

Mechanism of Microbial Detoxification of Heavy Metals: A Review

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Abstract

Heavy metal contamination from anthropogenic activities has an adverse effect on the environment due to its cytotoxicity, carcinogenicity and mutagenicity. Environment harbours microorganisms, some of which have been found to modify physico-chemical conditions of their surrounding environment through certain processes such as detoxification, metal homeostasis, precipitation, redox transformations etc. Investigations in the past have shown that short term contact with metals of certain bacteria causes the selection of resistant bacteria within weeks, while a prolonged exposure showed selected strains able to thrive better. Hence biotic methods could assist removal of heavy metals based on biosorption or bioaccumulation by microorganisms, which are cost-effective and environmental friendly in the long run. Microbial remediation is influenced by biotic and environmental factors as also the contamination site characteristics. The aim of this paper is to highlight and review some of the mechanisms of microbial remediation through techniques such as biostimulation, bioaugmentation etc.

Keywords: Heavy Metals, Biosorbents, Microbial Remediation

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Citation: Jayaram S, Ayyasamy PM, Aishwarya KP, Devi MP, Rajakumar S. Mechanism of Microbial Detoxification of Heavy Metals: A Review. *J Pure Appl Microbiol.* 2022;16(3):1562-1574. doi: 10.22207/JPAM.16.3.64

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INTRODUCTION

Microorganisms are widespread in all the realms of the biosphere growing in an extensive range of environmental conditions. The nutritional versatility of different microbes is being explored in the area of biodegradation of pollutants. Although industrialization and technological advancement in today's times is inevitable, multiple ecological and human catastrophes have continuously occurred over the past few decades that have resulted in the industries playing a huge anthropogenic factor for various reported environmental pollution episodes. Industrial discharges specifically impact the lotic water quality with the presence of toxic and hazardous persistent chemicals like lead, cadmium and mercury and toxic organic chemicals such as pesticides, PCBs, dioxins, polyaromatic hydrocarbons (PAHs), etc. which have a deleterious effect on human health.¹

The persistence of heavy metals in the environment and their toxic nature cause bioaccumulation thereby causing deleterious effects on organisms with an exposure to metals with high concentrations when enzymes, cellular proteins and nucleic acids react with them to form complexes.²

Toxic heavy metals have a tendency to bioconcentrate, bioaccumulate and thereby biomagnify thus causing ecological risks. Trophic transfers of four common toxic metals namely Cd, Cr, Cu and Hg investigated in the food webs in the Bohai Sea of North China exhibited Hg and Cr to be biomagnified between the trophic levels while Cu underwent biodilution and Cd exhibited no tendency to biomagnify though its presence in one species was shown to exceed the restrictive criteria which if consumed by another organism could pose sufficient ecological risks.³

Fish being a primary source of nourishment in many parts of the world, any bioaccumulation of toxic heavy metals in fish is of concern.⁴ High levels of heavy metals in fish may lead to toxicity in humans.⁵ Consumption of any fish growing in industrial effluent contaminated water bodies poses high human health risks.⁶ Likewise, heavy metal-contaminated crops if consumed as food could also lead to significant risks to human health.⁷ Agricultural fields if irrigated with sewage wastewater may cause heavy

metals to accumulate in soils and eventually they get transferred to food crops such as cereals and vegetables as well as to milk.⁸ Heavy metals tend to move from the abiotic to the biotic environment, accumulating in organisms at different trophic levels of the food chain or food web. Trophic transfer, bioaccumulation and biomagnifications of hazardous heavy metals in the nutrition network have important implications on wildlife and human health.⁹

Some heavy metals like Cr, Cu, As, Cd, Hg, Pb, Ni, Se, Zn, Ag etc. have also been reported to be toxic at low concentrations due to their cytotoxicity, carcinogenicity and mutagenicity.¹⁰ Studies conducted have also demonstrated that oxidative stress and reactive oxygen species (ROS) production are influenced by the toxicity and carcinogenicity of some heavy metals such as Cd, Cr, As, Pb and Hg, those that affect public health because of their toxic nature even in minor quantities.¹¹

Interactions of Microorganisms with Heavy Metals

Functional groups in the biomolecules of the cell are obstructed when excessive concentrations of metals may displace the enzyme metal ions and participate in cell reactions by competing with structurally related non-metals.¹² Metals in combination may have more negative impacts on the diversity of the microbial biomass in comparison to the high concentrations of single metals,¹³ even affecting the physiological functions in organisms when these form complex toxic compounds.¹⁴

Studies on certain soil bacteria have demonstrated that selection and growth of resistant bacteria can occur within weeks of a short term exposure of bacterial strains to heavy metals but only specific strains of resistant bacteria grow when exposed to metals for a longer duration.¹⁵ Some general effects of toxicity of heavy metals on microorganisms are listed in Table 1.

