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REVIEW ARTICLE **OPEN ACCESS**

Bacterial Assisted Bioremediation of Crude Oil Contaminated Environments Using *Pseudomonas Spp.*: A Review

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ABSTRACT
The development of petroleum industry into new frontiers and the apparent spillage occur during routine operations had invited more studies into oil pollution problems. It is in our hands to prevent these happenings. Biological weathering processes of oil degradation include evaporation, dissolution, dispersion, photochemical oxidation, water in oil emulsification, adsorption onto suspended particulate material, sinking and sedimentation. Chemical, thermal and biological methods are expensive, inadequate and unsatisfactory and may result in contamination of the environment, but then also they are the most widely used treatment procedures in oil spill clean-up. Bioremediation has received a considerable attention during the last few years. This review article discusses the chemicals released in oil pollution and the importance of applying bioremediation using Pseudomonas spp. as an alternative method in cleaning of oil polluted sites. Everyone has seen pictures of dying birds with feathers soaked in oil that cannot fly anymore and bays of dead fish because they cannot breathe or feed anymore. It is in our hands to prevent these happenings. Studies on this field may protect earth from this disaster.
Keywords: Bioremediation, Chemical, Thermal, Sedimentation, Photochemical oxidation.

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INTRODUCTION

Environmental pollution with petroleum and petrochemical products (complex mixtures of hydrocarbons) has been recognized as one of the most serious current problems, especially when associated with accidental spills on the large scale. If this occurs hydrocarbons may reach the water table before becoming immobilized in the soil. They spread horizontally on the ground water surface and continue to partition into ground water, soil pore space, air and to the surface of soil particles. Bioremediation has become an alternative way of remediation of oil polluted sites where the addition of specific microorganisms or enhancement of already present can improve biodegradation efficiency in both in-situ or ex-situ (in reactors) procedures [1], [2].

COMPOSITION OF CRUDE OIL

Crude oils are composed of mixtures of paraffin, alicyclic and aromatic hydrocarbons. Hydrocarbons are the simplest organic compounds. Containing only carbon and hydrogen, they can be straight chain, branched chain, or cyclic molecules. Carbon tends to form four bonds in a tetrahedral geometry. Hydrocarbon derivatives are formed when there is a substitution of a functional group at one or more of these positions.

Polycyclic aromatic hydrocarbons (PAHs) are prevalent contaminants in the environment as a result of fossil fuel combustion and by product waste from industrial activities. Many PAHs have mutagenic and carcinogenic properties and because they are only slightly soluble in water they are particularly recalcitrant [3].

BIOREMEDIATION

The key players in bioremediation are bacteria that live virtually everywhere. Microorganisms are suitable for contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily.In situ bioremediation can be regarded as an extension of the purpose that microorganisms have served in nature for billions of years: the breakdown of complex human, animal, and plant wastes so that life can continue from one generation to the next. Without the activity of microorganisms, the earth would literally be buried in wastes, and the nutrients necessary for the continuation of life would be locked up in detritus [4], [5].

Aerobic biodegradation of aliphatic hydrocarbons

Aerobic biodegradation of aliphatic hydrocarbons with bacterial strains depends on biological (enzymatic activity, steric hindrance, diffusion into the cells) and physiochemical parameters (solubility, emulsifier effect, surface tension). Most chemicals need intimate contact between bacteria and hydrocarbons. Bacteria are frequently seen attached to alkane droplets. The first step in the aerobic degradation of hydrocarbon is the incorporation of molecules of hydrocarbon. The most common pathway of alkane biodegradation is oxidation at the terminal methyl group. The alkane is oxidized first to an alcohol and then to the corresponding fatty acids. After formation of a carboxyl group the oxidation proceeds by successive removal of two carbon units through β oxidation, which is universal to most living systems. Under β oxidation the β methylene group is oxidized to a ketone group followed by the removal of a two carbon fragment from the compound [6],[7],[8].

Effects of oil spills on marine life

The earth has faced many disasters that have been caused by human species throughout the history. Today one of the most important hazards jeopardizing marine environments would be marine oil spills. Everyone has seen pictures of dying birds with feathers soaked in oil and that cannot fly anymore. It is in our hands to prevent these happening**.** Oil spills in the ocean have a negative effect on marine life, especially seabirds and filter-feeders. Seabirds, such as seagulls and ducks, spend most of their life on water and go to land only during their nesting period. The feathers of many seabirds are wettable and must be carefully dried for flight. If feathers come into contact with oil, the seabird ingests the oil while trying to dry. Filter-feeders, such as clams and oysters, take in surface water through their gills and filter it to take out microscopic food. If there is oil on the water, it gets concentrated within these shellfish, and then accumulates in any of their predators to a higher concentration [9],[10],[11].

