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Microbial Bioremediation of Heavy Metals-Process, Challenges and Future Aspect: A Comprehensive Review

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Abstract

Heavy metals have a prominent share as environmental pollutants owing to their toxicity, persistence in the environment and bio-accumulative nature. Cadmium (Cd), Copper (Cu), Lead (Pb), Selenium (Se) and Zinc (Zn) are few heavy metals that humans are primarily exposed to. These metals constitute a concern to human health because they can interfere with the operation of essential cellular components. The heavy metals can be created by nature through the weathering of metal bearing rocks and volcanic eruptions or by humans through mining and different industrial and agricultural operations. These heavy metals can be found in the soil, water and air. Heavy metals in the soil pose a significant danger to food security since their contamination poses risks to humans and the ecosystem by drinking contaminated groundwater, direct ingestion or the food chain and a decrease in food quality.

Present physical and chemical heavy metal remediation technologies, such as electrochemical treatment, ion exchange, precipitation and reverse osmosis are not cost effective, feasible time efficient or environmentally friendly; therefore, a biological approach could serve as an alternative heavy metal remediation technology.

By utilizing the innate biological capabilities of microorganisms, bioremediation appears as an efficient and environmentally acceptable approach of cleaning up areas that have been contaminated with heavy metals. This review examines the hazardous effects of heavy metal contamination and the environmental remediation techniques employed by microbes. This article examines the environmental impacts of heavy metals and how they can be efficiently remedied by the use of microorganisms.

The purpose of this review article is to highlight the significance of modern biotechnological processes and techniques for improving the ability of microbes to degrade heavy metals at a faster rate and to bring the major highlights of review articles in a more comprehensive manner. In this study, new breakthroughs in microbial bioremediation for the removal of heavy metals from the environment are discussed, along with their potential advantages and drawbacks.

Keywords: Heavy metal; Environmental pollution; Toxic, Microbial remediation; Bio-sorption

Introduction

Heavy metals are well-known contaminants found in the soil, water and air. Their presence is very toxic in nature even at very low concentrations [1]. A minute presence of these heavy metals such as arsenic, cadmium, cobalt, chromium, lead, mercury, nickel, selenium and zinc results in high toxicity owing to their high atomic weight and high density, five times greater than that of water. Natural and human actions discharge toxic heavy metals into the environment causing a load more than which can be effectively self-cleansed by the environment. Industrial units in large numbers discharge effluents containing heavy metals directly into fresh water without any adequate treatment leading to accumulation through the food chain and eventually to persistent and increasing ecological toxicity with assorted health problems [2]. Heavy metals get ingested into the human body through the contact with soil, water and air due to absorption by skin during agriculture, pharmaceutical, manufacturing, industrial or residential settings and turn toxic when their metabolism is incomplete. Toxic heavy metals such as Chromium (Cr), Nickel (Ni), Zinc (Zn), Arsenic (As), Selenium (Se), Silver (Ag), Cadmium (Cd), Gold (Au), Mercury (Hg), Lead (Pb) and Uranium (U) are capable of reducing plant growth due to reduced photosynthetic activities, plant mineral nutrition and reduced activity of essential enzymes.

The accumulation of heavy metals causes oxidative stress, which in turn generates free radicals such as the formation of Reactive Oxygen Species (ROS), which includes oxygen radicals (superoxide and hydroxyl) and non-radical derivatives of molecular Oxygen (O_2) such as Hydrogen Peroxide. (H_2O_2). Both the cell's ability to regenerate and the integrity of its DNA molecule are compromised as a result of this change [3].

Conventional methods of removal of heavy metals are expensive. There is a constant need and search for finding and developing low cost, ecofriendly method for removal of heavy metal from the environment to make mother earth free from the load of toxicity. Bio-remediation is a clean-up process either using naturally occurring or deliberately introduced microorganisms to consume and break down environmental pollutants and heavy metals from the pollution site.

Literature Review

Sources of heavy metals

Sources of heavy metals can be both natural and anthropogenic.

