aerobic and anaerobic condition but aerobic conditions prove to be faster compared to that of anaerobic condition (Jayasingh et al., 2005). The isolation of heavy metal resistant microorganisms and the understanding of the mechanisms they use in order to remove this kind of pollutants may contribute to the development of improved bioremediation processes (Vargas et al.,). Bioremediation is the use of biological interventions of biodiversity for mitigation of the environmental pollutants. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment (Asha et al., 2013). Bioremediation uses biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up contaminated soil and water (Kumar et al., 2011). Bioremediation can be used at the site of contamination (in situ) or on contamination removed from the original site (ex situ). In situ bioremediation involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and sorbed contaminants and use them as substrates. Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources. Bioremediation/ Phytoremediation and Rhizoremediation, Microflora associated with plants; endophytic bacteria, rhizosphere bacteria and mycorrhizae have the potential to degrade heavy metals in association with plants and this process is termed rhizoremediation. Thus bioremediation, phytoremediation and rhizoremediation contribute significantly to the fate of hazardous waste (heavy metals) and can be used to remove these unwanted compounds from the biosphere. Bioremediation processes can also be assessed through a multifaceted approach such as, Natural attenuation, sensing environmental pollution, metabolic pathway engineering, applying phyto and microbial diversity to problematic sites, plant-endophyte partnerships and systems biology (Asha et al., 2013). Enhancement of these polluted soil residues with different organic amendments like manure compost, biosolids, MSW will lead to increased bioavailability which in turn will act as nutrients for microorganisms and also a conditioner to improve the physical properties and fertility of the soils (Jin et al., 2010).The aim of this work is to study the ability of bioremediation process with microbial isolates and amendments to remove heavy metals from polluted soil residue.

2. Principle of Bioremediation

Recent studies in molecular biology and ecology offers numerous opportunities for more efficient biological process (Kumar et al., 2011). Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds (Asha et al., 2013). It uses naturally occurring bacteria, fungi or plants to degrade or detoxify components hazardous to human health and environment. The microorganisms may be indigenous to the contaminated area or they may be isolated from elsewhere and brought to the contaminated site (Kumar et al., 2011). Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules. Most important parameters for bioremediation are i) the nature of pollutants, ii) the soil structure, pH, Moisture contents and hydrogeology, iii) the nutritional state, microbial diversity of the site and iv)Temperature and oxidation-reduction (redox-Potential).In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Asha et al., 2013).

2.1 Agents of Bioremediation

Natural organisms, either indigenous or extraneous (introduced), are the prime agents used for bioremediation. The organisms that are utilized vary, depending on the nature of the polluting agents, and are to be selected carefully as they only survive within a limited range of contaminants. The first patent for a biological remediation agent was registered in 1974, being a strain of Pseudomonas putida that was able to degrade petroleum. Bioremediation can occur naturally or through intervention processes (Asha et al., 2013).

2.2 Classification of Microbes

Classifications of microbes are as (i) Aerobic: Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium.* These microbes have often been reported to degrade heavy metals, pesticides and hydrocarbons compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy. (ii) Anaerobic: Anaerobic bacteria are not as frequently used as aerobic bacteria for uptake of heavy metals. (iii) Ligninolytic fungi: Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs. (iv) 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 (Kumar et al., 2011).

2.3 Microbial Bioremediation of Heavy Metals

Microorganisms can be isolated from almost any environmental conditions. Microbes can adapt and grow at subzero temperatures, extreme heat, desert conditions, in water with an excess of oxygen and in anaerobic condition in presence of hazardous components or in any waste stream. The main requirement is an energy and carbon source (Vidali 2001). Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions. Other metals have no biological role e.g. cadmium

(Cd), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Soil micro-organisms have been shown to bio-accumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil. Oscillatoria spp. (a blue-green algae), Chlorella vulgaris & Chlamydomonas spp. (green algae).

