Indian Journal of Environmental Sciences 25(2) 2021, pp.101-106. ISSN 0971-8958
Green Earth Foundation

BIOREMEDIATION OF PETROLEUM WASTE CONTAMINATED SOILS: A REVIEW

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Received: 26th July 2021; Revised:30th July2021; Accepted : 31st August 2021

ABSTRACT

Since the industrial revolution, petroleum hydrocarbons are deleterious environmental contaminants which when introduced into the gleaming environment immediately diminishes its quality. The universal processes of extraction, refining, transporting and accidental spillage of petroleum contribute to soil pollution. Being toxic, mutagenic and carcinogenic, the petroleum hydrocarbons are a serious hazard to the environment. Bioremediation has appeared as one of the most promising treatment options carried out using microorganisms. It does not implement intensive chemical and mechanical treatments and has emerged as an environmental friendly and cost effective remediation approach for the removal of petroleum hydrocarbons. A successful bioremediation necessitates a better understanding of biodegradation mechanisms to accelerate transformation of petroleum hydrocarbon pollutants into less toxic and harmless by-products preserving environment and human health. This paper provides an overview of the recent literature inferring microbes as biodegraders, different approaches of bioremediation and provides suggestions for further developments.

Keywords: Bioremediation, biodegradation, microbes, petroleum hydrocarbons, pollution.

INTRODUCTION

Soil is one of the pristine natural resources which should be delicately and carefully protected. High levels of toxic contaminants pollute the soil. These soil pollutants not only diminish crop yields but also render it unsafe for consumption, thus reducing food security. Soil contaminated with petroleum hydrocarbons is a worldwide environmental problem. Petroleum is a complex mixture of hydrocarbons with different physical and chemical properties (Wang et al. 1998). Petroleum-based hydrocarbons are the major source of energy and raw materials for everyday use for industrial, commercial and domestic purposes. Anthropogenic activities such as mining, crude oil extraction etc. have revolutionized our society but our lives are made more comfortable at the cost of many folds increase in the environmental pollution.

The treatment and disposal of petroleum waste is a serious problem in recent years. The pollutants are released into nearby water bodies and soil due to lack of effective purification systems (Musa et al. 2015). Because of deleterious effect of these chemicals on human health and environment in the long term, they are classified as priority environmental pollutants by the US Environmental Protection Agency (1986). Not only accidental release of petroleum and petroleum products into the environment but also human activities concerning their production, refining, transportation, and storage, are the main cause of pollution (Varjani 2017, Yuniati 2018). The petroleum refineries effluent contains a huge quantity of petroleum byproducts, polycyclic and aromatic hydrocarbons, phenols, surfactants, sulfides, naphthaylenic acids and metals, including barium, lead, zinc, mercury, chromium, arsenic, and nickel. The constituents of petroleum industry effluents and oily sludge are hazardous to the environment as well as pose direct or indirect health risk to all life forms on the earth as they are toxic, mutagenic and carcinogenic (Varjani et al. 2019). Soil contamination with oil spills has been reported to be the most threatening and a major global concern today as it causes organic pollution of groundwater which limits its use, decreases the productivity of the agricultural soil and affects the indigenous organisms that dwell in the soil and destroy the food chain (Thapa et al. 2012).

Remediation of Petroleum Hydrocarbons

Several physical, chemical, and biological methods can be used for degradation of petroleum waste components (Singh et al. 2009). The best degradation technique is selected depending upon the quality and quantity of the contaminants, treatment costs, type of the soil and environmental conditions like temperature, pH etc. at the polluted sites (US. DOD 1994).

The physical methods such as activated carbon adsorption, membrane filtration, dissolved salts removal by reverse osmosis, ultra-filtration, evaporation etc. and chemical methods like precipitation, electrochemical lytic processes, Fenton process, and photo-catalytic degradation, ultrasonication, etc. have been implemented for petroleum refinery wastewater treatment. But these methods have various restrictions like production of voluminous sludge, high cost of equipment, high operating costs etc. which makes them less economical and feasible (Hu et al. 2015, Singh et al. 2017). Moreover, low solubility, nonpolarity, and hydrophobic nature of petroleum hydrocarbons lay more challenges in the way of remediation (Vidali 2001).

