Bacterial Exopolysaccharides; Nature's Solution from natural biopolymer for heavy metal sequestration

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Abstract:

Environment is affected from pollution which is causing difficulties to living organisms in worldwide and also became huge issues. Pollution is globally beginning huge issues in the environment and the deterioration of living organisms. The recent report on environmental pollution is on soil and water which is becoming contaminated by industrial pollution such as pesticide, and chemical pollution. Major contamination by heavy metals has been recognized as the major cause of public health issues, specifically the developing countries are more prominent with higher level of toxic metallic ions are well known. Hances, there should be an alternate approach to mitigate the arising issues of environmental pollutions. One such profound approach could be the natural and biodegradable biopolymer derived from biotransformation and consist with sugar molecules, proteins polysaccharides, uronic acid, lipid etc. Exopolysaccharides (EPSs) are one of the bacterially produced biopolymers secreted by microorganisms to cope with harsh environmental conditions. EPSs involved in the protection of microorganisms from different adverse environmental factors such as temperature, pH, antibiotics, pesticides and involve in host immune defences. Therefore, it exhibits excellent physiological, physicochemical and rheological properties and also EPSs display net anionic properties which allows the biopolymer to potentially aid in mitigation of positively charged heavy metal ions. Currently, heavy metal pollution has become a major disturbance, and researchers are focusing to solve this issue by seeking for potential EPS-producing microorganisms. In this review, the major focus is to study the EPS producing bacteria, which will be used to remove heavy metal contaminants in the soil and water. This will be provided new insights to an alternative natural biopolymer-based techniques in cost-effective tool to mitigate heavy metals pollution.

