Bioremediation of petroleum-contaminated soil: A Review

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Abstract. Petroleum is the major source of energy for various industries and daily life. Releasing petroleum into the environment whether accidentally or due to human activities is a main cause of soil pollution. Soil contaminated with petroleum has a serious hazard to human health and causes environmental problems as well. Petroleum pollutants, mainly hydrocarbon, are classified as priority pollutants. The application of microorganisms or microbial processes to remove or degrade contaminants from soil is called bioremediation. This microbiological decontamination is claimed to be an efficient, economic and versatile alternative to physicochemical treatment. This article presents an overview about bioremediation of petroleum-contaminated soil. It also includes an explanation about the types of bioremediation technologies as well as the processes.

1. Introduction
Petroleum is composed of hundreds or thousands of aliphatic, branched and aromatic hydrocarbons [1,2] and other organic compounds including some organometallic constituents [3]. Many activities such as industrial and municipal runoffs, effluent release, offshore and onshore petroleum industry activities as well as accidental spills cause petroleum hydrocarbon pollution. Most of them are toxic to humans, animals and vegetation [4-7]. In long term, this pollution affects the environment. Due to the adverse impact of these chemicals on human health and environment, they are classified as priority environmental pollutants by the US Environmental Protection Agency [8]. Releasing hydrocarbon pollutants through spillages and leakage from underground tanks, steamers, unplugging of oil wells, or abandoned oil refinery sites causes contamination of surface soil, groundwater and ocean [6,9-11].

2. Removal of petroleum hydrocarbon pollutants
Many methods for controlling oil contamination have been investigated including physicochemical and biological treatment. In physicochemical treatment, incineration, thermal desorption, coker, cement kiln, solvent extraction and land filling are used but they have some disadvantages [12]. Numerous physicochemical techniques decontamination methods are expensive due to the cost of excavation and transportation of large quantities of contaminated materials for ex-situ treatment. Green technologies for pollutant cleanup by biological means are used for bioremediation of petroleum polluted site(s) [13,14].

Bioremediation provides the most cost-effective and eco-friendly measurements for the remediation of petroleum contaminated soil and water to bring back its native environment [15]. Remediation refers to removing, degrading or transforming contaminants to harmless or less harmful substances. It includes methods that reduce mobility and migration of the contaminants, preventing their spreading to uncontaminated areas; toxicity of the contaminants remains unaltered, but the risk they pose to the environment is reduced [16]. The main molecules in crude oils and refine products are
Biodegradable, and they will eventually leave the environment as they are consumed by microbes. Bioremediation aims to stimulate the rate of this process [1].

Bioremediation is an innovative technique, which provides mitigation of microorganisms. It degrades or reduces hazardous organic pollutants to innocuous compounds such as CO₂, CH₄, H₂O and biomass without adversely affecting environment [17]. Hydrocarbons are natural energy-rich compounds. There are several hydrocarbon degrading or utilizing organisms available in nature. The use of individual indigenous microorganism or consortium as mitigation tool employs the catalytic abilities of living organisms to enhance the rate of pollutant degradation.

3. Bioremediation of petroleum hydrocarbons

Microbial bioremediation is a widely used technique for treating petroleum hydrocarbon pollution in both terrestrial and aquatic ecosystems [18-19]. The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities, is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry [20]. In addition, bioremediation technology is believed to be non-invasive and relatively cost-effective [21].

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) [22].

Bioremediation approaches are generally classified as in situ or ex situ. In situ bioremediation involves treating the polluted material at the site while ex situ involves the removal of the polluted material to be treated elsewhere [23]. In situ bioremediation can be described as the process whereby organic pollutants are biologically degraded under natural conditions to either carbon dioxide and water or an attenuated transformation product. It is a low-cost, low maintenance, environment-friendly and sustainable approach for the cleanup of polluted sites. With the need for excavation of the contaminated samples for treatment, the cost of ex situ bioremediation approaches can be higher than the cost of in situ methods. In addition, the rate of biodegradation and the consistency of the process outcome differ between the in situ- and ex situ bioremediation methods.