Bioremediation has been recognized as one of the most propitious treatment alternatives for oil contamination (Bragg et al. 1994). It is an inexpensive, effective and eco-friendly approach as compared to other treatments. The process of bioremediation is defined as the use of microorganisms in the removal of pollutants from soil and terrestrial environments. Microorganisms have infallible capabilities of degrading all conventionally occurring compounds. These metabolic and enzymatic capabilities are exploited to detoxify or mineralize pollutants. Biodegradation of petroleum hydrocarbons is a complex process that depends on the quality and quantity of hydrocarbons present.

Indigenous or native hydrocarbon degrading microorganisms exhibit an instrumental role in the bioremediation process (Wilkes et al. 2016). Bacteria are outlined as principal degraders and most active bioremediating agents (Dell'Anno 2012, Meckenstock et al. 2016). Myriad studies have divulged that umpteen native hydrocarbon-degrading bacteria are present in oil spill areas and oil reservoirs (Yang et al. 2015), and their abundance depends on the types of petroleum hydrocarbons and the environmental factors prevailing in the surrounding (Fuentes et al. 2015).

From current studies, approximately seventy nine bacterial genera have been identified that are capable of degrading petroleum hydrocarbons (Tremblay et al. 2017). Some of them which play pivotal role in bioremediation are *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Dietzia*, *Enterobacter*, *Marinobacter*, *Mycobacterium*, *Pandoraea*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Nocardia* and *Rhodococcus* (Roy et al. 2002, Foght 2008, Varjani 2017, Xu et al. 2018). It has been reported that the efficiency of biodegradation ranged from 0.13% to 50% for soil bacteria (Van Beilen et al. 2006). Microorganisms utilize petroleum hydrocarbons as a source of carbon and energy. Initially, dispelling of small chain (C1-C6) and saturated hydrocarbons occurs and then the aromatics are consumed during remediation (Das & Chandran 2011, Vandecasteele 2008). However, the feeble biodiversity of indigenous microflora and specialized microbes with complementary substrate specificity put limitation in the remediation of crude oil polluted sites (Ron & Rosenberg 2014). Thus, bioremediation involves the use of indigenous as well as introduced microorganisms to degrade environmental contaminants. The different approaches for bioremediation of crude oil polluted sites are (a) biostimulation, (b) bioaugmentation, (c) microbial consortium and (d) microbial cell immobilization systems.

(a). **Biostimulation** involves modification of the existing environmental conditions by the addition of substrates and oxygen that trigger microorganism activity to degrade the petroleum hydrocarbons faster (Basharudin 2008).

(b). **Bioaugmentation** implies addition of known oil degrading bacteria harboring specific catabolic abilities to complement the existing microbial population into a contaminated environment (Holliger et al.1997).

(c). **Consortium** is the mixed cultures of different hydrocarbon degrading microorganisms used together to clean the oil polluted sites. Different members of the consortium produce discrete enzymes that convert recalcitrant compounds to simpler form. Smaller compounds are again taken up by other series of microbes and degraded wholly. This implies that the complete remediation of petroleum hydrocarbon contamination requires the collaborative functioning of multifarious bacteria (Dombrowski et al. 2016). Based on this view, Varjani et al. (2015) constructed a halotolerant Hydrocarbon Utilizing Bacterial Consortium (HUBC) consisting of the bacterial isolates *Ochrobactrum* sp., *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* that was found to be good at degrading crude oil (3% v/v), with a degradation percentage as high as 83.49%. This has accredited to the fact that the metabolic versatility of mixed cultures is superior to pure cultures in utilizing hydrocarbon pollutants in petroleum crude as the sole carbon source (Varjani & Upasani 2013). A study conducted by Mandal and colleagues (2012) also reported that an indigenous microbial consortium of bacterial species when applied on the field scale at different oil refineries in India bioremediated 48,914 tons of different types of oily wastes successfully (total petroleum hydrocarbon (TPH)) to environment friendly end products.

(d) The use of immobilized microbial cell is another propitious approach that can be applied for bioremediation of petrowaste. Several methods used to prepare immobilized cells are physical adsorption, cross linking, covalent bonding, encapsulation and gel entrapment. The potential advantages of immobilization of cells using different carrier or support materials over free cells are maintaining sufficient microbial activity for a longer duration of time, are reusable, easily detached and show superior biodegradation efficiencies even at nonoptimal environmental conditions such as high pollutant concentrations, improper temperature, and pH (Partovinia and Rasekh 2018).

