

Innovative Trends In Biological Science

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Abstract:

The make use of microorganisms to reduce the concentration of harmful wastes on a contaminated site is called bioremediation. Such a biological treatment system has various applications counting cleanup of contaminated sites such as water, soils, sludges, and waste streams. Heavy metals are well thought-out one of the most common and hazardous pollutants in industrial effluents that might cause serious problems to the sewage network pipelines. The deleterious effects of heavy metals on biological processes are complex and generally related to species, solubility and concentration of the metal and the characteristics of the influent, such as pH as well as presence and concentration of other cations and molecules and suspended solids. Metal toxicity results from alterations in the conformational structure of nucleic acids, proteins or by interference with oxidative phosphorylation and osmotic balance. This paper outlines the various factors bioremediation of Pb using indigenous and non-indigenous bacteria.

Introduction

Bioremediation is defined as the increase of rate of the natural metabolic process using by microorganisms alter and break down organic molecules into other substances. According to the United States EPA, bioremediation defined as treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances. Bioremediation is an ecologically advanced technique that employs natural biological activities employing microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. With rapid industrialization all over the world, pollution rate also increases. One of the modes through which all types of pollutants enter the biosphere is that of industrial effluents.

To completely eliminate the toxic contaminants occurring in sludges, and ground water contaminated with petroleum hydrocarbons, solvents, pesticides, wood preservatives, non-halogenated SVOCs, and BTEX and other organic chemicals, especially effective for remediating low level residual contamination in conjunction with source removal. Compared with other technologies, such as thermal desorption and incineration (which require excavation and heating), thermally enhanced recovery (which requires heating), chemical treatment (which may require relatively expensive chemical reagents), and in situ soil flushing (which may require further management of the flushing water), bioremediation may enjoy a cost advantage. Not all contaminants, however, are easily treated by bioremediation using microorganisms. Bioremediation technologies are phytoremediation, bioleaching, landfarming, bioreactor, bioaugmentation, rhizofiltration, and biostimulation (Baker and Brooks, 1989). Bioremediation technologies can be generally classified as in situ or ex situ. In situ bioremediation involves the placement of conversion directly into contaminated area, whereas ex situ bioremediation transfers the contaminated source to a selected site for treatment. Biostimulation is one of the methods, by adding the amendments to accelerate of the process of indigenous microbial populations in the contaminant site. Recent progresses have also proven successful via the addition of indigenous microbe strains to the medium to boost the resident microbe population's ability to break down contaminants.

Heavy Metal Pollution

Heavy metals pollution has become one of the most serious ecological problems today. Many heavy metals with toxic properties have been brought into the environment through human activity. With the fast development of industries such as metal plating facilities, mining operations, fertilizer industries, tanneries, batteries, sheet manufacture and pesticides, etc. The toxicity level of the heavy metals is varied and its danger to human health. Heavy metals are directly or indirectly release into the environment progressively; especially in developing countries. Release of heavy metals without proper treatment pass a significant threat to public health because of its persistence and accumulation in the food chain. Unlike organic

