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Bioremediation: Current scenario and a necessity in immediate future

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ABSTRACT

Bioremediation refers to the use of microorganisms to degrade, sequester, or remove environment contaminants and is increasingly drawing attention. It offers an alternative, specific, cost-effective, and environment friendly technique over other conventional methods of pollutant cleans up. Three strategies (microbial-, phyto-, and nanotechnology based remediation) biodegrade xenobiotics and various recalcitrant compounds into simple organic compounds, carbon dioxide, water, salts, and other harmless substances. Bioaugmentation and biostimulation of oil and heavy metal contaminated soil and ground water; genetically engineered microorganisms for treating oil-spills, and for sequestering of heavy metals; genetically engineered microorganisms and transgenic plants for the treatment of chlorinated pollutants, including chlorinated solvents, polychlorinated phenols, and chlorinated herbicides are included. Enhanced bioremediation rates to many folds have been achieved with phages driven microbial loop. With the new development in this field and focus on interdisciplinary research, bioremediation technology will go a long way in cleaning our polluted environment in near future. Research on improved microbial strains, and bioanalytical methods for measuring the level of contaminants should be strengthen.

KEYWORDS

Bioremediation;
Pollutants;
Phytoremediation;
Transgenic plants;
Heavy metals;
Oil-spills.

INTRODUCTION

Bioremediation (also referred to as biotreatment, bioreclamation and biorestoration) utilizes the metabolic potential of living organisms such as green plants or their enzymes, bacteria, fungi, algae to clean up contaminated environments, to detoxify, degrade or remove environmental pollutants[1-3]. Bioremediation is the most promising, relatively efficient and cost-effective technology; and includes mechanisms like biostimulation, bioaugmentation, bioaccumulation, biosorption, phytoremediation and rhizoremediation[4]. Three essential components needed for bioremediation are micro organisms, food, and nutrients. Microorganism breaks down a variety of organic compounds to obtain nutrients, carbon, and energy for growth and survival. A contaminant if present provides a source of carbon needed for growth, and the microbes obtain energy by breaking chemical bonds and transferring electrons away from the contaminant. Microbial activity during bioremediation process is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities to increase its efficiency[5,6].

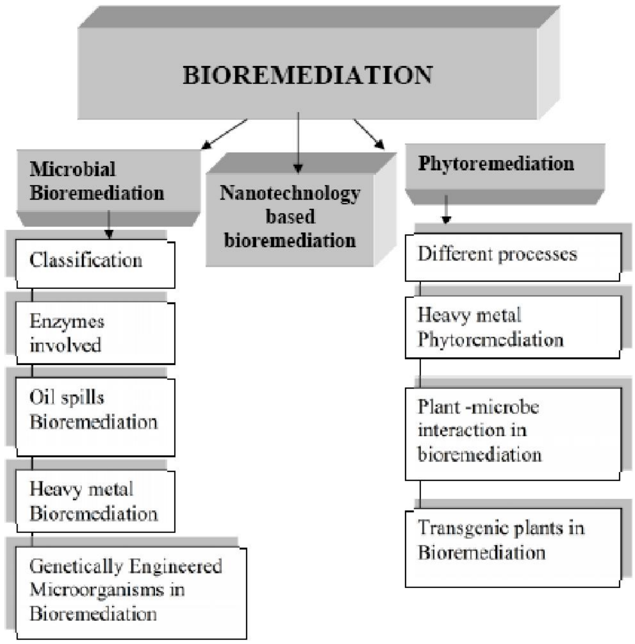

Figure 1 : Various aspects of bioremediation

Bioaugmentation and/or biostimulation, has emerged as the most advantageous soil and water clean-up technique for contaminated sites containing heavy metals and/or organic pollutants, as well as in situ remediation of contaminated soil[7]. Bioremediation works both under aerobic and anaerobic conditions but anaerobic bioremediation have an advantage of permitting microbes to degrade even recalcitrant compounds present in nature. However, various conditions affect the activity of microbe during degradation process. Most important parameters for bioremediation includes: the nature of pollutants, the soil structure, pH, moisture contents and hydrogeology; the nutritional state, microbial diversity of the site, temperature, oxidation-reduction potential and much more[8]. Biotechnological inputs in the field of bioremediation have lead to enhanced public acceptance and also compliance with environmental legislation[9]. Various parameters and optimum conditions for microbial activity during bioremediation [temperature: 15-45°C (mesophilic conditions); pH: 6.5 to 8; oxygen availability: aerobic, minimum air-filled pore space of 10%; nutrients: C:N:P = 100:10:1; type of soil: clay or sill content; soil moisture: 30–90%; type of metabolism: primary, secondary or co-metabolism; and contaminants: non-toxic conc. (xenobiotic, heavy metals) have been reported[10]. Various aspects of bioremediation are listed in Figure 1.