1. Introduction:

Over a year the environment is getting polluted and affecting the natural resource on the planet which are very valuable for the survival of living beings and are needed for the development of humankinds and natural biodiversity [1]. The environment became highly polluted due to various influenced activities by humans [2]. Due to uses of the various kinds of chemical-based pesticide, paint, the heavy metals are getting deposited into water bodied, soil, which is not an easy degradable element. Heavy metals like Pb2+, Cd2+, Ni2+, Cu2+, Zn2+, Hg2+, Cr2+, are natural element with high molecular density as compared to water. As some of the heavy metals are essential in a trace amount and promote the growth of plants and also vital for human physiology. However, toxicity can arise even at slightly higher concentrations due to the extensive industrial applications of this compound in form of pharmaceuticals, pesticides, rubbers, plastics, tanneries, variety of organic compounds and woody products. The non-degradable properties of heavy metals confirm its persistent existence and due to its high aquatic solubility triggers bioaccumulations. These accumulations are eventually leads to irrevocable and insidious health hazard such as potential carcinogenicity. Hence, entire globe is facing a huge issue regarding environmental pollution in soil and water bodies which is now entering into food chain and causing sever health related issues. The aftermath of the advancement of economics was not only the development and well-being, but it's also leads to causing massive contamination by industrialization, urbanization, and agricultural practices that slowly converted to turmoil and disasters[3, 4]. The release of toxic heavy metals seriously threatens natural biota, including human beings[5]. The effects of heavy metal pollution adding carcinogenic and mutagenic on a living being with major health issues such as eczema, skin irritations, respiratory tract disorders, mental disorders, Geno-toxicity, renal failure, reproductive damage, skeletal disorder, cancer, nephropathy, Alzheimer's disease, liver damage, chronic pulmonary problems, and osteoporosis which are becoming disaster to the human beings [6]. The pollution of heavy metals from industrial waste, and chemical particles dissolving in soil and water affect much aquatic life and terrestrial life throughout the globe[7]. The settling down of heavy metal at the bottom of the water bodies accumulates in the tissues of the aquatic biota, disturbing the photosynthesis and nitrogen fixation and causing death[8]. Therefore, contaminants of heavy metals are becoming stable in the environment which is causing several health issues and difficult to degrade or destroyed. To prevent catastrophes in the environment, many different techniques are utilized to eradicate environmental pollution. Consequently, many biological activities got approved in various procedures to enhance the cleanup of the environment. Recently multiple technologies developed in the biological method; among them bioremediation technology became more popular as it uses either biological molecules or microorganisms that are used to absorb or degrade pollutants and are also eco-friendly & cost-effective than expensive physical or chemical methods [9]. The excess amounts of polluted environmental conditions can be avoided by exploring more the uses of microorganism for bioremediation via extracellular biopolymers as exopolysaccharides (EPS) . Polysaccharide-based bioremediation have drawn attention as they offer several advantages including lower toxicity, higher degradability, and better compatibility with the environment[10]. EPS is now used in a wide range of commercial area including medical and industrial fields because of their potential in the field of the biotechnology sector, their pseudoplastic rheological behaviours, and their water-binding capacity to help nature. On the other hand, bioremediation is an environmentally beneficial method where toxins may be removed or detoxified from the soil and water, by utilizing microorganisms [11]. This alternative approach for mitigation of HMs that uses natural biological compounds which is having advantages over chemical treatment. To overcome this issue usage of Exopolysaccharides (EPSs) which are high molecular weight complex biopolymers constituted mostly of sugars and include non-carbohydrate molecules such as proteins, lipids, humic substances, or extracellular DNA as