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BIODEGRADATION OF CRUDE OIL BY BACTERIA ISOLATED FROM CRUDE OIL CONTAMINATED SOIL – A REVIEW

[1,*]Bharathi, B., [2]Gayathiri, E., [1]Natarajan, S., [1]Selvadhas, S. and [1]Kalaikandhan, R.

[1]Department of Plant Biology and Plant Biotechnology, Loganatha Narayanasamy Govt, College, (Autonomous), Ponneri

[2]Department of Plant Biology and Plant Biotechnology, G.S. Gill Research Institute, Guru Nanak College, Chennai

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ABSTRACT

Bioremediation of oil spills requires the identification of microbes which have the ability to degrade hydrocarbons present in the soil or water, so that in case of a large spill these can be stimulated further in order to clean-up the area. The world today is very much dependent on crude oil, either to fuel the vast majority of its mechanized transportation equipment or as the primary feedstock for many of the petrochemical industries. Biological cleaning approach is done by adding microbes which have the potential to consume hydrocarbons to use as a food source, giving out water and carbon dioxide as waste products into the biodegradability of the hydrocarbons in crude oil and oil sludge. The review can be utilized to assess the role of varies factors which controls the success of bioremediation. These factors include the availability of bacteria that can metabolize the contaminant and utilizing it as a sole carbon source under favorable conditions. Based on the review obtained from this research work, bioremediation could be considered as a key component in the clean-up strategy for crude oil and oily sludge contamination.

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INTRODUCTION

Bioremediation is the process been used to remediate crude oil hydrocarbon contaminants in soil in past. The major constituents of most crude oils are biodegradable, so bioremediation has proven to be cheap and efficient than other techniques. Different organisms are employed using various techniques of bioremediation according to hydrocarbon present in the contaminated soil. Oil spills that occur during discharge from the refineries, accidents of ships/tankers, their grounding, rupture on seabed and on shore pipelines, offshore oil production and exploration platforms do affect these habitats causing irreversible damage to the biodiversity. This paper provides information on microbial degradation of hydrocarbon contaminants towards the better understanding in bioremediation challenges.

***Corresponding author:* Bharathi, B.,**
Department of Plant Biology and Plant Biotechnology, Loganatha Narayanasamy Govt, College, (Autonomous), Ponneri.

Review of literature

Bioremediation of hydrocarbons in crude oil The bioremediation of heavy metals from soil and aquatic environment:

An overview of principles and criteria of fundamental processes heavy metals are natural constituents of the environment, but indiscriminate use for human purposes has altered their geochemical cycles and biochemical balance. This result in excess release of heavy metals such as cadmium, copper, lead, nickel, zinc etc., into natural resources like the soil and aquatic environments. Prolonged exposure and higher accumulation of such heavy metals can have deleterious health effects on human life and aquatic biota. The role of microorganisms and plants in biotransformation of heavy metals into non-toxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites.

In view of this, the present review investigates the abilities of microorganisms and plants in terms of tolerance and degradation of heavy metals. Also, advances in bioremediation technologies and strategies to explore these immense and valuable biological resources for bioremediation are discussed. An assessment of the current status of technology deployment and suggestions for future bioremediation research has also been included. Finally, there is a discussion of the genetic and molecular basis of metal tolerance in microbes, with special reference to the genomics of heavy metal accumulator plants and the identification of functional genes involved intolerance and detoxification. (Ruchita Dixit *et al.,* 2015)

Different algal species were screened for petroleum degrading algae. It was isolated for degradation of oil in oily water sewer, contaminated rain water sewer, sanitary waste pipes and blow down streams. TOC, TSS, BOD, COD etc. have been studied to quantify the total oil degradation. Algae has many advantages because of its cost effectiveness and heat generation is minimal is case of algal cultures. So, we need to shift our focus on algal degradation of petroleum wastes. (Aditi *et al.,*2015). Hydrocarbon bioremediation efficiency by five indigenous bacterial strains isolated from contaminated soils. Twenty hydrocarbon degrading microorganism were isolated from four hydrocarbon contaminated sites and were identified on the basis of morphological and biochemical characteristics as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The study revealed high density of bacteria acclimatized for biodegradation of hydrocarbon (Petrol) in soil. The isolates were examined for other hydrocarbon degradation in media supplemented with Benzene, Toluene, Xylene and Cyclohexane at three different concentrations viz 5%, 10% and 15% incubated for 3 different time intervals 5, 10 and 15 days. The results indicated that all the isolates possessed potential to degrade the wide variety of hydrocarbons. The most efficient among them was *Pseudomonas aeruginosa* which degraded all tested hydrocarbon showing maximum growth at 5% concentration and 10 days incubation. It could be concluded that native flora of hydrocarbon contaminated site adapt to the environmental condition and could be implicated to remove hydrocarbons. (Sudhir K Shekhar *et al.,* 2015)

