaminated soils, *in situ* bioremediation is only one of several possible technologies. Alternatives include:

(1) Excavation followed by sea disposal or incineration.
(2) On-site bioremediation using land-farming or fully enclosed soil cell techniques.
(3) Low temperature desorption.
(4) *In situ* vapour recovery.
(5) Containment using slurry walls and caps.

In situ methods (desorption, vapour recovery, containment, and bioremendiation) have the advantages of being minimally disruptive to the site and are potentially less expensive. Because *ex situ* methods require excavation, they disrupt the landscape, expose the contaminants, and require replacement of soils. For these reasons, *ex situ* methods are sometimes impracticable.

Potential advantages of bioremediation compared to other *in situ* methods include destruction rather than transfer of the contaminant to another medium; minimal exposure of the on-site workers to the contaminant; long-time protection of public health; and possible reduction in the duration of the remedial process. These advantages of the bioremediation systems over the other technologies have been summarised (Leavin and Gealt, 1993) as follows: can be done on site i.e. *in situ* application; keeps site destruction to a minimum; eliminates transportation costs and liabilities; eliminates long-term liability; biological systems are involved, hence often less expensive; and can be coupled with other treatment techniques to form a treatment train.

BIOREMEDIATION TECHNOLOGY

Basic understanding of bioremediation principles

Simply defined, bioremediation is the use of biological systems to destroy or reduce the concentrations of hazardous wastes from contaminated sites. Such systems have the potentially broad-spectrum site applications including ground water, soils, lagoons, sludge and process waste-streams, and it has been used in very large scale applications such as the shoreline cleanup efforts in Alaska, resulting from the oil tanker “Exxon Valdez” oil spill in 1989 (Caplan, 1993).

Bioremediation strategy can be as simple as applying a garden fertilizer to an oil-contaminated beach, or as complex as an engineered treatment “cell” where soils or other media are manipulated, aerated, heated, or treated with various chemical compounds to promote degradation (Hildebrandt and Wilson, 1991). The bioremediation strategy of choice ultimately will depend on the peculiarity of the contaminated site.

Many published articles have documented the potentials of microorganisms to degrade oil both in the laboratory and in field trials. A number of the scientific papers including several review articles covered aspects of the biodegradation process as well as results from controlled field experiments designed to evaluate degradation rates in various environments (Gunkel and Gassmann, 1980; Atlas, 1981; Halmos, 1985). Furthermore, some studies carried out following major oil spills like the Amoco Cadiz have assessed oil degradation in the environment and confirmed the reliability of bioremediation process.

Crude oil is a complex but biodegradable mixture of hydrocarbons, and the observation that hydrocarbon degraders can be enriched in many, if not most, types of environments (Atlas, 1981) have contributed to the development of oil bioremediation techniques (Margesin and Schinner, 1997). Although the optimum temperature for biodegradation of petroleum products has generally been found to be in the range of 20 – 30^{o}C (Atlas and Bartha, 1992), local environmental conditions may select for a population with a varying optimum temperature.

Practicality and application of bioremediation systems

Application of bioremediation techniques requires inputs from experts in microbiology, chemistry, geology, engineering, soil science, etc as the technologies are based on approaches common in environmental engineering, geology and soil science (Alexander, 1994). At specific sites where the contaminants are petroleum products, the spectrum of necessary professional expertise is greatly expanded. However, three important aspects are necessary in bioremediation studies, and these include microbial composition, contaminant type, geology of polluted site and chemical conditions at the contaminated site (Aichberger et al., 2005).

However, the key component in bioremediation is the microorganisms, which produce the enzymes involved in the degradative reactions leading to the elimination or detoxification of the chemical pollutant. Due to the expected superiority and metabolic versatility of mixed cultures over pure cultures, they are being applied for the treatment of petroleum wastes in fermentor-based systems (van Hamme et al., 2000). In such a situation, a pre-acclimated hydrocarbon-degrading culture may be subsequently exposed to a variety of heterogeneous hydrocarbon-contaminated waste streams. Different wastes may affect the structure and metabolic abilities of the microbial community. Venkateswaran and Harayama (1995) traced changes in a crude oil degrading mixed culture through six transfers onto the residual hydrocarbon extracts of the previous ten-day fermentation.