constituents the most significant and abundant class of chemicals, synthesized by bacteria, fungus, and algae. EPS produced by bacteria has a distinct advantage over polysaccharides derived from plants, animals, and algae as they are synthesized and released by the hosts cells to outside the cells, enabling EPS harvesting from the non-cell-based supernatant is straightforward and cost-effective while eliminating the need for ecologically destructive cell lysing chemicals & further offer faster manufacturing times and simplicity of expansion [11, 12]. The types of bacteria, the amount of time the biofilms have been around, and environmental factors all affect the composition of EPS. Extremophilic microorganisms that live in hazardous conditions create and surround themselves with EPS as a survival strategy. EPS protects bacteria from harsh environments such as temperature, drought, and dehydration [13, 14]. Many putative roles have been proposed for these polymers, such as protection against UV radiation, biomineralization, and phagocytosis. Moreover, toxic levels of heavy metals can interact with essential biomolecules in the cell, such as DNA and protein, leading to excess EPS formation, which internally also provides resistance to the extreme environment. Due to their role in the process of flocculation and their ability to bind metal ions from solutions, EPS is particularly relevant to the bioremediation procedure. Some studies also show the influence of heavy metals in EPS production and their ability for further metal removal it also plays a crucial role for the microbial cell, such as protection against biotic and abiotic stress. Many extremophilic bacteria such as *Halomonas eurihalina*, *Enterobacter cloacear*, and *Gordonia alkanivorans* produce EPS with emulsifying properties toward long-chain alkane, aromatic, and hydrocarbon mixtures. COO, HPO4, OH, and SO4 are metal cations with negatively charged functional groups that interact electrostatically to immobilize metal by EPS and have both adsorptive as well as adherent properties [10]. EPS from *Enterobacter cloacae* SUKCr1D was reported to decrease Cr (I) by 31.7% [15]. EPS generated by a halophilic bacterium flocculated with a dynamic similar to that of commercial cationic, non-ionic, and anionic synthetic polyelectrolytes in terms of turbidity and particle removal effectiveness. *Bacillus sp*. ATS-2 was able to bind Pb via hydroxyl and carboxyl groups as well as nitrogen-based bio-ligands such as amide and sulphonamide. The capacity of a *Pseudomonas aeruginosa* strain BU2's extracellular polysaccharide (EPS) to extract uranium from an aqueous solution was studied. The EPS has an acidic character and was discovered to be a powerful biosorbent for uranium (U), exhibiting pH dependency and quickly saturating metal sorption, with a maximum concentration (985 mg U g1 EPS) at pH 5.0. According to reports, marine isolates of sulfate-reducing bacteria (SRBs) produce EPS during growth, which forms a complex with Ni, Cr, and Mo and may be used for the bioremediation of marine ecosystems[16]. One biofilm-forming strain of *Herminiimonas arsenicoxydans* that was able to detoxify environments polluted with arsenic by oxidizing As (III) to As (V) and by scavenging arsenic ions in their EPS layer was found to exhibit EPS-mediated sorption of Arsenic. Still, our understanding of the mechanisms behind their production and secretion limits our ability to use them. EPS is divided into two types: homopolysaccharides and heteropolysaccharides. Homopolysaccharides are made up of only one type of monosaccharide, but heteropolysaccharides are made up of many monosaccharides synthesized within the cell, resulting in complex structures[17]. Most bacterial EPS are heteropolysaccharides. The availability of carbon (e.g. glucose, sucrose) and nitrogen (e.g. ammonium sulfate, peptone, sodium nitrate, yeast extract) are the most essential influencing factors for EPS synthesis and their generation is also affected by the growth phase, and the microorganism. Numerous research has shown the effectiveness of using pure EPS and those in biofilm, activated sludge, or bio granules in bioremediation, which has led to a lot of publications in support of this method. Microbially produced EPSs are non-toxic, biocompatible, and biodegradable polymers that have found excellent applications in various industries. Their utility as conjugates for drugs and vaccine-controlled delivery is well-recognized in the biomedical industry.

