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Pseudomonas Resistant to Heavy Metal Contamination Degrades and Mineralizes

Abhishek Dewividi
Department of Biology, Alagappa University, Tamil Nadu, INDIA.

Corresponding Author: dewivide_10@gmail.com

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ABSTRACT

Nowadays, due to industrialization and extraction of natural resources, soil and water pollution is one of the major global concerns. During the recent era of environmental protection, the use of microorganisms for the recovery of heavy metals from soil, sediments and water as well as employment of plants for landfill applications has generated growing attention. The role of microorganisms and plants in biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for bioremediation of metal-contaminated sites. The food and water we consume are often contaminated with a range of chemicals and heavy metals, such as gold, copper, nickel, zinc, lead, cadmium, arsenic, chromium, and mercury that are associated with numerous diseases. Human activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge these metals which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Many studies have demonstrated that microbes have the ability to remove heavy metals from contaminated soils. Among others some of the microorganisms that play great role in bioremediation of heavy metals are Pseudomonas spp. Alcaligenes spp., Arthrobacter spp.,

Keywords- Flavobacterium, Azotobacter, Agriculture.

I. INTRODUCTION

Nowadays, with growth of industrialization and extraction of natural resources, there has been a considerable increase in the discharge of industrial waste to the environment, mainly soil and water, which has led to the accumulation of heavy metals. Consequently, contamination of soils, groundwater, sediments, surface water, and air with hazardous heavy metals and toxic chemicals is one of the major threats facing the world, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. According to recent study by Asha et al., (2013), the need to remediate these natural resources has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal because of their potential to enter the food chain.

Scientific report reviled that metals when present in our body are capable of causing serious health problems, by interfering with our normal functions (Suranjana et al., 2009). Some of these metals are useful to the body in low concentration like arsenic, copper, iron, nickel, and the likes but are toxic at high concentration and are not only cytotoxic but also carcinogenic and mutagenic in nature (Salem et al., 2000). Because of human activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge a variety of metals such as Silver (Ag), Arsenic (As), Gold (Au), Cadmium (Cd), Cobalt (Co), Chromium (Cr), copper (Cu), mercury (Hg), Nickel (Ni), Lead (Pb), selenium (Se), and zinc (Zn), which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Some metals are required by plants in very small amounts for their growth and optimum performance. However, the increasing concentration of several metals in soil and waters due to industrial revolution has created an alarming situation for human life and aquatic biota.

Conventional methods to remediate heavy metals contaminated site are excavation and solidification/ stabilization, these technologies are suitable to control contamination but not permanently remove heavy metals (Bahi et al., 2012). However, they have some disadvantages, among them cost-effectiveness limitations, generation of hazardous by-products or inefficiency. On the other hand, biological methods potentially solve these drawbacks since they are easy to operate, do not produce secondary pollution. Heavy metals having relatively high density are toxic at low concentration. Microorganisms and plants are usually used for the removal of heavy metals. Process of involvement of microorganisms to reduce pollutant concentration is known as bioremediation which is a natural process and its importance of biodiversity (above or below the ground) is increasingly considered for clean-up of metal contaminated and polluted ecosystem. All the metals are toxic, but some of these are useful in low concentration. These metal toxicities cause serious morbidity and mortality (Surajana et al., 2009). Furthermore, Jin et al., (2011) reported that the bioavailability can be improved by addition of organic nutrients to the soil such as manure, compost, biosolids, which condition the soil and increases the fertility of soil. In order to make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements. There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment.

