A REVIEW OF BIOLOGICAL METHODS TO REMEDIATE CRUDE OIL POLLUTED SOIL

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ABSTRACT

Environmental pollution by crude oil can occur naturally through seepage or from anthropogenic sources via spillage, accidents or improper disposal of crude oil and related products. The main characteristic of pollution from refinery and petrochemical plants consists in that the pollution source is active, most often for a short period of time, but has an important intensity, polluting agent consisting generally of petroleum fractions. Also, in most cases of accidental spills of petroleum products, surface soil affected is much smaller than the first surface polluted aquifer pollution front met. Recent research has shown that biological methods to remediate crude oil polluted soil are a superior effective and much cheaper method compared with physicochemical methods. Biological methods are based on the activity of microorganisms to use crude oil as a source of carbon and energy. These methods are considered to be the most effective because it does not have irreversible effects on soil characteristics. Microorganisms such as bacteria, cyanobacteria, yeasts and fungi break down these dangerous chemicals into less toxic or non-toxic compounds.

INTRODUCTION

Recent research has shown that bioremediation is a superior method of remediating soils, effective and much cheaper compared with physicochemical methods. Bioremediation processes has many advantages compared to other soil remediation processes (solvent extraction, adding oxidizing agents, etc.) making it an effective method of treating polluted environments (Gogoi et al., 2003; Morelli et al. 2005).

In-situ bioremediation is based on the activity of microorganisms to use petroleum hydrocarbons as a source of carbon and energy. This method is considered to be the most effective because it does not have irreversible effects on soil characteristics and low cost. Microorganisms such as bacteria, cyanobacteria, yeasts and fungi break down these dangerous chemicals into less toxic or non-toxic compounds (Dart and Stretton, 1994).

To survive, microorganisms need nutrients (such as nitrogen, phosphorus, potassium, trace elements), carbon and energy source. Microorganisms which are found naturally in soil transforme a number of organic compounds in food and energy for its own ecosystem. For example, many bacterial species from the soil can use petroleum hydrocarbons as a source of energy and food. This natural process transforms petroleum hydrocarbons in less hazardous substances such as carbon dioxide, water and fatty acids. Biodegradation of organic components has become a great way for treatment of polluted soils (Atlas, 1981; Atlas and Bartha, 1992).

Testing bioremediation held for the first time in 1989 (Exxon Valdez incident), 40.9 million gallons of crude oil polluted the 2200 km coastline of Prince William Sound, Alaska (Shaw, 1992). Fined $ 900 million, Exxon Trust contacted the Environmental Protection Agency (EPA) to find an immediate and effective solution. Noting the presence of the taxonomic varieties rich in microorganisms and good aeration of the soil polluted conditions, the researchers decided to use the method of bioremediation by adding nutrients (nitrogen, phosphorus) to increase the rate of biodegradability. Decontamination was a success and was done in record time (Harvey et al., 1990).

MATERIAL AND METHOD. RESULTS.

The advantage of this method is inexpensive device that does not have negative effects on the environment. A disadvantage of this problem is the low speed of the degradation process. Figure 1 presents the elements involved in bioremediation, as it was called by Suthersan (1999) "biodegradation triangle ".

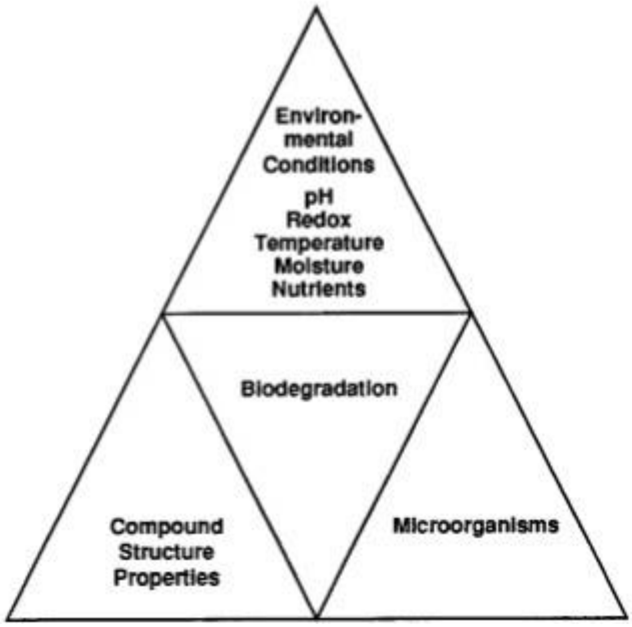

Figure 1 Elements involved in bioremediation ("Bioredegradation Triangle") (Suthersan, 1999)

Bacteria involved in biodegradation

Bacteria contain the necessary enzymatic equipment that breakdown petroleum hydrocarbons in soil. Microbial remediation of sites polluted with petroleum products and residues is due to a diverse group of microorganisms, particularly indigenous bacteria from soils affected by pollution (Farinazleen et al., 2004; dell'Abate et al., 2006). For example, the genus *Pseudomonas*, are a group of gram negative bacteria widely, with a remarkable ability to degrade a wide range of organic pollutants, including polycyclic aromatic hydrocarbons, halogenated and organic waste with recalcitrance molecular outstanding (Voiculescu et al. 2003).