2. Different biopolymers-based bioremediation

Polymers are a class of giant molecules consisting of discrete building blocks linked together to form long chains, simple building blocks are called monomers, while more complicated blocks are referred to as “repeat units” [18]. Humans have primarily utilized biopolymers throughout history as food or in the manufacture of clothes and furnishings. Biopolymers derived from renewable feedstocks have been developed in the last two decades in response to consumer demand for environment-friendly goods [19]. Biopolymers are large macromolecules that are composed of numerous repeat units and made from organisms that are fully biodegradable. The repeat unit in most biopolymers usually consists of molecules of either nucleic acids of nucleotides, amino acid proteins, or saccharides derived from sugars[20]. The monomer units present in these polymers are joined via covalent bonding to form large structures. Some biopolymers (DNA, RNA) can also transfer genetic material from one generation to another. The word biopolymer is derived from the Greek words bio and polymer in which the prefix ‘bio’ signifies that these polymers are inherently produced from living matter under natural conditions during the growth cycles of organisms or derived from renewable sources and shows the biological nature of the polymer to degrade naturally. Since they are made from living organisms, they and their derived materials are totally carbon neutral and can be easily recycled or renewed. The use of biopolymers minimizes carbon dioxide emissions, municipal solid waste, and reliance on petroleum-based resources. Moreover, biopolymers absorb the carbon dioxide plants emit instead of released into the atmosphere [19, 21]. Biopolymers are an example of biobased material which are referred to as products whose building blocks constitute substances procured from living matter that is either accessible naturally or in a form that is requisite of synthetization [20]. They can copy the properties of synthetic polymeric materials. Since biopolymers have low C-C bond energy, they involve the easy breakdown of large chains into smaller chains by the action of biological factors such as enzymes, moisture, and heat. They are formed within cells by complex metabolic processes. All the biopolymers are synthesized by enzymatic processes in the cytoplasm, in the various compartments or organelles of cells, at the cytoplasmic membrane or at cell wall components, at the surface of cells or even extracellularly, synthesis of a biopolymer may be initiated in one part of a cell and may be continued in another part as it occurs. Living matter is able to synthesize a wide range of different polymers and in most organisms, these biopolymers contribute the major fraction of cellular dry matter. Plants, animals, microorganisms, and agricultural wastes are examples of natural biological sources of biopolymers. Cattle are the most common animal sources, while corals, sponges, fish, lobster, and shrimp are the most common marine sources. Algae, fungus, and yeasts are the most common microbiological sources. There are different ways to produce biopolymers in order to make them available for different applications: isolation from plants and algae; isolation from extremely natural sources; in-vitro synthesis with isolated enzymes in cell-free systems; fermentative production as used in certain industries. Biopolymers are a new generation of smart textile materials based on petroleum, agricultural, or animal sources that offer a viable solution for the economy [22]. Biopolymers have caught a lot of interest in various applications where sustainable and biodegradable solutions are in high demand. Biopolymers are found to cover a considerable portion of present-day's produce, ranging from additives and blends in bioplastics to personal hygiene, edible goods, and medical products whilst offering the benefit of eco-friendly degradation. The feasible use of biopolymers in different fields relies on their functionality which in turn depends on factors such as the amount and quality/type of solvent; the purpose of selected biopolymer i.e. either for emulsion and foam surface activity or the viscosity forming bulk structure or gelation; the structure-developing process; and its collaboration with any additional formulated matter [20]. Several polymer industries have started working on biopolymers as an alternative source of synthetic raw materials. Imperial Chemical Industries initiated a program for the production of thermoplastic biopolymers called "BIOPOL". In the past few years, more energy has been required for the production of biopolymers as compared to synthetic polymers. But nowadays the upcoming scientific technique is heading towards the manufacture of low-energy-consuming and commercially viable biopolymers. The nature of biopolymer varies depending on the origin, extraction procedure, and adsorption characteristics. An approach to metalloid removal involves stable complex formation between metalloid ions and microbial polymers. Microbes have adopted diverse detoxifying mechanisms that include bioaccumulation, biomineralization, biosorption, and biotransformation for their survival which are often triggered and activated when the concentration of metalloids in their environment increases. Microbes secrete a large variety of metalloid-binding metabolites, ranging from simple organic acids, and alcohols to extracellular polymeric substances (EPS), slimes and sheaths, humic and fulvic acids, capsules, and biofilms which help in metalloid uptake from solution. These microbial products are superior to their chemical counterparts as they are biodegradable in nature and have low toxicity. Biosurfactants consist of several advantages over chemical surfactants which include: one or more chiral centres and functional groups; complex structural diversity; enhanced activity at extreme conditions of pH, salinity, and temperature; excellent ability to form molecular assembly and liquid crystals; gradual adsorption and continuing activity; advanced surface activity and lower critical micelle concentration; superior biodegradability and lower toxicity; versatile biological activity and production from renewable feedstocks. Biosurfactants act as a link between the fluid interfaces, due to their amphiphilic nature and thus reduce the surface tension. A decrease in the surface tension of water increases the mobilization of metalloid from unsaturated soils. Consequently, making the removal of metalloids possible. Bio flocculants are polymers produced by bacteria during growth and contain complex mixtures of macromolecular polyelectrolytes produced by different organisms. The formation of interface with metalloid ions was attributed to being the mechanism of metalloid removal by bio flocculant which depends upon the ionic groups present in the biopolymer which enables metal-floc interactions. It is a metabolic-independent process in which communication between metal ions and the flocculant functional groups can be established by complexation, immobilization, ion exchange physical adsorption, and precipitation processes [14, 23]. Gum kondagogu [Cochlospermum Gossypium] is an Indian tree gum that produces a carbohydrate biopolymer that is derived as an exudate and belongs to the family Bixaceae. In the recent past, gum kondagogu has been established as a biosorbent to remove toxic metals like lead, cadmium, nickel, and total chromium from aqueous solutions. The primary structure of this biopolymer contains sugar, galactose, mannose, glucuronic acid, and galacturonic acid. This gum has been grouped under substituted rhamnogalacturonans. The toxicological evaluation of gum kondagogu established that this gum was non-toxic and has potential application as a food additive. Gum kondagogu fulfils the requirements as a biosorbent as it contains many of the functional groups recognized earlier as being involved in metal binding. Thus, this gum has an impending application as a matrix for toxic metal bioremediation from industrial effluents [24]. Chitin is a natural polysaccharide that is also the second most abundant biopolymer on earth after cellulose, and it exhibits some unique features that make it a perfect candidate for the wastewater development process. This biopolymer consists of a β-(1,4)-N-acetyl-D-glucosamine and is found in the exoskeleton of arthropods and crustaceans. Chitosan has shown significant promise in the detoxification of polluted effluents. This can remove a variety of toxic metals such as Cu (II), Pb (II), Cr (IV), Hg (II), Mo (VI), As (V) as well as possesses the potential to remove inorganic species from water. Chitosan has been investigated as a sorbent against organic pollution in addition to removing inorganic pollution from water [Dhillon G. S. et al. (2012)]. Many bacterial polysaccharides have been shown to bind heavy metals with varying degrees of specificity and affinity. Novel formulations can be designed which enlarge the family of available bacterial biopolymers for metal binding and subsequent recovery [25]. The use of biopolymer for several application was shown in table.1.