Ways of demobilization of contaminants by microbes

Due to extraordinary genome and agile metabolism *Pseudomonas aeruginosa* is an extreme useful microorganism for Biotechnological applications, like crude oil degradation. The microbes may demobilize the contaminants well in 3 ways [12],[13].

- Microbial biomass can absorb hydrophobic organic molecules. Sufficient biomass grown in the path of contaminant migration could stop or slow contaminant movement. This concept is sometimes called a bio curtain.
- Microorganisms can produce reduced or oxidized species that cause metals to precipitate. Fe $(OH)_3$, FeS precipitates would be certain examples.
- Microorganisms can biodegrade organic compounds that bind with metals and keep the metals in solutions. Unbound metals often precipitate and are immobilized [14].

Pseudomonas

Pseudomonads are a large group of aerobic, nonsporing gram negative bacillus motile by polar flagella and belonging to the family *Pseudomonadaceae* containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches. They are ubiquitous, mostly saprophytic, being found in water, soil or other moist environments. Some of them are pathogenic to plants, insects and reptiles. A few cause human infection, typically opportunistic. Bacteria of the genus *Pseudomonas* belongs to the gamma subclass of the proteobacteria and is chemoorganotrophic, aerobic, and a respiratory rather than a fermentative metabolism [15].*Pseudomonas aeuroginosa* produces biofilms. Biofilms is a microcolonies which is enclosed in a polymeric substances [16].

Biofilm is a major response mechanism for the external stress factors by triggering phenotypic alterations like loss of motility, growth rate and susceptibility to host response [17],[18]. *Pseudomonas aeruginosa*'s ability to biodegrade hydrocarbons and other materials is mainly due to its genome. A major problem faced by scientists is that microorganisms cannot stick with the materials they need to degrade. Since *Pseudomonas aeruginosa* has the biofilm layer along with the fimbriae it has no problem attaching itself. The rhamnolipid biosurfactant produced by this organism influence various processes related to hydrocarbon degradation [19],[20].

The degradative plasmids

The degradative plasmids have been grouped into four categories

- OCT plasmid which degrade octane, hexane and decane [21],[22]
- XYL plasmid which degrade xylene and toluene [23],[24].
- CAM plasmid which degrade camphor [25],[26].
- NAH plasmids which degrade naphthalein [27],[28].

Characteristics of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a rapidly growing bacterium measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. Almost all strains are motile by means of a single polar flagellum, It will grow in the absence of O_2 if NO_3 is available as a respiratory electron acceptor. The typical *Pseudomonas* bacterium in nature might be found in a biofilm, attached to some surface or substrate, or in a planktonic form, as a unicellular organism, actively swimming by means of its flagellum. *Pseudomonas aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy five organic compounds for growth. Its optimum temperature for growth is 37°C and it is able to grow at temperatures as high as 42°C. It is resistant to high concentrations of salts and dyes, weak antiseptics and commonly used antibiotics [29],30].

Pigment production

Natural isolates from soil or water typically produce a small, rough colony. *Pseudomonasaeruginosa* strains produce two types of soluble pigments, the fluorescent pigment pyoverdin and the blue pigment Pyocyanin. The latter is produced abundantly in media of low iron content and functions in iron metabolism in the bacterium. *Pseudomonas aeruginosa* is otherwise called as *Pseudomonas pyocyanea* or *Bacillus pyocyaneus.*

According to Bergey's Manual of Systematic Bacteriology, organisms which produces pyoverdines belongs to the Pseudomonas RNA homology group 1 and it consists of different species of Pseudomonas like P. aeruginosa, P. chlororaphis, P. Fluorescens and P. syringae [31].The significant characteristics of Pseudomonas aeruginosa is the production of soluble pyocyanin pigments; it's a water soluble blue green phenazine compound [32].

Antibiotic resistance

It is notorious for its resistance to antibiotics and is therefore, a particularly dangerous and dreadful pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. *Pseudomonas spp*. are naturally resistant to penicillin and the related beta lactam antibiotics. It maintains antibiotic resistance plasmids, both R factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation [33].

USE AS BIOREMEDIATION AGENTS

It is possible that uneven distribution of water flow, nutrients, and microbial populations creates a dynamic spectrum of aerobic, microaerobic and anaerobic conditions. The ability of microorganisms to degrade hydrocarbons under strictly anaerobic conditions is very limited. It is practically absent for aliphatic compounds. Sequential biodegradation of hydrocarbons probably occurs in nature; the initial O_2 requiring oxidation occurs under aerobic or microaerobicconditions, and the subsequent mineralization may proceed even under anaerobic conditions. It should be noted that rhamnolipids are actively synthesized by stationary phase or resting *Pseudomonas aeruginosa*cultures. Therefore, they are also produced during the first stage of the proposed sequential bioremediation process and contribute to mobilizing and solubilizing the contaminants during the subsequent mineralization stage. Because *Pseudomonas aeruginosa* has these versatile, unique metabolic characteristics, it is the most suitable species for initial studies of sequential hydrocarbon metabolism [34],[35].