The survival of microorganisms in an environment polluted with petroleum hydrocarbons is a key factor in determining the rate of biodegradability (Ramos et al., 1991 Rippen et al., 1994). Li et al. (2000) conducted a study in which improved microbial existing table by adding biologically active carbon. The soil used in the experiment in-situ is located in the Zihe valley, near the Zibo town, in eastern China and is polluted with petroleum hydrocarbons at concentrations up to 200 $g \cdot kg^{-1}$ dry soil. Quantitative determinations of bacterial microflora values of order 10^7 $cfu \cdot g^{-1}$ dry soil. The dominant species were identified in polluted soil *Xanthomonas*, *Bacillus* and *Hyphomicrobium*.

It is also interesting to note the bacteria location phenomenon. In the aqueous medium are few bacteria are hardly noticeable under the microscope that the bacteria involves the affinity of the hydrocarbon oil and tend to survive in the interface between crude oil and water, as shown in Figure 2 (Li et al., 2000).

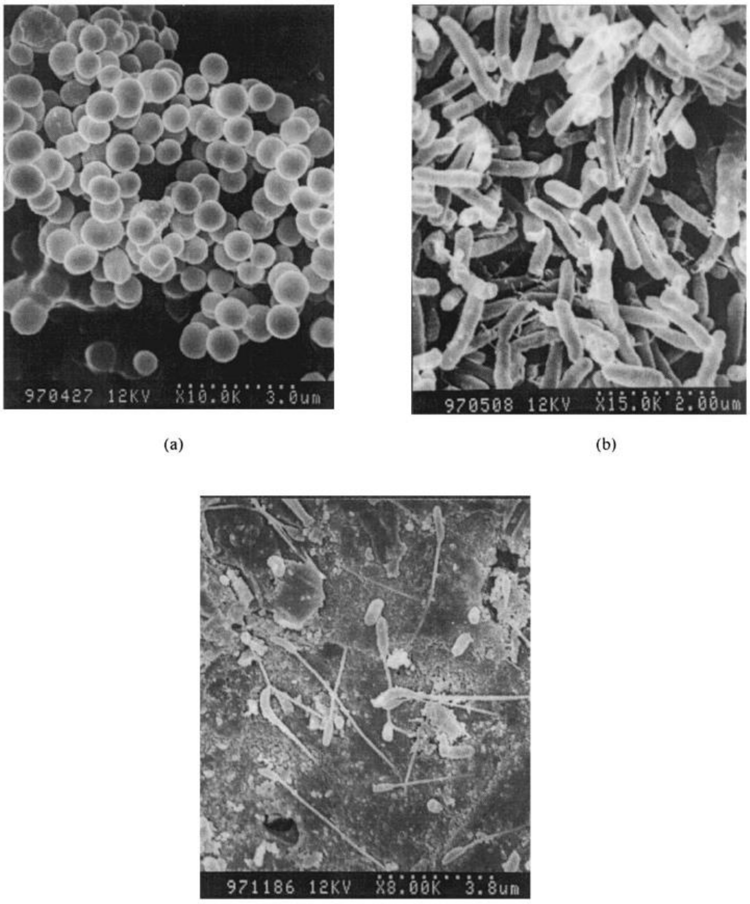

Figure 2 Images provided by electron microscope with different hydrocarbon degrading bacteria (Li et al., 2000)

Verma et al. (2006) conducted a study on the degradation of petroleum hydrocarbons in the presence of three bacterial species *Bacillus*, *Acinetobacter* and *Pseudomonas*. The 3 bacterial species were isolated from a soil polluted with crude oil in Ankleshwar, India. It was tested the ability to degrade bacterial species complex mixtures of petroleum hydrocarbons containing alkanes, aromatic hydrocarbons, resins and asphaltenes. Following investigations highest rate of biodegradability reached values of 59% in the presence of *Bacillus* species, followed by *Acinetobacter* and *Pseudomonas* where the values were 37% and, respectively, 35%.

Bacillus subtilis and *Pseudomonas aeruginoasa* efficiency on decreasing levels of petroleum hydrocarbons was studied by Das and Mukherjee (2007) on polluted soil from N-E India. The two types have proven very effective and can be used successfully in polluted soils with crude oil.