Table 1: Biopolymers from various source and their application in bioremediation

Biopolymer	Source	Bioremediation related Characteristics
Chitosan	Exoskeleton of arthropods, crustaceans, and fungi	Detoxification of polluted effluents, removal of a variety of toxic metals, biosorbents
Alginate	Brown Algae	Adsorbent for heavy metals
Cellulose	Green plants, Algae, and oomycetes	Adsorbent for heavy metal ions and other aquatic pollutants
Lignin	Vascular green plants	Extraction of heavy metals in various methods
Carbohydrate biopolymer (gum kondagogu)	Buttercup tree (*Cochlospermum gossypium*)	biosorbent for the removal of toxic metals
Biosurfactant	Bacteria, yeasts, and filamentous fungi	Metalloid complexing agent
Microbial flocculants	Bacteria	Metalloid removal

3. Mechanism action of bioremediation of heavy metals via EPS

Microorganisms play an important role in converting heavy toxic metals into non-toxic forms as microbes are everywhere in nature which is higher than heavy metals polluted sites. Microorganisms maintain two defense systems: 1. The targeted contaminants are cleaned by producing degradative enzymes. 2. The obstruction to applicable heavy metal [26]. The presence of extracellular polymeric substances on the cell wall of microbes that can be attached to the heavy metals by proton exchange or micro precipitation of metals is the mechanism of the bioremediation process [27]. The cell wall of bacteria is divided into two groups that are gram-positive bacteria and gram-negative bacteria. In gram-positive bacteria, the cell wall has a thick peptidoglycan layer crosslinked with an amino acid bridge, and in gram-negative bacteria, the cell wall has a thinner single layer of peptidoglycan layer which is not deeply crosslinked. The formation of crosslinking of peptidoglycan layers with the bacterial cell in the protective covering [28]. Before using bacterial species for bioremediation technology, it was exposed to contaminants for enzymatic induction. In recent days researchers are widely focusing on the developed mechanism of the bioremediation process by a bacterial agent which has heavy toxic metal resistance and remediation for their survival [29]. The EPS binding with the heavy toxic metals is not linked with the metabolic reaction of this interaction property between negatively charged EPS and positively charged metal ion. The bioremediation process takes place in various mechanisms, those are adsorption, biosorption, biotransformation, and bioaccumulation Figure 1.