Natural processes: Sea salt sprays, rock weathering, forest fires, volcanic eruptions, biogenic sources and wind borne soil particles are all natural sources of heavy metal emissions. Natural processes of weathering can result in the discharge of metals from their endemic spheres into other environmental compartments. Oxides, hydroxides, phosphates, sulphates, sulphides, silicates and organic molecules can all include heavy metals. The most common heavy metals are Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni) and Zinc (Zn). Heavy metals like those listed above can cause serious health problems in people and other mammals even if they are present in trace amounts.

Anthropogenic processes: Pollutants can also be released into various environmental source streams as a result of agricultural practices, wastewater treatment facilities, industrial operations, mining and metallurgical activities and runoffs. Some standout anthropogenic sources contributing significantly to heavy metal contamination in the environment include automobile exhaust releasing lead; smelting releasing copper, zinc and arsenic; insecticides releasing arsenic and the burning of fossil fuels releasing vanadium, nickel, selenium, tin and mercury. Due to common techniques in the production of industrial goods aimed at fulfilling the expectations of a large population, human activities contribute to environmental degradation to a greater extent [4].

Environmental impacts of heavy metals

Due to the increased usage and processing of heavy metals in a variety of activities to meet the demands of a rapidly expanding population, the presence of heavy metals in the environment is becoming a concern of growing significance on a global scale. The key environmental components *i.e.,* air; water and soil are getting affected by heavy metals pollution. Heavy metals such as Cobalt (Co), Copper (Cu), Iron (Fe), Manganese (Mn) and Molybdenum (Mo), while present in trace amounts, are necessary for the continued existence of living creatures; nevertheless, when present in higher concentrations, these elements are harmful. The heavy metals Ag, As, Au, Cd, Cr, Hg, Ni, U, Se and Zn are hazardous heavy metals.

Effect on soil: The vast majority of heavy metals cannot be broken down by microbes or chemicals due to the fact that they are non-degradable; as a consequence, their total concentrations continue to be present in the environment for a considerable amount of time after they have been released. Heavy metals cannot be broken down by living organisms, which makes it challenging to eliminate them from polluted biological tissues. This poses a significant risk to the general population's health. It has been discovered that soils are the primary repository for heavy metals that have been discharged into the environment as a result of the aforementioned anthropogenic activities. These metals were introduced into the ecosystem as a direct result of human activities. Because heavy metals have a propensity to accumulate in the food chain, the presence of these metals in the soil is a serious challenge that puts the overall health of the ecosystem at jeopardy. Heavy metals are toxic to a wide variety of organisms, including plants, humans, animals and even entire ecosystems and they do so in a number of different ways. These methods include absorption by plants, direct ingestion and presence in food chains, intake of polluted water and altering of the colour, porosity, soil's pH and natural chemistry, all of which effect the soil's quality.

Effects on water: Runoff from urban areas, municipalities and industrial sites can carry heavy metals to downstream locations. The majority of these metals end up becoming concentrated in the sediments and soil of various bodies of water. Even though they may only be present in trace amounts, heavy metals can nevertheless pose a significant risk to both human and environmental health if they are found in water sources. This is due to the fact that the toxicity level of a metal is dependent on variables such as the organisms that have been exposed to it, the nature of the metal, the role that the metal plays in the biological system and the amount of time that the organisms are exposed to the metal. In other words, the toxicity level of a metal can vary greatly depending on all of these factors. As an example of an organism that feeds at the greatest level, humans are more likely to have major health problems because the concentrations of heavy metals grow as you move up the food chain [5].

Effects on air: Industrialization and urbanization are the two primary contributors to air pollution, which is a significant environmental problem that affects the entire planet. Natural processes such as soil erosion, dust storms, rock weathering and volcanic eruptions are all examples of processes that release particulate matter into the air. On the other hand, human activities such as those related to industry and transportation contribute to the worsening of air pollution [6].