Cr and Cd have the ability to weaken the bioremediation capacity and denaturing microbial flora.¹⁶ Mutagenesis is found to be caused within the cell due to transcription and replication being affected when Cr (III) reacts with carboxyl and thiol groups thus changing the enzymatic structure and the cationic Cr (III) complexes formed within

the cell interact with phosphate groups of DNA which are anionic.¹⁹ Superoxide radicals that tend to damage the DNA structure are stabilized when in contact with Al.²³ Enzyme configurations could be altered through the allosteric effects by heavy metals that go into competitive or noncompetitive interactions with substrates thus affecting the vital enzymatic functions.²⁴ Ligand interactions and metal displacements from native binding sites in the presence of Cd and Pb cause damage to the DNA structure and cell membranes of the microorganism.²⁵ Microbial growth, metabolism and morphology are affected by a change in the nucleic acid structure with functional disturbances being caused thus affecting the physiology and disrupting the cell membranes and thereby inhibiting enzyme activity.²⁰

Microbial Remediation of Heavy Metals

Interactions of *Bacillus subtilis* with multiple metals like Cu, Fe, Mg, Au and Pb were studied in the early 1980s,²⁶⁻²⁸ wherein it was found that differences between the net anionic charge of the bacteria and the net positive charge of metals were what led to an interaction of the bacterial cell to the metals. The metals with the opposite charges were able to bind to the cell surface of the microbes on the nucleation sites which eventually caused precipitation of the metals on the cell wall.²⁹ Studies by the U.S. Geological Survey (USGS) revealed that interactions of certain species of bacteria and fungi with both metals and other toxic compounds led to the concept of bioremediation being explored further.^{30, 31}

USGS in 1992 explored the possibility of activating bacterial species in the soil by the addition of certain nutrients to the contaminated soils in Hanahan, SC.³⁰⁻³² Observations revealed that removal of about 75% of the toxic chemicals in the soil was effected within a span of a year. The utilization of natural microorganisms found in soil, water and sludge to eradicate pollutants thus spearheaded the study and further applications of bioremediation in the environment.³⁰ Even the genetically engineered microorganisms (GEM) were found to be capable of degrading the environmental toxins and bound metals as was found by the study conducted in the University of Tennessee and Oak Ridge National Laboratory

wherein the genetically engineered microorganism *Pseudomonas fluorescens* (KH44) designed in the laboratory was found to degrade toxic polycyclic aromatic hydrocarbons.^{30, 33}

Microbial cells through interactions can adsorb or absorb the heavy metals onto the binding sites on the cellular surface²¹ depending on the kinetic equilibrium and metal composition at the surface of the cell. Biosorption is thus affected through electrostatic interactions, redox processes, ion exchange, precipitation and surface complexation.³⁴ Cellular fragments, dead or live biomass can carry out biosorption through passive uptake via surface complexation (Figure 1) on to the cell wall and other outer layers. This process is not influenced by cell metabolic cycle.³⁶

During an active uptake, the heavy metal ions pass across the plasma membrane into the cytoplasm (method of bioaccumulation) during which the cell is influenced by physical, chemical, and biological processes including intracellular and extracellular processes.^{36, 37} An organism capable of bioaccumulation must be able to transform the metal, changing it from a toxic to a harmless form even when tolerating the high concentrations of one metal or a combination of metals. The surface structures of bacteria play a vital role in how the bacteria interacts with the surrounding environment. While the cell walls of the gram-negative bacteria contain lipopolysaccharide, phospholipids and peptidoglycan layers, those of the gram-positive bacteria have minor amounts of teichoic acid usually present along with the peptidoglycan in several layers, the latter forming as much as 90 % of the cell wall.^{38, 39}