2.4 Microbial Remediation of Toxic Metals

This occurs in Two Ways (i) Direct reduction by the activity of the bacteria decontamination, using bioreactors (pump & treat) and also for soils after excavation (pulping or heaping and inoculation with appropriate microbial consortium). These techniques are ex-situ methods, and very expensive and has low metal extraction efficiencies. (ii) Indirect reduction by biologically produced hydrogen sulfide (H2S) by sulfate reducing bacteria to reduce and precipitate the metals. This is an in-situ method, and an environmentally sound & inexpensive alternative to pump & treat (for contaminated groundwater) or excavate & treat (for contaminated soils). Microbial growth is induced in sub-surface zones by injecting substrates. The migrating metals are intercepted and immobilized by precipitation with biologically produced H2S (Asha et al.,2013).Toxic metals readily bind to sulfhydryl group of proteins. In-situ bioremediation of uranium contaminated sites have been conducted successfully with Desulfosphorosinus spp. and Closteridium spp. Table 1.1 shows the microorganisms and plant/fungi species that utilize heavy metal (Prasad et al., 2003)

Table 1.1: Microorganisms and Plants/Fungi that Utilize Heavy Metal

Sl.	Microorganisms	Elements	Plants/ Fungi	Elements
1	Bacillus Spp	Cu, Zn	Brassica juncea (L.) czern	Cd
2	Pseudomonas aeruginosa	U, Cu, Ni	B.juncea	Cr(IV)
3	Zooglea spp.	Co, Ni, Cd	B.juncea	Cu
4	Citrobacter spp.	Cd, U, Pb	B.juncea	Ni
5	Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg, Zn	Zea mays L, B. campetris L, B.juncea	Pb
6	Aspergillusniger	Cd, Zn, Ag, Th, U	B. chinesis L, B.juncea	U
7	Pleurotusostreatus	Cd, Cu, Zn	Avenasativa, B. juncea,napusL.Hordeumvulgare, B.rapa	Zn
8	Rhizopusarrhizus	Ag, Hg, P	Viola, Baoshanensis, Sediumalfredii,Rumexcrispus, Helianthus, Annus, B.juncea	Cd, Pb, Zn, As
9	Stereumhirsutum	Cd, Pb, Ca	Anthyllis, Vulneraria, Festuca, Arvernensis, Koeleria, Vallesiana	Cu Cr, Cd, Pb, Zn, As Ni,
10	Ganodermaapplantus	Cd,Pb		
11	Volvariellavolvacea	Cu, Hg, Pb		
12	Volvariellavolvacea	Zn, Pb, Cu		
13	Phormidiumvalderium	Cd, Co, Cu, Ni		

3. Types of Bioremediation

There are two approaches to bioremediation (i) **in situ bioremediation** involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and sorbed contaminants and use them as substrates for transformation. Since the in situ process is slow, it is not the best approach when immediate site cleanup is desired. (ii) **Ex situ bioremediation** is a different approach that utilizes specially constructed treatment facility. It is more expensive than in situ bioremediation (Sitinder et al., 2006).

3.1 In-Situ Bioremediation

In situ bioremediation is the application of biological treatment to the cleanup of hazardous chemicals present in the subsurface. 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. Generally, this technique includes conditions such as the infiltration of water containing nutrients and oxygen or other electron acceptors for groundwater treatment. The optimization and control of microbial transformations of organic contaminants require the integration of many scientific and engineering disciplines.

a) Biosparging

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

b) Bioventing

Bioventing is a promising new technology that stimulates the natural in-situ biodegradation of any aerobically degradable compounds in NAPL within the soil by providing oxygen to existing soil microorganisms. In contrast to soil-vapor extraction (SVE), Bioventing uses low air-flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil.

c) Bioaugmentation

Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. It is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentation is used to ensure that the in situ microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic. Monitoring of this system is difficult. Since the in situ process is slow, it is not the best approach when immediate site cleanup is desired.