In Romania, have been conducted studies on the role of heterotrophic bacteria present in deposits of crude oil, the chemical shifts of oil deposits in certain hydrogeological conditions: temperature, pH, depth, oxygen concentration. Following research have been identified a set of 23 taxonomic groups, mostly to the genus level, belonging to the family *Enterobacteriaceae* and genera *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Aeromonas*, *Vibrio*, *Microbacterium*, *Flavobacterium*, *Corynebacterium*, *Mycobacterium*, *Arthrobacter*, *Brevibacterium*, *Nocardia*, *Bacillus*, *Micrococcus*, *Staphylococcus* and *Sarcina* (Voiculescu et al., 2003). Among these as predominantly was *Pseudomonas* genus. The most common of nesporulate was *Arthrobacter* bacteria and representatives bacilli of *Bacillus cereus*. Hull are listed by genus *Micrococcus*.

Cyanobacteria involved in the biodegradation

Cyanobacteria are organisms with the simplest needed, as achieve synthesis of organic matter and fix molecular nitrogen (N_2) from the air. They are unicellular beings, bacillary or spherical, widespread because it can adapt to extremely varied ecological conditions. Rarely live isolated, frequently appear united in mobile filaments, sometimes included in a mucilaginous mass (Voiculescu et al., 2003).

According to research, cyanobacteria involved in the biodegradation of petroleum hydrocarbons are *Anabaena*, *Nostoc*, *Oscillatoria*.

Yeasts involved in the biodegradation

Yeasts are unicellular fungi that reproduce by burgeoning fermentation and the theoretically work represents an ideal material to elucidate concepts of cell biology, the eukaryotic organisms (Voiculescu et al., 2003). According to research yeasts that occur in the biodegradation of petroleum hydrocarbons are *Candida*, *Rhodotorula*, *Saccharomyces*. Joo et al. (2008) conducted a study of soils polluted with petroleum hydrocarbons using a yeast *Candida catenulata*. After 13 days from the inoculation was observed a 84% decrease of petroleum hydrocarbons from soil polluted with 2% THP.

Filamentous fungi involved in biodegradation

Recent research has shown that a number of filamentous fungi play a key role in the integrated in-situ biodegradation and are able to degrade the fractions of C12-C26 aliphatic hydrocarbons from crude oil (Foght et al., 1999). Also, by growing on culture with the only power source the carbon contained in hydrocarbons it has been proven the ability to biodegrade crude oil some strains of the genera *Aspergillus*, *Penicillium*, *Paecilimyces* and *Fusarium*, *Cladosporium*, *Tricodarma* (Lemos et al., 2002, Potin et al., 2004).

Once isolated and purified, fungi were tested for the ability to degrade hydrocarbons from the crude oil. Sensitive removal of mycelium and conidia to each of the strains isolated in the tubes, they were inoculated into the liquid mineral medium where was added a quantity of crude oil as the sole source of carbon and energy. Were selected nine filamentous fungi that have proven the ability to use hidorcarbons from the crude oil to grow. Experiment conclusions was that filamentous fungi actively engaged in crude oil biodegradation in soil are *Aspergillus niveus, A. niger, A. versicolor, A. terreus, A. fumigatus, Penicillinium corylophilium, Paecilomyces variotti, P. Niveus* şi *Fusarrium* sp. The most active species *Aspergillus versicolor* had the highest pollutant removal efficiency (10.8%) (Voiculescu et al., 2003).

Filamentous fungi possess a number of attributes that can be very good potential agents of biodegradation. Some fungi were identified in sugarcane and used in inoculum for soils polluted with polychlorinated biphenyls (Fernández-Sánchez et al., 2001), phenanthrene (Chávez-Gómez et al., 2003), and benzo (a) pyrene (Dzul-Puc et al., 2004). Dzul-Puc et al. (2004) reported that filamentous fungus, *Penicillium frequentans* isolated from soils polluted with petroleum hydrocarbons, developed sugarcane, added in a soil artificially polluted with 200 ppm phenanthrene, achieved a biodegradability of 74% in 7 days, while *Penicillium frequentans* and *Psedomonas pickettii* achieved 73.6% in 18 days.

CONCLUSIONS

To decontaminate soils polluted with petroleum hydrocarbons, can be used physical, chemical and biological methods. From these, research has shown that, bioremediation, especially in the case of crude oil pollution, it is a superior method, more efficient and much cheaper than chemical or physical methods. Indeed, in recent years, bioremediation of soils polluted with petroleum hydrocarbons is a challenge of modern scientific research (Rahman et al., 2003). Bioremediation can be: in-situ and ex-situ. Bioremediation is based on the ability of microorganisms to use petroleum hydrocarbons as a source of carbon and energy. It is considered to be the most effective because in addition to lower cost, has irreversible effects on soil characteristics affected pedogenetical (Voiculescu et al., 2003).

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