Bioremediation in Industrial Waste Treatment

Through bioremediation, industrial waste and wastewater treatment have been successfully adopted for multiple years. Waste treatment plants are known to employ specialized microbial populations in the treatment of industrial effluents. Microorganisms have been found to enzymatically metabolise many xenobiotics thereby degrading environmental contaminants.⁴⁰

A review done on the feasibility of microplastics removal through water treatment from water sources reveals that sludge and membranes carry more microplastics from water

Table 1. Toxicity of heavy metals to microorganisms

Heavy Metals	Sources	Effects on microbes	Citation
As	Contaminated soils, sediments, groundwater	Enzyme deactivation	[16, 17]
Cd	Sedimentary rocks, Anthropogenic inputs	Protein denaturation, nucleic acid damage, affects cell division and transcription	[16,18]
Cr	Anthropogenic sources	Growth suppression, inhibition of oxygen absorption	[16,19]
Cu	Rocks and soil	Cellular function disruption, inhibition of enzyme activities	[16, 20]
Se	Minerals	Hinders growth rate	[10, 16]
Pb	Anthropogenic sources	Protein/nucleic acid destruction, inhibition of enzyme action and transcription	[16, 20]
Hg	Natural sources and used in industrial applications	Protein denaturation, inhibition of enzyme activity, disrupts plasma membrane	[16, 20]
Ni	Sulphide ores and anthropogenic sources	Disrupts cell membrane, hinders enzyme functions and cause oxidative stress	[16, 20, 21]
Ag	Ores	Cell lysis, inhibition of cell growth	[16, 22]
Zn	Soil and sediments	Death and decline in biomass, inhibition of growth	[16, 21]

treatment. Primary treatment in a wastewater treatment plant was reported to remove 16.5 to 98.4% microplastics while secondary treatment showed a removal efficiency ranging from 78.1 to 100% with the tertiary treatment showing a removal of 87.3% to above 99.9% microplastics. It was however reported that a complete elimination of microplastics from the final treatment plant wastes was not feasible since the wastes removed from the processes get disposed back to the environment in the form of sludge and disposed membranes.⁴¹

Marine enzymes are successfully used as an alternative in terms of some kinds of industrial waste treatment. Industrial pollutants as those from metal smelting, petrochemical waste, mining wastes, paper and pulp industry wastes, chemical weapon producing industry wastes, wastes from dye industry, anthropogenic activity and agriculture tend to pollute the marine environment.⁴² Isolates of bacteria and fungi are found to remediate the dye effluents.⁴³ Partially degraded lignin and chlorinated phenolic compounds are sources of

pollutants released into the environment from paper and pulp industries. These wastes are bioremediated with fungal species that produce extracellular enzymes (oxidoreductase), viz., manganese peroxidases, lignin peroxidases, and laccase from fungal mycelium, being a quicker bioremediation process than bacteria.⁴⁴ Generally, enzymes are more active than microorganisms to utilize the substrate and its transfer from complex to simple form or smaller substances.⁴⁵ Most of the enzymes are ecologically beneficial and risk-free for the environment, being able to play a vital role in the bioremediation of adverse compounds in the environment.

Mechanism of Detoxification of Heavy Metal by Microbes

Degradation, removal, alteration, immobilization or detoxification of chemical and physical wastes through the action of microbial flora in the environment is the process of bioremediation, a technology that could also exploit naturally occurring mitigation measures

where no human intervention is required other than monitoring as is the case of natural attenuation which relies only on the natural conditions.⁴⁶

Degradation of a pollutant is encouraged when microorganisms play the role of catalysts by facilitating the progress of biochemical reactions that decompose the desired pollutant chemical through various enzymatic pathways.⁴⁶ The physical and chemical conditions of the immediate environment of the microorganisms are modified through processes like detoxification and redox transformations.^{47, 48} The processes of microbial interactions with metals and their locations in the bacterial cell are shown in Figure 2.