Moreover, most heavy metal salts are water-soluble and get dissolved in wastewater, which means they cannot be separated by physical separation methods ((Hussein et al., 2004). Additionally, physico-chemical methods are ineffective or expensive when the concentration of heavy metals is very low. Alternately, biological methods like biosorption and bioaccumulation for removal of heavy metals may be an attractive alternative to physico-chemical methods (Kapoor et al., 1995). Use of microorganisms and plants for remediation purposes is thus a possible solution for heavy metal pollution since it includes sustainable remediation technologies to rectify and re-establish the natural condition of soil. However, introduction of heavy metals into the soil causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms at relatively low concentrations (Doelman et al., 1994). Moreover, According to report by Wood and Wang, (1983) and Li et al., (1994),the modification of the microbial make up is mainly brought about by exerting an inhibitory action through blockage of essential functional groups, displacement of essential metal ions or modification of active conformations of biological molecules.Moreover, the response of microbial communities to heavy metals depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the medium and microbial species (Goblenz et al., 1994).

II. MATERIALS AND METHODS

2.1 The Concept of Bioremediation

The quality of life on Earth is linked to the overall quality of the environment. The problems associated with contaminated sites now assume increasing prominence in many countries. Enormous quantities of organic and inorganic compounds are released into the environment each year as a result of human activities. Contaminated lands generally result from industrial activities, use and disposal of hazardous substances, and the like. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of the sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use (Caimey, 1993, Damodaran and Suresh, 2011).

Bioremediation is an innovative and promising technology available for removal of heavy metals and recovery of the heavy metals in polluted water and lands. Since microorganisms have developed various strategies for their survival in heavy metal-polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorpti on , bioaccumulation, biotransformation and biomineralization.

Bioremediation is a general concept that includes all those processes and actions that take place in order to biotransform an environment, already altered by contaminants, to its original status. Adhikari et al., (2004) also defined as bioremediation is the process of cleaning up hazardous wastes with microorganisms or plants and is the safest method of clearing soil of pollutants. Bioremediation uses primarily microorganisms or microbial processes to degrade and transform environmental contaminants into harmless or less toxic forms (Garbisu and Alkorta, 2003).

2.2 Types of Bioremediation

According to EPA (2001 and 2002) on the basis of removal and transportation of wastes for treatment there are basically two methods. These are in-situ bioremediation ex-situ bioremediation.

2.3 In-situ Bioremediation

In-situ bioremediation is no need to excavate or remove soils or water in order to accomplish remediation. The pollution is eliminated directly at the place where it occurs or at the site of contamination so may be less expensive, create less dust, and it is possible to treat a large volume of soil and cause less release of contaminants. In-situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater (Vidali, 2001; Evans and Furlong, 2003).

Most often, in- situ bioremediation is applied to the degradation of contaminants in saturated soils and groundwater. It is a superior method to cleaning contaminated environments since it is cheaper and uses harmless microbial organisms to degrade the chemicals and also a safer method in degrading harmful compounds. In-situ bioremediation can be two types. These are intrinsic bioremediation and engineered in - situ bioremediation. In-situ bioremediation approach deals with stimulation of indigenous or naturally occurring microbial populations by feeding them nutrients and oxygen to increase their metabolic activity where as engineered in-situ bioremediation approach involves the introduction of certain microorganisms to the site of contamination. When site conditions are not suitable, engineered systems have to be introduced to that particular site. Engineered in situ bioremediation accelerates the degradation process by enhancing the physicochemical conditions to encourage the growth of microorganisms. Oxygen, electron acceptors and nutrients (nitrogen and phosphorus) promote microbial growth (Evans and Furlong 2003).

2.4 Advantage and Disadvantage of in- situ Bioremediation

This method have many potential advantages as it does not require excavation of the contaminated soil and hence proves to be cost effective, there is minimal site disruption, so the amount of dust created is less and simultaneous treatment of soil and groundwater is possible. It poses some disadvantages also as the method is time consuming compared to the other remedial methods, seasonal variation of the microbial activity due to direct exposure to changes in environmental factors that cannot be controlled and problematic application of treatment additives (EPA, 2003).

Microorganisms act well only when the waste materials present allow them to produce nutrients and energy for the development of more cells. When these conditions are not favorable then their capacity to degrade is reduced . In such cases genetically engineered microorganisms have to be used, although stimulating indigenous microorganisms is preferred (EPA, 2003).

