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Bioremediation of crude oil polluted soil and biological nitrogen fixation in association with indigenous oil degrading bacteria-A perspective analysis

Dr.Pushplata Jadhav[1], Dr. Purnima Sable[2]

Professor and Head,Department of Microbiology,Deogiri College, Aurangabad (MS) 431001,India[1]

Professor, Department of Botany, S.M.B.S.Thorat College, Sangamner, Maharashtra ,India[2]

Abstract: With the sharp increase in population and modernization of society, environmental pollution resulting from petroleum hydrocarbons has increased, resulting in an urgent need for remediation. Accidental release of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost – effective and will lead to complete mineralisation Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbondioxide, water, inorganic compounds and cell protein or transformation of complex organic contaminanats to other simpler organic compounds by biological agents like microorganisms. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. This chapter provides an overview of the recent literature to the usage of bacteria as biodegraders, discusses barriers regarding the implementation of this microbial technology, and provides suggestions for further developments.

Key Words: Petroleum hydrocarbon degrading bacteria, bioremediation, environmental pollution, carcinogens and neurotoxic.

I. INTRODUCTION

Crude oil is the primary energy source used in transportation industries and households. The activity of crude oil industry is a series of complex process from upstream to downstream.

The rapid progress in crude oil industry sector has both positive impact on the improved peoples wealth and negative side effect on the environmental pollution concurrently (Haris et al,2005). Leaks and accidental spills occur regularly during the exploration, production, refining, transport & storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons/year with a range of uncertainly of 200,000metric tons per year.

Release of hydrocarbons into the environment whether accidently or due to human activities is a main cause of water and soil pollution. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants.

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. In addition, bioremediation technology is believed to be noninvasive and relatively cost effective.

Biodegradation by natural populations of microorganisms represents one of the primary mechanism by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies.

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II. MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBONS

Soil must be treated well in order to preserve and maintain soil health so that it can sustain plant growth .One of the attempted efforts is to maintain soil health from crude oil exploration by human. Crude oil waste, which is disposed on the soil surface can lead to contamination of poisonous and hazardous compounds because of its hydrocarbon content. In order to curb the negative effect of crude oil, green technology of bioremediation with land farming system is necessary.

Biofertilizers are microbial inoculants which are artificially increased cultures of certain soil microorganisms that can enhance soil fertility and crop productivity ,biofertilizers are used to stimulate indigenous hydrocarbon degrading bacteria and fungi during bioremediation . Nitrogen fixing bacteria enrich the soil nutrient from oil killed microorganisms and the soil itself. The bacterial genera are Clostridium, Rhizobium, Azotobacter, Azospirillum and Beijirinkia.

Phosphate solubilising bacteria can be employed for the production of biofertilizesrs which actually improve the nutrient quality of soil.Example of the bacterial genera are Pseudomoanas, Bacillus, Flavobacteria, Aspergillus, Agrobacterium, Micrococcus, Achromobacter. Nitrogen fixing bacteria release the nitrogenase enzyme system which enhances bioremediation process of crude oil polluted soil.

The use of inorganic fertilizer (Ex. NPK) to enhance bioremediation , poses environmental and ecological challenges. There is need to employ remediation techniques that will restore contaminated media (eg.soil) to a state that can be used for agricultural and other important activities. The use of biofertilizer reduces the rate of environmental pollution .Commercial feasibility of biofertilizer production could have a price fall effects on chemical fertilizer.The processes facilitate soil enrichment and promote plants growth. It is also a sustainable and environmentally friendly approach to the remediation of hydrocarbons polluted media. It increases microbial populations in the soil by enhancing hydrocarbon utilizing bacteria.

III. METABOLIC RESTRICTION

The ability to biodegrade petroleum oil is associated with the concentration and composition of hydrocarbons. Extremely high levels of petroleum hydrocarbons strongly inhibit bacterial growth, resulting in poor biodegradation efficiency and even death of the bacteria (Ma et al.2015) . The key components of bacterial degradation of petroleum hydrocarbons are various specific enzymes(Wasmund et al, 2009; Uarjani,2017) . For ex.,the enzymes alkane 1- monooxygenase, acohol dehydrogenase ,cyclohexanol –dehydogenase ,methane monooxygenase and cyclohexanone 1,2 monoxygenase are involved in degradation of alkanes , while nathalene 1,2 dioxygenase ferrodoxin reducatse component , cis- 2,3 –dihydrobiphenyl-2,3-diol dehydrogenase and salicylaldehyde dehydrogenase are associated with naphthalene degradation and benzene dioxygenase, toluene dioxygenase and ethylbenzene dioxygenase work on other petroleum hydrocarbons (Bacosa et al. 2018).

IV. TIME CONSUMING

The core element of bioremediation is functional microorganisms that need sufficient nutrients and suitable environmental conditions. In general , petroleum oil hydrocarbons are not necessary substrates for hydrocarbons degrading bacteria and they utilize these compounds as alternative carbon and energy sources, especially in the absence of their preferable substrates. The function of hydrocarbons degrading bacteria mainly depends on their hydrocarbons degrading enzymes , the expression and activity of which are closely related to the physiological activity of bacteria (Mukherjee et al 2017; Song et al -2017) ; sufficient time is needed to synthesize hydrocarbons degrading enzymes because of the requirement of bacterial growth and synthetic metabolism.