In bioaccumulation, the uptake mechanisms of metal into the bacterial cell have been studied to be through processes as passive diffusion, facilitated diffusion and active transport.⁵⁰ Heavy metal polluted environments, specifically soil and water have been bioremediated through the use of *Cupriavidus metallidurans* CH34 which has the capacity to bioconcentrate Se and Au and to volatilize Hg through multiple reactions.^{39, 51, 52} *Pseudomonas stutzeri*, a microorganism isolated from a foundry soil demonstrated good resistance to the toxicity of Cr to a concentration of up to 1mM. It was also found to reduce Cr (VI) up to 100 μ M anaerobically.⁵³

Processes such as ion exchange,

adsorption, and covalent bonding that occur due to the chemiosmotic gradient across the cell without the use of ATP are found to be the crux of biosorption.^{54, 55} Biosorption mechanisms are formulated on two factors; dependence on cell metabolism and area inside the cell from where the metal is eliminated.⁵⁶

Biostimulation is when the activities of indigenous microorganisms are stimulated through the use of specific nutrients into the soil or water so as to encourage the removal of the metal from the site.⁴⁶ Application of plant nutrients, growth supplements and essential trace elements as also changes made to the conditions like pH, oxygen and temperature will help accelerate the rate of metabolic reactions and the metabolic pathways,^{57, 58} and thus stimulate the naturally existing bacterial and fungal communities. The population of microorganisms in contaminated soil also needs to be in large numbers so as to bring about effective bioremediation. Small concentrations of pollutant can stimulate the microorganisms by activating the operons for the bioremediation enzymes. The use of nutrients like N, P and C will encourage the microorganisms to create the basic environment needed for pollutant degradation.^{46, 59}

Large array of physical, chemical and biological processes that contribute to the eradication of the pollutants from surroundings

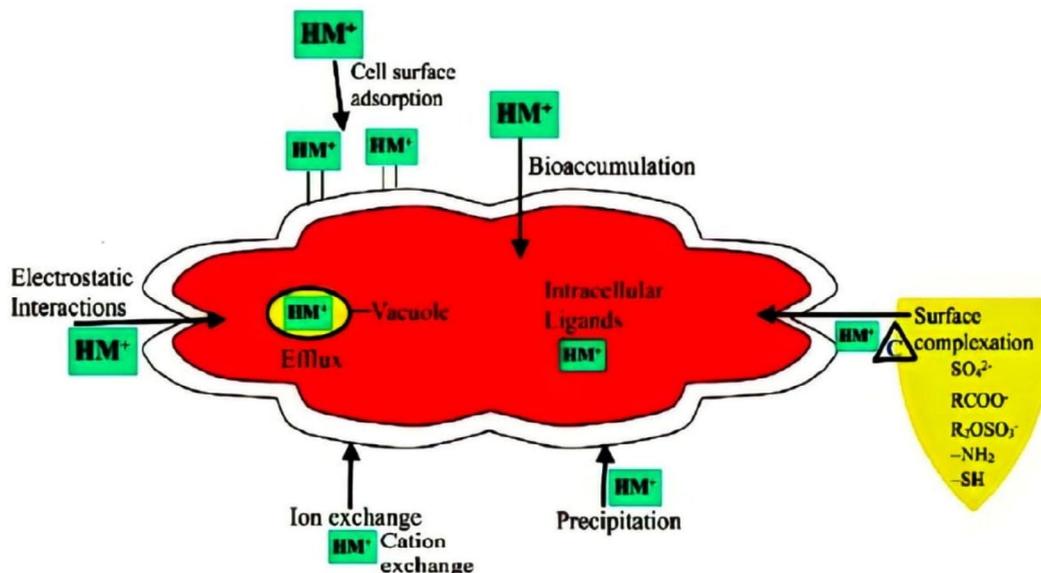


Figure 1. Mechanisms of heavy metal uptake by microorganisms. [35]

through processes such as biodegradation (aerobic and anaerobic), uptake of chemicals by biota, physical phenomena such as diffusion, dispersion, dilution, sorption/desorption, volatilization and reactions such as ion exchange and complexation is the basis of bioattenuation,⁶⁰ which ultimately help reduce the toxicity, mass or volume and concentration of contaminants in the environment.