CLASSIFICATION OF BIOREMEDIATION

Bioremediation can broadly be classified into *in situ and ex situ* bioremediation. In the *in-situ* techniques, the polluted site is treated in place without excavation, however, in *ex-situ* techniques; samples from polluted sites are collected and transferred to laboratory for treatment.

In situ bioremediation

In situ biodegradation used for soil and groundwater remediation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants[10]. Various techniques such as bioventing, biaugmentation and biosparging utilized in advanced *in situ* bioremediation are given in TABLE 1.

Intrinsic bioremediation

This approach involves the process of stimulation of indigenous or naturally occurring microbial populations (biostimulation/ bioaugmentation) by feeding them nutrients and oxygen to increase their metabolic activity.

Engineered in situ bioremediation

This 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 accelerated the degradation process by enhancing the physicochemical conditions to encourage the growth of microorganisms[11].

Ex situ bioremediation

This process requires excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. Depending on the state of the contaminant to be removed, *ex situ* bioremediation is classified as:a) Solid phase system (including land treatment and soil piles). b) Slurry phase systems (including solid-liquid suspensions in bioreactors). *Ex situ* bioremediation depends upon techniques such as land farming, composting and biopiles. Also bioreactors are used as latest more efficient and controlled way of bioremediation[4].

TABLE 1 : Various techniques used in bioremediation

Technology	Types	Advantage	Disadvantage	Applications
In situ[7,12-15]	a) Biosparging b) Bioventing c) Bioaugmentation d) Biostimulation	Most cost efficient Non-invasive Relatively passive Natural attenuation Processes. Treats soil and water. Hastened the bioremediation rates	Environmental constraints. Extended treatment time. Monitoring difficulties, Strain selection Loss of stimulant due to diffusion, microbial ecology, type of contaminant, environmental constraints, and procedures of culture introduction	Biodegradative abilities of indigenous microorganisms Presence of metals and other Inorganic. Environmental parameters. Biodegradability of pollutant, Chemical solubility. Geological factors. Distribution of pollutants Biodegradative abilities of native microorganisms Nutrient additions, metabolisms
Ex situ[16-18]	a) Land farming (Solid-phase treatment system)	Cost efficient, Simple procedure, Inexpensive, self-heating	Space requirements Slow degradation rates, Long incubation periods	Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling.
	b) Composting (Anaerobic, convert's solid organic wastes into humus-like material)	Low cost, Rapid reaction rate, Inexpensive self heating	Extended treatment time, Requires nitrogen supplementation, incubation periods months to years	Better growth of plants, good alternative to land filling or incinerating. Practical and convenient.
	c) Biopiles	Can be done on site.	Need to control abiotic loss. Mass transfer problem, bioavailability limitation.	Surface application, agricultural to municipal waste.
Bioreactors[4,19,20]	a) Slurry reactors	Rapid degradation kinetic, Optimized environmental Parameters	Soil requires excavation	Bioaugmentation Toxicity of amendments
	b) Aqueous reactors	Enhances mass transfer, Effective use of inoculants and surfactant	Relatively high cost capital, Relatively high operating cost	Toxic concentrations of Contaminants
Precipitation or Flocculation[4,21]	Non-directed physico-chemical complex -ation reaction between dissolved contaminants and	Cost-effective	Yet to be exploited commercially	Removal of heavy Metals

Technology	Types	Advantage	Disadvantage	Applications
Precipitation or Flocculation[4,21]	Non-directed physico-chemical complex -ation reaction between dissolved contaminants and charged cellular components (dead biomass) biomass)	Cost-effective	Yet to be exploited commercially	Removal of heavy Metals
Microfiltration[4]	Microfiltration membranes are used at a constant pressure	Remove dissolved solids rapidly	Yet to be exploited commercially	Waste water treatment; recovery and reuse of more than 90% of original wastewater
Electrodialysis[4,22]	Uses cation and anion exchangers	Withstand high temperature and can be reused	Yet to be exploited commercially	Removal of dissolved solids efficiently