contaminants, heavy metals are not biodegradable and tend to accumulate in living organisms and abundant heavy metal ions are recognized to be poisonous or carcinogenic (Medici et al., 2010; Yang et al., 2009). Each heavy metal has unique toxicity or function. For examples zinc and copper can enhance microbial development at low concentrations, but represses growth at high concentrations (Ge et al., 2009). Heavy metals such as copper, iron, chromium and nickel are fundamental minerals since they play an important role in biological systems, where cadmium and lead are non-major metals, they are toxic, even in trace amounts (Ibrahim et al., 2013). Some conventional methods to remediate sites contaminated with heavy metals are excavation and solidification/ stabilization which are suitable in controlling contamination but not permanently remove heavy metals (Bahi et. al., 2012). However, these technologies are expensive and can lead to incomplete decomposition of contaminants. There for unconventional methods like using microorganisms which help in reducing the toxicity of harmful effluents has been explored. Microbial communities respond to heavy metals depending upon the concentration and availability of heavy metals and are also a complex process which is controlled by factors such as, the type of metal, the nature of the medium and microbial species (Goblentz et.al., 1994). Frequent attempts have been made to design genetically modified microorganisms for environmental release as agents for the bioremediation of heavy metal pollutants. However, these microorganisms do not behave in a predictable fashion under conditions that are quite different from the controlled ones of the laboratory. Bioremediation Of Heavy Metals Heavy metals are chemical elements with a specific gravity that is at least five times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Some well-known toxic metallic elements are arsenic, cadmium, iron, lead, and mercury. Based on the toxicological point of view, heavy metals can be divided into two types. The first type is an essential heavy metal, where its presence in a certain amount is needed by living organisms, but in excessive quantities can cause toxic effects. Examples of the first kind is Pb, Zn, Cu, Fe, Co, Mn, etc., while the second type includes the heavy metals that are not essential and toxic, whose presence in the body has no known benefits or may even be toxic, such as Hg, Cd, Pb, Cr

and others. Heavy metals can affect human health effects depending on which part of heavy metals are bound in the body. Various organisms have the ability to bind metals with very high capacity, namely marine algae, fungi and molds that have been reported to be able to accumulate various metals.

Toxicity of Pb

Lead (Pb) has been commonly used since ancient time and plays important role in the industrial economy. Lead (Pb) is known as toxic element in environment. Besides, its spreading in the environment is also connected to both agricultural and urban activities, such as land application of sewage sludge, smelting operations and use of leaded petrol (Iain et al., 2001; Marquita, 2004; International Lead association, 2009). Therefore, to control environmental pollution by Pb, it is necessary to restrict maximum content of Pb in the waste water that discharged into the environment. The concentration of Pb higher than the standards would be harmful to living organisms, especially indirect impact on the human health, it can damage the brain which reduce the intelligence of children (Ekere et al., 2014). Lead cause interference on nervous system, reproductive system and urinary tract (Stancheva et al., 2013). Besides lead is a neurotoxic metal, affecting visual/motor performance, memory, attention and verbal comprehension. Subtle changes in neuropsychological function have in fact been seen in inorganic lead workers with blood lead levels as low as 40µg/100ml. Moreover, chronic workplace exposure increases the likelihood of high blood pressure, can damage the nervous system and kidneys, and sometimes leads to anaemia and infertility (Marquita, 2004; Gidlow, 2004). Because the body treats Pb much as it does with calcium, lead accumulates in the skeleton and can remain in the bones for decades. While blood level can show recent exposure, bone lead level reflects exposure over a life time (Collins et al., 2004). Actually about 90% of a person's lead intake is eventually stored in the skeleton and lead levels in modern human skeletons and teeth are hundreds of times greater than those found in pre-industrial-age skeletons (Marquita 2004). Lead is only weakly mutagenic, but in vitro it inhibits DNA repair and acts synergistically with other mutagens. Nevertheless, there are at present insufficient data for suggesting that lead compounds are

carcinogenic in humans (Gidlow, 2004). Lead enters the waters through efflorescence in the air with the help of rain water (Widiyanti et al., 2005). Alternative treatments should be done to avoid such health problems, especially treatment for waste problem.

Researchers have demonstrated the successful use of biosurfactants for facilitating the degradation of organic pollutants in soil and water. The assessment of efficiency of biosurfactants (rhamnolipid) producing microorganisms (*Pseudomonas* sp.) isolated from heavy metal contaminated site has been reported (Jayabarath et al., 2009). There lease of heavy metals into the environment, mainly as a consequence of anthropogenic activities, constitutes a worldwide environmental pollution problem. Bioremediation of heavy metals is considered to be economically viable alternative to conventional methods of heavy metal clearance. Soil bioremediation is a complex and costly process that aims to restore contaminated sites to environmentally sustainable conditions using microorganisms.