ORDER OF BIODEGRADATION OF PETROLEUM PRODUCTS

The order of decreasing susceptibility to biodegradation of petroleum constituents has been n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes > high molecular weight alkanes > polar compounds. The order of biodegradation of petroleum products was hexadecane = naphthalene >pristane>phytane>fluorenes>dibenzothiophene = phenanthrene> chrysene [36].

FACTORS AFFECTING BIOREMEDIATION

There are certain environmental factors that affect the bioremediation processes, such as temperature, pressure, oxygen level, salinity, pH, turbulence, back ground concentration of inorganic nutrients and the type of contaminants. The principal forces limiting the biodegradation of polluted petroleum in the sea are the resistant and toxic components of oil itself. Low winter temperature can limit rates of hydrocarbon biodegradation, increasing resident time of oil pollutant [37]. Other factors includes:

- Microbial population; Aerobic, anaerobic, ligninolytic fungi and methylotrophs.
- Environmental factors ; Nutrients, soil moisture, type of soil, soil PH, heavy metals [38],[39].

MAJOR APPROACHES FOR BIOREMEDIATION

There are three major approaches used for bioremediation of marine oil spills: Stimulation of indigenous microorganisms through addition of nutrients (fertilization), introduction of special assemblages of naturally occurring oil degrading microorganisms (seeding) and introduction of genetically engineered microorganisms (GEMs) with special oil degrading properties.Bacterial biodegradation of crude oil using local Isolates was done [40]. This experimental study was undertaken to assess the efficiency of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Acinetobacterlwoffi* isolated from petroleum contaminated water and soil samples to degrade crude oil, separately and in a mixed bacterial consortium. Enhanced crude oil hydrocarbon degradation by self-immobilized bacterial consortium culture on sawdust and oil palm empty fruit bunch was done [37]. The study demonstrated that bacteria immobilized onto carrier materials increases crude oil degradation by increasing production of biosurfactants.Aerobic degradation of petroleum components by microbial consortia done [36]. He isolated numerous microorganisms and studied their phylogeny and metabolic capacity to degrade a variety of aliphatic and aromatic hydrocarbons. *Pseudomonas i*solate P19 was able to degrade 56% of n-Hexadecane in 24 days [41].

A new method for the detection of oil degrading *Pseudomonas aeruginosa* based on transformation and PCR hybridization was done [42]. Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus* was done [43].

CONCLUSION

Oil-impacted soils already contain adequate indigenous microorganisms capable of degradation under suitable environmental conditions. Nutrient addition, especially nitrogen, may increase the rate of oil biodegradation when sediment nutrient levels are low, but O_2 availability appears to be the most important variable controlling oil degradation in marsh soils. Oil impacts on sediment demand $O_2$and restriction in O_2 exchange at the sediment–water interface can alter biogeochemical processes and gaseous exchange (CO_2 and CH_4) with the atmosphere [44].

Pseudomonas aeruginosa could be used widely for the degradation of oils. In the case of oil spills these organisms could be applied to the field. Because it can survive high stress conditions and also it possess oil degrading plasmids as well as it has only minute nutritional requirements. As this is a naturally occurring one it can be introduced easily into the oil spilled areas. *Pseudomonas spp.* is a natural competitor to control other pathogens in agriculture and aquaculture and it is suggested that *Pseudomonas* can be an ideal bio-control agent as it was a nonpathogenic and ideal competitor to control even *Actinomycetes,* fungi and viral pathogens [45].

The earth has faced many disasters that have been caused by human species throughout the history. Today one of the most important problem in the marine environments would be marine oil spills. Studies on this field may protect earth at least from this disaster. Research indicates that *Pseudomonas spp.* reserves the property to prevent the contamination of oil polluted areas of to a certain extent. Crude oil and n-Hexadecane degrading *Pseudomonas* spp. present on the most of the coastal areas. Presence of these organisms on the marine environments will reduce the pollution in water by accident oil spills to some extent. In accidental oil spill cases by the addition of nutrients (biostimulation), the growth of indigenous organisms can be stimulated to prevent oil pollution. The efficacy of biodegradation depends not only on chemical structure of contaminants but also on catabolic potential of microorganisms.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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