MICROBES IN BIOREMEDIATION

Microorganisms, used for bioremediation or degradation of xenobiotics may be broadly divided in three categories: (i) Autochthonous (indigenous) organisms, (ii) Allochthonous (non-indigenous) organisms, and (iii) genetically modified organisms. Bioremediation with microorganisms is an attractive alternative to conventional techniques, such as incineration and chemical treatment for pollutant disposal[23]. The xenobiotic generally serves as a storehouse of carbon, energy and other macronutrients such as nitrogen, phosphorous, sulphur, etc. for microbes to enhance degradation process. The main mechanisms involved in xenobiotic degradation involve ability of microbes to enzymatically degrade the specific xenobiotic or absorption of that xenobiotic in their biomass. Single or group of microbes/living organism called as microbial consortia can be used depending upon type of pollutant and other conditions. Bioremediation when used in conjunction with other physical and chemical treatment methodologies can effectively degrade recalcitrant xenobiotics[2]. Further, consortia even play a crucial role in the human gut microbiome[24] and are also known to heavily influence the ecological dynamics of the marine community[25]. The complex relcalcitrant compounds may be degraded by employing two species to complete metabolic reactions from which neither species would gain energy without the cooperation, known as syntrophic degradation[26].

Biological mechanisms behind remediation include: (i) use of microorganisms to detoxify the metals by valence transformation, extracellular chemical precipitation, or volatilization and (ii) use of specific plants to decontaminate soil or water by inactivating metals in the rhizosphere or translocating them in the aerial parts. This approach, called phytoremediation, is considered as a new and highly promising technology for the reclamation of polluted sites and cheaper than physicochemical approaches[27]. Detoxification and biotransformation of chlorinated nitroaromatic compounds have also been studied in various fungi, actinomycetes and bacteria[28]. Atrazine contaminated soils have been treated using bioremediation by employing *Pseudomonas* sp. along with different methods[29]. Complete degradation can be done by consortium of microbes in natural environment which may include synergism or co-metabolism. Most commonly used microorganisms include *Acinetobacter, Actinobacter, Acaligines, Arthrobacter, Bacillus, Berijerinckia, Flavobacterium, Methylosinus, Mycobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete,Pseudomonas, Rhizocotania, Serratio, Trametes and Xanthofacter*[30].

ENZYMES IN BIOREMEDIATION

Enzymatic bioremediation uses enzyme preparations rather than the microorganisms themselves to do the job more quickly and efficiently. Enzymes are proving invaluable to the bioremediation of polluted water and pesticide contaminated soil[31]. Bioremediation is a cost effective and nature friendly biotechnology that is powered by microbial enzymes. The various enzymes produced by different microorganisms in degradation of pollutants ranging from aliphatic to recalcitrant aromatic branched chain hydrocarbon includes, microbial oxidoreductases [microbial monooxygenases and dioxygenases, microbial laccases, microbial peroxidases], microbial lipases, microbial cellulases, and microbial proteases. These enzymes have specific mode of action for degradation of specific pollutant[32]. Enzymes released by the microbe break the contaminant down into digestible pieces and the contaminant is consumed as food by the cell. Many bacteria and fungi depend upon the participation of different intracellular and extracellular enzymes respectively for the remediation of recalcitrant and lignin and organopollutants present in nature by aerobic or anaerobic ways[10,33]. An enzyme-based product, Landguard™, reduced organophosphate levels in cotton irrigation wastewater by 90 per cent within 10 minutes and in used sheep dip by 99 per cent within 30 minutes[34].

Biocatalysis introduces new ways to improve the development of bioremediation strategies. Enzymatic remediation is a valuable alternative as it can be easier to work with than whole organisms, especially in extreme environments. Furthermore, the use of free enzymes avoids the release of exotic or genetically modified organisms (GMO) in the environment[35]. Bioremediation technologies rely on the activity of microbial or plant enzymes involved in the metabolic and co-metabolic transformation of a variety of organic substrates. Hydrolases from *Pseudomonas spp.* and other bacteria have been shown to hydrolyze and detoxify organophosphate pesticides. Several fungal phenoloxidases effectively oxidized xenobiotic phenols and anilines to reactive intermediates that subsequently were detoxified through polymerization or binding to humus[36]. An initial field trial with an enzyme-based product demonstrated that the technology was technically capable of remediating water bodies contaminated with the most common triazine herbicide, atrazine[37]. Information on the enzymes from various microorganisms involved in the biodegradation of wide range of pollutants, applications, and suggestions required to overcome the limitations of their efficient use were reported[38,39].

