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Bioaugmentation-A Strategy For Cleaning Up Soil

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Abstract : **The contamination of soil with aromatic compounds is a serious environmental concern as it results in carcinogenic and mutagenic properties. One of the methods of their removal from soil is bio augmentation, defined as a technique for improvement of the degradative capacity of contaminated areas by introduction of specific microorganisms. Addition of pre-grown microbial cultures is done to enhance the degradation of organic or heavy metal compounds. The most important factor is the selection of proper microorganisms that can not only degrade contaminants but can also successfully compete with indigenous micro flora. Several strategies are being developed to make it a successful technology without degrading indigenous microorganisms. These approaches also involve the use of genetically engineered microorganisms. Bioaugmentation is commonly used in municipal wastewater treatment .As a result, remediation industry practices have shifted toward a more prescriptive approach to bio augmentation to achieve cost-savings and accelerate site remediation.**

Keyword: Bioaugmentation, Microorganisms, activated sludge, site remediation.

1. INTRODUCTION

The production of chemicals from industries and their negligent disposal has resulted in contamination. The pollutants are released from oil refineries, gas stations, and use of wood preservatives, pharmaceuticals and petrochemical industries. The presence of these chemicals poses a serious threat to human kind because of their ability to accumulate in the food chain for a long period of time. Many manmade aromatic compounds like polycyclic aromatic hydrocarbons (PAH) and BTEX compounds have carcinogenic and mutagenic properties. (Budavari 1996)

These pollutants can be removed by both physio-chemical and biological approaches. Physio- chemical methods are expensive then the latter and require high energy demand and consumption of many chemical reagents. This is a reason why use of microorganisms is preferred for degrading the toxic compounds (Hamdi et. al2007).

One of the biological (*insitu)* techniques is bioaugmentation which improves the capacity of contaminated sites by introducing single strains of microorganisms with desired biodegradable capabilities. Few genetically engineered microorganisms (GEMs) exhibiting amplified catalytic capabilities can also degrade aromatic hydrocarbons and these groups of bacteria can be used in soil bioaugmentation (A. Mrozika, Z. P. Segetb 2009).

Bioaugmentation is generally practiced in the areas where bioactivity is minimal or the area has difficulty to reduce pollutants which needs to be controlled.

2. STRATEGIES APPLIED

The strategies used for bioaugmentation are: (1) Firstly, pre-adapted pure bacterial strain is added. (2) Secondly, pre-adapted consortium is added;(3) Thirdly, genetically engineered bacteria is introduced; and (4) Fourthly, biodegradation relevant genes packaged is added in a vector in order to transfer by conjugation into indigenous microorganisms (El Fantroussi and Agathos 2005).Microorganism with higher metabolic potential should be selected to retain essential features intact in harsh environmental conditions.(Thompson e t al.2005; van der Gast et al. 2004). For co-contamination of high metal concentrations and organic pollutants, the microbial population should degrade the organic compounds that inhibit by the co-contaminants (Roane et al. 2001).The above said strategies, in such cases, have involved the use of multi component systems such as a microbial association, which is a better illustration of a real environment than models based on single-component

systems (Ledin 2000).According to Alisi et al. 2009).Using a microbial association rather than a pure culture for the bioaugmentation is generally more advantageous as it provides the metabolic diversity needed for field applications.(Rahman et al. 2002a, b; Nyer et al.2002).Alisi et al. (2009) successfully obtained complete degradation of diesel oil and phenanthrene; a reduction of 60% of isoprenoids; and an overall reduction of about 75% of the total hydrocarbons in42 days, using a microbial formula made with selected native strains. Li et al. (2009) also stated that indigenous microbes could degrade PAH in aged contaminated soil. Albeit, adding microbial consortia five fungi-*Phanerochaetechrysosporium*, *Cuuninghamella sp*, *Alternaria alternate (fr.) Keissler*, *Penicillium chrysogenum*, and *Aspergillus niger*; and three bacteria:

Bacillus sp., Zoogloea sp. and *Flavobacterium* enhanced the degradation rate significantly (41.3%) (Li et al, 2009).

The inoculums to be used in the soil for bioaugmentation are produced in bioreactors; the transfer and survival still remain a questionable issue. Microbial inoculants are homogeneous cell suspensions which are produced under optimum conditions, they often undergoes stress when in contact with the complexity of the nature. A biotic and biotic stresses effect the introduced population in a way that the number starts decreasing. The factors that hinder microbial growth are: (1) fluctuations in temperature,(2) water content, (3)pH, (4) reduction in nutrients(5)increase in potentially toxic pollutant levels in the contaminated soil(Gentry e t al.2004), (Goldstein et al.(1985).