Most of the microorganisms that are frequently identified as active members of bioremediation microbial consortia belong to the genera *Acinetobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Berjerinckia*, *Flavobacterium*, *Methylosinus*, *Mycobacterium*, *Mycococcus*, *Nitrosomonas*, *Nocardia*, *Penicillium*, Phanerochaete, *Pseudomonas*, *Rhizoctonia*, *Serratia*, *Trametes*, and *Xanthobacter*. Detailed report of these and other organisms have been reported elsewhere (Okoh, 2001; Barth, 2003; Lliros et al., 2003; Chaillana et al., 2004). Several strains of fungi and actinomycetes were also confirmed to be important agents for bioremediation of hydrocarbon contaminated sites (April et al., 2000; Zhang et al., 2006). Soil fauna have also been implicated in bioremediation as they redistribute microbes or help reintroduce them from less contaminated soil layers. Particularly, worms of various sizes also mix the soil and make it more porous, and thereby improve aeration (Romantschuk et al., 2001) which is necessary for effective bioremediation. Different aspects of soil fauna in relation to bioremediation is reviewed in depth elsewhere (Haimi, 2000). For these biological entities to carry out effective biodegradation, certain requirements are necessary viz.: the presence of the organisms in appropriate densities with the capability to degrade the target compound(s); the chemical substrate (contaminants) must be accessible to the organisms in a form that it can be used as energy and carbon source; the presence of an inducer to cause the synthesis of specific enzymes for the target compound(s); the presence of an appropriate electron acceptor-donor system; favourable environmental conditions for enzymatic catalysed reactions (moisture and pH); availability of nutrients necessary to support the microbial growth and enzyme production (nitrogen and phosphorus are essential); temperature ranges that supports microbial activity and enzymatic reactions; absence of toxic substances; environmental conditions that limit the growth of competitive organisms in favour of those conducting the desired reactions (MDA, 2006; Vidali, 2001; Graham et al., 1999). A careful and balanced adjustment of these requirements is important for a successful bioremediation to take place.

The type of the contaminant (pollutant) is also a very important factor to consider in bioremediation studies (Trindale et al., 2005), and information about site characterisation need be examined for proper evaluation of the feasibility of a bioremediation technology. Crude oil contamination of the environment is mostly derived from exploratory activities, spillages, tanker accidents etc, and simple hydrocarbons have been known to degrade faster than complex ones.

Bioremediation strategies

The goal of bioremediation is to degrade organic pollutants to concentrations that are undetectable, or if detectable, to concentrations below the limits established as safe or acceptable by regulatory agencies. Bioremediation has been used for the degradation of chemicals in soils, groundwater, wastewater, sludge, industrial wastewater systems, and gases. The list of compounds that may be subjected to biological decontamination by one or other bioremediation system is long. However, because they are widespread, constituting health or ecological hazards, and are susceptible to microbial detoxification, greater interest has been directed at oil and oil products, gasoline and its constituents, polycyclic aromatic hydrocarbons, and halogenated hydrocarbons. A variety of different bioremediation strategies and procedures are currently being used, and a number of new and promising approaches have been suggested or have reached advanced stages of development. Some of these are *in situ* while the others are *ex situ*.

Microorganisms in soil have a broad array of catabolic activities, and simple ways of degrading pollutants is to add the compounds or materials containing them to the soil and rely on the indigenous microflora. This procedure is called “land farming” or “land treatment”, and has been frequently used by the oil industry to decontaminate oily wastes. It has been utilised for many years. It is also employed if oily or hydrocarbon-rich materials are spilled on soil. In most cases, nutrients especially nitrogen and phosphorus are added in one form or the other, and the need for supplemental oxygen is met by mixing the soil in some ways, sometimes by simple ploughing and sometimes by more thorough mixing. Because surface soil often dries out, arrangements are made to provide water to maintain optimum moisture levels for aerobic organisms (National Research Council, 1993).