Advantages of using enzymes over microorganisms include: no requirement of nutrients, biomass acclimation, no formation of metabolic by-products, significantly lowered mass transfer limitation on contaminants, easy-to-control process and effective in small quantity, applicable to recalcitrant compounds and more harsh operational conditions such as contaminant concentration, pH, temperature, and salinity.

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 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.

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 micro organisms (*Pseudomonas sp.*) isolated from heavy metal contaminated site has been reported[40]. The release 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. The process relies upon the ability of microorganisms to degrade organic molecules, but it also depends on the microorganisms coming into contact with the contaminants, and the environment in the contaminated soil being conducive to the survival of the bacteria. A wide variety of techniques have been developed to ensure that these latter two constraints are overcome to enhance contaminant biodegradation[41]. Few of the microorganisms such as algae including cyanobacteria, fungi and bacteria have a tendency to grow in heavy metal contaminated waters indicating that these are able to resist metal toxicity. Endophytes have also been employed for metal removal system in heavy metal remediation even at low concentrations[42]. The potential and limitations of bioremediation for Cr and U toxic metals highlighted the importance of biologically mediated transformation, immobilization, and mineralization of toxic metals during the course of remediation[43]. Minerals support microbial growth by providing essential nutrients, and microbial activity altered mineral solubility and the oxidation state of certain constituent elements. Microbially mediated dissolution, precipitation, and transformation of minerals are either directly controlled by microorganisms or induced by biochemical reactions that usually take place outside the cell. All these reactions alter metal mobility, leading to the release or sequestration of heavy metals and radionuclides. These processes therefore have implications for ore formation and the bioremediation of contaminated sites[44]. The outlook of bioremediation for arsenic and the issues and realms which call for more researches in the future were discussed[45].

The use of algae to remove pollutants particularly heavy metals is called as algal bioremediation. The cosmopolitan nature of the macroalgae and their ability to grow and concentrate a suite of heavy metals from industrial wastes, paves a way towards better bioremediation practices[46]. *In situ* bioremediation of uranium by microbial reduction of soluble U (VI) to insoluble U (IV) has been shown. The use of field-based uranium immunosensors, and a more sophisticated approach to maintain a metal-reducing microbial community were considered among few futuristic techniques[47]. Biosorption of heavy metals using dried algal biomass has been extensively described[46]. Different micro organisms used for bioremediation of heavy metals are summarised in TABLE 2.

TABLE 2 : List of microorganisms used for bioremediation of heavy metals

Organisms	Genus/species	Reference
Yeast	*Candida utilis*	[48]
	Hansenula anomala	[49]
	Rhodotorula mucilaginosa	[50]
	Rhodotorula rubra GVa5	[51]
	Saccharomyces cerevisiae	[50-54]
Fungi	*Aspergillus terreus*	[55]
	Dunaliella (alga)	[56]
	Arbuscular mycorrhiza	[57]
	Penicillium chrysogenum	[50]
Edible Fungi	*Arthrobacter*	[58,59].
Bacteria	*Bacillus*	[52] [60]
	Citrobacter	[61]
	Serratia	[53]
	Cupriavidus metallidurans	[59]
	Cyanobacteria	[52]
	Enterobacter cloacae	[52,62]
	Pseudomonas	[50] [29,63]
	Streptomyces	[50]
	Zoogloea ramigera	[52]
Archea	Filo *Crenarchaeota*	[64]
	Phanerochaete chrysosporium	[65]

Bioremediation of oil spills

The petroleum industry effluents, oily sludge and oil spills cause a serious threat to the environment as they are toxic, mutagenic and carcinogenic. Conventional methods are not safe and environment friendly. Oil contamination has severe impacts on the plant as well as animal ecosystem including human health[66,67].