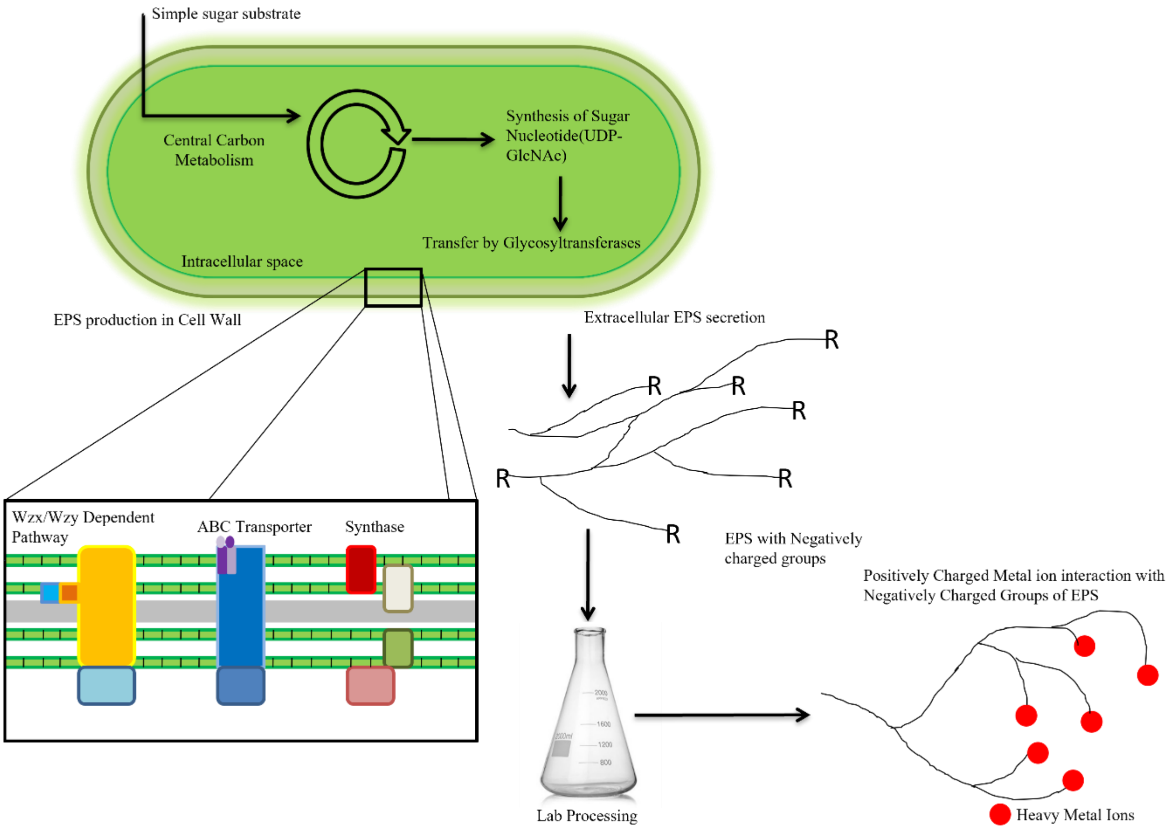

Figure 2: Biosynthesis of exopolysaccharides in bacteria: The starting sugar material as substrate which used up by the central carbon metabolic pathway which further undergoes for series s of enzymatic reactions and sugar bioconversion within the intracellular environment, facilitate for the assembly of monomer of sugar into polysaccharide and export to the extracellular surface via pathway; Wzx/Wzy depended pathway, ABC transporter pathway, enzymatic synthase pathway. The extracellular secreted exopolymer further used for heavy metal sequestration via exploring its charge based functional groups.

3.1. Mechanism of Biosorption

Biosorption is the process used to solve environmental problems and the main goal of biosorption technology is to remove heavy metals from wastewater and soil as it is cost-effective (Calderon O. A. R. et al.; 2020). The mechanism of biosorption is a complex process to utilize biosorbents for binding sorbates onto it. Biosorption is a phenomenon that interacts between positive charge metals with negative charge EPS of the cell wall to remove contaminants [10]. In both live and dead organisms cell surfaces present of negative charge functional group that uptake the heavy metals simultaneously. The entire process of this mechanism takes place through microprecipitation figure 2.