The efficacy of "land treatment" for spills of oil and oil products has been confirmed in carefully controlled experiments in the laboratory and in the field. Hydrocarbons in gasoline, jet fuel, and heating oil were found to be extensively degraded in soils in the laboratory that were treated with fertilisers, lime, and simulated tilling compared to soil not receiving these treatments. Land treatment is also a means to dispose off contaminating water (Lynch and Genes, 1989).

A variation of land farming technique is the so-called "prepared bed reactor" which included additional systems to provide irrigation water and nutrients, a liner at the bottom of the soil, and a means to collect leachate. Either clay or a synthetic material acts as the liner. These reactors are used at many Superfund sites in which bioremediation have been tried. Often, the contaminants are polycyclic aromatic hydrocarbons, BTEX (benzene, toluene, ethylbenzene, and xylenes) or both. The liner and a system to collect leachates are included because of the concern that conventional land treatment may result in contamination of the underlying groundwater with the parent compounds or products of microbial transformation that are carried downward with percolating water. Land treatment could also be applied to dispose contaminated water (Lynch and Genes, 1989).

Another method (solid-phase treatment) involves the same approach as land farming but relies on a different way of providing oxygen. Additional air is usually provided by vacuum extraction of oil above the water table, thereby supplying the terminal electron acceptor that is needed by the aerobic bacteria. This process, which is designed for hydrocarbon-contaminated sites, is termed bioventing or simply venting (Hinchee et al., 1991).

Adaptation of bioremediation for marine oil spills

Interest in the cleanup of oil spill in marine, estuarine, and fresh waters, and in the use of microorganisms for freeing the adjacent shorelines of oil, has existed for many years. However, it was not until the tanker "Exxon Valdez" was grounded on a reef in Prince Williams Sound, Alaska, that a major bioremediation of oil in surface waters was undertaken (Swannell et al., 1996). Early studies showed that although hydrocarbon-oxidising bacteria were widespread, nitrogen and phosphorus limitation occurs when oil was introduced into the water. This limitation could however, be corrected using formulations containing oleophilic fertilizers.

The occurrence of marine pollution has prompted the development and refinement of techniques for dealing with oil pollution both at sea and on shorelines. These include physical, chemical and biological methods. A number of different technologies may fall into the category of biological methods. These include the use of straw or plant material as an absorbent of oil (Tookey and Abbott, 1991); biosurfactants to cleanup oiled surfaces (Banat et al., 1991); biological polymers to coat surfaces to prevent oil adhesion; and the addition of materials to encourage microbiological biodegradation of oil (USCOTA, 1991), which has received the most attention, notably after the "Exxon Valdez" incident (Swannel et al., 1996). Biological methods can be most effective in the removal of thin oil films spread on the surface of water, where physical or chemical methods are not effective. However, most marine environmental conditions, such as low water temperature and low concentration of oil degrading microorganisms and inorganic nutrients, are not favourable for bioremediation (Oh et al., 2000).

To overcome these difficulties, bioremediation methods have focused on the addition of microorganisms or nutriaents. However, to counteract the effect of dilution in open-water systems, which were observed in the study by Tagger et al. (1983), most studies have tried to develop or evaluate oleophilic formulations which maintain nutrients or microorganisms at the oil-water interface, where oil biodegradation actively occurs (Oliviera et al., 1978). Recently, immobilisations of hydrocarbon-absorbing materials such as alginate (Li et al., 1995), wax (Rasnick, 1998), a microcapsule system, and polyurethane foam (Oh, et al., 2000) have been used to encourage the microbial biodegradation of oil.