It is difficult to eliminate heavy metals from polluted biological tissues due to the toxic nature of heavy metals and their lethality is a major cause for concern for global health especially since Heavy metals are unable to biodegrade. Heavy metals such as Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu) and Molybdenum (Mo), while present in trace amounts, are necessary for the continued existence of living creatures; but their higher concentrations, these elements are harmful [7]. The heavy metals Cr, Ni, Zn, As, Se, Ag, Cd, Au, Hg and U are hazardous heavy metals. They contaminate the environment and have detrimental consequences on soil quality, crop production and public health [8-11]. In water the maximum permissible concentration of heavy metals like Ar (0.01 mgL^{-1}), Cr (0.01 mgL^{-1}), Ag (0.05 mgL^{-1}), Cd (0.05 mgL^{-1}), Hg (0.002 mgL^{-1}) and Pb (0.015 mgL^{-1}) (by Comprehensive Environmental Response Compensation and Liability Act, USA) [12]. These pollutants are significant contributors to life-threatening degenerative diseases, including Alzheimer's, atherosclerosis, cancer, Parkinson's disease, etc. [13]. Heavy metal toxicity greatly affects the normal physiological activities of plants. The processes such as cell division, electron transport chain, photosynthesis and respiration are adversely affected by elevated levels of heavy metals [14].

Exposure to heavy metals will adversely affect the plant growth and metabolism as high metal toxicity will inhibit cytoplasmic enzymes in plant cells causing damage to cell structures due to oxidative stress [15]. High levels of following heavy metals causes adverse effects; Lead (Pb) could cause serious health problem such as lack of coordination and paralysis, Cadmium (Cd) can cause damage to the internal organs of the body such as the kidney, liver and cardiac tissues [16]. Arsenic (As) causes acute heavy metal poisoning in adults and children and could result in respiratory diseases such as reduced pulmonary function or lung cancer [17]. Mercury (Hg) is a neurotoxic that inhibits speech and hearing as well as causes muscle weakness. In aquatic environments, it builds up in the cells of bacteria where it is converted to methyl mercury and becomes poisonous to aquatic life.

Bioremediation-A “clean-up” process

Bioremediation is a cutting-edge technique that is rapidly gaining popularity as a method for removing environmental toxins from their natural habitat. The reduction in the solubility of environmental contaminants through pH adjustment, redox reactions and adsorption from a polluted environment are the fundamental principles that underpin bioremediation [18]. Bioremediation is the preferred approach for converting hazardous heavy metals into less damaging forms by utilizing microbes or its enzymes to flush polluted environmental choke points. The method is both environmentally beneficial and economically viable, allowing the ecosystem to be restored to a more pristine state [19].

The process of bioremediation is based on redox reactions which involve chemical transformation of harmful contaminants into less toxic compounds that are more stable, less mobile or inert [20]. It comprises changing the chemistry and microbiology of hazardous metals by infusing selected reagents into a polluted site in order to accelerate breakdown and extraction through *in situ* chemical oxidation/reduction processes. This is done to remove hazardous metals more effectively [21]. It plays a crucial role in the transformation of harmful heavy metals in soils and sediments, particularly As, Cr, Hg and Se, into less toxic or innocuous forms. [22,23]. Redox reactions in contaminated soil sediments and groundwater are frequently influenced by the medium's physicochemical qualities, however this can be adjusted by the addition of composts and biochar [24,25]. Addition of compost cause difference in the soil microbial population and increases available nutrients in metal-contaminated soils by changing pH and decreases the solubility of heavy metals. Biochar, a byproduct of the pyrolysis of biomass derived from agricultural residue, manure and solid waste, can be utilised to promote bioremediation by creating a more conducive environment for microorganisms. Biochar is an established and effective agent in immobilization of metals. It is capable of donating, accepting or transferring electrons within its environment *via* biological pathways. It may also facilitate microbial electron shuttling processes as they display similar functional characteristics to soil redox-active organic matter. A blend of biochar and chitosan is developed to remove heavy metal from synthetic and waste water. Redox reactions regulate the oxidation state of numerous elements, including chromium, copper, nickel, arsenic, selenium and lead, as well as their mobility and toxicity. About 99% As (III) is removed from contaminated water using clay-supported zerovalent iron nanoparticles by mixing ferric nitrate with liquor of commercially available tea. The process involved oxidative route for transformation of As (III) to As (V) reported by Tandon.