Generally, bioremediation technologies can be executed as *in situ* or *ex situ*. *In situ* remediation, the polluted soil is treated as it is at the original site under natural conditions. It is a cost effective, effortless, ecofriendly and tenable approach for the restoration of polluted sites (Partovinia et al. 2010). However, in *ex situ* bioremediation involve excavation and transportation of the polluted soil for treatment at above-ground facilities which makes the process costly, but as stated earlier *ex situ* bioremediation may be advantageously performed when the environmental conditions at the original polluted soil are suboptimal for microbial activity, for eg, the temperature is too low or if the nutrients are unevenly distributed. Several studies have mentioned various *in-situ* and *ex-situ* remediation techniques such as biosparging, bioventing, land farming, composting, soil biopiles, and biotrickling (Boopathy 2000, Partovinia et al. 2010).

Factors Affecting Biodegradation of Petroleum Hydrocarbon

The biodegradation of petroleum hydrocarbons is a complicated process controlled by various factors. The native structure and composition of the petroleum hydrocarbon pollutant is the prime factor (Okoh 2006). Linear and branched chain hydrocarbons with simpler structure can be easily biodegraded but the structural complexity of hydrocarbon makes them less susceptible to degradation. Alkylated aromatics, monoaromatics, cyclic alkanes > polyaromatics hydrocarbons (PAHs) are degradation resistant in increasing order (Ulrici 2000).

The microorganisms with suitable metabolic capabilities are another key requirement. The optimal rates of microbial growth and hydrocarbon biodegradation depend upon environmental conditions like temperature, pH, adequate concentrations of nutrients and oxygen availability. The most of oil degrading microorganisms are mesothermic degrading maximum at 30°C-40°C. At lower temperatures, the oil viscosity increases, making the oil more toxic and less appealing to degrading microbes (Atlas 1975). The optimal pH for oil degradation lies between 6 and 9. Therefore, pH of most of the petro waste contaminated sites is preferably adjusted to this optimal range adding lime (Alexander 1995). The microbial requirement for minerals like nitrogen, phosphorus and potassium is fulfilled by addition of urea, phosphate, N-P-K fertilizers, ammonium and phosphate salts to speed up the biodegradation of petroleum hydrocarbon pollutants (Boopathy 2000, Ron & Rosenberg 2014).The success of bioremediation depends on having the appropriate microorganisms in place under suitable environmental conditions and composition of the contaminant.

Petroleum oil degradation by bacteria can occur under both aerobic and anaerobic conditions (Zengler et al. 1999). Under aerobic conditions, O_2 acts as a powerful oxidant which oxidizes and cleaves the ring of aromatic petrochemical compounds. Oxygen serves as the final electron acceptor and a co-substrate for some key catabolic processes (Singh et al. 2017). The enzyme oxygenase plays key role in incorporation of molecular oxygen into the reduced substrate (Li & Liu 2002). Aliphatic hydrocarbons are initially converted into alcohols which are oxidized sequentially via dehydrogenases to carboxylic acids, then undergo â-oxidation to yield acetyl CoA, which is metabolised in the usual manner (Abbasian et al. 2015). In aromatic and polycyclic aromatic hydrocarbons (PAHs), the enzyme mono- or dioxygenase causes hydroxylation of the ring to form diols which then cleaved and further degraded (Goyal and Zylstra 1997).

Biosurfactants have been outlined as another factor playing a key role in bioremediation of oil sludge (Cameotra & Singh. 2008). These are divergent groups of surface active chemical compounds produced by a variety of microorganisms which increase bioavailability of pollutants by solubilizing them.

CONCLUSION

The remediation of petroleum hydrocarbon pollutants in the environment is a matter of concern. Bioremediation has turned up as an efficient, cost effective and universal substitute for physicochemical techniques. By using microorganisms either individually or within consortium, harmful petroleum pollutants can be transformed to nontoxic compounds. Therefore, some of the approaches should be focused on narrowing the gaps in the field. Foremost the screening of the microbial strains suitable for environmental conditions at the contaminated site and establishing criterion for their introduction to the site should be done. Further, construction of novel bacterial strains using genetic engineering would give more potency for petroleum hydrocarbon degradation. The exploration of biocompatible surfactants for enhancing contact between bacteria and petroleum hydrocarbons is another requisite**.** Successful implementation of these strategies would help in achieving a significant reduction in petroleum hydrocarbon pollution and enhancing soil quality, protecting the environment and human health.

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