2.5 Ex- situ Bioremediation

This process requires excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. This technique has more disadvantages than advantages. Ex- situ bioremediation techniques involve the excavation or removal of contaminated soil fromground. Depending on the state of the contaminant to be removed, ex- situ bioremediation is classified as solid phase system and slurry phase systems.

The Solid phase treatment includes organic wastes such as leaves, animal manures and agricultural wastes and problematic wastes like domestic and industrial wastes, sewage sludge and municipal solid wastes. Solid phase soil treatment processes include land farming, soil biopiles, and composting.

III. RESULTS AND DISCUSSION

3.1 Microorganisms used in Bioremediation

The bioremediation processes may be conducted by the autochthonous microorganisms, which naturally inhabit the soil/water environment undergoing purification, or by other microorganisms, that derive from different environments. There are a number of microorganisms that can be used to remove metal from environment, such bacteria, fungi, yeast and algae (White et al., 1997 and Vieira and Volesky, 2000).

Microorganisms can be isolated from almost any environmental conditions. Microbes canadapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. The main requirements are an energy source and a carbon source (Vidali., 2001). Because of the adaptability of microbes and other biologicalsystems, these can be used to degrade or remediate environmental hazards. Natural organisms, either indigenous or extraneous (introduced), are the prime agents used for bioremediation (Prescott et al., 2002). The organisms that are utilized vary, depending onthe chemical nature of the polluting agents, and are to be selected carefully as they onlysurvive within a limited range of chemical contaminants (Prescott et al., 2002ˇ Dubey, 2004). Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be required for effective mediation (Watanabe et al., 2001).

Table 1: Microorganisms Having Biodegradation Potential for Xenobiotics.

Microorganisms	Toxic Chemicals	Reference
Pseudomonas spp.	Benzene, anthracene, hydrocarbons, PCBs	Cybulskiet al, 2003
Alcaligenes spp.	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	Kapleyet al., 1999
Arthrobacter spp .	Benzene,hydrocarbons,pentachlorop henol, phenoxyacetate, polycyclic aromatic, Aromatics, long chain alkanes, phenol, Cresol	Jogdand, 1995
Bacillus spp.	Halogenated hydrocarbons, Phenoxyacetates	Cybulskiet al.,2003
Corynebacterium spp.	Aromatics	Jogdand, 1995
Flavobacterium spp.	Aromatics Naphthalene, biphenyl	Jogdand, 1995

Azotobacter spp.	Aromatics, branched hydrocarbons benzene,cycloparaffins	Jogdand, 1995 DeanRosset al.,2002
Rhodococcus spp.	Hydrocarbons Aromatics	DeanRosset al.,2002
Mycobacterium spp.	Aromatics Hydrocarbons, polycyclic hydrocarbons	Park et al., 1998
Nocardia spp .	Phenoxyacetate, halogenated hydrocarbon diazinon	Jogdand, 1995
Methosinus sp.	PCBs, formaldehyde	Ijah, 1998
Methanogens	PCBs, polycyclic aromatics, biphenyl	Jogdand, 1995

These microorganisms can be subdivided into the following groups:

1. Aerobic:

Pseudomonas , Alcaligenes , Sphingomonas, Rhodococcus and Mycobacterium. These microbes have often been reported to degrade pesticides and hydrocarbons, bothalkanes and polyaromatic compounds. Many of these bacteria use the contaminant asthe sole source of carbon and energy.

2. Anaerobic:

There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

3. Ligninolytic Fungi:

Fungi such as the white rot fungus Phanaerochaete chrysosporium have the ability to degrade a nextremely diverse range of pers is tent or toxicenvironmental pollutants. Common substrates used include straw, saw dust, or corncobs.

3.2 Methylotrophs

Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1, 2-dichloroethane (EPA, 2003).

Bioremediation is not effective only for the degradation of pollutants but it can also be used to clean unwanted substances from air, soil, water and raw materials form industrial waste. With this in view, though many engineered processes for applying bioremediation have been developed but the inexpensive treatment of such sites has remained an elusive goal (Zeyaullah et al.,2009).