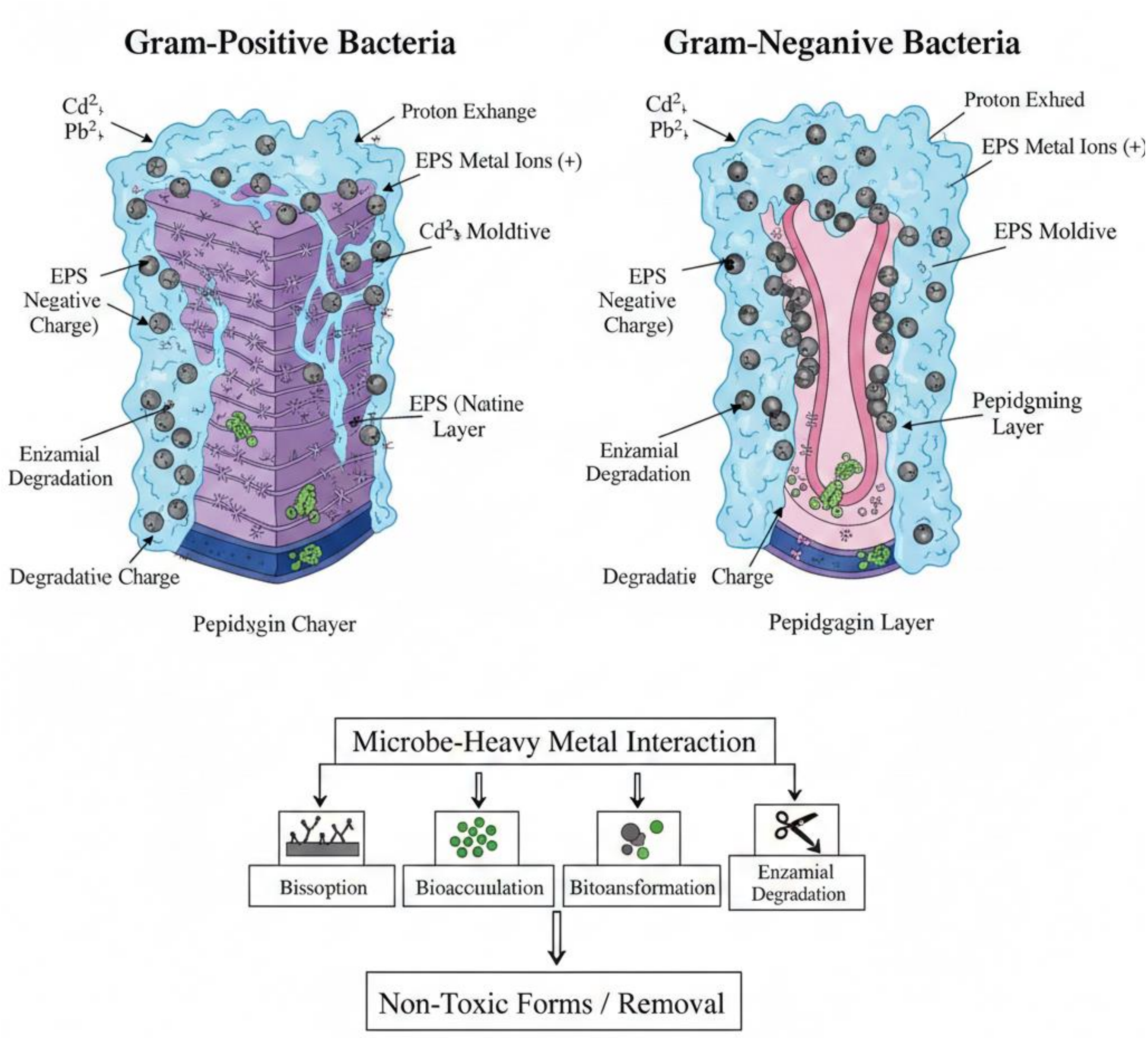

Figure 2. Mechanisms of Heavy metal biosorption via microbial EPS and cell walls

The mechanism of adsorption and biosorption through EPS is an essential process for removing heavy toxic metal contaminants from the environment and is also friendly to nature & cost-effective to the global.