Bioremediation Techniques

Bioremediation strategies employed for in situ bioremediation; bio stimulation and bioaugmentation - Bio-stimulation: Bio-stimulation in which the biodegradation is accelerated by the addition of amendments to contaminated water or soil to encourage the growth and activity of bacteria already existing in the contaminant environment. Amendments include air (oxygen), added by bioventing; oxygen-releasing compounds, which keep the contaminated media aerobic; and reducing agents, such as carbon-rich vegetable oil and molasses, which promote growth of anaerobic microbial populations. wastewater treatment facilities. To date several studies have been focused on the degradative capacities of bacterial population in polluted environments (Cavalca et al., 2000; Juck et al., 2000; Bundy et al., 2002). The important objective was the determination of physiology and function of such diverse catabolic populations in the bioremediation process. However, all the environmental bacteria cannot be cultured yet by conventional laboratory techniques (Torsvik et al., 2002). Therefor encourage the growth and activity of bacteria existing in the native environments.

Microbial communities can adapt to contaminants after prolonged exposure by changing their composition on the native

ecosystem. Hence, assessment of the structure of microbial communities is an important step to determine possible indicators of heavy metals. In this aspect, some studies investigated the changes in the indigenous bacterial community structure for addressing the impact of contamination on the microbiology of ecosystems (Macnaughton et al., 1999; Ogino et al., 2001).

Frequently bioremediation has been studied in polluted marine environment and bio-stimulation studies have indicated that can efficiently promote biodegradation (Venosa et al., 1999). However, current knowledge of the impact of this process on the ecosystem is limited. Therefore, a detailed study of the contaminated site in relation to the pollutant, environmental conditions and the microbial community is still necessary for in-situ bio-stimulation to be considered reliable and safe cleanup technologies (Iwamoto, et al., 2001). To date, this method has not been very successful when done at the site of the contamination because it is difficult to control site conditions for the optimal growth of the microorganisms added. Scientists have yet to completely understand all the mechanisms involved in bioremediation, and organisms introduced into a foreign environment may have a hard time surviving (Dejonghe et al., 2001). Factors influencing bio-stimulation Heavy metal biodegradation can be limited by many factors, including nutrients, pH, temperature, moisture, oxygen, soil properties and contaminant presence (Atagana 2008, Al Sulaimani 2010;). This can be done by addition of various forms of limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g., in the form of molasses), which are otherwise available in quantities low enough to constrain microbial activity (Elektorowicz, 1994; Piehler et al., 1999; Rhykerd et. al., 1999). Previously Perfumo et al., (2007) as the addition of nutrients, oxygen or other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation. They opined that bio-stimulation can be considered as an appropriate remediation technique for heavy metal removal in soil and requires the evaluation of both the intrinsic degradation capacities of the autochthonous microflora and

the environmental parameters involved in the kinetics of the in-situ process.

The primary advantage of bio-stimulation is that bioremediation will be undertaken by already present native microorganisms that are well-suited to the subsurface environment, and are well distributed spatially within the subsurface. The primary challenge is that the delivery of additives in a manner that allows the additives to be readily available to subsurface microorganisms is based on the local geology of the subsurface. Tight, impermeable subsurface lithology (tight clays or other fine-grained material) make it difficult to spread additives throughout the affected area. Fractures in the subsurface create preferential pathways in the subsurface which additives preferentially follow, preventing even distribution of additives. Addition of nutrients might also promote the growth of heterotrophic microorganisms which are not innate degraders of heavy metal thereby creating a competition between the resident micro flora (Adams, 2014).

Bio-augmentation:

Bio augmentation, which involves the addition of genetically engineered microorganisms or microorganisms with enhanced degradation capabilities to the contaminated site. Bioremediation can also be accelerated through injection of native or non-native microbes (bioaugmentation) into a contaminated area. Bioaugmentation has been proven successful in cleaning up of sites contaminated with aromatic compounds but still faces many environmental problems. One of the most difficult issues is survival of strains introduced to soil. It has been observed that the number of exogenous microorganisms has decreased shortly after soil inoculation. Many studies have shown that both abiotic and biotic factors influence the effectiveness of bioaugmentation (Cho et al., 2000; Bento et al., 2005; Wolski et al., 2006).