Although some bacteria have been reported to mineralize petroleum hydrocarbons completely within several days ,or even less than one day under culture conditions, the degradation efficiency of these bacteria makes it difficult to meet the expected effects in practical usage (Chen et al ,2017 ; Zheng et al, 2018). The complex combination of various biological and abiotic factors limits the function of, petroleum hydrocarbons degrading bacteria in many ways (Zhao et al , 2017 ; Wang Y. et al 2018).

V. ADVANTAGES OF BIOREMEDIATION

The process of biodegradation involves the use of natural processes ; it is a considered as a green method that brings about no harm to the environment . The bioremediation takes place on site which contains naturally occurring microbial species , thus it does's not disturb the neighbouring microbial communities. The bioremediation process cheaper and economical over other methods.

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VI. GENETICALLY MODIFIED BACTERIA

Applications for genetically engineered microorganisms (GEMs) in bioremediation have received a great deal of attention to improve the degradation of hazardous wastes under laboratory conditions. These are reports on the degradation of environmental pollutants by different bacteria. The genetically engineered bacteria showed higher degradative capacity.

VII. ENVIRONMENTAL CONSTRAINTS

Many environmental factors such as temperature, nutrients, electron acceptors and substrates play vital roles in bioremediation and influence biodegradation reaction (Varjani and Upasani ,2017) . Indeed temperature can affect bacterial growth and metabolism , the soil matrix and the mode of occurrence of pollutants, thereby indirectly affecting biodegradation efficiency (Abed et al, 2015). It is well known that the growth of bacteria requires sufficient carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorous and various trace elements.

However, the main compounds of petroleum hydrocarbons are only hydrogen and carbon, Therefore the environment must have enough other nutrient elements to ensure growth of bacterial degraders. It is estimated that approximately 150 gm. of nitrogen and 30 gm.of phosphorous are consumed to convert 1 kg of hydrocarbons in bacterial cells (Ron and Rosenberg ,2014).Extensive laboratory and field studies have been devoted to solving this problem. The addition of fertilizers containing bioavailable nitrogen and phosphorous has been successfully applied to stimulate petroleum oil biodegradation on a number of different shorelines and sandy beaches (Roling et al 2002; Hazen et al ;2016) . Soluble and non soluble nutrients suffer from problems in the actual remediation , leading to low bioremediation efficiencies (Ron and Rosenberg ,2014). Researchers have found that using nitrogen fixing hydrocarbons degrading bacteria to improve the bioremediation efficiency was another good strategy instead of providing nitrogen sources (Thavasi et al 2006). For aerobic degradation procecesses , using oxygen as an electron acceptor is quite important , but it is usually not adequate in petroleum oil in contaminated environments because of the limited air permeability. Gogoi et al ,2003 reported that upto 75% of the hydrocarbons contaminants were degraded within one year in field tests by controlling and regulating aerations. However , providing a sufficient oxygen supply to stimulates the bioremediation of petroleum pollutants in the environment is rather expensive and not feasible . Hence the application of bulking agents such as saw dust into the soil to increase permeability or other electron acceptors (Nitrate, Iron, or manganese) into anoxic environments to stimulate anaerobic microorganisms is often more economical than oxygen supplementation (Zedelius et al 2011; Brown et al 2017).

VIII. SEEDING WITH NATURALLY OCCURRING MICROORGANISMS (BIOAUGMENTATION)

Onwurah (1999) noted that role of diazotrophic bacteria in the bioremediation of crude oil polluted soil. Nitrogen is one of the most limiting inorganic nutrients in the process of bioremediation of crude oil polluted environments . Enhanced remediation of crude oil polluted soil was achieved in situ by accelerating the biodegradation process through seeding with adapted *Azotobacter* which not only acted as supplier of mixed nitrogen to the indigenous crude oil degrading bacteria, but also performed some cometabolic activities that are useful in the bioremediation of crude oil polluted soil and biological Nitrogen fixation when in association with indigenous oil degrading bacteria.

Other Approach involves the inoculation of naturally occurring microorganisms to a polluted environment that promote increased rates of biodegradation. The inoculums may be a blend of non –indigenous microbes from various polluted environments specially selected and cultivated for their degrading characteristics, or it may be a mix of oil degrading microbes selected from the site to be remediate and mass cultured in the laboratory or in on-site bioreactors. The purpose for adding microorganisms to the spill site is that the indigenous microbial community may not include the diversity of oil degraders .The inoculated microbes not only have to degrade petroleum hydrocarbons but they would also compete with the mixed indigenous population that are well adapted to their environment . Along with this they would have to cope with the physical conditions (temperature, water and salinity) and predation by other species. The time required for introduced microbes to begin metabolizing hydrocarbons is also important .The inoculated cultures must be genetically stable, must not be pathogenic or produce toxic metabolites. Some laboratory and small scale experiments in controlled environments have demonstrated that seeding can promote biodegradation .

XI. CONCLUSION

Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants .Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy .The use of genetically modified (GM) bacteria represents a research frontier with broad implications . The potential benefits of using genetically modified bacteria are significant.

RECOMMENDATIONS

Government agencies must be heartened to sponsor field executions for application of biofertilizer made with nitrogen fixing and phosphate solubilising bacteria should be provided to farmers for agricultural practices instead of chemical fertilizers.

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