Bioremediation may be performed *in situ* or *ex situ*. During *in situ* bioremediation, contaminated environments are cleaned up by supplementing contaminated soils with nutrients to stimulate microorganisms' ability to degrade contaminants, by adding new microorganisms to the environment or genetically engineering the indigenous microorganisms to degrade specific contaminants. *Ex situ* bioremediation is more advanced than *in situ* bioremediation since it transports contaminated media to a different area for treatment dependent on treatment cost, depth of contamination, pollutant type and extent of pollution, geographical location and geology of the polluted site.

Remediation and de-pollution are both processes for metal removal basis metal-microbial interaction. Utilizing solid-state electrodes as electron donors or acceptors to induce microbial growth has resulted in the development of novel Microbial-Electrochemical Technologies (METs). The application of microorganisms as a green approach for the synthesis of metallic Nanoparticles (NPs) has been well reported. Genetically modified microorganisms have also been used for remediation techniques [26].

Agents of bioremediation

For bioremediation, natural organisms, either indigenous or exotic (introduced), serve as the primary agents. The organisms deployed vary based on the type of the pollutant agents and must be carefully chosen because they can only survive in a narrow range of contaminants. In 1974, the first patent for the use of a biological remediation agent was filed for a strain of *Pseudomonas putida* capable of degrading petroleum.

Microbes can be classified as aerobic: Aerobic bacteria recognized for their degradability are, *Alcaligenes, Mycobacterium, Pseudomonas, Rhodococcus* and *Sphingomonas*. These microbes have often been reported to degrade hydrocarbon compounds, pesticides and heavy metals. The contamination is the only source of carbon and energy for many of these bacteria, so they rely on it exclusively. Anaerobic: when it comes to the uptake of heavy metals, aerobic bacteria are utilised far more frequently than their anaerobic counterparts. Ligninolytic fungi: During the degradation process, certain types of fungi, such as the white rot fungus *Phanaerochaete* chrysosporium, are able to use common substrates like straw, saw dust or corn cobs enabling the fungi to digest an unusually wide variety of persistent or harmful environmental contaminants. Methylotrophs: Aerobic bacteria that thrive by extracting carbon and energy from methane as a source.

Types of bioremediation

There are two approaches to bioremediation *in situ* bioremediation includes the process of treating pollutants at the sites where they are found. In this scenario, the microbes have direct contact with the dissolved and absorbed pollutants and they employ those contaminants as substrates for the transformation process. Due to the time consuming nature of the *in situ* method, it may not be the method of choice when quick site cleanup is required. *Ex situ* bioremediation is a different approach that utilizes specially constructed treatment facility. It is more expensive than *in situ* bioremediation.

Key mechanisms used in microbial bioremediation

Biosorption mechanism: The term "biosorption" refers to the passive removal of toxic heavy metals such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+} and Hg^{2+}, chemical and particles from a solution by inexpensive biomaterials. It is the collection of all techniques that can remove heavy metals and other contaminants from solutions using either living or decomposing biomass. By using low-cost biological microorganisms, such as dead mass or natural materials with greater degradative ability. The mechanisms for biosorption are either linked to the cell's metabolism or to the process of removing metals, which is a separate metabolism.

It has been observed that negatively charged functional groups including hydroxyl, phosphate and carbonyl groups found on the surfaces of microbial cell walls readily attach to heavy metal ions. There are two modes of heavy metal uptake by microbial cells *via* biosorption mechanisms: independent biosorption, which takes place predominantly on the surface of the cells methodological approaches that are based on metabolism and include sequestration, redox reaction and species-transformation processes.

