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Abstract

The idea of bioremediation is with the nature itself. Owing to contamination in a particular region, some organisms may die; growth of few others might on the contaminants by metabolizing it. Bioremediation would thrive well on the contaminants by metabolizing it. Bioremediation would involve identification of such organisms and fostering their growth, naturally or by inoculation, so as to breakdown the contaminants into less harmful metabolites. This technology being cheaper and nature friendly is certainly a technology for the future. But, like other technologies, this too is not a panacea to all the maladies of environmental contaminants; toxic metals like cadmium obliterate complete flora and fauna of the contaminated area, and hence, it is not possible to use biological agents to treat them. Microbes require oxygen as an electron acceptor hence in aqueous phase; oxygen concentration below 1 mg/l restricts the process of bioremediation.

6.1 Introduction

Even if we travel to the Mars, send rockets to the new planet, manufacture the super computers cloning the human beings, and do all unimaginable things, still we have many difficulties to clean the soil, air, and water we use. In many parts of the globe, the accessibility of water is a critical and vital issue and, even more so, the clean hygiene water. The environmental pollution is the most terrible and nasty ecological catastrophe that man is facing today. The environmental pollution is a global threat, and it is increasing day by day and also becomes a today's world's scare word. Rapid increase in human populations fueled by technological advancements in agriculture

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and health has led to speedy enhancement in the environmental pollution. The extraordinary and unmatched population growth and industrial development during the twenty-first century have not only increased usual liquid and solid waste pollutants to a hazardous level but also generated a range of earlier unknown chemical pollution problems for which human society was unsuspecting. Growth of world population, the installation of numerous industries, and the wide application of chemical fertilizers and pesticides in modern agriculture have burdened not only the clean water resources but also the ambiance and the soil with toxic pollutants.

6.2 Bioremediation of Petroleum Contaminants

Petroleum and its components drive the present civilization and are the major energy sources. But, where there is use, there is a chance for abuse too. Hence, being the prime source of energy, petroleum is also a major environment pollutant. Since 1992, there have been 21 major oil spills causing huge economic and immeasurable noneconomic losses (Sarkar et al. 2005. <http://www.endgame.org/oilspills.htm>). Petroleum contamination is quite harmful for the higher organisms (Cheong et al. 2011; Janjua et al. 2006; Lyons et al. 1999), but it is fortunate that microorganisms can thrive on it and assimilate (Atlas 1995; de Oliveira et al. 2012). Soon after major oil spill incident is reported, the efforts are concentrated at physical removal of oil, but they rarely achieve complete clean up. As per Office of Technology Assessment (OTA; USA), such mechanical methods are efficient at removing no more than 10–15% of oil after a major spill. In such cases, bioremediation has a major role to play in neutralizing the harmful effects of oil in the open environment. The basic principle is to use organisms that can use petroleum as carbon source and, hence, break them down to harmless end products.

Like any other technology that uses biological agents, success of bioremediation of petroleum contamination also depends on establishing and maintaining conditions that favor proliferation of petroleum scavenging microorganisms. Bio augmentation and bio stimulation are the two main approaches followed in this regard. Bio augmentation refers to inoculating the affected area with degrading microorganisms, while bio stimulation would require favoring growth of such microorganism through addition of nutrients or by providing other growth-limiting substrates (e.g., oxygen, surf washing, etc.). As petroleum is hydrophobic in nature, its bioavailability becomes a major constraint in the process of bioremediation. The use of biosurfactants is a common approach to increase the bioavailability. Requirements of a successful bioremediation process of petroleum contamination are as follows.

The very first requirement is the availability of microorganisms that can utilize oil as a metabolic substrate. Finding and transplanting such an organism to the site of contamination would be the first approach. Jones et al. in 1983 reported for the first time biodegraded petroleum by-products in marine sediments (Das and Chandran 2011). Enzymatic degradation of petroleum can be achieved by bacteria, algae, or fungi. Different organisms have varied degradation capabilities and act on different

substrates. As petroleum is an assortment of different components, it is advisable to use a cocktail of organisms to affect remediation. Bacteria are the most efficient of all organisms that can degrade hydrocarbons (Rahman et al. 2003; Brooijmans et al. 2009). Floodgate (1984) mentioned 25 genera of hydrocarbon-degrading bacteria and 25 genera of hydrocarbon-degrading fungi which were isolated from marine environment.

Some of the bacteria recognized as hydrocarbon degrading are *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococciis*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffii*, *Flavobacterium* sp., *Micrococcus roseus*, and *Corynebacterium* sp. (Jones et al. 1983; Adebusoye et al. 2007). Some fungal genera utilized for this purpose are *Amorphoteca*, *Neosartorya*, *Tal arontyces*, *Graphium*, *Candida lipolytica*, *Yarrowia*, *Pichia*, *Aspergillus*, *Cephalosporium*, *Rhodotorula mucilaginoso*, *Geotrichum* sp., *Trichosporon mucoides*, and *Penicillium* (Boguslawska Was and Dabrowski 2001; Chaillan et al. 2004; Singh 2006). After the potential scavengers have been identified, the conditions for their survival and proliferation have to be ascertained.

