ROLE OF BACTERIA IN BIODEGRADATION OF PAHS: MINI REVIEW

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Abstract
Environmental pollution with petroleum hydrocarbons has been recognized as a prevalent and serious problem. Several methods has been employed for decontaminating the environment. Hydrocarbon interact with the terrestrial ecosystem and soil microorganisms shaping the destiny of the toxic contaminants comparative to their chemical nature and microbial degradative capabilities, respectively. Bioremediation by microbial degradation of petroleum hydrocarbons is considered a non-destructive, cost-effective, and sometimes logistically favorable cleanup technology. Bioremediation attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions. The most commonly reported genera of hydrocarbon-degraders include *Pseudomonas, Acinetobacter, Nocardia, Vibrio* and *Achromobacter.* This review emphasis on the bioremediation of petroleum contaminated soil and the role ofbacteria on degradation of polyaromatic hydrocarbons.

Keywords: *Environmental Pollution, Hydrocarbons, Microorganisms, Bioremediation, Biodegradation.*

Introduction

Pollution of the biosphere has increased strongly since the commencement of the industrial revolution.Hydrocarbon compounds for instance petroleum are essential elements of life. Since they do not naturally come about in the forms most useful to humans, they can be hazardous. Fuel and engine oil spills have develop into a major environmental hazard to-date [1]. The toxicity of petroleum hydrocarbons be determined through solubility and the bioavailability of the hydrocarbons. In the past, it was implicit that the water soluble fractions of the aromatics and polyaromatics are assumed to be mutagenic, teratogenic and carcinogenic in nature[2]. Conventional remediation approaches include physical removal of contaminated material through manual and chemicals, which can't be able to remove detrimental effects of organic pollutants[3].Biodegradation is an important practice in petroleum toxicology because it changes both the nature and concentration of the toxic chemical compounds.Biodegradation of such compounds is requisite by convention method that is affordable and ecofriendly [4, 5].Diverse range of microorganisms has the ability to clean up the hydrocarbon contaminated sites [6]. Microbes convert the chemical compounds into energy, cell mass and biological waste products[7]. Microorganisms have the ability to degrade popular hydrocarbon components, the saturated and unsaturated alkanes, monoaromatic and low molecular weight polycyclic aromatic hydrocarbons (PAHs).

Many microorganisms including bacteria, yeasts and fungi contribute to hydrocarbon degradation in the environment [8, 9]. In fact, approximately 79 bacterial genera have been reported to utilize hydrocarbons as a sole source of carbon and energy [8].Microorganisms, namely, *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus*were found to be involved for alkylaromatic degradation. Nine bacterial strains, namely, *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus* sp., *Alcaligenes*sp., *Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium* sp. were isolatedfrom the polluted stream which could degrade crude oil. *Acinetobacter* sp. wasfound to be capable of utilizing n-alkanes of chain length C10–C40 as a sole source of carbon [10]. Bacterial genera, namely, *Gordonia*, *Brevibacterium*, *Aeromicrobium, Dietzia*, *Burkholderia,* and *Mycobacterium* isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation[11].

The present review was aimed study the potent use of microorganisms and their activities for removal of soil pollution also leading to the recommendation of possible processes to improve soil biotreatability.

Bacterial species involved in Bioremediation

Most bacterial polycyclic aromatic hydrocarbon degraders have been isolated from contaminated/terrestrial environments[12-15]. Many microbial strains capable of degrading a specific compound are available commercially for bioremediation [16-18]. However, oil sludge is a complex mixtureof alkane, aromatic NSO (Nitrogen, Sulfur, Oxygen, containing compounds), and asphaltene fractions. A single bacterial species has only limited capacity to degrade all the fractions of hydrocarbons presents [19-22].

Various class of bacteria previously isolated from PAHs contaminated soil belong to *Pseudomonas* [23], *Vibrio*[24], *Mycobacterium*[25],*Comamonas*[26],*Arthrobacter*[27],*Burkholderia*[28],*Flavobacterium*[29],*Sphingomonas*[30].

Other species of PAHdegrading bacteria isolated from marine environment were reported as *Cycloclasticus*,*Moraxella* and*Marinobacter*[31].

Table 1: List of Bacteria capable of degrading petroleum hydrocarbonsCompound Microorganism

Compound	**Microorganism**
Alkanes	*Pseudomonas* sp., *Bacillus* sp., *Acinetobacter calcoaceticus*, *Micrococcus* sp., *Candida Antarctica, Nocardiaerythroplis*, *Ochrobactrum*sp., *Acinetobacter* sp., *Serratiamarcescens*, *Candidatropicalis*, *Alcaligenesodorans*, *Arthrobacter*sp., *Rhodococcus*sp.
Mono-Aromatic hydrocarbons	*Brevibacillus*sp., *Pseudomonas* sp., *Bacillus* sp.,*B. stereothermophilus*, *Vibrio* sp., *Corynebacterium* sp., *Ochrobactrum* sp., *Achromobacter*sp.
Poly-aromatic hydrocarbons	*Alcaligenesodorans, Sphingomonaspaucimobilis, Achromobacter*sp., *Mycobacterium* sp.,*Pseudomonas* sp., *Mycobacterium flavescens, Rhodococcus*sp., *Arthrobacter*sp., *Bacillus* sp., *Burkholderiacepacia., Xanthomonas*sp., *Alcaligenes*
Resins	*Pseudomonas* sp., Members of Vibrionaceae.,Enterobacteriaceea., *Moraxella*sp**.**

Hydrocarbon Degradation

There are two principle routes by which complex mixtures of hydrocarbons are degraded in nature.