The hazardous oily waste is basically composed of total petroleum hydrocarbons (TPH), water, and sediments. The TPH constitutes a complex mixture of alkane; aromatic nitrogen, sulfur, and oxygen containing compounds; and asphaltene fractions[68]. Biological methods involve development of indigenous microbial consortium which could biodegrade the components of total petroleum hydrocarbon (TPH) of the oily waste into environment friendly end products. It has been shown that the bioremediated soil was non-toxic and natural vegetation can grow on the same[69]. Bioremediation efficiency can be increased, in some serious cases of oil spills by addition of fertilizers because it hastened the degradation rates[70]. Bioaugmentation followed by biostimulation using consortium of oil degrading microbes in soils contaminated with oil sludge has been reported as an effective way of bioremediation[71].

Researchers reported the microbial communities of a Gulf of Mexico coastal salt marsh during and after the influx of petroleum hydrocarbons following the *Deepwater Horizon* oil spill. The relative richness and abundance of phyla containing previously described hydrocarbon-degrading bacteria (*Proteobacteria, Bacteroids,* and *Actinobacteria*) increased in hydrocarbon-contaminated sediments and then decreased once hydrocarbons were below detection. A greater decrease in hydrocarbon concentrations among marsh grass sediments compared to inlet sediments (lacking marsh grass) suggests that the marsh rhizosphere microbial communities could also be contributing to hydrocarbon degradation[72]. Since nature of hydrocarbon polluted soil is complex, so it may be necessary to apply several remediation techniques including various physicochemical and biological methods to reduce the concentrations of petroleum hydrocarbons to acceptable levels[73]. The microorganisms used for oil bioremediation include *Alcanivorazx borkumensis, Cycloclasticus, Oleispira, Colwellia (Genus), Neptunomonas (Genus).* The usage of fungi/mushrooms and beeswax, has also been reported[67].

PHYTOREMEDIATION

Plants act as natural filters and metabolize substances in the natural ecosystems. The process of pollutant removal by plants is called as phytoremediation[74]. Phytotechnology is a set of technologies using plants (roots, shoots, tissues, and leaves) to remove, transfer, stabilize, or destroy contaminants in soil sediments and groundwater. The basic aim of phytoremediation involves containment -stabilization, sequestration, assimilation, reduction, detoxification, degradation, mobilization, and /or mineralization using plants[75].

The main advantages of phytoremediation over other bioremediation methods include: simple and less costly, easily monitored, possibility of the recovery and re-use of valuable products, preserves the natural state of the environment etc. The various plants used for this process include: *Viola baoshanensis, Sedum alfredii, Rumex crispus, Helianthus annus, Alfalfa, poplar,* juniper, fescue, Indian mustard *Elodea Canadensis, Pueraria thunbergiana, Helianthus annus, Duckweed parrotfeather*, Hybrid poplar. *Brassica juncea, Anthyllis vulneraria, Festuca arvernensis, Koeleria vallesiana, Armeria arenaria, Lupinus albus, cabbage,Stanleya pinnata, Zea mays etc*[4].

There are different main five categories of phytoremediation. These are: phytoextraction, phytofiltration, phytostabilization, phytovolatization and phytodegradation. Phytoextraction involves the use of plants to remove contaminants from soil. The metal ion accumulated in the aerial parts that can be removed to dispose or burnt to recover metals. Phytofiltration utilizes the plant roots or seedling for removal of metals from aqueous wastes. In phytostabilization, the plant roots absorb the pollutants from the soil and keep them in the rhizosphere, making them harmless by preventing them from leaching. Phytovolatization involves the use of plants to volatilize pollutants like Se and Hg. Phytodegradation, the use of plants and associated microorganisms to degrade organic pollutants depends upon different plant cultivars, which process or processes it includes during phytoremediation[76]. Methods used to phytoremediate metal contaminants (phytoextraction, rhizofilteration, phytostabilization) are slightly different to those used to remediate sites polluted with organic contaminants. Different methods processes involved in phytoremediation are summarized in TABLE 3. The mechanisms of phytoremediation include biophysical and biochemical processes like adsorption, transport and translocation, as well as transformation and mineralization by plant enzymes[77].