Intracellular sequestration: The process of intracellular sequestration refers to the complexation of metal ions by a range of compounds that are located within the cytoplasm of the cell. A rise in the concentration of metals within microbial cells can be caused when metals bind with surface ligands, which is then followed by a slow transport process into the cell. It is a phenomenon that has been extensively studied and recorded in clinical settings, most notably in the context of effluent treatment, that bacterial cells have the ability to accumulate metals within their own cells. With the assistance of cysteine rich low molecular weight proteins, the cadmium resistant *P. putida* strain has been shown to have the capability of intracellular sequestration of copper, cadmium and zinc ions. Additionally, it was shown that glutathione was responsible for the intracellular sequestration of cadmium ions in *Rhizobium leguminosarum* cells.

Extracellular sequestration: In the process of extracellular sequestration, metal ions are either complexed into insoluble compounds or accumulated in the periplasm of the cell by cellular components. Both of these procedures are carried out outside of the cell. Copper resistant Pseudomonas syringae strains developed copper inducible proteins CopA, CopB (periplasmic proteins) and CopC (outer membrane protein). These proteins are able to bind copper ions and microbial colonies. Metal ions are expelled from the cytoplasm of bacteria and taken up by the periplasm, where they are stored safely. Zinc ions are able to move out of the cytoplasm and into the periplasm of the synechocystis PCC 6803 strain due to the efflux mechanism.

Extracellular sequestration can be illustrated through the process of metal precipitation. Bacteria that decrease iron, like *Geobacter spp*., and bacteria that reduce sulphur, like *Desulfuromonas spp.*, are able to convert potentially dangerous metals into metals that are less toxic or even nontoxic. *G. metallireducens*, a stringent anaerobe, is able to reduce Manganese (Mn) from lethal Mn (IV) to Mn (II) and Uranium (U) from poisonous U (VI) to U (IV) (IV). Both *G. sulfurreducens* and *G. metallireducens* have the ability to convert the extremely hazardous form of Chromium (Cr) known as Cr (VI) into the less harmful form of Cr (III). Sulfate-reducing bacteria are responsible for the production of significant quantities of hydrogen sulphide, which results in the precipitation of metal cations. Under anaerobic conditions, the *Klebsiella planticola* strain produces hydrogen sulphide from thiosulfate and the resulting reaction precipitates cadmium ions as insoluble sulphides. Additionally, under aerobic conditions, the *P. aeruginosa* strain causes cadmium to get precipitated. Utilization of the *Vibrio harveyi* strain to precipitate soluble divalent lead as complex lead phosphate salt heavy metal removal using bacterial remediation. The biosorptive capacities of microbial biomass are extremely diverse and also highly variable amongst different types of microorganisms.

Heavy metal remediation using micro-organisms

The mechanism involves the utilization of microorganisms that utilize heavy metals and trace elements as terminal electron acceptors. These bacteria then gain the necessary energy to detoxify metals *via* processes that are either enzymatic or non-enzymatic. Metals can be decontaminated by using microorganisms by chemical precipitation, coagulation, electro-dialysis, ion exchange, evaporative recovery, flocculation, floatation, reverse osmosis, nano-filteration, ultra-filtration, etc., in addition to physico-chemical techniques including extraction, stabilization, immobilization, soil washing, etc. All viable process techniques, even if effective, are prohibitively expensive due to high energy and chemical reagent demands, in addition to the development of secondary toxic byproducts. Due to the presence of negative charge on their cell surface because of the anionic structures, microbes bind to metal cations. Amine, alcohol, carboxyl, hydroxyl, ester, sulfonate, sulfhydryl, thiol and thioether groups are the negatively charged sites of microbes which are involved in the metal adsorption process. Heavy metals and trace elements are used by microbes as terminal electron acceptors, providing them with the energy they need to detoxify metals using enzymatic and non-enzymatic methods.

The biosorbents, which can be biomass of microorganisms, are a byproduct of the sewage or pharmaceutical industries and sewage treatment operations. They consist of microorganisms that have been cultivated and propagated on a special base to indicate their potential to efficiently handle metals (as crust-rich tannins, humus, moss peat, nutshell, sea plants etc.)