4. Application of EPS in Bioremediation

Recently the environment aroused contamination of heavy metals and caused a high risk to human health [30]. In the environment, there is an accumulation of heavy metals due to an excess amount of metal mining operations such as electrical machinery, municipal sewage, chemical fertilizers, and disposal pesticides. Overcoming these environmental contaminants by using microorganisms in absorption and biosorption processes which are the recent biotechnology tactics for bioremediation. Hazardous metal can be removed by physical and chemical processes in comparison to bioremediation by microbes that can be helpful to the environment [31, 32]. Conventional remediation is cheaper and does not produce dangerous by-products that can affect the environment. The researcher is curious about microorganisms EPS due to their structure, physical and chemical diversity, and cleaning the environment through the most demanding bioremediation technology [9]. Removing the heavy metal by using it as a novel source of EPS in various sources, adsorption of various heavy metals by bacterial EPS such as *Pseudomonas pachastrellae, Bacillus firmus, and Bacillus cereus* will absorb the heavy metals toxically and clean up the environment. EPS is used for toxic chemical removal from the soil by flocculating metal sorption process using bacteria *Rhizobium sp*. that shows great metal binding capacity. EPS is also used for oil spills in soil and water which can be removed by using different EPS in the biodegradation process by removing the oil and oil aggregate formation. There is another resource of our life is textiles from textile industries a lot of polluted dye Methylene Blue is released into soil and water which causes major environmental pollution [33]. To remove the pollution from soil and water using a bioremediation process by microorganism EPS will degrade the methylene blue by biosorption which is effective and environmentally safe for the biodegradation of hazardous textile dye. There is another way to clean the environment pollution heavy metal toxicity by using a gel composed of fluid crosslinked with EPS to form a three-dimensional polymeric network in a gel matrix that can absorb the huge biological fluids contaminants from the soil and water. Hydrogel can be used as a platform for drug release and proliferation [34]. Fibers mimic the extracellular matrix and can be used as antibacterial, wound dressing materials widely proposed. Biological modification is a potential alternative way to improve pollutant environmental conditions. Microorganisms are genetically engineered to increase the binding sites of the microbes for the sorption of the metals. This will also improve the environmental conditions. However, there are multiple ways to clean up the environment from heavy toxicity by using the EPS with the combination of a bioremediation process that is effective and eco-friendly. Therefore, using bioremediation is an attractive and time-consuming process.

5. Conclusion

Heavy metal contamination has emerged as one of the most critical environmental challenges due to rapid industrialization, urbanization, agricultural intensification and improper waste management. Heavy toxic metals like lead, cadmium, chromium, mercury and nickel persist in soil and water bodies because of their non-biodegradable nature, leading to bioaccumulation through the food chain. Hence, their accumulation poses sever ecological disturbance and significant human health risks, including carcinogenic, mutagenic, renal, neurological and severe respiratory disorders. Conventional physical and chemical remediation techniques, although effective to some extent, often involve high operational costs, secondary pollution, and generation of hazardous by-products. In contrast, bioremediation has emerged as a sustainable, eco-friendly, and cost-effective alternative. Microorganisms possess remarkable adaptive mechanisms to survive in heavy metal–contaminated environments through processes such as biosorption, bioaccumulation, biotransformation, biomineralization, and enzymatic detoxification. Among these strategies, extracellular polymeric substances (EPS) play a central role due to their high molecular weight structure, abundance of functional groups (carboxyl, hydroxyl, phosphate, sulphate), and strong affinity toward positively charged metal ions. In conclusion, microbial biopolymers and EPS-based bioremediation represent a promising, sustainable, and environmentally responsible strategy for mitigating heavy metal pollution. With continued technological advancements and interdisciplinary research, bioremediation has the potential to become a primary approach for restoring contaminated ecosystems and safeguarding human health.

Competing interests

The authors report no conflicts of interest

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