A laboratory scale study was conducted to investigate the effect of temperature on bioremediation of Libyan crude oil contaminated soil under mesophilic and thermophilic conditions. The initial crude oil concentration in the soil was determined to be 38,075 mg/kg of dry soil. The contaminated soil mixed with urea (as an inorganic amendment), hydrocarbon degrader bacteria plus urea or with wastewater activated sludge (as an organic amendment and microbial flora) was incubated at either 25°Cor 45°Cfor 12 weeks. A contaminated soil with no amendment was used as a control. The total petroleum hydrocarbons (TPHs) removal was observed regardless of the added amendments was 79.8% and 62.4% at mesophilic and at thermophilic temperatures, respectively. The maximum TPHs removal was obtained with the contaminated soil treated with urea plus hydrocarbon degrading bacteria. The lowest TPHs removal (<35%) was observed in the control treatment. The addition of activated sludge showed a very minor effect on the biodegradation rate compared to the control treatment. In short, the results show that:1) mesophilic temperature was more beneficial for biodegradation than thermophilic; 2) bioaugmentation of indigenous bacteria in the presence of nutrients enhanced TPHs removal; and 3) addition of activated sludge had no significant effect on biodegradation. (Rafik M.Hesnawi *et al*., 2013). Optimum petroleum rates by aerobic bacteria occur in temperature ranging from 15 to 30 °C (1993). (Englert *et al.,* 1993). Polycyclic aromatic hydrocarbons (PAHs) are the one of the most prevalent contaminants found in soil. The origin of PAH contaminated soils include anthropogenic sources such as abandoned manufactured gas sites, leaking underground storage tanks, wood treatment sites, and current industrial process (Breedveld *et al.*, 1994). PAH contamination can also occur “naturally” as a result of forest fires and volcanic activity. Due to the carcinogenic and recalcitrant nature of many of the PAHs, there is considerable concern over this wide spread contamination. (Harvey 1991). Biodegradation of accidentally spilled crude oil in aquatic and terrestrial environments has been the subject of recent reviews. (Tennyson *et al*., 1993). Unrefined crude oil accounts for majority of oil spills is the principle cargo of Supertankers, which hold up to 84 million gallons of crude oil; common sources of these are storage tank bottoms, oil- water clarifiers, the cleaning of processing equipment and biological waste water treatments.(Raghavan *et al.*,1999)

Microorganisms are considered to be the only biological source for degradation of hydrocarbons bacterial species have been shown to degrade hydrocarbon. The degradation was reported to be stimulated with fertilizer in laboratory (Scherrer *et al*., 1989). Crude oil causes imbalances in the C-N ratio at the spilled site. For efficient growth of bacteria, the C-N ratio should be around 60-100:1 (Dibble *et al*., 1979). Urea is added at the oil- spilled site to correct deficiencies in the nitrogen content of the soil, whether it is slower or greater, the growth of bacteria will be retarded. (Jobson *et al.,* 1972). Bioremediation can be used successfully to clean rocky, cobble and coarse sand shorelines. Field experiments were also focused on the ability of bioremediation of shoreline sediments. Methods that have been proved most effective in overcoming environmental limitations include oxygenation and nutrition. (Bragg *et al.,* 1994). Ward and Brock, (1978).Coast *et al.,* (1997) and Caldwell *et al.,* (1998) demonstrated that anaerobic hydrocarbon metabolism may be an important process in contaminated anoxic environments. (Ward *et al*., 1978).