Composting as a bioremediation process (solid phase treatment)

In composting as a treatment procedure, the polluted material is mixed together in a pile with solid organic substance that is itself reasonably readily degraded, such as fresh straw, wood chips, wood bark, or straw that had been used for livestock bedding. The pile is often supplemented with nitrogen, phosphorus, and possibly other inorganic nutrients. The material is placed in a single heap, formed in long rows known as windrows, or introduced into a large vessel equipped with some means of aeration. Moisture must be maintained, and aeration is provided either by mechanical mixing or by some aeration device. A container vessel is desirable when the compost contains hazardous chemicals. Heat released during microbial growth on the solid organic material is not adequately dissipated, and hence the temperature rises. The higher temperatures (50 - 60^{o}C) are often more favourable to biodegradation than the lower temperatures that are maintained in some composts (Alexander, 1994).

Slurry reactors

Bioremediation can be effected by a series of procedures in which contaminated soils are mixed with a liquid in a slurry-phase treatment. The system may be reasonably unsophisticated and entail introduction of the contaminated soil, sludge, or sediment into a lagoon that has been constructed with a liner or it may be a sophisticated reactor in which the contaminated materials are mixed. The operation in many ways resembles the activated sludge procedure that is common for the treatment of municipal wastes, and it allows for aeration, adequate mixing, and control of many of the factors affecting biodegradation. The level of dissolved oxygen, the pH, and the concentration of inorganic nutrients may be monitored and controlled. Some bioreactors are inoculated with a single species or a mixture of microorganisms able to function effectively under the controlled conditions. Slurry phased treatment has been extensively utilised for the bioremediation of various categories of organic pollutants (Mueller et al., 1991; Aronstein and Alexander, 1992). Slurry-phase procedures may also be combined with a washing technique to remove contaminants from soil (Compeau et al., 1991).

In situ groundwater biorestoration

A common procedure for *in situ* bioremediation entails the introduction of nutrients and oxygen into the subsurface aquifers, relying on the indigenous microflora to destroy the unwanted molecules. This process is sometimes called biorestoration (Testa and Winegardner, 1991). Most of the contaminated sites treated so far contain petroleum hydrocarbons as the contaminants. Leakage from underground storage tanks containing gasoline results in the appearance of benzene, toluene, ethylbenzene, and xylenes (BTEX). Although these BTEX compounds were initially in the gasoline phase, particular attention was given to them because they are toxic and because they could enter the aqueous phase in the form of a sustained release.

Initially, as much of the free oil or hydrocarbon as possible is removed by one of the several physical means. Bioremediation without such would be unreasonable because the bulk source would continue to add new chemical to the groundwater. The three nutrients that are commonly required for optimal activity are nitrogen, phosphorus, and oxygen, which are typically the factors that limit activities of indigenous microflora (Devine, 1992). The nitrogen, and phosphorus salts are usually dissolved in the groundwater that is circulated through injection wells into the saturated zones. The water is recovered from production wells, often amended with nutrients and re-circulated, or in some cases disposed of at the surface (Thomas et al., 1992).

The success of biorestoration depends on the hydrogeology of the site. If the hydrogeology is complex, success is problematic, and bioremediation sometimes will be of dubious value. Moreover, the subsurface environment must be sufficiently permeable to permit the transport of the added nitrogen, phosphorus, and oxygen to the microorganisms situated at the various subsurface sites containing the contaminants. This water movement, referred to as hydraulic conductivity, is critical to a positive outcome (Thomas et al., 1992).

CONCLUSION

The quest for a cheap source of energy coupled with the extensive rate of industrialization has expanded the frontiers of petroleum hydrocarbon exploration with its attendant negative consequence being the pollution of the environment. Several remediation alternatives have been in use for the restoration of polluted systems. Bioremediation, which exploits the biodegradative abilities of, live organisms and/or their products have proven to be the preferred alternative in the long-term restoration of petroleum hydrocarbon polluted systems, with the added advantage of cost efficiency and environmental friendliness. However, there is the need for further studies towards optimizing the process conditions for the application of bioremediation strategies in diverse climatic zones especially in extreme environments.

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