Numerous studies have investigated the creative use of microbes to sequester, precipitate or alter the oxidation state of numerous heavy metals. A wide variety of microorganisms is capable of dissimilatory Fe (III) reduction which is the early form of microbial respiration. These bacteria use molecular hydrogen, lactate, pyruvate or acetate as their electron donor and Fe (III) as electron acceptor. Many of them are also able to use Mn (IV) as electron acceptor, reducing it to Mn (II).

Rather than employing a single strain culture, bioremediation of heavy metals will be successful if multiple bacterial strains are employed. In the study of Kang et al., the effect of bacterial combinations on the bioremediation of a mixture of Pb, Cd and Cu from contaminated soils was tested using four strains: *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47. After 48 hours, it was observed that the bacterial combinations were more resistant and effective at removing heavy metals than single strain cultures, with remediation efficiencies of 98.3% for Pb, 85.4% for Cd and 5.6% for Cu being Siderophores are organic compounds with low molecular masses metal chelating agents that are produced by microorganisms and plants growing under low iron conditions. They function as biocontrols, biosensors and bioremediation and chelation agents. The role of siderophores is primarily to scavenge Fe and to form complexes with other essential elements (*i.e.* Mo, Mn, Co and Ni) in the environment and make them available for microbial cells.

Bacteria, algae, fungi, endophytes, aquatic plants and agrowastes as source of adsorbent in adsorption process for removal of heavy metals from aquatic medium.

Bio-remediation of heavy metal by bacteria: Bacteria are significant biosorbents due to their uniqueness, size and ability to grow under controlled settings and resistance to the effects of the surrounding environment. In addition to meso-di-aminopimelic acid and teichoic acid, the cell walls of gram-positive bacteria are composed of layers of peptidoglycan that contain the amino acids alanine and glutamic acid. On the other hand, gram-negative bacteria have cell walls that are composed of enzymes, lipoproteins, glycoproteins, lipopolysaccharides and phospholipids. In bacteria, these components of the cell wall transform into the active sites for the binding activities as a result of their role as ligands in the process of binding metal ions, which ultimately leads to the removal of these metals from polluted environments.

Bhattacharya studied the feasibility of a newly isolated strain Acinetobacter sp. B9 for concurrent removal of phenol and Cr (VI) from wastewater. The strain was found to utilize phenol as sole carbon and energy source while no Cr (VI) removal was observed. Bacteria, because of their ability to develop under regulated settings and their capability to survive extreme environmental conditions, are crucial biosorbents for the treatment of polluted environments. Bacteria are essential biosorbents for the treatment of polluted habitats. They are effective biosorbents for heavy metals that can be found in a variety of contaminated environments.

Mercury-resistant bacteria such as *Alcaligenes faecalis, Bacillus pumilus, Pseudomonas aeruginosa* and *Brevi bacterium* iodinium were utilised by De Jaysankar and his coauthors. They studied to remove heavy metals such as Cadmium (Cd) Lead (Pb) and Mercury (Hg) using 13 marine bacterial strains that are highly resistant to mercury.

It has been observed that use of indigenous facultative anaerobic *Bacillus cereus* can be made for detoxifying hexavalent chromium. The bacteria are able to reduce Cr (VI) throughout a broad temperature range (25°C to 40°C) and a wide pH range (from 6 to 10), although they perform best at 37 degrees celsius and an initial pH of 8.0. It has been discovered that bacteria fare better and are more likely to survive when they are a part of a mixed culture.

Bacillus subtilis, Bacillus megaterium, Aspergillus niger and *Penicillium* sp were the microorganisms that were used in this study by Abioye and his colleagues to evaluate the biosorption of Lead (Pb), Chromium (Cr) and Cadmium (Cd) on tannery effluents. The amount of Lead (Pb) in the water was reduced from 2.13 mg/L to 0.03 mg/L by B. megaterium, which was followed by *B. subtilis*, which reduced it from 2.13 mg/L to 0.04 mg/L.

Ashruta and his coworkers reported approximately 75% to 85% of chromium, cobalt, copper; zinc, cadmium and lead were removed by bacterial consortiums within two hours of interaction.