3.2 Ex Situ Bioremediation

This technique has more disadvantages than advantages. Ex situ bioremediation techniques involve the excavation or removal of contaminated soil from ground. Depending on the state of the contaminant to be removed, ex situ bioremediation is classified as Solid phase system (including land treatment and soil piles) and Slurry phase systems (including solid- liquid suspensions in bio reactors)

a) Phytoremediation

Phytoremediation is the use of higher plants to bio remediates contamination in soil, water, or sediments. Variations of phytoremediation that have been used in the past include to treat heavy metals from soil and water environment, municipal sewage or neutralize acidic mine drainage. Phytoremediation are classified into four types (Asha et al., 2013).

b) Phytodegradation

Specifically, phytodegradation, also called "phytotransform with the subsequent breakdown, mineralization, or metabolization by the plant itself through variousinternal enzymatic reactions and metabolic processes. Depending on factors such as the concentration and composition, plant species, and soil conditions, contaminants may be able to pass through the rhizosphere only partially or negligibly impeded by phytosequestration and/or rhizodegradation. In this case, the contaminant may then be subject to biological processes occurring within the plant itself, assuming it is dissolved in the transpiration stream and can be phytoextracted.

c) Phytovolatilization

Phytovolatilization is the volatilization of contaminants from the plant either from the leaf stomata or from plant stems. Chemical characteristics such as the, Henry‟s co contaminants to volatilize. In some cases, a breakdown product derived from the rhizodegradation and/or phytodegradation of the parent contaminant along the transpiration pathway may be the phytovolatilized constituent.This effect was studied for the uptake and phytovolatilization of trichloroethene (TCE) or its breakdown products in poplars. Similarly, certain inorganic constituents such as mercury may be volatilized as well. Specifically, tobacco plants have been modified to be able to take up the highly toxic methyl-mercury, alter the chemical speciation, and phytovolatilize relatively safe levels of the less toxic elemental mercury into the atmosphere. Once volatilized, many chemicals that are recalcitrant in the subsurface environment react rapidly in the atmosphere with hydroxyl radicals, an oxidant formed during the photochemical cycle.

d) Phytostabilization

Phytostabilization refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils. Establishment of rooted vegetation prevents windblown dust, an important pathway for human exposure at hazardous waste sites. Hydraulic control is possible, in some cases, due to the large volume of water that is transpired through plants which prevents migration of leach ate towards groundwater or receiving waters. Phytostabilization is especially applicable for metal contaminants at waste sites where the best alternative is often to hold contaminants in place. Metals do not ultimately degrade, so capturing them in situ is the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas where a large-scale removal action or other in situ remediation is not feasible.

e) Phytoextraction

Phytoextraction refers to the ability of plants to take up contaminants into the roots and translocate them to the aboveground shoots or leaves. For contaminants to be extracted by plants, the constituent must be dissolved in the soil water and come into contact with the plant roots through the transpiration stream. Alternatively, the uptake may occur through vapor adsorption onto the organic root membrane in the vadose zone. Once adsorbed, the contaminant may dissolve into the transpiration water or be actively taken up through plant transport mechanisms.

f) Rhizofiltration

Rhizofiltration can be defined as the use of plant roots to absorb, concentrate, and/or precipitate hazardous compounds, particularly heavy metals or radionuclides, from aqueous solutions. Rhizofiltration is effective in cases where wetlands can be created and all of the contaminated water is allowed to come in contact with roots. Roots of plants are capable of sorbing large quantities of lead and chromium from soil water or from water that is passed through the root zone of densely growing vegetation.