Bioaugmentation is a method of biodegradation wherein the biodegradable microorganisms, be it engineered, exotic or natural, are introduced into the environment to aid in natural microorganism population growth to increase the capacity of biodegradation of the native microbial populations thus effecting enhanced degradation through preferential feeding of the metal contaminants on site. Microorganisms collected from the contaminated site can be cultured or genetically modified before being introduced back to the site.⁴⁶ Through this process, even complex pollutants can be eliminated quickly.⁶⁰

Studies conducted using crude oil as a growth substrate showed that microbial consortia from oil- contaminated sites efficiently degraded

complex hydrocarbons.⁶¹ The degradative efficiency is more enhanced in the genetically modified microorganisms due to diverse metabolic profiles that can aid it in biodegradation.^{62, 63} Research has shown that effective degradation of a wider range of chemical and physical environmental pollutants has been possible using genetically engineered microorganisms in bioremediating soil, groundwater and activated sludge.^{60, 64}

Mobility of microbial cells or enzymes can be arrested by adopting techniques by still preserving its viability and catalytic functions through the process of immobilization.⁶⁵⁻⁷⁰ This brings about numerous benefits like greater degradative efficiency of pollutants, less probability of alterations in the gene, resistance of biocatalysts to changes in the immediate environment and tolerance of the biocatalysts to the excessive pollutant concentrations.^{65, 69-72}

Some techniques of immobilization^{65, 70,72} identified have been adsorption, electrostatic or covalent binding on the surface, flocculation, encapsulation and entrapment (Figure 3). Immobilization of the cells and enzymes of microorganisms through the technique of

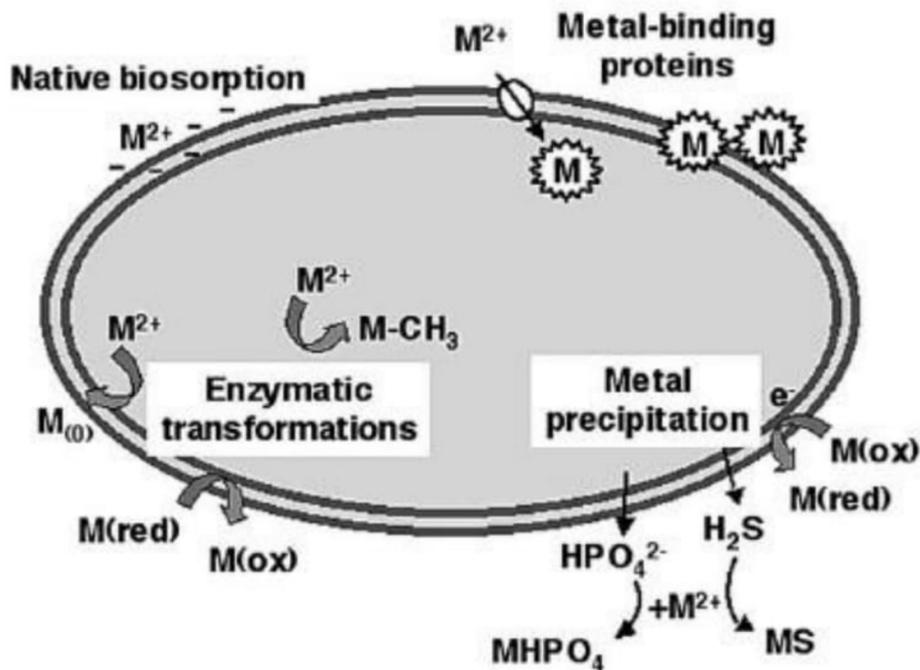


Figure 2. Bacterial interaction with heavy metal ions (M^{2+}) in the environment, with reference to the cellular compartment where bacteria response happens. [49]

adsorption is achieved through physical interaction with the external surface of water-insoluble carrier molecules employed in bioremediation forming weak bonds.⁷⁰ In electrostatic binding, the water insoluble carriers' surfaces are washed with a buffer solution. This makes the surface hydrophilic that promotes the attraction of negatively charged cells or enzymes.^{70,73,74} With the technique of entrapment in bioremediation, microorganisms are entrapped in a porous matrix thus restricting their mobility within the carrier. The advantage with this is that there is limited exchange of nutrients and metabolites and thus no leaks from the system to the environment. The entrapped microbes are physiologically varied with the cells closer to the surface showing more metabolic activity as opposed to the starved cells seen in the inside of the heterogenous carrier.^{65,69,70,72,75} Entrapment is a quick, nontoxic, economical method. The entrapped microorganisms are guarded against any change in the environmental factors.^{69, 70} The use of a semi-permeable membrane to set apart the immobilized particles from the external environment is the process termed as encapsulation.⁷⁰ Though the microorganisms are significantly protected against the unfavourable conditions of the external environment, their use in ex-situ bioremediation is limited due to the probable damage that could be caused to the growing cells.^{65, 70, 72, 76}