PAH bioremediation approaches used in soils, which include tillage, nitrogen, and phosphorus addition and microbe inoculation. However, these approaches did not include the solution to the problem of unavailability of those compounds to microbial degradation. (Abbandanzi *et al.,* 2004). Bioavailability is determined by the rate of substrate mass transfer into microbial cells relative to their intrinsic catabolic and excretion activity. (Johnsen *et al.,* 2005). Recalcitrant components of fuels, such as di-benzothiophenes, polycyclic aromatic hydrocarbons (PAH), and compounds found in the unresolved complex mixture (UCM) will not be readily degraded during bioremediation. (Alexander, 1999). Soil contamination is a typical side-effect of industrial activity. Among the technologies available to deal with contaminated soils, bioremediation based on the metabolic activity of microorganisms has certain advantages; also it is cheaper than many other remediation technologies. Nevertheless, to assess whether a biological treatment of contaminated soil is appropriate, every location relating to its microbial populations and the biodegradability of its contaminants needs to be characterized. In addition, given the diversity of effects of additives such as fertilizers, inoculum is necessary to evaluate the influence of various factors that may affect the bioremediation process (Exner *et al.*, 1994). Petroleum transformation has employed mixed bacterial or fungal cultures in efforts to maximize biodegradation and demonstrated a consortium of 8 strains made up of members of 6 genera to be able to effectively degrading crude oil. Interestingly, only 5 of these strains were able to grow in pure cultures using a variety of hydrocarbons. However, when the other 3 strains were removed from the consortium, the effectiveness of the mixed culture was remarkably reduced. This further supports the theory that each member in a microbial community has significant roles and may need to depend on the presence of other species or strains to be able to survive when the source of energy is limited and confined to complex carbons. (Rambeloarisoa *et al.,* 1984)

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amounts of the hydrocarbons present. Bacteria are the most active agents in petroleum biodegradation and there is evidence of spilled oil in environment. However, one of the factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. (Geerdink *et al.,* 1996) Polycyclic aromatic hydrocarbons (PAH) constitute an important fraction of petroleum hydrocarbons and they are considered among the major contaminants in soil and water environments because many of these compounds have been found to be cytotoxic, mutagenic and potentially carcinogenic. They are exceedingly recalcitrant to degradation due to their inhibitory nature and their very low aqueous solubility. (Tsai *et al*., 2001)

The effectiveness of microbial inoculation in soil and sediments for bioremediation and pollution control may be determined by the following factors:

- Concentration of active inoculants
- Interaction between added micro- organisms and indigenous populations.
- Nutrient (including electron acceptor) supplies to the target microorganism.
- Availability of target compound to the added microorganism and
- Effects of heterogeneous materials on the biodegradation process. Manipulation of these factors has become a critical issue in an inoculation practice. (Lin *et al.*, 1993)

Soils and sediments contaminated with PAH, the degradation of high molecular weight compounds (those with four or more rings) by indigenous microorganisms is usually much slower or less extensive than the degradation of low molecular weight PAH. (Herbes *et al.,* 1978). The ability of indigenous microbial communities to mineralize or otherwise degrade the high molecular weight compounds also appear to the site specific. (Grosser *et al.,* 1991). There are several possible explanations for the apparent recalcitrance of compounds and the variability between sites, including effects of bioavailability, the occurrence of degrades of a specific compounds, or complex interactions among substrates leading to the inhibition of the degradation of one PAH in the presence of other PAH. (Weissenfels *et al.,* 1992)

Bacteria, algae and fungi are capable of degrading many PAHs have been isolated and characterized. (Cerniglia, 1992); Bacteria that can degrade NHA, such as carbazole, have been isolated, and intermediates of carbazole degradation have been identified, including 3-hydroxy-carbazole, anthranilic acid, and catechol carbazole degradation by *Pseudomonas sp*. CA_{10} and LD_2 has been proposed to occur through carbazole 1, 9 a-dioxygenase and catecholCarbazole degradation by 2'-aminophenyl 1-2-3 dihydrated has been isolated from cultures of CA_{10} Carbazole degradation by strain LD_2 may include a second pathway involving indole-3-acetic acid.(Resnick *et al.,* 1993). Isolation and characterization of intermediates of carbazole degradation using an isolated soil bacterium, *Ralstonia sp*. Strain RJG11.123 used in the bioremediation of NHA from contaminated soils. (Gieg et al., 1996). Polycyclic aromatic hydrocarbons (PAHs) of natural and anthropogenic origin were widely distributed in soils and aquatic environments. The occurrence of PAHs in aquatic and terrestrial ecosystems has received considerable attention since. Many of these compounds are recognized as mutagens and carcinogens. (Blumer *et al.,* 1976)