3.3 Phytoremediation

Plants can also be used to clean up soil, water or air; this is called phytoremediation. For instance, plants like locoweed remove large amounts of the toxic element Selenium. The Selenium is stored in plant tissues where it poses no harm until and unless the plant is eaten. Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (Glick, 2003). This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Phytoremediation applications can also be classified based on the mechanisms involved. Such mechanisms include extraction of contaminants from soil or groundwater; concentration of contaminants in plant tissue; degradation of contaminants by various biotic or abiotic processes; volatilization or transpiration of volatile contaminants from plants to the air; immobilization of contaminants in the root zone; hydraulic control of contaminated groundwater (plume control); and control of runoff, erosion, and infiltration by vegetative covers (Macek et al., 2000).

3.4 Degradation

Plants may enhance degradation in the rhizosphere (root zone of influence). Microbial counts in rhizosphere soils can be one or two orders of magnitude greater than in non-rhizosphere soils. It is due to microbial or fungal symbiosis with the plant, plant exudates including enzymes, or other physical or chemical effects in the root zone. Contaminants like trinitrotoluene (TNT), petroleum hydrocarbons (PH), pentachlorophenol (PCP), and polynuclear aromatic hydrocarbons (PAH) are degraded in the root zone of planted areas. Another possible mechanism for contaminant degradation is metabolism within the plant. Some plants may be able to take in toxic compounds and in the process of metabolizing the available nutrients, detoxify them. Trichloroethylene (TCE) is possibly degraded in poplar trees and the carbon used for tissue growth while the chloride is expelled through the roots (EPA, 2003).

3.5 Extraction

Phytoextraction, or phytomining, is the process of planting a crop of a species that is known to accumulate contaminants in the shoots and leaves of the plants, and then harvesting the crop and removing the contaminant from the site. Unlike the destructive degradation mechanisms, this technique yields a mass of plant and contaminant (typically metals) that must be transported for disposal or recycling. This is a concentration technology that leaves a much smaller mass to be disposed of when compared to excavation and land filling (EPA, 2003).

Volatilization or transpiration through plants into the atmosphere is another possible mechanism for removing a contaminant from the soil or water of a site. Mercury (Hg) has been shown to move through a plant and into the air in a plant that was genetically altered to allow it to do so. The thought behind this media switching is that elemental Hg in the air poses less risk than other Hg forms in the soil (EPA, 2003).

Advantages and Disadvantage of Bioremediation:

Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies (Vidali, 2001 and Zeyaullah et al., 2009).

Like other technologies, bioremediation also has its own limitations. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded (EPA, 2003). There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biological processes are often highly specific.

3.6 Treatment of Heavy Metals

According to Sharm and Rehman (2009) heavy metals are normally regarded as metals with an atomic number 22 to 92 in all groups from period 3 to 7 in the periodic table. Some of the metals such as Cu, Zn, Cd, Pb, Fe, Cr, Co, Ni, Mn, Mo, V, Se is essential in trace quantities for the general wellbeing of living organism but an excess of these can be lethal. Costa and Duta (2001) reported that heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants. Their presence in soil and water, even in traces, can cause serious problems to all organisms. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality (safety and marketability), crop growth (due to phytotoxicity) and environmental health. Adhikariet al.,(2004) also added heavy metals at higher concentrations are toxic in nature to higher life forms because lead to biomagnifications and their pollution deteriorates the quality of soil and crops produced. Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes namely inhalation and ingestion, and with ingestion being the main route of exposure to these elements in human population. Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries (Costa and Duta, 2001).

IV. CONCLUSION

Bioremediation provides a technique for cleaning up pollution by enhancing the natural biodegradation processes. So by developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting field trials of new bioremediation techniques which are cost effective, and dedicating sites which are set aside for long term research purpose, these opportunities offer potential for significant advances. There is no doubt that bioremediation is in the process of paving a way to greener pastures. Regardless of which aspect of bioremediation that is used, this technology offers an efficient and cost effective way to treat contaminated ground water and soil.

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