Non-Biological

Many of the hydrocarbons undergo auto-oxidation by free-radical formation during exposure to UV light (< 400 nm), heat or metal ions. Free radical chain reactions lead to the formation of hydro peroxides that are most unstable and hence rapidly oxidized subsequently (Figure1).

Initiation	$RH \rightarrow R^{\bullet} + (H^{\bullet})$
Propagation	$R^{\bullet} + O_2 \rightarrow RO^{\bullet}_2$
	$RO^{\bullet}_2 + RH \rightarrow RO_2H + R^{\bullet}$
Termination	$R^{\bullet} + R^{\bullet} \rightarrow RH + R\ (-H)$
	$RO^{\bullet}_2 + {}^{\bullet}OH \rightarrow ROH + O_2$
	$R^{\bullet} + R^{\bullet} \rightarrow R - R$

Figure1: Free radical mechanism of hydrocarbon decay

At ambient temperature, the tertiary –CH groups in alkanes are oxidized more easily than the primary groups. Alkenes are preferentially degraded over alkanes except at double bonds. Many of the phenolic and some of the heterocyclic components of petroleum inhibit the rate of decay whereas some of the metal ions and organometallic compounds stimulate it.

Biological

There is a broad spectrum of distribution of microorganisms that have ability to degrade or transform hydrocarbons in nature.

Aliphatic hydrocarbons are preferentially utilized by many bacteria than aromatics. A generalized sequence of petroleumcomponents in order of decreasing biodegradability is representedas follows [32]*n*-alkanes > branched-chain alkanes >branched alkenes > low-molecular-weight *n*-alkyl aromatics>monoaromatics> cyclic alkanes >polynuclear aromatics>>asphaltenes.

Hydrocarbon degradation cannot occur extracellularly since membrane bound enzymes are involved in the degradation process. In order to metabolize the hydrocarbons, the microorganisms must attach to the oil molecule, which is followed by uptake through the cell membrane to the metabolic enzymes in the cell [33].

Two general biological strategies have been reported for enhancing contact between the bacteria and the substrate: specific adhesion mechanisms and emulsification or solubilization of hydrocarbons. Microorganism with high cell surface hydrophobicity can adhere to the hydrocarbons. This surface hydrophobicity is attained by the organism as a result of a variety of microstructures, i.e., fimbriae, fibrils, surface proteins and lipids [34]. The second strategy involves production of extra cellular solubilizing and emulsifying agents that may induce microbial growth on hydrocarbons. In some instances, the mechanism of substrate contact may consist of non-specific, passive diffusion of the substrate through the cellular envelope followed by specific interaction of the substrate with the enzyme responsible for the first step of its catabolism [33].

Aerobic Biodegradation

Aerobic biodegradation is the most efficient form of PAH bioremediation. The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Complete contaminant degradation results in the formation of CO_2 and H_2O. 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. The degradation of petroleum hydrocarbons can be mediated by specific enzyme system. Other mechanisms involved are attachment of microbial cells to the substrates and (2) production of biosurfactants . The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied.

The factors that may prevent microbial degradation and bioremediation listed as follows: 1) chemical concentrations that may be toxic to microorganisms; 2) conditions that are too acidic or alkaline; 3) lack of essential nutrients such as nitrogen, phosphorous, potassium, sulfur, and/or trace elements; 4) unfavorable moisture conditions (too wet or too dry); and 5) lack of oxygen or other electron acceptors. Typically, a lack of nutrients and/or oxygen would be a rate limiting parameter at the beginning of an *in situ* PAH bioremediation project. As time progresses, the total mass of readily degradable constituents are decreased and thus the oxygen requirements for active bioremediation are also decreased. Over time, dissolved oxygen supplied by natural ground water advection will be an adequate source of oxygen for continued in situ bioremediation [35].

Anaerobic Biodegradation

Although desirable for active hydrocarbon biodegradation, completely aerobic conditions are hard to implement in the field because of the low solubility of oxygen in water. It is possible that uneven distribution of water flow, nutrients and microbial populations creates a dynamic spectrum of aerobic, micro aerobic and anaerobic conditions. A basic idea for augmenting bioremediation under anaerobic conditions is to make electron acceptors available at concentrations higher than that of dissolved oxygen from air [36]. The ability of microorganism to degrade hydrocarbons under strictly anaerobic conditions is limited to a few strains and is typically much slower than aerobic degradation [37]. Although other oxidants such as nitrate, Fe (III), Mn (IV), sulphate and CO_2 can be used by many microorganisms as terminal electron acceptors that replace oxygen in respiration, they cannot replace oxygen as a direct reactant [38]. Sequential biodegradation of hydrocarbons may be used as an advance bioremediation approach. The initial oxidation (transformation) is affected by aerobic resting cells in a first remediation stage without promoting active growth that may cause increased oxygen demand. The oxidized metabolites are then degraded under the conditions of stimulated microbial activities where the respiration needs are meet by adding a water-soluble alternative electron acceptor, such as nitrate [38].Since hydrocarbon metabolites are potentially toxic, the ability of indigenous microorganisms to further degrade aerobic metabolites under denitrifying conditions is also important for the ultimate success of nitrate based bioremediation [39].

A common finding from most studies on biodegradation of aromatic hydrocarbons is that transformation under aerobic conditions is substantially faster than under anaerobic conditions [40].

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