

Bioremediation of Contaminants

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Abstract: The idea of bioremediation is with the nature itself. Due 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 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 1mg/l restricts the process of bioremediation.

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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 non-economic losses (Cedre, 2012. <http://www.endgame.org/oilspills.htm>). Petroleum contamination is quite harmful for the higher organisms (Cheong *et al.*, 2011; Janjua, Kasi Nawaz, Farooqui, & Khuwaja, 2006; Lyons, Temple, Evans, Fone, & Palmer, 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 per cent 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 favour proliferation of petroleum scavenging microorganisms. Bioaugmentation and Biostimulation are the two main approaches followed in this regard. Bioaugmentation refers to inoculating

the affected area with degrading microorganisms while biostimulation would require favouring 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. 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 byproducts in marine sediments (Das & Chandran, 2010). 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 effect remediation. Bacteria are the most efficient of all organisms that can degrade hydrocarbons (Rahman *et al.*, 2003; Brooijmans 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.aerugi.nosa, *Bacillus subtilis*, *Bacillus sp.*, *Alcaligenes sp.*, *Acinetobacter lwoffii*, *Flavobacterium sp.*, *Micrococcus roseus*, and *Corynebacterium sp.* (Jones *et al.*, 1983; Adebuseye *et al.*, 2007). Some fungal genera utilized for this purpose are *Amorphoteca*, *Neosartorya*, *Talaromyces*, *Graphium*, *Candida lipolytica*, *Yarrowia*, *Pichia*, *Aspergillus*, *Cephalosporium*, *Rhizoglyphus muciliginosa*, *Geotrichum sp.*, *Trichosporon rrucooides* and *Penicillium* (Boguslawska.-Was & 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 & Chandran, 2010). 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 delaying 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 favour 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 in very high salinities. However, some bacteria like *Streptomyces albaxialis* (Kuznetsov *et al.* 1992) for crude oil degradation and *Halobacterium spp.* (Kulichevskaya, Milekhina, Borzenkov, Zvyagintseva, & Belyaev, 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 upto 6 per cent NaCl.

pH also had an implication on biodegradation rates. The rates were found to be highest at neutral pH (Leahy & Colwell, 1990). Lower pH at around 5.0 (Patrick Jr & DeLaune,

1977) as seen in salt marshes reduces oil mineralization but the rates were satisfactory at pH above 6.5 (Hambrick, DeLaune, & Patrick, 1980). Octadecane mineralization improved further at pH 8.0 (Leahy & Colwell 1990).

Bioavailability of petroleum is a major problem that limits the rate of biodegradation. In order to enhance bioavailability, it is must that solubilization be increased. Such a task is accomplished by certain microorganisms that secrete surfactants which is a group of surface active chemicals that increase the bioavailability of petroleum floating on the water column by increasing their solubilization. (Das & Chandran, 2010). 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 & Singh, 2008). A consortium of bacteria was used for evaluation of surfactants and their composition by Cameotra & 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 (Rahman *et al.*, 2007; Cameotra & 2008, Beal & Betts, 2000; Pornsunthorntaweew *et al.*, 2008).

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 non-polar solvent medium of which only 5 per cent 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 ground water 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 organochlorides 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, including 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 non-toxic 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.

Conclusion:

Industrial revolution and increased handling of heavy metals, POPs and similar hazardous chemicals has 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 chemical 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 ecofriendly, 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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