Bio-remediation of heavy metal by fungi: Due to their capacity to both absorb and recover metals, fungi are frequently utilised as biosorbents to eliminate harmful metals. Numerous investigations revealed that fungal cells, both living and dead, are essential for the adhesion of inorganic compounds. Through active accumulation, intracellular and extracellular precipitation and valence transformation, fungi may tolerate and detoxify metal ions. Heavy metals can be absorbed by fungi into their mycelium and spores. As a consequence of this, they have the potential to function as biocatalysts in the process of the bioremediation of heavy metals. Yeast (*Sacharomyces cerevisiae*) can also be used as effective bioremediation agents because it can remove toxic metals from polluted wastewater by biosorption through the ion exchange mechanism.

The effectiveness of *Aspergillus* sp. employed to remove chromium from tannery effluent was reported by Srivastava and Thakur. In a bioreactor system, 85% of the chromium from the synthetic medium was eliminated at pH 6.

Park and his coauthors reported using dead fungal biomass of *Aspergillusniger, Rhizopusoryzae, Saccharomyces cerevisiae* and *Penicillium chrysogenum* for converting toxic Cr (VI) to less toxic or nontoxic Cr (III).

Luna, observed that Candida sphaerica produced biosurfactants with a removal efficiency of 95%, 90% and 79% for Fe (iron), zinc (Zn) and lead (Pb) respectively. Meanwhile, usage of *Candida* spp. Accumulated substantial quantity of nickel Ni (57%-71%) and copper Cu (52%-68 %).

Multiple strains of yeast such as *Hansenula polymorpha, S. cerevisiae, Yarrowialipolytica, Rhodotorula pilimanae, Pichia guilliermondii* and *Rhodotorula mucilage* have been used for bio-converting Cr (VI) to Cr (III).

Removal of heavy metal using biofilm: The elimination of heavy metals can be accomplished by biofilms in a variety of different ways. Biofilm is an effective technique for bioremediation and it also functions as a biological agent that can stabilize given environment. It is worth noting that biofilms have a very high tolerance to harmful inorganic elements, even at quantities that are deemed to be lethal. According to research conducted on *Rhodotorula mucilaginosa*, the efficacy of metal removal increased from 4.79% to 10.25% for planktonic cells and it went from 91.71% to 95.39% for biofilm cells. The bioremediation process in biofilms can take place either through the biosorbent mechanism or by the exopolymeric compounds that are present on biofilms and whose molecules have surfactant or emulsifier capabilities.

Bio-remediation of heavy metal by algae: Algae are considered one of the most promising types of biosorbents which are interest in search for and development as a new biosorbent materials because of they have high sorption capacity and are readily available in a large quantity in seas and oceans.

Algae are autotrophic and hence require fewer nutrients than other microbial bio sorbents while producing a tremendous biomass. Biomass of algae is utilized for bioremediation of wastewater contaminated with heavy metals *via* adsorption or incorporation into the cells. Numerous chemical groups, including amide, carboxyl, hydroxyl and phosphate serve as metal binding sites on the surface of algae. They use phytoremediation to remove the heavy metals by or to degradation of toxicant.

The biosorption effectiveness of algae was measured at 15.3%-84.6% by Mustapha and Halimoon, which is higher than the efficiency, measured using other types of microbial biosorbents. Ion exchange processes are responsible for this phenomenon. It has also been demonstrated that brown sea algae can effectively remove heavy metals including Cd, Ni and Pb by using chemical groups such as carboxyl, sulfonate, amino and sulfhydryl on their surfaces. Groups were among these chemical groups. Because algae produce a vast biomass, these biosorbents have a high sorption capacity in comparison to those produced by other microorganisms.

Bioremediation by microbial genetic engineering: Microbes are now being designed with desirable traits, such as the ability to accumulate metals and the capacity to resist metal stress, owing to developments in genetic engineering.

Microorganisms were genetically modified by Frederick et al. to produce trehalose and they demonstrated that this process reduced Cr (VI) to Cr (III). Encapsulation of Agrobacterium biomass in alginate with iron oxide nanoparticles resulted in an adsorption capacity for Lead (Pb) that was effective for five consecutive cycles.