PHYTOREMEDIATION AND HEAVY METALS

Polluted soils and waters pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology. Scientists have shown that the *Typha domingensis* decreased heavy metals from municipal wastewater[88]. More than 400 plant species have been identified to have potential for soil and water

TABLE 3 : Different phytoremediation processes for removal of different types of pollutants

Phytoremediation processes	Function	Pollutant	Plants	References
Phytoextraction	Remove metals pollutants that accumulate in plants. Remove organics from soil by concentrating them in plant parts	Cd, Pb, Zn, As, Petroleum, Hydrocarbons and radionuclides in soil and groundwater	*Viola baoshanensis, Sedum alfredii, Rumex crispus, Helianthus annus, Alfalfa, poplar,* juniper, fescue, Indian mustard, cabbage,*Thlaspi caerulescens, Viola calaminaria*	[78] [79] [80]
Phytostabilization (Immobilization)	Use of plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn, present in soil	*Anthyllis vulneraria, Festuca arvernensis, Koeleria vallesiana Armeria arenaria, Lupinus albus* Hybrid poplar, Grasses	[81,82]
Rhizofiltration	Roots absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams	Zn, Pb, Cd, As and Radionuclei in groundwater	*Helianthus annus* (Sunflowers), *Brassica juncea*	[82] [83]
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT, Explosives, waste and Nitrates Groundwater	*Elodea Canadensis, Pueraria thunbergiana, Duckweed parrotfeather*, Hybrid poplar	[84] [85]
Phytovolatilization/ rhizovolatilization	Use of plants to volatilize pollutants	Se, CCl4, EDB, TCE	*Stanleya pinnata, Zea mays Brassica sp.*	[86].
Phytotranformation	Plant uptake and degradation of organic Compounds	xenobiotic substances in soil	*Cannas*	[87]

remediation. Among them, *Thlaspi*, *Brassica*, *Sedum alfredii* H., and *Arabidopsis* species have been mostly studied. Recent progresses in research and practical applications of phytoremediation for soil and water resources were reported[89]. The approaches used for plant-assisted bioremediation of heavy metal contaminated soils and aquifers were reported[90,91].

Using plants for heavy metal clearance depends upon genetic variations among plant species and even among the cultivar of the same species. The mechanisms of metal uptake, accumulation, exclusion, translocation, osmoregulation and copartmentation vary with each plant species and determine its specific role in phytoremediation. The recent advances in plant biotechnology have created a new hope for the development of hyperaccumulating species[92]. The latest developments are taking place in bioremediation by utilizing rhizoremediation, protein engineering, metabolic engineering, whole-transcriptome profiling, and proteomics for the degradation of recalcitrant pollutants such as chlorinated aliphatic and polychlorinated biphenyl as well as for binding heavy metals[93]. Cell surface expression of specific proteins allowed the engineered microorganisms to transport, bioaccumulate and/or detoxify heavy metals as well as to degrade xenobiotics[94]. The drawbacks of phytoremediation includes the slow detoxification of organic pollutants and if decomposition is not complete, toxic compounds may accumulate in plant tissue and can be released to the environment or enter food-chains[95]. Examples of plants called hyperaccumulators used to extract heavy metals include Indian mustard *Brassica juncea*, *Thlaspi*

TABLE 4 : Plants and microbe interaction in remediation of heavy metal contaminated soils

Pollutant	Microbe/ Microbial communities	Characteristics properties of microbe	Plant	Soil nature	Reference
Ni	*Chlorella vulgaris Holophaga/ Acidobacterium division and α-proteobacteria, Methylobacterium oryzae, Methylobacterium mesophilicum, Sphingomonas*	Bacterial Ni Solubilisation	*Thlaspi goesingense, Lycopersicon esculentom Alyssum murale*	Ni-rich Serpentine soils, Gontobiotics, pot culture experiments	[102-105]
Zn	*Bacillus spp*	Bacterially mediated dissolution of Zn from non labile phase	*Salix caprea, Anthyllis vulneraria Lupinus albus, Thlaspi caerulescens*	Pot Experiments	[106-109]
Cu	*Bacillus spp*	Dissolution of Cu by addition of rhizobacterial strain MS12 & ampicillin 0.1mg/g, Cu tolerant, exopolymer producing bacterial communities, predominantly, Bacillus	*Elsholtzia splendens* Willow *(Salix viminalis)*	Cu- contaminated soil (Near Cu mines)	[110]
U	*Pseudomonas aeruginosa Citrobacter spp.*		Sun flower, *Phragmites sp*.		[43,57,111-113]
Co	*Zooglea spp.*				[114]
As	*Arthrobacter, Ochrobactrum Bacillus, Serratia sp Pseudomonads*		*Helianthus annuus, Agrostis tenuis*, Chinese Brake fern *Pteris vittata* (in its leaves)	As containinated cattle dip sites	[45,115-120]
Cd	*Bacillus subtilis Citrobacter spp pseudomonad strains (MKRh1, MKRh3, and MKRh4)* Blue green alga Hapalosiphon Welwitschii Nagel	Coinoculation of *Brevibacillus* sp. and AM Fungus, Cadmium resistant bacterial strains inoculated to plants. (Indole acetic acid as auxin produced by the isolates for tolerance)	*Trifolium repens, Brassica napus Salix viminalis), Thlaspi caerulescens,* Willow *(Salix viminalis), Populus canadensis*		[121-125]
Hg	*Pseudomas fluorescens*		Soybean In green-house		[126]
Se		Bacteria volatilizes Se into nontoxic forms, such as dimethylselenide	*Brassica juncea*		[127]
Au	*Chlorella vulgaris*				[114]