Oily sludge

Oil is both the principle source of energy for man and an important environmental pollutant. In addition to accidental contamination of ecosystems by oil spills, vast amounts of oily sludge generated in refineries from accumulated oily waste materials in the bottom of storage tanks and water-oil separation systems pose great challenges because of the expense of disposal (Ferrari *et al.,* 1996). Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be eliminated from the environment. (Leahy and Colwell, 1990) Land farming of petroleum hydrocarbon wastes involves the use of the natural biological, chemical, and physical process in the petroleum-contaminated soil to transform the organic contaminants of concern (Harmsen, 1991)

Biological activity apparently accounts for most of the transformation of organic contaminants in oil, although physical and chemical mechanisms may provide significant pathways for some compounds. Land farming concept serves as a basis for the design and operation of soil bioremediation technologies in a large number of waste sites requiring cleanup. (Pope *et al.*, 1993). Accidental release of petroleum hydrocarbons are of particular concern in Arctic environments. Cold temperatures, short summers, and remote locations combine to make remediation of these sites especially challenging. Over the past several years, a number of studies in both regions have shown that microorganisms naturally occurring in these harsh environments are capable of degrading petroleum hydrocarbons. However, the slow degradation rates measured under ambient conditions generally preclude natural contaminant attenuation within reasonable time frames (Rike *et al.,*2003). Oily sludge is much more difficult for the bioremediation. Numerous researches have demonstrated high bioremediation efficiency for oil polluted soils, but these methods have limitations for the oily sludge mainly dealt with extremely high pollution level and recalcitrance of contaminants for biodegradation. The branched alkanes are more resistant to microbial degradation than n-alkanes due to their molecular structure.

Furthermore, Larger molecular are considered to be less degradable than smaller ones (Gogoi *et al.,* 2003). Land farming also known as land treatment is a treatment technology that involves the controlled application of a waste on the soil surface and the incorporation of that waste into the upper soil zone. Saudi Arabia has the largest oil reserves in the world and produces approximately 8 million barrels of crude oil every day. With seven refineries, 22 bulk plants, several terminals and operating tank farms, oily sludge is one of the largest categories of generated industrial wastes. (American Petroleum Institute, 1983). Oil production processing and important quantities of oily sludges or slops are generated, which represent a source of pollution for the environment and, at the same time, block important spaces in tanks or where they are stored. The oily sludge, frequently present in oil production or processing sites, contain different concentrations of waste oil 40-60%., waste water 30-90% and mineral particles 5-40% (Francis and Stehmeyer, 1989). Bioremediation of polluted sites is considered an effective biotechnology with a range of advantages compared to traditional technologies, as bioremediation is based on the ability of microorganisms to degrade the waste hydrocarbons to non-toxic products such as HO, CO and biomass (Leahy and Colwell, 1990).

Bioremediation could be a cost- effective clean-up technology to treat oily sludge and sediments containing biodegradation hydrocarbons and indigenous specialized microorganisms (Jackson *et al.,* 1996). Bioremediation has become a major method employed in the restoration of oil polluted environments, attempts to accelerate the natural hydrocarbon degradation rates by overcoming factors that limit bacterial hydrocarbon degrading activities. Biodegradation of petroleum hydrocarbons is complex process that depends and the nature amount of oil or hydrocarbon present. (Atlas and Bartha, 1992) Indigenous populations of microorganisms, which are ubiquitous in soil and soil and ground water and self-adapted to hard conditions, actually grow by using the carbon from the pollutants as energy source and cells building blocks. This break down the contaminants into carbon- di- oxide and water as end products. Despite decade of research successful bio treatment of petroleum hydrocarbon contaminated sites remains a challenge and several factors must be fulfilled and optimized to determine the outcome of the biodegradation process such as: biomass concentration, population diversity, bacterial growth, metabolic pathways nature and concentration of pollutants, chemical structure of organic compounds, and toxicity of contaminants and presence of nutrients. (Scrag, 1999).