With the introduction of selected

reagents into metal-polluted water, the redox reactions involved modifies the chemistry of the water on-site thus enhancing the decomposition and extraction of various contaminants.^{56, 77} Studies have shown that heavy metals with high toxicity like As, Cr, Hg and Se in soils or sediments have also been transformed into innocuous forms with less toxicity through the oxidation-reduction reactions happening in situ.⁷⁸⁻

⁸⁰ Oxidation-reduction reactions in contaminated environments like groundwater, soil and sediments are often influenced by the physical and chemical properties of the medium, but adding substances like composts and biochar or any other organic and inorganic amendments can help manipulate the oxidation reduction reactions.^{80, 81} The toxicity as well as mobility of many elements like As, Cr, Hg, Se, Pb, Ni and Cu are also found to be influenced by redox reactions since the chemical reactions are influenced by the oxidation states of these metals.^{77,82} Some specific microbes that serve as bioremediators for heavy metals are listed in Table 2.

Role of Genetically Engineered Microorganisms (GEMs) in Bioremediation

GEMs are studied to have more degradative capabilities than many natural microbes, in removing persistent compounds in the natural environmental conditions.⁹⁵⁻⁹⁷ With the application of various genetic engineering

Table 2. Microorganisms used for bioremediation process

Microorganisms	Element/compound	Citation
<i>Saccharomyces cerevisiae</i>	Heavy metals, Pb, Hg, Ni	[83, 84, 85]
<i>Cunninghamella elegans</i>	Heavy metals	[85]
<i>Pseudomonas fluorescens</i> and <i>Pseudomonas aeruginosa</i>	Fe ²⁺ , Zn ²⁺ , Pb ²⁺ , Mn ²⁺ , Cu ²⁺	[86]
<i>Lysinibacillus sphaericus</i> CBAM5	Co, Cu, Cr, Pb	[87]
<i>Microbacterium profundum</i> strain Shh49T	Fe	[88]
<i>Aspergillus versicolor</i> , <i>A. fumigatus</i> , <i>Paecilomyces sp.</i> , <i>Paecilomyces sp.</i> , <i>Terichoderma sp.</i> , <i>Microsporium sp.</i> , <i>Cladosporium sp.</i>	Cd	[89]
<i>Geobacter sp.</i>	Fe (III), U (VI)	[90]
<i>Bacillus safensis</i> (JX126862) strain (PB-5 and RSA-4)	Cd	[91]
<i>Pseudomonas aeruginosa</i> , <i>Aeromonas sp.</i>	U, Cu, Ni, Cr	[92]
<i>Aerococcus sp.</i> , <i>Rhodospseudomonas palustris</i>	Pb, Cr, Cd	[93, 94]