Pollutant	Microbe/ Microbial communities	Characteristics properties of microbe	Plant	Soil nature	Reference
Pb	*Cupriavidus taiwanensis TJ208* *Bacillus megaterium HKP-1*		*Mimosa pudica*, Indian mustard (*Brassica juncea*), Ragweed (*Ambrosia artemisiifolia*), Hemp Dogbane (*Apocynum cannabinum*), or Poplar trees, which sequester- lead in its biomass	Experiments in green house	[128,129]

(lead) *caerulessces* (zinc /cadmium), *ipomea alpine* (copper), *Haumaniastrum roberti* (cobalt), *Astragalus racemosus* (selenium), *Sebertia acuminita* (nickel)[96]. Halophytes offer a greater potential for phytoremediation research for the decontamination of heavy metal polluted soils. Recently, the use of salt-accumulating halophytes for soil desalination in arid and semiarid regions has been suggested[97]. Present usage of phytoremediation in heavy metals contaminated soils have been reported[98,99].

During rhizofilteration, there is an interaction between microbe present in the soil and plant root system to enhance biodegradation of heavy metals. Phytoremediation holds great promise for *in situ* treatment of heavy metal contaminated soils. The benefits of combining siderophore-producing bacteria with plants for metal removal (particularly iron) from contaminated soils have been demonstrated[100]. Enhanced bioremediation can be done by exploiting plant-microbe interaction using transgenic science[101].

TRANSGENIC PLANTS IN PHYTOREMEDIATION

Transgenic plants for enhanced bioremediation utilize various biotechnological techniques to engineer plants which are capable of remediating contaminated soils and groundwater in better ways. Various transgenic plants have been generated in order to modify the tolerance, uptake or homeostasis of trace elements[130]. Phytoremediation of herbicides present in soil and water can be done by using transgenic plants[131]. The main approaches used for the development of transgenic plants for phytoremediation include: transformation with genes from other organisms (mammals, bacteria, etc), transformation with genes from other plant species; and over expression of genes from the same plant species[132]. The development of transgenic plants to clean up environmental pollution caused by the wastes of heavy metal mining is a promising method for removing metal pollutants from soils[133]. Transgenic alfalfa plants have a great potential for phytoremediation of mixed environmental contaminants[134]. De-esterification is an important degradation or detoxification mechanism of sulfonylurea herbicide in microbes and plants. Construction of sulfonylurea herbicide-resistant transgenic crops helps in understanding the various mechanisms of degradation of herbicide through metabolism studies and detoxification analysis through de-esterification which further implicates development of bioremediation methods of sulfonylurea herbicide-contaminated environments[135].

Transgenic plants and associated bacteria constitute a new generation of genetically modified organisms for efficient and environmental-friendly treatment of soil and water contaminated with polychlorinated biphenyls (PCBs). Bacterial genes such as biphenyl dioxygenases have been introduced into higher plants, to develop transgenic crops having better PCB degrading capability. Also bacterias have been genetically modified that exhibit improved biodegradation capabilities and were found able to maintain stable relationships with plants. Transgenic plants and associated bacteria bring hope for a broader and more efficient application of phytoremediation for the treatment of PCBs[136].

GENETICALLY ENGINEERED MICROORGANISMS (GEM) AND BIOREMEDIATION

The first genetically engineered organism for bioremediation was *Pseudomonas*. This along with several other microbes was claimed to be effective in treating oil spills. The use of genetic engineering to enhance the natural capacity of microorganisms for remediation has become very promising[137]. Genetically engineered microorganisms have shown a great potential for bioremediation applications in soil, groundwater, exhibiting enhanced degradative capabilities covering a wide range of chemical contaminants.