Enumeration of oil degrading bacteria usually involves growth on a medium that contains crude oil or a refined petroleum product as the selective substrate. Because these complex substrate contain both aliphatic and aromatic compounds. Refinery processing of crude oil generates oil sludge as waste end products, which are poorly biodegradable, constituting environmental problems, with substantial implications for economic development and human health oil sludge bioremediation may be cost effective and environmentally sustainable (Mulkis-Philips *et al.,* 1974). The effect and possible use for corn waste in the ex-situ bioremediation of an oil sludge – contaminated soil, showed that optimal microbial biodegradation can be achieved using a bioaugmentation + biostimulation system, with corn waste as an absorbent. (Cunningham *et al*., 2000)

Bio surfactants

Surfactants constitute an important class of industrial chemical widely used in almost every sector of modern industry, are surface active compounds capable of reducing surface and interfacial tension between liquids, solids and gases. Most of the surface active compounds currently in us are chemically synthesized. However, increasing environmental awareness has led to serious consideration of biological surfactant as possible alternatives to existing products. (Desai *et al*, 1997) Microbial produced surfactants offer several advantages over their chemical counterparts. Since microorganisms have diverse synthetic capability, they can produce a range of biosurfactants. Novel compounds providing new possibilities for industrial application. However the most important advantage of bio surfactant is probably an ecological acceptance. Biosurfactants are biodegradable and can be produced from renewable substrates (Parra *et al*., 1989). Microbial surfactants are complex molecules, comprising the wide variety of chemical structures, such as glycolipids, lipopeptides, fatty acids, polysaccharide protein complexes, peptides, phospholipids and neutrolipids. Rhamnolipids produced by *Pseudomonas aeruginosa* have been widely studied. (Lang and Wullbrandt, 1999)

Biosurfactants and bioemulsifiers are not only of interest for bioremediation process in the petroleum industry. These compounds can be used to enhance oil recovery from wells, reduce the heavy oil viscosity, clean oil storage tank, increase flow through pipelines and stabilize fuel water-oil emulsions (Ghurye *et al.,* 1994). *Pseudomonas aeruginosa* UG2 was isolated from soil contaminated with oily wastes and was found to produce highly emulsifying activity when grown on various substrates (Berg *et al.,* 1990). Biosurfactants are compounds produced on microbial cell surfaces or executed extra cellular which contain both hydrophilic and hydrophobic moieties. Many used have been proposed for bio surfactants including remediation of hydrophobic compounds from soil. (Van Dyke *et al.,* 1991)

Bio surfactants are known to be produced by hydro carbonolistic micro-organisms during their growth on hydrocarbons and carbohydrates. The formation of biosurfactants by different species of *Pseudomonas* from various carbon sources such as glucose, glycerol and hydrocarbons has been reported (Jayani and Joshi, 1992). Poly aromatic hydrocarbons are the most obvious pollutant in both terrestrial and aquatic realm. Its damage in the agricultural fields is normal water systems are well documented. PAHs pollution leads to drastic effecting flora and fauna of the aquatic systems and its directly affecting the human life, because of this carcinogenicity and mutagenicity biodegradation appears to be the best method in controlling PAH pollution effectively. Biosurfactants are produced during hydrocarbon degradation by bacteria and they influence the degradation rate. (Mahesh *et al.,* 2006)

DISCUSSION

The reviews presented above are expected to provide a meaningful insight into the biodegradability of the hydrocarbons in crude oil and oil sludge. The experimental results of this study can be utilized to assess the role of varies factors which controls the success of bioremediation. The initial population of bacteria and catabolic activities in a community are important factors in determining its efficacy for petroleum degradation. Optimization of the relative number of bacterial population may be almost impossible, but the strategy and review taken in this study, namely enrichment of bacteria by growth on crude oil may be one way to select the microbial population for better crude oil degradation.

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