3. FACTORS INFLUENCING BIOAUGMENTATION

Table 1:

Factors	Description	References
Cell death during inoculation	extreme environmental change	Van Veen et al.(1997)
Death after inoculation	Insufficient nutrients/toxicity	Goldstein et al.(1985)
Nutrient availability	Competition for nutrients by microbes	Thompson et al. (2005), El Fantroussi and Agathos (2005)
Threat of other organisms	Bacterial population can be over shadowed by protozoa growth	Bouchez et al. (2000)
pH	High pH inhibit microbial degradation process	Dibble and Bartha (1979)
Temp	Effects microbial growth and degradation process	Atlas (1981)
Moisture	Low level influences microbial growth, higher soil aeration	Dibble and Barth (1979), Leahy and Colwell (1990)

4. MICROORGANISMS IN BIOAUGMENTATION

Materials which are waste for humans and higher vertebrates become a useful food substrate for the microorganisms. In both natural and engineered treatment systems microorganisms such as bacteria, fungi, and protozoa play an essential role in conversion of organic waste to more stable less polluting substances? Similarly, several bacterial strains have been reported to have the properties required for the degradation of toxic compounds namely the species of *Pseudomonas, Mycobacterium, Haemophilus, Rhodococcus,*

Paenibacillus and *Ralstonia*.(Farhadian et al. 2008; Haritash and Kaushik 2009; de Carvalho et al. 2005).These strains have the potential to degrade petroleum hydrocarbons and aromatic hydrocarbons such as benzene, toluene, ethyl benzene and xylene and PAHs(poly aromatic compounds) which include naphthalene(*Pseudomonas*),phenanthrene (*Pseudomonas and Haemophilus*), anthracene(*Rhodococcus*), pyrene (*Haemophilus and Mycobacterium)* and the highly carcinogenic benzo[a]pyrene (Rhodococcus and Mycobacterium). M. Tyagi, M. Manuela R. da Fonseca, Carla C.C. R. de Carvalho 2011).

Bioaugmentation of soil requires knowledge of the soil type and volume of particular contaminant and the suitable microbial strain. While selecting the strain of microorganisms following features should be analyzed: fast growth, ability to resist higher concentrations of contaminants and the ability to survive in harsh conditions (Singer e t al.2005; Thompson et al.2005). Bacterial colonies can be isolated from given contaminated site and after culturing the bacteria under laboratory conditions the pre-adapted pure bacterial strains should be returned to the same soil. This approach is called reinoculation of soil with indigenous micro- organisms. Many studies have indicated that using a group of bacterial cultures has been more effective in removing pollutants as compared to selected single strain (Ghazali et al, 2004; Goux et al. 2003)

Experiments were also carried on gram-negative bacteria belonging to genus *Pseudomonas* (Heinaruetal. 2005), *Flavobacterium* (CrawfordandMohn1985), *Sphingobium* (Dams etal.2007), *Alcaligenes* (Halusˇkaetal.1995) and *Achromobacter* (Ronen e t al.2000). More attention is needed towards gram-positive bacteria belonging to the genera *Rhodococcus* (Brigliaetal.1990), *Mycobacterium* (Jacquesetal.2008) and *Bacillus* (Silva e t al.2009b). Fungi is potentially useful for bioaugmentation that are represented by species from genus *Absidia*(Garonetal.2004),*Achremonium*(Silvaetal.2009b), *Aspergillus* (dos Santosetal. 2008), *Verticillium* (Silva etal.2009b),*Penicillium* (Mancera-Lo´pezetal.2008) and *Mucor*(Szewczyk).No microorganisms or their groups are universally applicable to bioaugmentation. Many microorganisms are able to degrade a wide range of substrates.

5. CONCLUDING REMARKS

Augmentation approaches appears to have a great potential for cleaning of aromatic compounds. The most important step in bioaugmentation is selection of microbial strains. The principle of bioaugmentation can be achieved by using microbial inoculants isolated from environments where contamination had occurred since ages. The success rate is largely influenced by the availability of inoculates and their survival in contaminated soil. As the interactions between inoculated strain or consortia and indigenous microbial communities are not known so further studies are required especially at the field level. Other approaches that are considered to be more effective than cell bioaugmentation involve immobilized microorganisms, activated soil and gene bioaugmentation. Also research should be done on extraction of the groups of microbes that can degrade variety of substrates.

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