4. Factors affecting bioremediation of petroleum hydrocarbon pollutants

Different factors influencing hydrocarbon degradation have been reported by many authors and resumed on Table 1.

| Factor          | Effect                                                                 | Reference |
|-----------------|------------------------------------------------------------------------|-----------|
| Temperature     | Pollutants persist longer at lower temperature.                        | [24]      |
|                 | At low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation. | [25]      |
|                 | Temperature also affects the solubility of hydrocarbons. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. | [26]      |
|                 | Elevated temperature increases solubility of hydrocarbon pollutants, decreases viscosity and transfers long chain n-alkanes from solid phase to water phase. | [27,28]   |
|                 | Highest degradation rates that generally occur in the range 30–40°C in soil environments. | [29,30]   |
High temperatures and salinity hamper microbial growth and their products. [31]

Soil pH
- Microorganisms and enzymes exhibit pH-dependent activity. [24]
- pH is between 6 and 9

Oxygen availability
- Aerobic condition

Water content
- Transport of pollutants and the degraded products; degradation of pollutants. [24]

Reduction / oxidation potential
- Concentrations and ratios of electron donors/acceptors determine pathways and efficiency of degradation. [24]

Nutrients
- The acceleration of microbial turnover of chemical pollutants generally depends on the supply of carbon nutrients such as N and P. [34,35]
- To stimulate microbial degradation, nutrients in the form of fertilizers (water soluble (e.g., KNO₃, NaNO₃, NH₃NO₃, K₂HPO₄ and MgNH₄PO₄), slow release and oleophilic are added [36]

Type of pollutant / hydrocarbons
- The pollutants as substrates must be available and accessible either to microorganisms or to their extra cellular enzymes for metabolism to occur. [37]
- Biodegradability of hydrocarbons can be ranked as: linear alkanes > branched alkanes > low-molecular-weight alkylaromatics > monoaromatics > cyclic alkanes > polyaromatics_asphaltenes [38,39]
- Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all. [40]

Site condition
The soil properties and the indigenous soil microbial population affect the degree of biodegradation. [41]

Microbial communities
A successful strategy for in situ bioremediation can be the combination, in a single bacterial strain or in a syntrophic bacterial consortium, of different degrading abilities with genetic traits that provide selective advantages in a given environment. [42]

Organic matter
Influence degradation and sorption/entrapment. [24]

Co-contaminant / the presence of possible inhibitors
To know whether bioremediation itself is appropriate or not. [43]

5. Mechanism of petroleum hydrocarbon degradation
The most rapid and complete degradation of the majority of organic pollutants is brought under aerobic conditions. The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. Peripheral degradation pathways convert organic pollutants step by step into
intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis [33]. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants [44].

6. The possibility of bioremediation of petroleum-contaminated soil by using Material Preservation of Microorganism (MPMO)

It was previously reported that Material Preservation of Microorganism (MPMO) could be used to degrade organic wastewater from textile industry [45] and also as a starter in the processing of organic waste water of sugar factory [46]. The results could improve the quality of effluent and increase its environmental certificate of wastewater treatment plant.

Material Preservation of Microorganism (MPMO) is a storage system (in the form of tablet) of waste water decomposer bacteria that can survive for 1-5 years. Calcium bentonite acts as a medium (shell) to isolate bacteria. Breeding microorganisms are aerobic bacteria, Bacillus licheniformis. MPMO tablet provides the ease to microorganism users, especially in biological wastewater treatment.

Bacteria, as primary degraders of spilled oil in the environment, are the most active agents in petroleum degradation [47, 48]. It reported that the efficiency of biodegradation ranged from 0.13% [49] to 50% [50] for soil bacteria. Based on the ability of MPMO as a storage system, there is a possibility to store bacteria that can remediate petroleum-contaminated soil in MPMO.

7. Conclusions

Bioremediation is the main natural mechanism than can clean up the petroleum hydrocarbon pollutants from the environment. The process uses microscopic organisms (primarily bacteria) that live on soil and ‘eat’ petroleum hydrocarbon. A number of influencing degradation factors has been identified to reduce the toxicity of oil contamination in the environment by removing, degrading or transforming contaminants. Therefore, a successful bioremediation treatment requires understanding of those factors.

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