GEM possessing metallothionein, provided mercury resistance and accumulation provided a viable technology for mercury bioremediation[138]. The use of genetic engineering to produce microorganisms capable of degrading specific contaminants 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[139]. 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[140], Due to eco-friendly approach and lesser health hazards as compared to physico-chemical based strategies to combat heavy metal pollution; GEM 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 biosafety and environmental concerns like genetic pollution, caused by using GEM should be well accounted before releasing into environment[141]. Future bioremediation approaches need emphasis on application of technologies discussed in this review to decontaminate e-waste from the soil-water environment. The hazardous effects of e-waste, Indian and global scenario and innovative bioremediation technologies to remove it from environment have been reported[142].

NANOTECHNOLOGY BASED BIOREMEDIATION

Nanotechnology is an emerging area in the field of soil remediation[143]. The ability of nanotechnology to abate pollution production is in progress and presents a number of potential environmental benefits[144]. Microbe utilization for intracellular/extracellular nanoparticles synthesis with different chemical composition, size/shapes and controlled monodispersity has been shown as a novel, economically viable and eco-friendly strategy that reduced toxic chemicals in the conventional protocol[145,146]. The bacteria cultures exposed to HgS nanoparticles methylated mercury at a rate slower than cultures exposed to dissolved forms of mercury. Further, the methylation potential of HgS nanoparticles decreased with storage time of the nanoparticles in their original stock solution[147].

Enzymes short lifetime is one of the major concerns in their environmental applications. Studies on trypsin and peroxidase attached to uniform core-shell magnetic nanoparticles (MNP's) indicated that the lifetime and activity of enzymes increased dramatically from a few hours to weeks and that MNP-enzyme conjugates were more stable, efficient, and economical[148]. Researchers have caged single enzymes to create a new class of catalysts called "Single enzyme nanoparticles" (SENs)[149,150]. The combination of SENs and nanostructured matrices has potential to make a great impact in bioremediation[151].

BACTERIOPHAGES IN BIOREMEDIATION

Current methods of bioremediation in oil spills often require the introduction of exogenous bacteria, which cause imbalance of delicate marine microcosms. Modification of a phage that is endogenous to the environment, in which it exists, can infect its normal hosts, enabling them to produce compounds such as nitrates, sulfates, and ferric irons to bio-degrade hydrocarbons. The use of phage, as opposed to widespread dumping of these substances in affected areas, is self sustaining, less severe on the environment, and facilitates efforts in areas that may be inaccessible to prolonged human activity. However, careful monitoring of any side-effects of introducing the re-engineered phage to experimental microcosms should be explored. Phages driven microbial loop enhanced bioremediation rates to many folds. Efficiency of bioremediation in petroleum and other hydrocarbons contaminated water has shown to increase when phages along with bacteria were employed[152]. It is desirable that bacteria must die after performing its function so modified systems can be developed by using phages with either a holin or the holin–endolysin pair under the control of an inducible promoter which was found sensitive to the specific substance. These can be developed by providing with a suitable controlled system[153].

CONCLUSIONS AND PERSPECTIVES

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. Generally bacteria aids bioremediation by transforming a specific contaminant. There are many advantages as well as limitations of this process. Bioremediation advantages outweigh the disadvantages, as it offers an efficient and cost effective way to treat contaminated ground water and soil.

Molecular biology and biotechnological methods can help creating stronger microbes and plants with better bioremediation capacity. Genetic modification offers a new hope for phytoremediation as GM approaches can be used to over express the enzymes involved in the existing plant metabolic pathways or to introduce new pathways into plants. The various obstacles faced in uplifting the process are current technologies and also ethical, legal, and social issues involved this technology. With the exciting new development in this field and focus on interdisciplinary research, bioremediation technology will go a long way in cleaning our polluted environment in near future. Improving microbial strains; improving bioanalytical methods for measuring the level of contaminants. However, there are a number of problems which are encountered with bioremediation as well. First, organism's population must increase. For this, their growth conditions must be determined and maintained at the contaminated sites. Even in an ideal environment an organism may prefer to metabolize other more readily available nutrients within a contaminated area, or the pollutant may be completely or partially inaccessible to the degrading organism. The environment may contain substances or organisms that inhibit growth of the degrading population.

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