Among the physical factors, temperature is most important one determining the survival of microorganisms and composition of the hydrocarbons (Das and Chandran 2011). At higher temperature, some fraction may get evaporated and the oil would tend to spread, while in low temperature, the slick would be more viscous, and retention of otherwise volatile fractions thereby delays the bioremediation process. For freshwater, bioremediation process 20–30 °C is the ideal temperature, while for marine 15–20 °C is recommended. For high molecular weight polycyclic hydrocarbons, which are otherwise difficult to degrade, higher temperatures may be required (Bartha and Bossert 1984; Cooney 1984). As temperature has effect on enzymatic turnover rate “ Q^{10} ” hence, higher temperature would favor bioremediation. It was reported that the rate of hydrocarbon remediation was maximum in the range of 30–40 °C in general, and above this, the membrane toxicity effect of hydrocarbons was found to inhibit the survival of microorganisms (Bartha and Bossert 1984). As there is a close relationship between temperature and oil bioremediation, it is easy to understand why an oil leak disaster would be dangerous in polar regions.

The first step in degrading hydrocarbons is action of oxygenase which microbial communities are in very high salinities. However, some bacteria like *Streptomyces albaxialis* (Kuznetsov et al. 1992) for crude oil degradation and *Halobacterium* spp. (Kulichevskaya et al. 1992) for degradation of n-alkanes (C10–C30) have been identified. Kapley et al. (1999) cloned *E. coli* pro U operon, which is responsible for osmoregulation, into some bacterial consortium which can attack various fractions of crude oil making them salinity tolerant up to 6% NaCl.

pH also had an implication on biodegradation rates. The rates were found to be highest at neutral pH (Leahy and Colwell 1990). Lower pH at around 5.0 (Patrick and DeLaune 1977) as seen in salt marshes reduces oil mineralization, but the rates were satisfactory at pH above 6.5 (Hambrick et al. 1980). Octadecane mineralization improved further at pH 8.0 (Leahy and Colwell 1990).

Bioavailability of petroleum is a major problem that limits the rate of biodegradation. In order to enhance bioavailability, it is a must that solubilization be increased. Such a task is accomplished by certain microorganisms that secrete surfactants which are a group of surface active chemicals that increase the bioavailability of petroleum floating on the water column by increasing their solubilization (Das and Chandran 2011). Biosurfactants increase the oil surface area and, hence, the amount of oil that is actually available for degradation to the bacteria. Due to this property of enhancing biodegradation of oil, such surfactant-producing bacteria have potential to be used in bioremediation (Cameotra and Singh 2008). A consortium of bacteria was used for evaluation of surfactants and their composition by Cameotra and Singh (2008). The surfactant was found to be a conglomerate of 11 rhamnolipid family members and found that crude biosurfactant addition to the oil contamination was very effective in degradation process. Genus *Pseudomonas* is widely known for efficient surfactant production properties (Rhaman et al. 2007; Cameotra and 2008; Beal and Betts 2000; Pornsunthorntawe et al. 2008).

6.3 Bioremediation of Pesticides

Today, intensification of agriculture has increased the risk of losses due to improper crop health making agriculture sector heavily dependent upon the use of pesticides to prevent losses from pests. Pesticides are usually applied as a spray over the crop in aqueous or some nonpolar solvent medium of which only 5% is estimated to be utilized for the intended purpose, and the rest remains in the environment as residues. These residues may get washed off and either seep into the groundwater or reach water bodies along with the runoff. Once reaching the water bodies, the process of biomagnification begins. Vaccari et al. (2006) estimated that pesticide dichlorodiphenyldichloroethane (DDD) may get accumulated 85,000 times more in a predatory fish than at concentration it enters in water.

Some pesticides may get decomposed sooner after they are dissolved in a solvent, but the most commonly used organochlorines have a very long half-life making them threatening to the ecosystem and human beings. Pesticides may get accumulated in the human adipose tissue which enter the system orally, through inhalation, and some are even absorbed dermally. In humans, pesticides may cause irritation, affect mental health, affect digestion, and even cause carcinosis (Green and Hoffnagle 2004). Concern of this chapter would only be limited to persistent organic pesticides which have a very long half-life and are recalcitrant. UNEP's (United Nations Environment Programme) list of persistent organic pollutants includes aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzenes, mirex, and toxaphene.

Sometimes the pesticide used may be less toxic than the degraded product that is produced from it. Hence, an effective bioremediation technique would be one that acts fast so as to prevent the degradation process, and the end product that results from bioremediation is either nontoxic or less toxic. Bioremediation of metal contaminants or hydrocarbon contaminants is easier as the organisms that can survive in excess of metals and hydrocarbons can be naturally found, but this is

not the case with pesticide as these are artificial chemicals intended to kill. Hence, identification of organisms that may help in bioremediation process is crucial. Usually, four remediation technologies are followed at the pesticide-contaminated regions – low temperature desorption, incineration, bioremediation, and phytoremediation. All these techniques have their own advantages and disadvantages. While incineration and low temperature desorption are faster technologies, they are usually very expensive. Bioremediation and phytoremediation on the other hand are very efficient and cheaper technologies but the time taken for remediation.

6.4 Conclusion

Industrial revolution and increased handling of heavy metals, POPs, and similar hazardous chemicals have raised the chances of accidents. Nuclear disasters at Fukushima and other previous similar incidents have raised lot of questions about the usage of such hazardous chemicals. One thing is clear that with rising population and rising demand for food and energy will certainly rule out utilization of hazardous metals and other chemicals, but we can certainly develop technologies that can help in mopping them up so that impact on biodiversity and on human population can be minimized. Bioremediation is one such method which is eco-friendly, cost-effective, and cleans up the contaminants to quite an extent efficiently. But, side pace and threat from genetically modified organisms to biodiversity may be deterrents to this technology. Bioremediation by itself may not be a complete solution to the problem of contamination, but mixing physical and chemical remediation techniques with bioremediation may be an answer to complete remediation of natural resources.

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