Bioaugmentation should be applied in soils (1) with low or non-detectable number of contaminant-degrading microbes, (2) containing compounds requiring multi-process remediation, including processes detrimental or toxic to microbes and (3) for small-scale sites on which cost of non biological methods exceed cost for bioaugmentation. Moreover, the introduction of microorganisms into soil is particularly recommended for areas

polluted with compounds requiring long acclimation or adaptation period of time. This review addresses the bioaugmentation of soils polluted with aromatic compounds; however, it should be noted that this strategy may be also effective for cleaning up diverse biotops contaminated with heavy metals (Je'ze'quel and Lebeau 2008; Lebeau et al., 2008; Beolchini et al., 2009) and radionuclides (Kumar et al., 2007; Gavrilesco et al., 2009).

Advance Molecular ecological techniques will be useful for the analysis of the diversity of pollutant degrading microorganisms, and for the development of strategies to improve bioremediation (Watanabe, 2001; Macnaughton et al., 1999). According to Forsyth et al., (1995)- the use of genetic engineering to produce microorganisms capable of convert the heavy metal or to enhance such processes in native organisms with such capabilities has become a popular way of increasing the efficiency of bioremediation in laboratory studies. Techniques used can include engineering with single genes, pathway construction, and alteration of the sequences of existing coding and regulatory genes (Perpetuo et al., 2001). These applications could further be extended to greenhouse gas control, carbon sequestration, or conversion of wastes to value added eco-friendly products. Regardless, there remains the need for a regulatory, safety, or costs benefit-driving force to make these potentials a reality (Sayler and Ripp, 2000), Due to eco-friendly approach and lesser health hazards as compared to physio-chemical based strategies to combat heavy metal pollution; genetic engineering microbes-based remediation offered a more promising field. Good microbiological and ecological knowledge, biochemical mechanisms and field engineering designs would be an essential element for successful in situ bioremediation in contaminated sites using engineered bacteria. Various bio safety and environmental concerns like genetic pollution, caused by using genetic engineering microbes should be well accounted before releasing into environment (Singh et al.,2011).

Factors influencing bioaugmentation

The most important abiotic factors such as temperature, moisture, pH and organic matter content are discussed; however, aeration, nutrient content and soil type also determine the efficiency of bioaugmentation. There are many examples proved the pH

moisture, aeration, nutrient content and soil type are playing important role in bioaugmentation techniques. For example, Hong et al., (2007), studying the effect of temperature and pH on fenitrothion (nitro phenolic pesticide) degradation by inoculated *Burkholderia* sp. FDS-1, found that optimal parameters for bacteria activity were 30°C and slightly alkaline pH, whereas 10 and 50°C and highly acidic condition were unsuitable for pesticide detoxification. The effect of water content on the survival of *Achromobacter piechaundii* TBPZ and degradation of tribromophenol (TBP) in soil samples were reported by Ronenet al. (2000). Their results indicated that, at 25% and 50% water content, TBP degradation was rapid whereas in soil with only 10% moisture the degradation proceeded to a small extent. Water potential has been reported to have significant influence on survival and degradative activity of *Pseudomonas stutzeri* P16 lux AB4 in sterile and non-sterile soil amended with phenanthrene (Mashreghi and Prosser 2006). The discussed results indicated that bioaugmentation is not always the best method for cleaning up of contaminated soils and it is difficult to predict the final results of this process. One of the problems connected with soil inoculation is how to deliver the suitable microorganisms to the desired sites. It is easy to disperse inoculants into surface soil but it is difficult or even impossible to do it in subsurface environments. Soils have potential for microbial transport but cell adhesion to organic matter strongly limits their distribution. To avoid these constraints, surfactants, foams and strains resistant to adhesion may be applied (Wang and Mulligan 2004; Franzetti et al. 2009). Recently, bioaugmentations with encapsulated or immobilized cells for various purposes have been tested (Cassidy et al., 1996, 1997; Gardin and Paus 2001; Gentili et al., 2006). Microorganisms for bioaugmentation