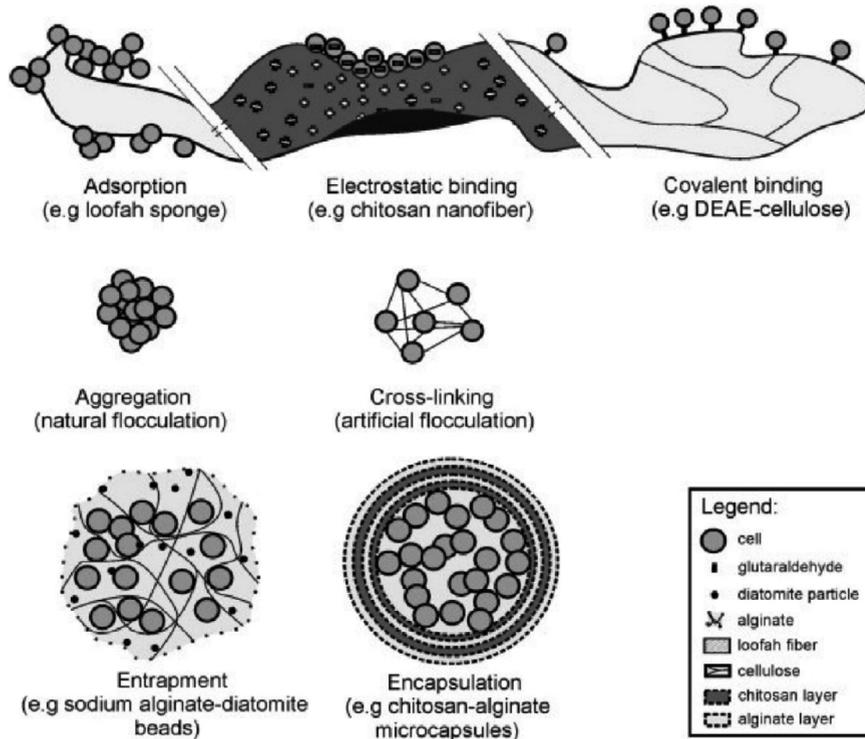


Figure 3. Methods of immobilization. [70]

approaches, the modified genome of the microorganisms have been found to be more efficient in enhancing bioremediation. Methods as single gene editing, pathway construction and change of existing gene sequences aid in modifying rate-limiting stages of the metabolic process.^{95, 98} GEMs have thus been studied to assist in the removal of heavy metals such as Fe, Cd, As, Cu, Hg and Ni.^{95, 99-101}

GEMs of certain bacteria, fungi and algae have been employed to degrade organic pollutants as oil spills, camphor, hexane, naphthalene, toluene, octane, xylene, halobenzoates, trichloroethylene etc. They are found to be more potent than the natural strains with greater degradative efficiencies and a quick acclimatization to the pollutant medium as substrates.¹⁰²

Beneficial microorganisms are used widely in various practices. There is a lot of development in technological innovations for safe and environmental friendly disposal of sewage sludge. One such technology referred

to as Effective Microorganisms (EM), a brand name developed by Dr. Teruo Higa, a Professor of Horticulture at the University of Ryukus, Okinawa, Japan^{103, 104} is now being put to use in organic farming, medicine, environment, livestock sector, forestry and agriculture.^{103, 105} EM is a microbial composition in liquid form consisting of a mix of beneficial and nonpathogenic microbes that coexist between aerobic and anaerobic modes. Currently, investigations are underway for its use in water quality restoration, wastewater treatment, sludge treatment and composting.^{103, 104}

Advantages of Bioremediation

Bioremediation is a universally acceptable biological treatment process for polluted and contaminated environments since the end products are non-toxic and mainly consist of the cell biomass, carbon dioxide and water.¹⁰⁶ Complete degradation of a range of pollutants is viable and the products from the process are safe for disposal into the environment.¹⁰⁷

Bioremediation is a long-term sustainable, cost-effective, eco-friendly process in comparison to the other technologies employed in the removal of hazardous waste. This can be carried out effortlessly in situ, reducing the threat to the biota and environment in general.¹⁰⁶

Disadvantages of Bioremediation

Bioremediation is only applicable to the removal of biodegradable contaminants.¹⁰⁷ leaving the non-biodegradable pollutants like plastic persisting in the environment and posing a risk. Biological processes are highly influenced by many factors like the presence of metabolically active microorganisms, suitable environmental factors which can aid microbial growth and an appropriate level of nutrients and contaminants. The survival of microorganisms is based purely on the environmental conditions in which they thrive.¹⁰⁸ Extrapolation from the pilot-scale studies to field operations is not ideal in certain conditions thus making it critical to carry out on-site research to initiate bioremediation technologies appropriate for sites with a complex mix of unevenly dispersed pollutants in the environment.¹⁰⁸

CONCLUSION

The technique of biotechnology is environment friendly and cheaper when compared to many pollutant removal mechanisms which are used to transform pollutants into benign substances thereby helping to develop production and disposal processes that are environmentally safe. The application of genetic engineering through exploitation of microorganisms to reduce the toxic load in the environment is paving the way for the future of maintaining the environmental quality thus proving to be a very rewarding option to remediate and manage the polluted environment through microorganisms.

Enhancing the natural biodegradation processes proves to be an effective cost-efficient way of treatment. More potential for advances in this field through more knowledge of how microorganisms respond to different specific environments and assist in degradation will help in reducing the impacts of heavy metal pollution on the environment.

ACKNOWLEDGMENTS

The authors would like to thank Bharathidasan University for providing with all the facilities and resources used for the making of this article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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