Genetically engineered microbes used for heavy metal remediation involved using *Escherichia coli* (*E. coli* ArsR (ELP153AR)) to target As (III) and *Saccharomyces cerevisiae* (CP2 HP3) to target $Cd^{2+}$and Zn^{2+}. Similarly, *Coryne bacterium glutamicum* was genetically modified using expression of arsoperons (ars1 and ars2) to decontaminate as-contaminated sites.

Discussion

Drawback/Limitations of bioremediation technique

The process of bioremediation is associated with certain limitations that need to be answered scientifically through a holistic approach. Nature of the microorganisms being used is major cause of concern. The micro-organism degrades the pollutant for its survival to obtain the energy necessary for its metabolic activity. For this process certain conditions are required to enhance the development of these organisms. For the survival of these organisms (bacteria or fungi) oxygen or fertilizers are added into the contaminated soil which can disturb the habitat of the pre-existing indigenous species.

Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation. The process is very specific. The rate of degradation of a pollutant is highly dependent on the initial concentration and the toxicity of the pollutants present to microorganisms, the biodegradability of the pollutants, the properties of the contaminated soil and the selected treatment technique.

The effectiveness of bioremediation is limited at sites with high concentrations of metals, highly chlorinated organics and inorganic organic salts that are toxic to the microorganisms. Bioremediation often takes longer time than other treatment options, such as excavation and removal of soil or incineration. Compared to *ex situ* technique, *in situ* techniques require long periods of time to have an effect, whereas *ex situ* treatments give results more rapidly.

In situ bioremediation is also more effective for sites with relatively permeable soil (sand) rather than sites situated on clay. Since clay has a low porosity, therefore oxygen, which is a much needed ingredient for organism development, is not easily circulated through the contaminated area.

Generally, it is carried out in mesophilic or thermophilic habitats *i.e.,* at above freezing temperatures which is again a limitation of the process.

Another major limitation of this technique is that the byproducts left by the organism biodegrading the pollutant can be as or even more toxic than the original contaminant.

Conclusion

Bioremediation is an environmentally benign and cost effective technological approach for the removal of heavy metals from the ecosystem. Microbes which are able to degrade the pollutant increase in number and as pollutant gets completely degraded their population declines, generally living behind nontoxic and harmless by-products such as water, carbon dioxide and cell biomass.

Numerous natural bio-sorbents derived from microorganisms have been reported to possess effective bio-sorption properties. These biosorbents are successful in removing metals across a wide range of pH, solution conditions and temperatures and could serve a desired commercial usage. Bioremediation targets complete destruction of the contaminant transferring contaminants from one environmental medium to another and eliminates the chances of future liability of treatment and disposal of harmful pollutant.

It can often be carried out on site, without causing a major disturbance to normal activities. This eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

This comprehensive report presents the current state of bioremediation of heavy metals, which reveals significant potential for the bio sorption and detoxification of metals, particularly from genetically engineered bacteria and biofilm. The purpose of this review paper is to illustrate the significance of bacteria, viruses, fungi, microbial cells, biofilms and their metabolites in the removal of heavy metals and the study of environmental issues.

The removal of heavy metals through bioremediation is not without its drawbacks. The creation of harmful compounds by bacteria and the inability of heavy metals to biodegrade are two very important problems which are need to be answered with sound research and scientific analysis and thought process.

Methods that are based on biofilms, the transfer of genes between microbes and the use of microbial fuel cells have emerged as potentially useful candidates for ongoing and upcoming research. An active binding site for increased metal uptake can be found in the peptidoglycan and polysaccharide component of the cell wall that is found in bio-sorbents. This approach if adopted will provide benefits such as faster kinetics and high metal binding throughout a wide pH and temperature range.

Increased efforts should be made in future in the areas such as biofilms mediated bioremediation techniques, genetically engineered microorganisms and Microbial Fuel Cell (MFC) to remediate heavy metals from the ecosystem at the same time maintaining balance and harmony with the system and surroundings.

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