There are several approaches that allow selection of microorganisms useful for bioaugmentation. Bacteria for this purpose may be isolated from given contaminated soils and after culturing under laboratory conditions pre-adapted pure bacterial strains return to the same soil. Most experiments dealing with bioaugmentation were carried out using gram-negative bacteria belonging to genus *Pseudomonas* (Heinaru et al., 2005), *Flavobacterium* (Crawford and Mohn 1985), *Sphingobium* (Dams et

al., 2007), *Alcaligenes* (Haluřka et al., 1995) and *Achromobacter* (Ronen et al., 2000). Increasing attention also needs to be directed to gram-positive bacteria belonging to the genera *Rhodococcus* (Briglia et al., 1990), *Mycobacterium* (Jacques et al., 2008) and *Bacillus* (Silva et al., 2009). In turn, fungi potentially useful for bioaugmentation are represented by species from genus *Absidia* (Garon et al., 2004), *Achremonium* (Silva et al., 2009b), *Aspergillus* (dos Santos et al., 2008), *Verticillium* (Silva et al., 2009b), *Penicillium* (Mancera-Lo'pez et al., 2008) and *Mucor* (Szewczyk and Długon'ski 2009). There are no microorganisms or their groups universally applicable to bioaugmentation. Many microorganisms are metabolically versatile and are capable of degrading a wide spectrum of substrates.

Bioaugmentation with GMOs and genes

Indigenous microorganisms during long-term exposure to xenobiotics evolve to create a capacity to degrade these compounds. The evolution, involving mutations and horizontal gene transfer (HGT), takes place constantly but is relatively slow in nature. Due to this there is a need to improve microbial degradative activity using molecular biology, which offers numerous technologies for engineering or enhancing remediation genes (Halden et al., 1999; Liphay et al., 2001; Rodrigues et al., 2006). Recently, special attention has been focused on enhancing the biodegradative potential of microorganisms by transfer of packaged catabolic genes from one or more donor strains to indigenous microflora existing in contaminated areas. Many catabolic pathways are located on plasmids such as TOL/pWW0, TOL/pWW53, TOL/pDK1, BPH/pWW110, NAH/NAH7 and PHE/pVI150, transposons or other mobile and/or integrative elements (Top et al., 2002; Jussila et al., 2007). Plasmid-encoded pathways are beneficial since they present genetically flexible systems and can be transferred between bacteria species or even genera (Sayler et al., 1990; Reineke 1998; Sayler and Ripp 2000). Because most of the degradative plasmids are self-transmissible, conjugation has the highest significance in widespread catabolic genes with respect to bioaugmentation. However, other mechanisms of HGT such as transformation and transduction play important roles in the development and adaptation of microorganism.

Conclusions

Increasing awareness and concern of environmental issues has forced humanity to think above conventional methods of waste treatment. Bioremediation, a need of present and immediate future, is a powerful tool available to clean up contaminated sites. Success of bioremediation strategies depends on the amenability of the pollutant to get biologically transformed; the accessibility or bioavailability of the contaminant to microorganisms; and the opportunity for optimization of biological activity. It is important to ensure that the contaminated material is suitably detoxified at the end of the treatment. The degradation with molecular approaches with help of microbial populations have already begun. Recent innovative breakthroughs in molecular and ‘-omics’ technologies such as molecular profiling, ultrafast pyro-sequencing, microarrays, mass spectrometry, meta transcriptomics and meta-proteomics, transcriptome and proteome analyses of entire community along with bioinformatics tools have potential to gain insights of indigenous microbial communities and their mechanism in bioremediation of environmental pollutants. In future, genetically modified organisms can be developed with chemotaxis power that helps them to approach and degrade toxic compounds in the environment. Therefore, in the future a combination of techniques/microbes can be used for bioremediation purpose. Bioremediation depends for its success on selling the results which not only provide benefit but also remediate the wastes.

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