4. Microbial Remediation and its Application

Microbial bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade the hazardous contaminants to environmentally safe levels in soil, subsurface materials, water, sludge and residues. Microbial activity is proved to play an important role in remediating metals in soil residues. Studies on interaction of microorganisms with heavy metals have an increasing interest in recent years. Microbial metal uptake can either occur actively (bioaccumulation) or passively (biosorption). Study carried out by Irma et al., (2013) revealed that the Aspergillus fumigatus fungal isolated from contaminated site has good biosorption capacity towards selected heavy metals. Vargas et al., (2009) showed efficient detoxification of multi polluted heavy metals by fungi isolated from compost. According to Jin et al., (2011), the study relies on soil microorganisms bioavailability can enhance the bioremediation process and this can be done by addition on organic amendments such as biosolids, compost, MSW, compost to the soil. Hadis et al., (2011) studied bioremediation by isolating arsenite-resistant bacteria from arsenic contaminated soil and the investigation of arsenite bioremediation efficiency by the most resistant isolates. The maximum percentage of arsenite removal potential (92%) and arsenite bioaccumulation (36%) by B. macerans was found. Narayanan et al., (2011) studied the bioremediation on effluents from magnasite and bauxite mines using thiobacillus spp and pseudomonas spp. The results of biosorption process showed the T. ferrooxidans reduced/absorbed some heavy metals from mines (Cd, Ca, Zn, Cr, Mn and Pb) and P. aeruginosa absorbed most of the metals than T. ferrooxidans. Both species effectively absorbed Cd, Ca, Zn followed by Pb. Muhammad et al., (2007) conducted a study on ability of loofa sponge-immobilized fungal biomass to remove lead ions from aqueous solution. A new biosorbent was developed by immobilizing a white rot basidiomycete Phanerochaete chrysosporium within low cost and easily available matrix of loofa sponge. The Fungal biomass immobilized on loofa sponge (FBILS) adsorbed Pb (II) very efficiently from aqueous solution and biosorption equilibrium was established in about 1 h. No loss to biosorption capacity of FBILS was found due to the presence of loofa sponge as compared to free fungal biomass (FFB) an increase of 24.27% was noted in the biosorption capacity of FBILS. Pb (II)-laden FBILS was regenerated using HCl, with up to 99% recovery and reused in seven biosorption-desorption cycles without any significant loss in biosorption capacity. FBILS were found to very strong, both physically and chemically, and can resist a wide variation in pH, temperature and agitation without any visible change in shape, structure or texture. His study reports that FBILS have a high biosorption capacity to Pb (II) and can be used as an effective biosorbent for the removal of Pb (II) or other heavy metals. J. Jeyasingh and Ligy Philip (2005), has revealed that bacterial strains that were isolated and enriched from the contaminated site show high Cr (VI) reduction potential. He evaluated Cr (VI) reduction both in aerobic and anaerobic conditions. Though the aerobic system performed better than the anaerobic one, he carried further study in the anaerobic condition due to its economic viability. At higher initial concentration, Cr (VI) reduction was not complete even after 108 h, however, specific Cr (VI) reduction, unit weight of Cr reduced/unit weight of biomass was greater at higher concentration. It was found that a bacterial concentration of 15 ± 1.0 mg/g of soil (wet weight) 50 mg of molasses/g of soil as carbon source were required for the maximum Cr(VI) reduction. The bioreactor operated at these conditions could reduce entire Cr (VI) (5.6 mg Cr (VI)/g of soil) in 20 days. This study showed that bioremediation is a viable, environmental friendly technology.

5. Conclusion

Bioremediation offers a viable alternative to the regular use of physicochemical methods of decontamination which are not generally cost effective. The bioremediation process is influenced by various factors such as soil type, pH, temperature, nutrient, amendments and oxygen. Bioremediation has the capability to detoxify inorganic pollutants like metals by methods of adsorption, uptake or accumulation by microbes. A good bioremediation process and approach will involve strategic use of all native/ indigenous microorganisms in an engineered way to achieve the best possible detoxification levels. In summary, bioremediation technologies have been successfully employed in the field and are gaining more importance with increased acceptance of eco-friendly remediation solutions.

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